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Original article

Development of simultaneous antioxidant and visual pH-sensing films based on guar gum loaded with *Aronia melanocarpa* extract

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Summary Anthocyanins have attracted increasing attention for different packaging systems due to having functional features such as biocompatibility, antioxidative activity, visible colour response at varying pH values. In this study, the extract of *Aronia melanocarpa* as anthocyanins source was incorporated into guar gum films to take advantage of both antioxidant and pH responsive attributes. Aronia addition did not affect the thermal stability of guar gum films. Radical scavenging activity of the films (%) was measured by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay, and aronia added films exhibited very strong antioxidant activity (up to 85%). The colour of aronia solution varied from pink to brown with pH ranging from 1 to 13. Similarly, when the films were immersed in buffer solutions at different pH values, the visual colour varied from pink to brownish yellow. ΔE values of GR_AR_3 (guar gum films having the highest aronia concentration) ranged between 23.31 and 40.62 at pH 1–13. This result proved colour change of the films can be even detected by untrained consumers. Furthermore, the films were found to be very sensitive to ammonia vapour. Aronia incorporated guar gum films could be suggested as both antioxidant films to prevent foods oxidation from oxidation and promising intelligent films to monitor the deterioration of foods.

Keywords anthocyanin, antioxidant activity, *Aronia melanocarpa* extract, guar gum film, intelligent films, pH indicator.

Introduction

Packaging has a vital role in protection of food from ambient conditions, providing shelf life and advertising. However, over the past years, the expectance from packaging has evolved and according to customers, just maintenance of food product quality has not been seen satisfying. Depending on consumer demands, extension of shelf life and monitoring food quality are the new missions of the food packages. In that concept, intelligent packaging could be defined as a packaging system that indicates the quality of foods before consumption during storage and give information to customers (Kuswandi *et al.*, 2011). To design an intelligent package, a kind of indicators showing time–temperature relation, humidity data, freshness labels, and biosensors are added to the packaging system. Among them, freshness indicators provide sign of microbial and chemical deterioration during shelf life. Rather than sensors, the basic indicators usually depend on colorimetric changes as a sign of the temperature, time, and pH, oxygen. pH of foods is the most significant response to deterioration due to undesired microbial growth and chemical changes. In general, pH indicators consist of a dye changing colour with respect to pH which has the advantages of sensitivity, low cost, and quick answer. pH-sensitive dyes could be composed of synthetic and natural pigments. The common synthetic pigments that can be used as pH-sensitive dye are bromocresol purple, cresol red, bromocresol green, methyl orange, bromophenol blue,

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and chlorophenol red. However, due to the possible toxicity, customers could keep their distance from this kind of intelligent film. Thus, there is an increasing trend to study the applicability of natural plant-origin pigments as colorimetric pH indicators instead of synthetic ones (Lee *et al.*, 2015; Balbinot-Alfaro *et al.*, 2019). It is noteworthy that as plant-origin pigments, anthocyanins step forward due to having high sensitivity to colour change at the pH range. Anthocyanins are plant-based, water-soluble, nontoxic pigments having purple, blue and red colour. Due to their conjugate structure, anthocyanins show sensitive colour reactions in the function of pH and it makes them a great option as indicators to be used in intelligent film development (Ma & Wang, 2016; Ma *et al.*, 2017b). In recent years, several studies have reported the applicability of different kinds of anthocyanins extracted from purple sweet potato (Choi *et al.*, 2017; Li *et al.*, 2019), black rice bran (Wu *et al.*, 2019), red cabbage (Pourjavaher *et al.*, 2017; Liang *et al.*, 2019), grape skins (Ma & Wang, 2016), purple tomato (Li *et al.*, 2021), and black carrot (Moazami *et al.*, 2020) among others for the development of intelligent packaging.

Black chokeberry or aronia (*Aronia melanocarpa*) belongs to the Rosaceae family which is a plant originating from North America (Kapci *et al.*, 2013). Aronia has been recently grown in the eastern European countries and today it is transferred to Turkey which has a temperate climate. Aronia fruits show high resistance to damage during transportation, cold storage and frosting so the cultivation popularity of aronia has increased among the countries having convenient climate conditions (Tolić *et al.*, 2015). Anthocyanins, phenolic acids, flavanols, and proanthocyanidins are the types of polyphenols in the aronia fruits. The strong anthocyanin content comes from mostly cyanidin-3-galactoside and cyanidin-3-arabinoside, followed by cyanidin-3-glucoside, and cyanidin-3-xyloside (Denev *et al.*, 2012). Moreover, flavanols in aronia compose of mainly quercetin derivatives (quercetin-3-glucoside, 3-O-vicianoside, 3-O-robinobioside, 3-rutinoside, 3-galactoside) (Nawirska-Olszanska *et al.*, 2020). Aronia is rich in phenolic acids, especially chlorogenic and neochlorogenic acids (Veberic *et al.*, 2015). Due to high polyphenol content, aronia fruits show high antioxidative potential, which expand their usage for food products. In addition to showing strong antioxidant ability, aronia fruits display biological activities including anticancer, antiinflammatory, anticarcinogenic, antiatherogenic and antidiabetic effects (Kapci *et al.*, 2013). Nowadays, aronia is utilised in different fields of the food industry such as juice (Kardum & Takic, 2014; Vagiri & Jensen, 2017), dried fruits (Šavikin *et al.*, 2016; Nawirska-Olszanska *et al.*, 2020), wine (Gumienna *et al.*, 2011; Lachowicz *et al.*, 2017). Besides food products, due to the high anthocyanin content, aronia is a good alternative to produce intelligent film. In the literature, Halász & Csóka (2018) immobilised the extract of aronia into chitosan films to obtain an intelligent package, which had the colour response depending on the pH. Moreover, high phenolic content of aronia also could be used for the design of active film that could increase the shelf life of foods.

Various biodegradable polymers have been used to immobilise extracted anthocyanin, such as gellan gum (Wei *et al.*, 2017), cassava starch (Luchese *et al.*, 2018; Qin *et al.*, 2019), chitosan (Vo & Dang, 2019; Ebrahimi *et al.*, 2022), κ-carrageenan (Sun *et al.*, 2020). Guar gum is a water-soluble natural polysaccharide obtained from the seeds of *Cyamopsis tetragonolobus*. It was used to immobilise grape pomace as active packaging material (Saurabh *et al.*, 2015). Guar gum is as a galactomannan and consists of long linear β-(1.4)-mannose backbone to which α-(1.6)-linked galactose residues. It is produced from endosperm of *Cyamopsis tetragonoloba* (Cunha *et al.*, 2005; Saurabh *et al.*, 2015).

