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Physical, mechanical, and antioxidant properties of alginate/pectin edible films with incorporated chokeberry and wild thyme extracts

Svetla Maksimova Dyankova, Ayten Osman Solak*

Department of Cryobiology and Biologically active substances, Institute of Cryobiology and Food Technologies – Agricultural Academy, Sofia, Bulgaria

**Corresponding author: svetla.diankova@ikht.bg*

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Abstract: The purpose of the present study was to develop edible composite films based on sodium alginate and pectin with incorporated *Aronia melanocarpa* (Michx.) Elliot and *Thymus serpyllum* L. extracts. The influence of the extracts on the physicochemical, optical, mechanical, and antioxidant properties of the films was investigated. The addition of the extracts changed the colour parameters and significantly increased the barrier properties to UV and visible light. This effect is more pronounced in the film with chokeberry extract and is due to the anthocyanins contained in it (75.43 μg cyd eq·g^{-1}, cyd eq – cyanidin equivalents). The antioxidant activity of films with incorporated extracts was 4-fold (thyme) to 7-fold (chokeberry) higher than the control alginate/pectin film. A significant improvement in the mechanical characteristics of the films with extracts was found. The values for tensile strength were 9.41 MPa (chokeberry) and 9.54 MPa (thyme), while for the film without extract – 4.63 MPa. The resulting films could find potential application as active packaging with antioxidant properties, which could increase the quality and extend the shelf life of the foods packaged in them.

Keywords: active food packaging; antioxidant activity; sodium alginate

The development of edible films and coatings incorporating biologically active substances from plant extracts has undergone significant development in recent decades. The term edible films and coatings defines structures applied to the surface of the food product and intended to act as a barrier between the food and the surrounding environment, supporting the outer packaging in its protective role (Nogueira et al. 2019). They are made from natural biopolymers and can be consumed with the packaged food. As food hydrocolloids with film-forming properties, pectin and sodium alginate can serve as matrices for the production of biodegradable, environmentally friendly and edible packaging films (Yong and Liu 2020).

The inclusion of active components in the polymer matrix gives new functionality to the packaging film

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– antimicrobial and/or antioxidant properties, desirable colour, aroma, and taste, thereby expanding its applicability as an active packaging (Azeredo et al. 2016).

The first active packaging systems made of synthetic materials were developed as early as the 1980s and have a wide range of applications, both in terms of functions and technological solutions (Ahmed et al. 2022).

Active packaging, in addition to the traditional protective function of packaging, can serve to extend the shelf life and improve the quality, safety or sensory properties of the packaged food. Currently, much research is focused on the development of active packaging with antimicrobial and/or antioxidant properties by incorporating extracts of medicinal or edible plants into biodegradable materials of natural origin (Talón et al. 2017; Ahmed et al. 2022).

Lipid oxidation is one of the main causes of quality deterioration in foods with high lipid content such as nuts, vegetable oils, meat or fish products. Oxidation of lipids leads to rancidity and the appearance of an unpleasant taste and smell, formation of toxic aldehydes, and loss of nutritional value. These characteristics make the product unacceptable for human consumption. One of the strategies to reduce lipid oxidation is by including antioxidant agents in the package. The increased interest in the use of antioxidants from natural sources is driven by concerns about the safety of synthetic antioxidants such as butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT) (Gómez-Estaca et al. 2014).

In recent years, increasing attention has been paid to the development of active and intelligent biopolymer packaging films incorporating anthocyanins from various plant sources (Wang et al. 2019; Yong and Liu 2020). The use of fruit purees, juices, and fruit extracts in edible biopolymer films of starch, pectin, and chitosan has been researched (Yoshida et al. 2014; Azeredo et al. 2016; Nogueira et al. 2019). The inclusion of anthocyanin-rich extracts leads to an increase in the antioxidant capacity of the films, which can be used as active packaging to protect foods with a high-fat content from oxidation (Wang et al. 2019).

A significant amount of research has been done to obtain edible films with active components from medicinal plants. Herb and spice extracts are good sources of a variety of bioactive compounds, including polyphenols, terpenoids, and flavonoids, which have high antioxidant and antimicrobial activity (Nogueira et al. 2020). Mahcene et al. (2020) developed sodium alginate films incorporating essential oils from *Rosmarinus officinalis* L., *Artemisia herba-alba* Asso, *Ocimum basilicum* L., and *Mentha pulegium* L. A high antibacterial activity and a strong increase in the antioxidant capacity were found compared to the control films. Edible chitosan films were obtained with *Pistacia terebinthus* extracts, which showed strong antioxidant and antimicrobial effects (Kaya et al. 2018). The addition of rosemary extract to cassava starch films leads to an increase in barrier properties against UV radiation (Piñeros-Hernandez et al. 2017). Similar effects were also found with a gelatin film with included *Ginkgo biloba* extract (Hu et al. 2019). However, most researchers report that the addition of plant extracts rich in polyphenols and flavonoids in the biopolymer matrix, besides increasing the antioxidant capacity of the film, can also affect (in some cases negatively) its mechanical and optical properties (Halász and Csóka 2018; Hu et al. 2019; Nogueira et al. 2019).

The aim of the present study was to develop edible composite films based on sodium alginate and pectin with included chokeberry and thyme extracts, and to evaluate their physico-chemical, optical, mechanical, and antioxidant properties.

MATERIAL AND METHODS

Material. Sodium alginate was supplied by Biosynth AG (Switzerland), and high methoxyl apple pectin (DE 69.20%) – by Obipektin (Switzerland). Fresh fruits of *Aronia melanocarpa* (Michx.) Elliot, cultivated in the area of the village of Baylovo, Sofia region, were used to obtain the water-ethanol extract. For the preparation of water-ethanol extract of wild thyme *(Thymus serpyllum* L.), dried aerial parts of the herb, purchased from a herbal pharmacy in the city of Sofia, were used.

Calcium chloride, sodium acetate, potassium persulfate, and hydrochloric acid were supplied by Merck (Germany). Ethanol, glycerol, and sodium carbonate were from Valerus (Bulgaria). Methanol, gallic acid, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), and Folin-Ciocalteu's phenol reagent provided by Sigma-Aldrich (Germany) were used to carry out the analyses. All reagents and chemicals used were analytical grade.

Preparation of chokeberry extract. Prior to extraction, the chokeberry fruit was pressed to separate the juice from the pomace. The pomace extraction was performed with 75% *v*/*v* ethanol, acidified with 0.1% HCl and plant material : solvent ratio – 1 : 2. Samples were sonicated for 20 min (Ultrasonic System, M 7652; DIMOFF, Bulgaria) and then left in the dark at room temperature for 48 h (Solak et al. 2023). The extracts

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obtained were filtered through a 100 µm sintered glass filter and stored in the dark at 4 °C.

Preparation of wild thyme extract. The extraction was carried out with 70% *v*/*v* ethanol at room temperature for 7 days with plant material : solvent ratio – 1:10 (Solak et al. 2022). The extracts obtained were filtered through a 100 µm sintered glass filter and stored in the dark at 4 °C.

Determination of dry matter content in the extracts. Dry matter was determined according to BDS EN 12145:2000 and expressed in %.

Determination of total phenolic compounds. The content of total phenolic substances (*TPC*) in the extracts was determined by spectrophotometric method with Folin-Ciocalteu reagent and expressed as gallic acid equivalents (mg GAE·mL^{-1}, GAE – gallic acid equivalents) (Singleton et al. 1999).

Total monomeric anthocyanins content in the chokeberry extract. The content of total monomeric anthocyanins (*TMA*) in the chokeberry extract was determined by the pH-differential method (Lee et al. 2005). The absorbance of samples in buffers with pH 1.0 and 4.5 and wavelength 520 nm and 700 nm were measured. The calculations were made according to Equation 1, and the results were presented as mg cyd eq·L^{-1} (for the extract) or µg cyd eq·g^{-1} (for the film) (cyd eq – cyanidin equivalents).

$$TMA = \frac{A \times Mw \times DF \times 10^3}{\varepsilon \times l} \quad (1)$$

where: *TMA* – content of total monomeric anthocyanins; *A* – absorbance of samples, [(A_{520}–A_{700} nm) pH 1.0 – (A_{520}–A_{700} nm) pH 4.5]; *Mw* – molecular weight [449.2 g·mol^{-1} for cyanidin-3-glucoside (cyd-3-glu)]; *DF* – dilution factor; 10^3 – factor for conversion from g to mg; ε – molar extinction (26 900 for cyd-3-glu); *l* – pathlength (cm).