This study focused on developing a novel colorimetric pH indicator film. Anthocyanins extracted from aronia was incorporated into guar gum matrix. The sensing abilities of guar gum–aronia films to different pH values and ammonia were assessed. Furthermore, total phenolic content and antioxidant activity of the films were investigated in addition to thermal behaviour of the films.

Materials and methods

Materials

Guar gum was supplied from Katkı Dünyası Gemici Gıda Ticaret Ltd. Şti (İstanbul, Turkey) and aronia powder was bought from Liya Hanım Çiftliği (Canakkale, Turkey). Glycerol (≥99%), sodium carbonate Folin-Ciocalteau reagent, 2,2-diphenyl-1-picrylhydrazyl, hydrochloric acid, sodium bromide and sodium hydroxide were purchased from Sigma-Aldrich Chemie Gmbh (Darmstadt, Germany).

Methods

Film preparation

Guar gum (1% w/v) was dissolved in distilled water using magnetic stirrer at 500 r.p.m. for 4 h. To extract the phenolics, different concentrations of powder (5%, 10% and 15%) were mixed with ethanol:water (4:1) solution by using magnetic stirrer at 750 r.p.m. for 2 h at room temperature. Then, the suspension was centrifuged (Nüve, NF 800, Turkey) at 4000 r.p.m. for 15 min. To arrange the ratio of guar gum:aronia as 1:1, 1:2, 1:3 (w/w), guar gum solution was mixed with aronia solution. Glycerol at 0.15% (w/v) as plasticizer was added to guar gum/aronia solution. Guar gum/aronia solution (20 mL) was poured into petri dishes

(diameter is 9 cm) and dried for 48 h at room temperature in a chamber. The films were placed in desiccators having 57.7% RH (adjusted by saturated NaBr solution) at 25 °C (Greenspan, 1977). Guar gum/aronia films were coded as GR_AR_1, GR_AR_2, GR_AR_3, and only guar gum and glycerol containing film was used as control (labelled as GR).

Total phenolic content (TPC) of films

The TPC of guar gum/aronia films were determined by the modified Folin–Ciocalteu method. The film (0.1 g) was immersed in 10 mL ethanol:water (4:1) for 1 h and it was filtrated to collect supernatant. Then, the sample of 1 mL was mixed with 2.5 mL of Folin–Ciocalteu reagent (0.2 N) and left for 5 min in the dark. After that, 2 mL of sodium carbonate solution (7.5% w/v) was added to the previous mixture. Samples were stored in the dark for 1 h, and finally, the absorbance was measured at 760 nm by using a spectrophotometer (UV 1800, Shimadzu, Kyoto, Japan). Different concentrations of the gallic acid solutions (10, 20, 40, 60, 80 100, 133 mg/L) were prepared as described above for the calibration curve. Finally, TPC of films were calculated with eqn 1 as gallic acid equivalents (GAE) in milligrams per gram dry weight.

$$\text{TPC (mg GAE/g film)} = \frac{C \times V \times D}{W_S} \quad (1)$$

where V, D, C and W_s are the volume of the solution (L), the dilution factor, the concentration determined from the calibration curve (mg/L), and the weight of the film (g), respectively.

Antioxidant activity of films

The antioxidant activity of guar gum/aronia films was measured as the scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay (Aydogdu *et al.*, 2019). To carry out the analysis, first, 0.1 g of film was dissolved in 10 mL of ethanol/water (80/20). Then, 1 mL of the diluted sample was mixed with 3 mL of DPPH solution (25 ppm), and stored in the dark for 1 h. The absorbance of samples was measured at 517 nm. The antioxidant activity (% AA) of films was calculated by eqn 2.

$$\text{AA (\%)} = \frac{A_{\text{control}} - A_{\text{film}}}{A_{\text{control}}} \times 100 \quad (2)$$

where A_{control} and A_{film} refer to the absorbance of the DPPH solution without and with the dissolved films, respectively.

Thermogravimetric properties

The thermogravimetric analysis (TGA) and derivative thermogravimetry (DTG) of films were perfomed by using the SII Exstar TG/DTA 6300 (RTI Instruments, Inc., Woodland, USA) to measure thermal degradation temperatures of the samples. About 10-mg film pieces subjected to heating up to 450 °C at a rate of 10 °C/min under nitrogen purge at a flow rate of 50 mL/min.

Absorbance spectrum and response of guar gum/aronia solutions and films at different pH levels

Aronia is a pH-sensitive compound and its colour changes accordingly. It is known that each colour has a distinct characteristic peak at different nm. Therefore, to analyse the max absorbance peak of the aronia solutions at different pHs, this analysis was carried out.

The response of the guar gum–aronia solutions to the different buffer solutions (pH 1–13) was measured spectrophotometrically by using UV 1800, Shimadzu, Columbia, USA, wavelength between 300 and 600 nm.

Similarly, the colour response of the selected indicator film (GR_AR_3) to different buffer solutions (pH 1–13) was measured by the CIE Lab method as described by Wu *et al.* (2019).

First, 1 mL of each buffer solutions was dropped into the films with 1 cm x 1 cm dimensions. After 20 min, films were placed on a white plate and the CIELab colour parameters (L^*, a^*, and b^*) were measured with a Chroma Meter CR400 colorimeter (Konica Minolta, Inc., Tokyo, Japan). The total colour difference (ΔE) was calculated as follows:

$$\Delta E = \sqrt{(L_0{}^* - L^*)^2 + (a_0{}^* - a^*)^2 + (b_0{}^* - b^*)^2} \quad (3)$$

where $L_0{}^*$, $a_0{}^*$ and $b_0{}^*$ are the colour parameters of the film before soaking.

Sensitivity to ammonia

To illustrate possible colour changes of aronia-incorporated films when it is applied to a real food system, films were exposed to ammonia vapour as described by Yildiz *et al.* (2021) with some modifications. Different aronia concentration containing films covered the top (4.90 cm^2) of an Erlenmeyer flask containing 80 mL of ammonia solution (0.32 M). Colour changes of films were recorded in every 3 min for 30 min by colorimeter Chroma Meter CR400 (Konica Minolta, Inc., Japan) in terms of L^*, a^*, b^*. Then, using MATLAB 9.0 (R 2016a), the colour date was converted to RGB colour scale. Sensitivity of the films was calculated using the eqn 4 (Mohammadalinejhad *et al.*, 2020)

$$S_{\text{RGB}} = \frac{(R_a - R_b) + (G_a - G_b) + (B_a - B_b)}{(R_a + G_a + B_a)} \times 100 \quad (4)$$

where R, G, and B refer to the red, green and blue colour, respectively. Subscripts a and b represent the initial and final colour measurements, respectively.