Preparation of films. Sodium alginate (3.0% *w*/*v*) and pectin (4.0% *w*/*v*) were dissolved separately in distilled water. Film forming solution was prepared by mixing sodium alginate and high methoxyl pectin solutions at the ratio of 3:1. The mixture was homogenised, and glycerol was used as a plasticiser (Solak et al. 2023). To obtain the test groups of films, 4 mL of chokeberry extract (FA) or 4 mL of thyme extract (FT) respectively, were added to 96 g of the film-forming solution. For the control group (F0), extracts were replaced with the corresponding amount of 50% ethanol. The prepared mixtures were sonicated for 10 min and treated under a vacuum to remove air bubbles. A quantity of 90 mL of each mixture was poured onto Petri dishes (*d* = 14 cm) and dried under vacuum (20 kPa, SPT-200 Vacuum Drier; Valerus, Bulgaria) at 35 °C. Dried samples were immersed for 30 min in 0.3 M $CaCl_2$ solution to allow cross-linking (Solak et al. 2022), washed with distilled water and dried at 25 °C.

Moisture content. The moisture content (%) of film samples was measured with Sartorius Thermo Control YTC 01L balances (Sartorius AG, Germany).

Film thickness. The film thickness was determined with a digital micrometer (IP65; Mitutoyo, Japan) with an accuracy of 0.01 mm ± 5% in five randomly selected sections of the film.

Mechanical properties of films. The tensile strength (*TS*) and elongation at break (*E*) were determined according to BDS EN ISO 527-3:2003 with macro mechanical and tribology tester UMT:2M (CETR, USA). Six samples from control and test film groups, with dimensions of 55 mm by 6 mm were examined. The test was conducted at room temperature (20 ± 2 °C), speed – 0.017 mm·s^{-1}, sensor – 1 000 N. The results are presented as average values ± SD (standard deviation) of 6 measurements for each sample.

Optical properties of films. The colour parameters of the extracts and developed films were evaluated with NR200 portable digital colourimeter (Huanyu, China), using the International Commission on Illumination (CIE) Lab scale. The values of lightness (*L*), redness (*a*), and yellowness (*b*) of the films were measured. The background value was estimated to L^* = 96.05, a^* = 0.04, and b^* = –1.99. Measurements were performed on five random points of each film. The parameters of total colour difference (Δ*E*) were calculated by Equation 2:

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

Light transmission (LT) of films was determined on a UV-Vis spectrophotometer (Libra S 22 UV-Vis; Biochrom, USA) at wavelengths – 200, 280, 350, 400, 540, 600, 660, and 700 nm. Three measurements of the three film samples from the test and control groups were performed.

Antioxidant activity. The antioxidant activity of the extracts was evaluated by the DPPH method (Brand-Williams et al. 1995) with a slight modification: 0.6 mL of 0.2 mM DPPH solution in methanol

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was mixed with 0.9 mL of methanol and 0.5 mL of the corresponding dilution of the extract in 80% methanol. The samples were incubated for 60 min in the dark at room temperature, and the decrease in absorbance at 517 nm was recorded. In the control, the sample solution is replaced by 0.5 mL of 80% methanol.

To determine the antioxidant activity of the films, samples of 0.5 g were ground and mixed with 10 mL of 50% *v*/*v* methanol. After stirring for 1 h, the mixtures were centrifuged at 3 000 g for 10 min (Beckman J2-21M; Beckman Coulter, USA) and the supernatants were analysed according to the described procedure. Antioxidant activity was calculated against a Trolox standard curve (1.0 to 15.0 μg·m^{-1}) and was expressed as Trolox equivalents – μg TE·mL^{-1} (for the extract) or μg TE·g^{-1} (for the film).

Statistical analysis. All experiments were performed with three or six replicates. The data were analysed with Microsoft Excel (2016 software). Analysis of variance and post hoc Tukey test were used. A significance level of $P < 0.05$ was adopted for all comparisons. The results were presented as mean ± SD.

RESULTS AND DISCUSSION

Characterisation of chokeberry and thyme extracts

The obtained extracts were free-flowing liquids with a characteristic odour and a dark red (chokeberry) or dark brown (thyme) colour. Visual observation was confirmed by the instrumentally determined colour parameters (Table 1). In the chokeberry extract the red (+*a*) and to a lesser extent the blue (–*b*) components prevailed, while in the thyme extract the chromaticity parameters were in the yellow (+*b*) and green (–*a*) zones. The dark colour of both extracts was in concordance with low lightness values (*L*) (22.80 and 24.93 respectively). The dark red colour of the *A. melanocarpa* extract is due to the high content of anthocyanins (1 746.7 mg cyd eq·L^{-1}). *A. melanocarpa* is one of the fruits with the highest content of polyphenols and anthocyanins (Kim et al. 2021). In the present work, extraction of wet pomace, remaining after separation of the juice, was carried out. It was reported that the content of polyphenols and anthocyanins in pomace remaining after juice production is significantly higher compared to juice and whole fruits (Kaloudi et al. 2022).