Statistical analysis

Data were analysed by using MINITAB (version 16; State College, PA, USA). Analysis of variance (ANOVA)

was applied, and Tukey's Multiple Comparison Test was used for identification of the significance of differences among values ($P \leq 0.05$). Results were expressed as the arithmetical mean and the standard deviation (±) (SD) for 3 replicates.

Results and discussion

Moisture content, water vapour permeability (WVP), total phenolic content (TPC) and antioxidant activity of films

It is important to note that the moisture content can affect the film's physical, mechanical, and barrier properties, so it is essential to consider the intended use and storage conditions when designing films. The moisture content in guar gum films represents the water trapped within the film matrix. The range of moisture content observed in the film samples tested was from 19.37% to 21.76%. The addition of aronia did not have any significant effect on the moisture content of the films. This is because the hydrophilic compounds in the film containing extract can retain water molecules in their structure (Ebrahimi *et al.*, 2022). Other researchers have conducted studies that revealed that adding various extracts did not have a significant impact on the moisture content of films based on biopolymers (Qin *et al.*, 2019; Xue Mei *et al.*, 2020). The water vapour permeability (WVP) of a film determines its ability to prevent the transfer of water vapour between two sides of the film. To be effective for packaging purposes, it is important for a film to have a low WVP value to limit the passage of water vapour through the film (Kilic *et al.*, 2022b). WVP of the films have not been changed after aronia incorporation into guar gum film matrix. In other studies, it has been observed that the addition of phenolic compounds does not affect the water vapour permeability of the films (Andretta *et al.*, 2019; Ezati & Rhim, 2020).

Free radicals play a significant role in oxidative degradation and quality loss of foods. Due to showing radical scavenging activity, antioxidant compounds could be either incorporated to active packaging materials or added directly to food products. The films having antioxidative compounds are effective in reducing lipid oxidation and providing preferred food quality during shelf life (Nwude *et al.*, 2022). Table 1 indicats the TPC and antioxidant activity of guar gum films containing aronia. As expected, aronia incorporation increased TPC of films significantly ($P < 0.05$). Aronia is rich in terms of phenolic acids and flavonoids. The main phenolic compounds in aronia fruits are hydroxycinnamic derivatives (chlorogenic and neochlorogenic acids) and flavonoids (Quercetin 3-O-arabinoglucoside, Quercetin 3-O-galactoside, Quercetin 3-O-glucoside) (Romani *et al.*, 2016) and anthocyanins are accounted 57% of total phenolic content of aronia (Tian *et al.*, 2017).

The antioxidant activities of the films were determined by the DPPH scavenging method, which is based on the discoloration of DPPH. Films with higher phenolic content caused more discoloration of DPPH, and this resulted in lower absorbance value (Siripatrawan & Vitchayakitti, 2016). Phenolic compounds are active agents, and a significant relation was observed between phenolic content and the antioxidant activity of films. While GR_AR_1 film showed 16.22 mg GAE/g film TPC and 62.07% antioxidant activity, TPC and antioxidant activity of GR_AR_3 films were 52.92 mg GAE/g film and 85.39%, respectively. The phenolic compounds in aronia extract is the reason why guar gum films had an antioxidant activity. Oszmiański & Lachowicz (2016) also reported significant antioxidant activity of aronia extract due to having rich phenolic compounds. A similar observation was found in several studies that investigated films including phenolic sources could be regarded as antioxidant film. For example, Kim *et al.* (2020) incorporated goji berry extract into starch films and the films showing antioxidant activity were obtained. In another study, Nwude *et al.* (2022) showed that chitosan films having rice berry extract showed antioxidant activity of about 60%.

TGA analysis of films

TGA analysis records the weight loss of the material components as a consequence of the increase in temperature, giving information about the thermal

Table 1 Moisture content, water vapour permeability (WVP), total phenolic content (TPC) and DPPH free radical antioxidant capacity (%) of aronia–guar gum films

Film	Moisture content (%)	WVP × 10^{10} (g m^{-1} s^{-1} Pa^{-1})	TPC (mg GAE/g film)	Antioxidant activity (%)
GR_AR_1	20.12 ± 1.04^{a}	5.7581 ± 0.1841^{a}	16.22 ± 0.16^{c}	62.07 ± 0.88^{c}
GR_AR_2	21.76 ± 1.10^{a}	5.8989 ± 0.1852^{a}	28.40 ± 1.09^{b}	74.51 ± 1.17^{b}
GR_AR_3	19.37 ± 1.16^{a}	5.7629 ± 0.2611^{a}	52.92 ± 0.14^{a}	85.39 ± 1.02^{a}
GR	20.55 ± 1.13^{a}	5.2755 ± 0.1351^{a}		

The mean and the SD for $n = 3$ are reported. Different letters in the same column indicate significant differences among the means ($P \leq 0.05$).

stability. The thermal degradation curves of films based on guar gum and aronia incorporated guar gum films are shown in Fig. 1. Between 50 and 125 °C, all films exhibited approximately 10% weight loss due to the loss of moisture (Yildiz *et al.*, 2021). All the films had two stages of degradation and the first degradation temperature was observed at around 210 °C attributed to the decomposition of glycerol used as a plasticizer (Ezati & Rhim, 2020). Similar behaviour was observed in several studies about the packaging material design when glycerol is used as plasticizer (Ma & Wang, 2016; Nwude *et al.*, 2022). The second degradation stage of films was observed at around 275–325 °C mainly due to guar gum backbone degradation (Elsaeed *et al.*, 2021). According to the results, the incorporation of aronia did not significantly affect thermal stability of guar gum films. Similar observations were reported in the studies of Qin *et al.* (2019) and Liang *et al.* (2019). In these studies, incorporation anthocyanins extracted from fruits of *Lycium ruthenicum* and red cabbage did not affect thermal stability of gum and starch films, respectively. Therefore, aronia–guar gum films had similar thermal stability as reported in the literature.

Colour response of solutions and films at different pH values

Colour response to pH change is real-time monitoring of freshness and spoilage of packed foods. Anthocyanins are sensitive to pH, and their colour changes with respect to pH. This characteristic of antocyaninds make them one of the best option for the intelligent film aiming monitoring deterioration of foods (Oladzadabbasabadi *et al.*, 2022). However, anthocyanins decompose easily and their stability is affected from temperature, pH, light, oxygen. Furthermore, the stability of anthocyanins depends on the concentration and structure of them (Li *et al.*, 2021). The colour change of aronia solution at different pH values (pH 1–13) is shown in Fig. 2. As stated before, aronia is a good source of anthocyanins so aronia solutions represented a clear colour change under different pH values. At different pH values, aronia loaded in films resulted in distinct colour change from pink to brown. At acidic pH values, the colour of solutions was pink, and it turned to purple at pH 5–7. As the pH increased up to 8, the solution turned to dark green, then at basic pH values, the colour of solutions was brown. As stated in many studies, the main coloration compounds are flavylium cations, carbinol pseudobase, chalcones and quinone base in anthocyanin extract solutions. These four compounds are in pH-dependent equilibria (Li *et al.*, 2019). In the pH range of 1–3, the flavyliums cations were dominant and they gave the red colour to solution. When the pH of solution raised to 5, purple coloured carbinol was formed due to the nucleophilic reaction between flavonoid cations and water. Then, raising pH to alkaline conditions, by loss of cations from carbon and oxygen, dark purple blue quinoid anions reacted with phenolics, and a dark green colour is observed. At pH 12–13, further deprotonation resulted in chalcone formation that gave the brown colour to aronia loaded films (Li *et al.*, 2021; Roy & Rhim, 2021). Similar observations were previously reported by Nwude *et al.* (2022) and Liang *et al.* (2019) for rice berry and red cabbage phenolic extract in response to pH variation. In the study of Nwude *et al.* (2022), rice berry extract solutions showed pink colour at pH values of 2 and 4 and brown colour at pH 12. (Liang *et al.*, 2019) observes the colour change of red cabbage extract from pink to dark green with increasing pH 3 to 10. The visible spectra of aronia solutions at different pH values are

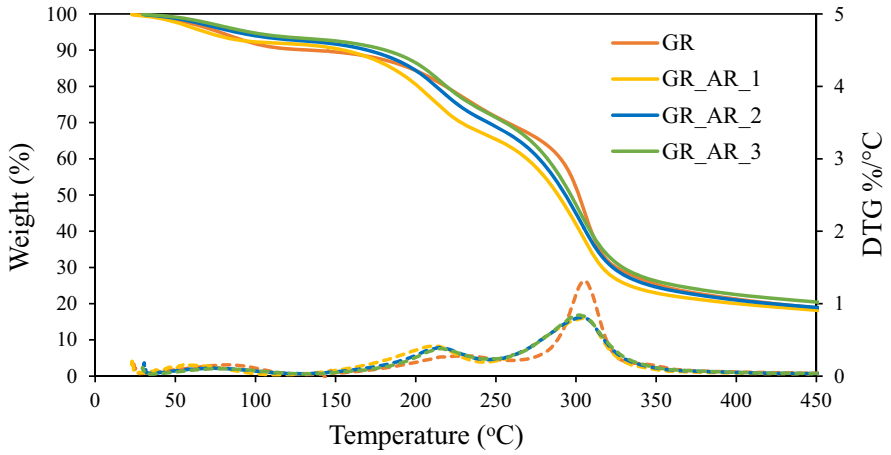

Figure 1 TGA curves of guar gum and aronia incorporated guar gum films (DTG refers to derivative thermo-gravimetric curve).

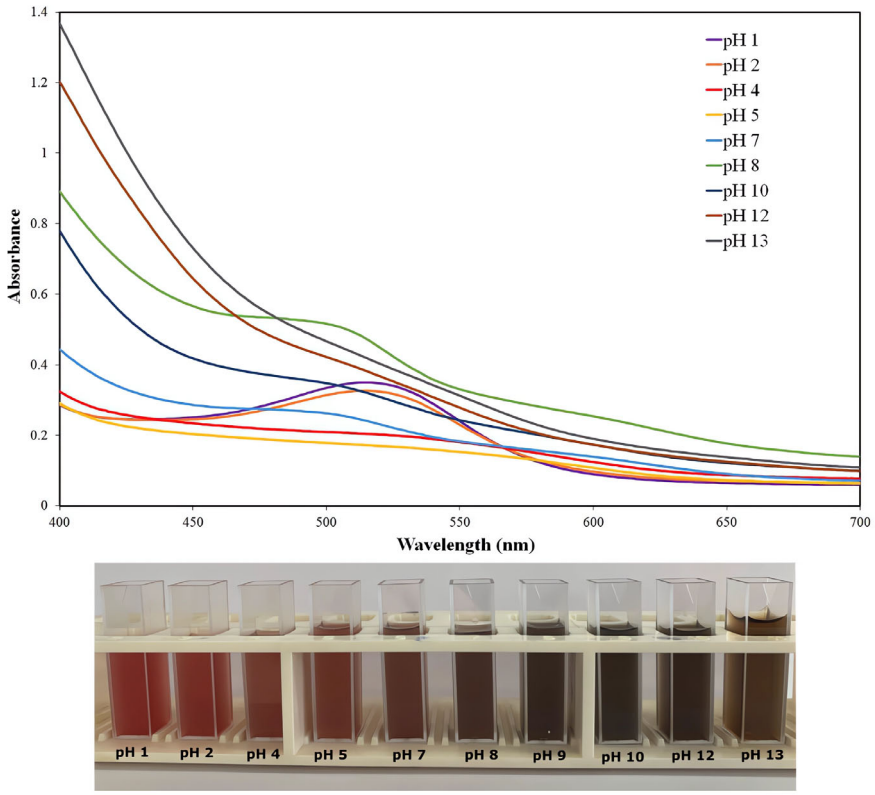

Figure 2 Spectra and colours of aronia solutions recorded from the 1.0–13.0 pH range.

shown in Fig. 2. The maximum absorption peak was observed at 530 nm at pH 1 and pH 2, which was attributed to flavylium cations (Liang *et al.*, 2019). As pH increased, the peak almost disappeared. At higher pH, flavylium cations undergo nucleophilic attack and form pseudo-base colourless carbinol and then chalcone. At basic pH, due to the deprotonation, flavylium cation forms neutral quinoidal base (Neuenfeldt *et al.*, 2022). Similar trend was observed in the studies of Ma & Wang (2016) and Sun *et al.* (2020) for extract of grape skins and *Prunus maackii* pomace, respectively.