Both chokeberry and thyme extracts had a high phenolic content – 29.21 mg GAE·mL^{-1} and 18.83 mg GAE·mL^{-1}, respectively. According to Kaloudi et al. (2022) besides anthocyanins, the other polyphenolic compounds in chokeberry extract are polymeric proanthocyanins, quercetin glycosides, and phenolic acids (chlorogenic and neochlorogenic acid). In the ethanol extracts of thyme, the main phenolic components are rosmarinic, chlorogenic, caffeic and p-coumaric acid, luteolin glycosides, apigenin, naringenin and thymol (Janiak et al. 2017).

The antioxidant activity of the examined extracts was evaluated by determining their radical scavenging ability against DPPH. This assay is widely used to determine the antioxidant activity of crude extracts or purified compounds from plants. From the results in Table 1, it can be seen that the chokeberry extract has significantly higher antioxidant activity compared to the thyme extract.

Properties of sodium alginate/pectin composite films

Colour and optical properties. The obtained edible alginate/pectin films were homogeneous, elastic without brittle areas. Control film samples without extract (F0) were transparent with a slight yellowish tint. Films with chokeberry extract (FA) were translucent with a cherry-red colour, and films with thyme extract (FT) were translucent with a yellow-brownish colour (Figure 1).

The colour parameters of films are shown in Table 2. The *L* of F0 was 90.87, but it decreased significantly ($P < 0.05$) to 48.61 and 77.18 after blending with chokeberry and thyme extracts, respectively. The *a* and *b*-values of FA and FT were also significantly different from those of the control film ($P < 0.05$). Consequently, the total Δ*E* of FA and FT increased remarkably (56.34 and 35.13, respectively) compared with the control films (9.20). This result is in good accordance with the visible observation.

Table 1. Physico-chemical characterisation of extracts

Extracts	Colour parameters			Extractive substances (%)	*TPC* (mg GAE·mL^{-1})	*TMA* (mg cyd eq·L^{-1})	Antioxidant activity (mg TE·mL^{-1})
	L	*a*	*b*				
Chokeberry	22.80	9.45	3.70	7.19 ± 0.02	29.21 ± 0.82	1 746.7 ± 68.5	10.54 ± 0.47
Wild thyme	24.93	–2.35	5.77	2.32 ± 0.03	18.83 ± 0.76	–	2.82 ± 0.08

The results are presented as mean ± SD; *L* – lightness; *a* – redness; *b* – yellowness; *TPC* – total phenolic substances; *TMA* – total monomeric anthocyanins; GAE – gallic acid equivalents; cyd eq – cyanidin equivalents; TE – Trolox equivalents

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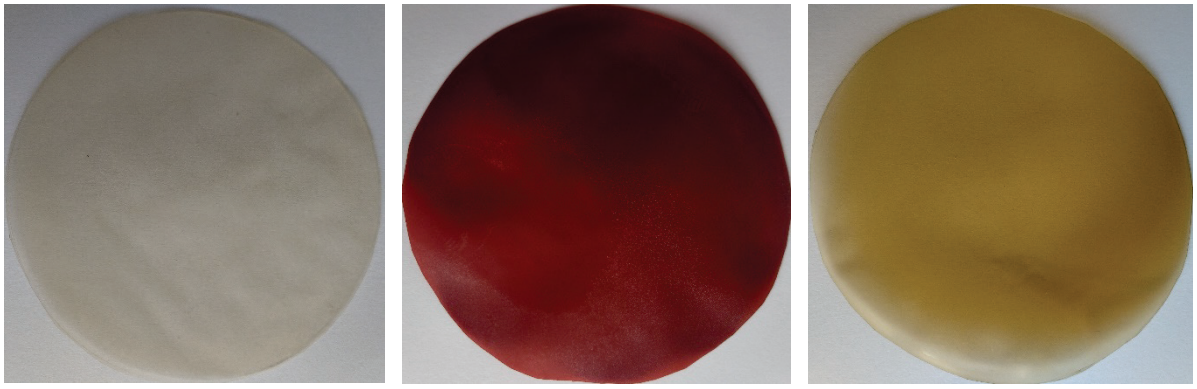