The colour response parameters (L^*, a^*, b^* and ΔE) of aronia/guar gum films are shown in Table 2 and the pH sensitivity of GR_AR_3 films is indicated in Fig. 3. When aronia added films were dipped into the buffer at pH 1 and pH 2, a^* values became higher as indicator of red colour due to the dominant characteristics of flavylium cations in acidic medium. Films containing higher amounts of aronia showed higher redness value. When pH changed from acidic to alkaline conditions, the a^* values decreased gradually as evidence of greenness. At pH 8–10, the colour of aronia-incorporated guar gum films shifted through purple to grey due to the formation of quinonoidal bases or colourless carbinol pseudo-bases (Mohammadalinejhad *et al.*, 2020). With an increase in alkalinity, a very noticeable brownish yellow colour appeared and b^* value gradually increased from 8.97 to 43.77 for GR_AR_3. The colour change of barberry incorporated methyl cellulose/chitosan films also followed very similar trend. Its colour ranged from reddish (pH 5), pale pink (pH 6–8) and finally yellow (Alizadeh-Sani *et al.*, 2021). Likewise, in the study of paper flower extract incorporated potato starch films, yellowness (b^*) values of the samples start to increase when pH reached 9 (Naghdi *et al.*, 2021).

ΔE representing colour change is a significant parameter that examines the applicability of intelligent pH indicators. To detect the colour response according to pH change visually, ΔE must be higher than 5 (Prietto *et al.*, 2017). Larger ΔE values mean that aronia incorporated guar gum films have good colour variation depending on pH. As stated in Table 2, ΔE was found strongly dependent on aronia amount and GR_AR_3 films showed the highest colour change. Therefore, it is obvious that GR_AR films are potential in a sensitive visual pH indicator.

Table 2 CIELab colour parameters and the change in colour (ΔE) of aronia-incorporated guar gum films as a function of the pH values (1–13) of soaking buffers

Film	pH value	L^*	a^*	b^*	ΔE^*
GR_AR_1	1	90.52	8.87	4.45	11.26
	2	90.37	7.67	4.69	10.38
	4	92.80	2.51	4.68	5.42
	5	92.62	1.96	4.71	5.32
	7	92.05	1.11	5.17	5.37
	8	90.53	0.04	6.195	6.44
	10	92.20	1.42	7.75	4.93
	12	87.97	1.79	20.83	16.39
	13	88.10	1.51	22.71	17.89
GR_AR_2	1	85.78	14.79	7.24	18.50
	2	86.12	13.48	7.43	17.27
	4	89.79	4.66	7.32	8.50
	5	89.53	3.84	7.62	8.29
	7	88.05	2.16	7.79	9.12
	8	90.91	0.47	9.93	6.61
	10	88.23	3.05	12.93	10.83
	12	83.01	1.67	33.08	29.42
	13	82.89	1.99	37.21	33.17
GR_AR_3	1	82.14	19.24	8.97	24.31
	2	82.67	17.18	9.45	22.41
	4	86.74	6.59	9.57	12.33
	5	87.46	5.32	9.46	11.06
	7	85.64	3.26	10.22	12.11
	8	82.34	0.96	12.07	15.38
	10	84.82	4.66	16.22	15.76
	12	78.26	2.59	39.43	37.32
	13	79.43	2.75	43.77	40.62

Ammonium response of films

During the spoilage of protein rich foods such as chicken, pork, shrimp, beef and fish, volatile basic amines (trimethylaime, dimethylamine and ammonia) are produced due to enzymatic and bacterial activities. Therefore, these volatile nitrogen compounds amines are held responsible from the colour change of the intelligent packaging. Ammonia is selected as a representative compound to illustrate this alteration due to its lowest boiling point among the mentioned amines (Kilic *et al.*, 2022a). Gaseous ammonia diffuses in to the aronia incorporated guar gum film matrix and form hydrated structure ($NH_3{\cdot}H_2O$), then it produces ammonium ions (NH_4^+) and hydroxyl ions (OH^-). Ammonium ions led the formation of alkaline environment on the film surface and hydroxyl ions forms bond with hydroxyl groups of anthocyanin to produce oxygen anion. At the end, the change in ionic structure of the compound resulted in colour change (Koshy *et al.*, 2021).

The colour change of aronia-incorporated guar gum films at different ammonia concentration with respect to time is shown in Fig. 4. In previous studies showed that the colour change of the potential intelligent films depends on the exposed ammonia concentration, relative humidity and therefore, film forming matrix (Ma *et al.*, 2017a; Zhai *et al.*, 2020; Naghdi *et al.*, 2021). However, apart from these factors, this study also showed that the sensitivity and response time of the films to the ammonia vapours depend on the aronia concentration, in other words active compounds concentration. Sensitivity of the GR_AR_1 and GR_AR_2 increased after 12 and 9 min exposures, respectively. However, the sensitivity of GR_AR_3 films exhibited very sharp increment after 3 min compared to the other films. The gradual raise was reported until 9 min and after that sensitivity remained constant.

The colour response of the film should be fast enough to be suggested as a potential intelligent film layer. Therefore, GR_AR_3 could be advised for the potential applications.

Conclusion

In this study, different concentrations of aronia (5%, 10% and 15%) have been incorporated into the guar gum film matrix [guar gum:aronia as 1:1, 1:2, 1:3 (w/w)] to obtain both antioxidant and intelligent films. Films exhibited great radical scavenging activity due to high polyphenol content of aronia. Antioxidant activity of the films depended on the aronia concentration and activity ranged from 62% to 85%. The colour responses of films to the change in pH were also analysed. The visual colour changes from pink to yellow in the pH range of 1–13. Finally, films were exposed to 0.32 M ammonia solution to mimic the behaviour of basic amines that are produced during deterioration of high protein foods. The results showed that the response time and sensitivity of the films to ammonia depended on the aronia concentration in the film. As a result, this study showed that aronia incorporated guar gum colorimetric films can be suggested as a visual indicator for food quality assurance and antioxidant film layer material.

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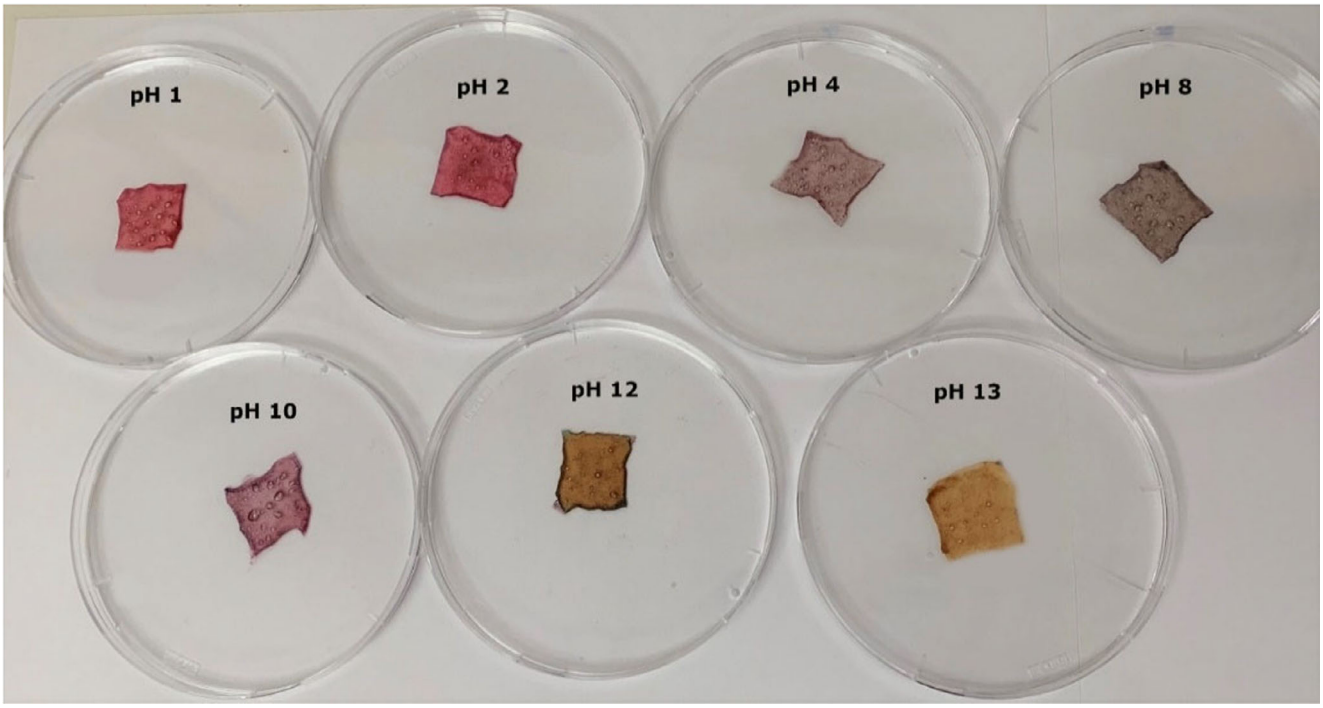

Figure 3 The colour change of GR_AR_3 films obtained after soaking in the pH buffer solutions indicated in each image.

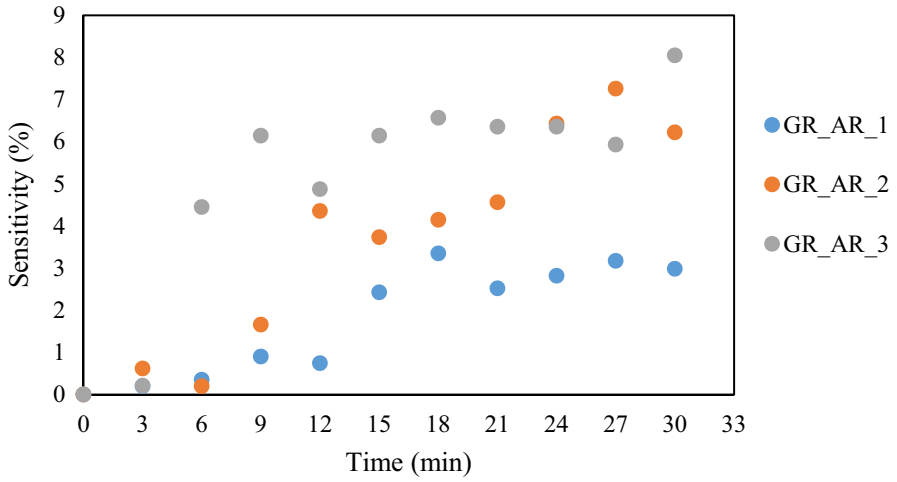

Figure 4 Sensitivity of the films towards 0.32 M ammonium solution.

Author contributions

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Conflict of interest

The authors declare no conflicting interests.

Peer review

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Alizadeh-Sani, M., Tavassoli, M., Mohammadian, E. *et al.* (2021). pH-responsive color indicator films based on methylcellulose/chitosan nanofiber and barberry anthocyanins for real-time monitoring of meat freshness. *International Journal of Biological Macromolecules*, **166**, 741–750.

Andretta, R., Luchese, C.L., Tessaro, I.C. & Spada, J.C. (2019). Development and characterization of pH-indicator films based on cassava starch and blueberry residue by thermocompression. *Food Hydrocolloids*, **93**, 317–324.

Aydogdu, A., Yildiz, E., Aydogdu, Y., Sumnu, G., Sahin, S. & Ayhan, Z. (2019). Enhancing oxidative stability of walnuts by using gallic acid loaded lentil flour based electrospun nanofibers as active packaging material. *Food Hydrocolloids*, **95**, 245–255.

This article is important for this manuscript. Because the antioxidant activity of guar gum/aronia films were done according to this article.

Balbinot-Alfaro, E., Craveiro, D.V., Lima, K.O., Costa, H.L.G., Lopes, D.R. & Prentice, C. (2019). Intelligent packaging with pH indicator potential. *Food Engineering Reviews*, **11**, 235–244.

Choi, I., Lee, J.Y., Lacroix, M. & Han, J. (2017). Intelligent pH indicator film composed of agar/potato starch and anthocyanin extracts from purple sweet potato. *Food Chemistry*, **218**, 122–128.

Cunha, P.L.R., Castro, R.R., Rocha, F.A.C., de Paula, R.C.M. & Feitosa, J.P.A. (2005). Low viscosity hydrogel of guar gum: preparation and physicochemical characterization. *International Journal of Biological Macromolecules*, **37**, 99–104.