Figure 1. Photographs of alginate/pectin based edible films

F0 – control film; FA – film with chokeberry extract; FT – films with thyme extract

Many authors have reported similar changes in the colour parameters of biopolymer films after the incorporation of anthocyanin extracts obtained from different sources: grape skins (Ma and Wang 2016), red cabbage (Pourjavaher et al. 2017) or mulberry (Liu et al. 2019). When examining alginate films with chokeberry extract, Kim et al. (2018) also found a significant decrease in *L* and an increase in *a* compared to the control. In FT, the colour change was expressed in a strong increase in *b* (yellowness) compared to F0, which is in agreement with the results obtained for biopolymer films incorporated with thyme extracts (Talón et al. 2017; Olcay and Sarıçoban 2022).

UV-Vis light transmission at specific wavelengths (200–700 nm) of the films is presented in Table 3. Barrier properties to light are an important parameter that shows how well the packaging material can prevent the photooxidation of lipids, vitamins, and pigments in the food and, consequently, the loss of nutrients in the product. The films with extracts had a significantly lower light transmission at wavelengths from 200 to 400 nm (UV region) than the control film. The presence of phenolic compounds responsible for the absorption of UV radiation may explain this result. FA is a very good barrier at UV-B and UV-A wavelengths (280 and 350 nm, respectively). Compared to FA, the light transmission values for FT were slightly higher at 200 and 280 nm and even lower at 350 nm (1.7%). FA also showed good barrier properties to visible light (400–700 nm). Other authors also found better barrier properties to light in edible films with included anthocyanins (Kim et al. 2018; Yun et al. 2019).

Table 2. Colour parameters of the control (F0) and experimental (FA and FT) films

Film sample	*L*	*a*	*b*	Δ*E*
F0	90.87 ± 0.35^{a}	0.55 ± 0.12^{a}	5.66 ± 0.46^{a}	9.20 ± 0.69^{a}
FA	48.61 ± 2.87^{b}	21.11 ± 0.27^{b}	19.85 ± 0.24^{b}	56.34 ± 2.48^{b}
FT	77.18 ± 0.90^{c}	5.77 ± 0.35^{c}	27.08 ± 1.17^{c}	35.13 ± 1.50^{c}

$^{a–c}$ any values in the same column followed by the different letters are significantly ($P < 0.05$) different; each value is the mean of five replicates with the standard deviation; *L* – lightness; *a* – redness; *b* – yellowness; Δ*E* – total colour difference; F0 – control film; FA – film with chokeberry extract; FT – films with thyme extract

Table 3. UV-Vis light transmission (%) of the control (F0) and test (FA and FT) films

Film sample	Wavelength (nm)							
	200	280	350	400	500	540	600	700
F0	62.9	51.8	48.2	62.5	78.2	81.1	81.9	83.7
FA	13.4	7.6	1.9	10.0	21.3	29.9	39.5	45.0
FT	26.5	16.4	1.7	17.4	51.4	59.9	67.0	70.1

F0 – control film; FA – film with chokeberry extract; FT – films with thyme extract

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Table 4. Moisture content (*MC*), anthocyanin content (*TMA*), and antioxidant activity of the control (F0) and test (FA and FT) films

Film sample	*MC* (%)	*TMA* (μg cyd eq·g^{-1})	Antioxidant activity (μg TE·g^{-1})
F0	9.56 ± 0.83^{a}	–	2.11 ± 0.10^{a}
FA	11.24 ± 0.53^{b}	75.43 ± 1.3	14.71 ± 0.14^{b}
FT	10.29 ± 0.72^{a}	–	8.86 ± 0.27^{c}

$^{a–c}$ means with different letters in the same column are significantly ($P < 0.05$) different; each value is the mean of three replicates with the standard deviation; F0 – control film; FA – film with chokeberry extract; FT – films with thyme extract; cyd eq – cyanidin equivalents; TE – Trolox equivalents

Moisture content, anthocyanin content, and antioxidant activity. The addition of chokeberry extract significantly affected the moisture content (*MC*) ($P < 0.05$) of the film compared to the control (Table 4). A slight increase in *MC* was also observed for the film with thyme extract, but the result was not significant ($P > 0.05$). According to data cited in the literature, the *MC* in anthocyanin-enriched biopolymer films is affected by both the source and concentration of the anthocyanins, as well as the nature of the biopolymer and storage conditions (Yong and Liu 2020). Liu et al. (2019) reported decreased *MC* in k-carrageenan films with included mulberry extracts. On the other hand, Kim et al. (2018) found a reliable increase in *MC* in alginate films with chokeberry extract.