Denev, P.N., Kratchanov, C.G., Ciz, M., Lojek, A. & Kratchanova, M.G. (2012). Bioavailability and antioxidant activity of black chokeberry (*Aronia melanocarpa*) polyphenols: *In vitro* and *in vivo* evidences and possible mechanisms of action: a review. *Comprehensive Reviews in Food Science and Food Safety*, **11**, 471–489.

Ebrahimi, V., Mohammadi, A., Bolandi, M. & Baghaei, H. (2022). Fabrication and characterization of a pH-sensitive indicator film by purple basil leaves extract to monitor the freshness of chicken fillets. *Food Packaging and Shelf Life*, **34**, 100946.

Elsaeed, S.M., Zaki, E.G., Omar, W.A.E., Soliman, A.A. & Attia, A.M. (2021). Guar gum-based hydrogels as potent green polymers for enhanced oil recovery in high-salinity reservoirs. *ACS Omega*, **6**, 23421–23431.

Ezati, P. & Rhim, J.W. (2020). pH-responsive chitosan-based film incorporated with alizarin for intelligent packaging applications. *Food Hydrocolloids*, **102**, 105629.

Greenspan, L. (1977). Humidity fixed points of binary saturated aqueous solutions. *Journal of research of the National Bureau of Standards. Section A, Physics and chemistry*, **81A**, 89–96.

Gumienna, M., Lasik, M. & Czarnecki, Z. (2011). Bioconversion of grape and chokeberry wine polyphenols during simulated gastrointestinal *in vitro* digestion. *International Journal of Food Sciences and Nutrition*, **62**, 226–233.

Halász, K. & Csóka, L. (2018). Black chokeberry (*Aronia melanocarpa*) pomace extract immobilized in chitosan for colorimetric pH indicator film application. *Food Packaging and Shelf Life*, **16**, 185–193.

Kapci, B., Neradová, E., Čížková, H., Voldřich, M., Rajchl, A. & Capanoglu, E. (2013). Investigating the antioxidant potential of chokeberry (*Aronia melanocarpa*) products. *Journal of Food and Nutrition Research*, **52**, 219–229.

Kardum, N. & Takic, M. (2014). Effects of polyphenol-rich chokeberry juice on cellular antioxidant enzymes and membrane lipid status in healthy women. *Journal of Functional Foods*, **9**, 89–97.

Kilic, B., Dogan, V., Kilic, V. & Kahyaoglu, L.N. (2022). Colorimetric food spoilage monitoring with carbon dot and UV light reinforced fish gelatin films using a smartphone application. *International Journal of Biological Macromolecules*, **209**, 1562–1572.

Kim, S., Kang, J. & Song, K.B. (2020). Development of a sword bean (*Canavalia gladiata*) starch film containing goji berry extract. *Food and Bioprocess Technology*, **13**, 911–921.

Koshy, R.R., Koshy, J.T., Mary, S.K., Sadanandan, S., Jisha, S. & Pothan, L.A. (2021). Preparation of pH sensitive film based on starch / carbon nano dots incorporating anthocyanin for monitoring spoilage of pork. *Food Control*, **126**, 108039.

Kuswandi, B., Wicaksono, Y., Jayus, Abdullah, A., Heng, L.Y. & Ahmad, M. (2011). Smart packaging: sensors for monitoring of food quality and safety. *Sensing and Instrumentation for Food Quality and Safety*, **5**, 137–146.

Lachowicz, S., Wojdyøo, A., Chmielewska, J. & Oszmian, J. (2017). The influence of yeast type and storage temperature on content of phenolic compounds, antioxidant activity, colour and sensory attributes of chokeberry wine. *European Food Research and Technology*, **243**, 2199–2209.

Lee, S.Y., Lee, S.J., Choi, D.S. & Hur, S.J. (2015). Current topics in active and intelligent food packaging for preservation of fresh foods. *Journal of the Science of Food and Agriculture*, **95**, 2799–2810.

Li, Y., Wu, K., Wang, B. & Li, X. (2021). Colorimetric indicator based on purple tomato anthocyanins and chitosan for application in intelligent packaging. *International Journal of Biological Macromolecules*, **174**, 370–376.

Li, Y., Ying, Y., Zhou, Y. *et al.* (2019). A pH-indicating intelligent packaging composed of chitosan-purple potato extractions strength by surface-deacetylated chitin nano fibers. *International Journal of Biological Macromolecules*, **127**, 376–384.

Liang, T., Sun, G., Cao, L., Li, J. & Wang, L. (2019). A pH and NH3 sensing intelligent film based on Artemisia sphaerocephala Krasch. Gum and red cabbage anthocyanins anchored by carboxymethyl cellulose sodium added as a host complex. *Food Hydrocolloids*, **87**, 858–868.

This article is important for this manuscript. Because the TGA analysis results of our manuscript were compared with those of this article.

Luchese, L., Abdalla, V.F., Spada, J.C. & Tessaro, I.C. (2018). Evaluation of blueberry residue incorporated cassava starch film as pH indicator in different simulants and foodstuffs. *Food Hydrocolloids*, **82**, 209–218.

Ma, Q., Du, L. & Wang, L. (2017a). Tara gum/polyvinyl alcohol-based colorimetric NH3 indicator films incorporating curcumin for intelligent packaging. *Sensors and Actuators, B: Chemical*, **244**, 759–766.

Ma, Q., Ren, Y., Gu, Z. & Wang, L. (2017b). Developing an intelligent film containing *Vitis amurensis* husk extracts: the effects of pH value of the film-forming solution. *Journal of Cleaner Production*, **166**, 851–859.

Ma, Q. & Wang, L. (2016). Preparation of a visual pH-sensing film based on tara gum incorporating cellulose and extracts from grape skins. *Sensors & Actuators: B. Chemical*, **235**, 401–407.

Moazami, M., Moradi, M., Tajik, H., Forough, M., Ezati, P. & Kuswandi, B. (2020). Development of an easy-to-use colorimetric pH label with starch and carrot anthocyanins for milk shelf life assessment. *International Journal of Biological Macromolecules*, **153**, 240–247.

Mohammadalinejhad, S., Almasi, H. & Moradi, M. (2020). Immobilization of *Echium amoenum* anthocyanins into bacterial cellulose film: a novel colorimetric pH indicator for freshness/spoilage monitoring of shrimp. *Food Control*, **113**, 107169.

Naghdi, S., Rezaei, M. & Abdollahi, M. (2021). A starch-based pH-sensing and ammonia detector film containing betacyanin of paperflower for application in intelligent packaging of fish. *International Journal of Biological Macromolecules*, **191**, 161–170.

This article is important for this manuscript. Because it was used to discuss the relationship between the color response parameters and pH.

Nawirska-Olszanska, A., Pasøawska, M., Stepien, B., Oziembøowski, M., Sala, K. & Smorowska, A. (2020). Effect of vacuum impregnation with apple-pear juice on content of bioactive compounds and antioxidant activity of dried chokeberry fruit. *Food*, **9**, 1–11.

Neuenfeldt, N.H., Moraes, D.P., de Deus, C., de Barcia, M.T. & de Menezes, C.R. (2022). Blueberry phenolic composition and improved stability by microencapsulation. *Food and Bioprocess Technology*, **15**, 750–767.

Nwude, F., John, T. & Singh, S. (2022). Fabrication of intelligent pH-sensing films with antioxidant potential for monitoring shrimp freshness via the fortification of chitosan matrix with broken riceberry phenolic extract. *Food Chemistry*, **366**, 130574.

Oladzadabbasabadi, N., Mohammadi Nafchi, A., Ghasemlou, M., Ariffin, F., Singh, Z. & Al-Hassan, A.A. (2022). Natural anthocyanins: sources, extraction, characterization, and suitability for smart packaging. *Food Packaging and Shelf Life*, **33**, 100872.

Oszmiański, J. & Lachowicz, S. (2016). Effect of the production of dried fruits and juice from chokeberry (*Aronia melanocarpa* L.) on the content and antioxidative activity of bioactive compounds. *Molecules*, **21**, 1098.

This article is important for this manuscript. Because it has been used as a supporting article on the phenolic compound content and antioxidant activity of aronia extract.

Pourjavaher, S., Almasi, H., Meshkini, S., Pirsa, S. & Parandi, E. (2017). Development of a colorimetric pH indicator based on bacterial cellulose nanofibers and red cabbage (*Brassica oleraceae*) extract. *Carbohydrate Polymers*, **156**, 193–201.

Prietto, L., Correa, T., Fernanda, J. *et al.* (2017). pH-sensitive films containing anthocyanins extracted from black bean seed coat and red cabbage. *LWT*, **80**, 492–500.

Qin, Y., Liu, Y., Yong, H., Liu, J., Zhang, X. & Liu, J. (2019). Preparation and characterization of active and intelligent packaging films based on cassava starch and anthocyanins from *Lycium ruthenicum* Murr. *International Journal of Biological Macromolecules*, **134**, 80–90.

Romani, A., Vignolini, P., Ieri, F. & Heimler, D. (2016). Polyphenols and volatile compounds in commercial chokeberry (*Aronia melanocarpa*) products. *Natural Product Communications*, **11**, 99–102.

Roy, S. & Rhim, J.W. (2021). Anthocyanin food colorant and its application in pH-responsive color change indicator films. *Critical Reviews in Food Science and Nutrition*, **61**, 2297–2325.

Saurabh, C.K., Gupta, S., Bahadur, J., Mazumder, S., Variyar, P.S. & Sharma, A. (2015). Mechanical and barrier properties of guar gum based nano-composite films. *Carbohydrate Polymers*, **124**, 77–84.

Šavikin, K., Jankovic, T., Pljevljakušic, D., Zdunic, G. & Nada, C. (2016). Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. *Food*, **194**, 135–142.

Siripatrawan, U. & Vitchayakitti, W. (2016). Improving functional properties of chitosan films as active food packaging by incorporating with propolis. *Food Hydrocolloids*, **61**, 695–702.

Sun, G., Chi, W., Xu, S. & Wang, L. (2020). Developing a simultaneously antioxidant and pH-responsive κ-carrageenan/hydroxypropyl methylcellulose film blended with *Prunus maackii* extract. *International Journal of Biological Macromolecules*, **155**, 1393–1400.

Tian, Y., Liimatainen, J., Alanne, A.L. *et al.* (2017). Phenolic compounds extracted by acidic aqueous ethanol from berries and leaves of different berry plants. *Food Chemistry*, **220**, 266–281.

Tolić, M.T., Jurčević, I.L., Krbavčić, I.P., Marković, K. & Vahčić, N. (2015). Phenolic content, antioxidant capacity and quality of chokeberry (*Aronia melanocarpa*) products. *Food Technology and Biotechnology*, **53**, 171–179.

Vagiri, M. & Jensen, M. (2017). Influence of juice processing factors on quality of black chokeberry pomace as a future resource for colour extraction. *Food Chemistry*, **217**, 409–417.

Veberic, R., Slatnar, A., Bizjak, J., Stampar, F. & Mikulic-Petkovsek, M. (2015). Anthocyanin composition of different wild and cultivated berry species. *LWT*, **60**, 509–517.

Vo, T. & Dang, T. (2019). Chitosan/poly (vinyl alcohol)/anthocyanin extracted from red cabbage. *Polymers*, **11**, 1–12.

Wei, Y., Cheng, C., Ho, Y. & Tsai, M. (2017). Active gellan gum/purple sweet potato composite films capable of monitoring pH variations. *Food Hydrocolloids*, **69**, 491–502.

Wu, C., Sun, J., Zheng, P. *et al.* (2019). Preparation of an intelligent film based on chitosan/oxidized chitin nanocrystals incorporating black rice bran anthocyanins for seafood spoilage monitoring. *Carbohydrate Polymers*, **222**, 115006.

Xue Mei, L., Mohammadi Nafchi, A., Ghasemipour, F., Mat Easa, A., Jafarzadeh, S. & Al-Hassan, A.A. (2020). Characterization of pH sensitive sago starch films enriched with anthocyanin-rich torch ginger extract. *International Journal of Biological Macromolecules*, **164**, 4603–4612.

Yildiz, E., Sumnu, G. & Kahyaoglu, L.N. (2021). Monitoring freshness of chicken breast by using natural halochromic curcumin loaded chitosan/PEO nanofibers as an intelligent package. *International Journal of Biological Macromolecules*, **170**, 437–446.

Zhai, X., Wang, X., Zhang, J. *et al.* (2020). Extruded low density polyethylene-curcumin film: a hydrophobic ammonia sensor for intelligent food packaging. *Food Packaging and Shelf Life*, **26**, 100595.