The amount of *TMA* in FA was relatively high – 75.43 μg cyd eq·g^{-1}. Anthocyanins in FA not only cause changes in the colour and light transmission but also increase its antioxidant activity.

The control film F0 showed weak antioxidant activity (2.11 μg TE·g^{-1}), most likely due to the pectin component. In a comparative study of the antioxidant activity of 5 polysaccharides, Ro et al. (2013) reported the highest values for high methoxyl apple pectin.

The inclusion of the plant extracts resulted in a significant ($P < 0.05$) increase in the antioxidant activity of the films (8.86 μg TE·g^{-1} for FT and 14.71 μg TE·g^{-1} for FA), which agrees with the results observed by many researchers (Piñeros-Hernandez et al. 2017; Talón et al. 2017; Kim et al. 2018). The use of biopolymer films with antioxidant activity in model food systems, shows an extension of storage stability. Wang et al. (2019) reported significantly delayed olive oil oxidative deterioration when using a film composed of gelatin and anthocyanins loaded chitosan hydrochloride/carboxymethyl chitosan nanocomplexes.

Thickness and mechanical properties. The thickness of FT was higher than that of F0 and FA, but the difference was not significant ($P > 0.05$) (Table 5). The mechanical properties of packaging films directly affect the quality and their ability to maintain the integrity of the packaged product. The parameters investigated in this test were tensile strength (*TS*) and elongation at break (*E*). The inclusion of extracts had a significant effect ($P < 0.05$) on the *TS* of the resulting films. In FA, the increase in *TS* was more than two-fold (9.41 MPa). Many researchers have reported that the addition of anthocyanins significantly increases *TS* of the films, which is related to the formation of a more stable and denser film network (Koosha and Hamedi 2019; Liu et al. 2019; Yong and Liu 2020). However, some researchers have found a decrease in *TS* of anthocyanin-rich films, and in these cases the sources of anthocyanins were not water-ethanol extracts, but fruit juices or pulp mixed directly with the film-forming suspensions (Azeredo et al. 2016; Nogueira et al. 2019). Results for FT were similar to those obtained for FA. Again, a more than two-fold increase in *TS* (9.54 MPa) was observed compared to F0. Talón et al. (2017) also found an increase in *TS* in chitosan films with incorporated thyme extract.

Table 5. Thickness, tensile strength (*TS*), elongation at break (*E*), and Young's modulus (*YM*) of films based on alginate/pectin as the control (F0) and test (FA and FT) films

Film sample	Thickness (mm)	*TS* (MPa)	*YM* (MPa)	*E* (%)
F0	0.29 ± 0.07^{a}	4.63 ± 0.55^{a}	5.46 ± 1.30^{a}	87.65 ± 17.90^{a}
FA	0.29 ± 0.06^{a}	9.41 ± 1.17^{b}	10.57 ± 2.03^{b}	71.27 ± 7.87^{a}
FT	0.32 ± 0.04^{a}	9.54 ± 0.89^{b}	7.92 ± 0.87^{c}	82.11 ± 5.23^{a}

$^{a–c}$ means with different letters in the same column are significantly ($P < 0.05$) different; each value is the mean of six replicates with the standard deviation; F0 – control film; FA – film with chokeberry extract; FT – films with thyme extract

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Elongation at break is the maximum capability of the films to resist changes in their length. The high values for *E* in the alginate/pectin film without extract (87.65%) are due to the plasticiser used (glycerin). FA and FT showed slightly lower *E* than those of the control film, but the differences were not significant ($P > 0.05$).

CONCLUSION

In this study, edible films based on sodium alginate and pectin with incorporated water-ethanol extracts of chokeberry and thyme were successfully developed. Films with plant extracts were homogeneous, elastic, and translucent with a cherry-red colour (chokeberry) or a yellow-brownish colour (thyme). The addition of chokeberry and thyme extracts significantly increased the light barrier properties of the obtained films, especially in the UV region. A 4-fold (film with thyme extract) to 7-fold (film with chokeberry extract) increase in antioxidant activity compared to the control was observed. A significant improvement was found in the mechanical properties of the films with incorporated extracts. The resulting films can be used as active packaging with antioxidant properties, capable of inhibiting lipid oxidation, and leading to improved quality and extended shelf life of packaged foods.

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