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Antioxidant capacity and light-aging study of HPMC films functionalized with natural plant extract

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ABSTRACT

The aims of this work were to functionalize edible hydroxypropyl methylcellulose (HPMC) films with natural coloring biomolecules having antioxidant capacity and to study their photo-aging stability in the films. HPMC films containing a natural red color compound (NRC) at the level of 1, 2, 3 or 4% (v/v) were prepared by a casting method. A slight degradation of films color was observed after 20 days of continuous light exposure. The antioxidant activity of NRC incorporated films was stable during different steps of film formation and 20 days of dark storage. On the other hand, antioxidant activity of samples stored under light was significantly affected after 20 days. FTIR (Fourier Transformed Infrared) spectroscopy was used to characterize the new phenolic polymeric structures and to study the photo-degradation of films. The results showed a good polymerization phenomenon between NRC and HPMC in polymer matrix giving a natural color to the films. NRC showed an ability to protect pure HPMC films against photo-degradation. This phenomenon was directly proportional to the concentration of NRC.

1. Introduction

Environment and food safety have been at the forefront of research concern in recent years. Currently, there is an increasing trend to employ environmental friendly materials with the intention of substituting non-degradable materials. To deal with environmental issues, extend the food quality and to reduce non-degradable packaging wastes has catalyzed the use of new bio-based packaging materials in edible food packaging (Burke, 2006). In edible coating, use of bio-degradable polymers such as polysaccharides, proteins, lipids and their complexes derived from natural origin (Ray & Bousmina, 2005), depends on their barrier properties against light, water vapor and oxygen (Turhan & Sahbaz, 2004).

Cellulose based materials are widely used due to their biocompatibility, edibility, barrier properties, non-polluting and being more economical (Vasconez, Flores, Campos, Alvarado, & Gerschenson, 2009). The use of hydroxypropyl methylcellulose is attractive because it is a readily available non-ionic edible plant derivative shown to form transparent, odourless, tasteless, oil resistant, and water soluble edible films (Akhtar et al., 2010). HPMC is approved for food uses by the FDA (21 CFR 172.874) and the EU (EC 1995); its safety in food use has been affirmed by the JECFA (Burdock, 2007). The tensile strength of HPMC films is high and flexibility neither too high nor too fragile, which make them suitable for edible coating purposes (Brindle & Krochta, 2008).

In the scope of natural active agents, recently, fruit and vegetable extracts have gained a considerable market in food industries (Stintzing & Carle, 2004). To consider the natural bioactive colors as the colorants, stability, yield and price are mostly constrains. The natural coloring agents in comparison with artificial colors show less stability against light, oxidation, temperature or pH change and other factors (Fabre et al., 1993; Laleh, Frydoonfar, Heidary, Jameei, & Zare, 2006). In spite of such factors, these natural colorants are gaining importance due to their coloring potential, hygiene, nutrition, pharmaceutical activities, bioactivity and environmental consciousness, which indicates relative dependence on natural products (Frank et al., 2005; Hari, Patel, & Martin, 1994).

Although anthocyanins are less stable in various environmental conditions, they include varieties of colors such as orange, red, maroon and blue which make them an attractive alternative as coloring agents in food industries (Markakis, 1982). Moreover, anthocyanins have many health benefits, including reduced risk of cardiovascular diseases (Bell & Gochenaur, 2006) and decreased risk of cancer (Dai, Patel, & Mumper, 2007). These benefits make them essential to provide a healthier food for consumers. Several studies on the antioxidant and antiradical activity of betalains (mainly betanin) from red beetroot extract (*Beta vulgaris* L.) have been published (Escribano, Pedreño, Garcia-Carmona, & Munoz,

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1998; Kanner, Harel, & Granit, 2001; Pedreño & Escribano, 2000). In addition to their coloring properties, they are supposed to provide protection against oxidation stress related disorders in humans when being part of the regular diet (Kanner et al., 2001). Betalains are reported to exhibit anti-inflammatory effects (Gentile, Tesoriere, Allegra, Livrea, & Alessio, 2004) and antiradical activities (Cai, Sun, & Corke, 2003; Stintzing & Carle, 2004). These bioactive color compounds can be added into edible films to give them additional properties such as color, antioxidant and gas barrier capacity. As previously reported, the red color of HPMC films allows very good control against photo-oxidation of polyunsaturated fatty acids (PUFA) in salmon oil (Akhtar et al., 2010). Such edible films would also provide additional benefits to traditional edible film forming materials by providing unique sensory and antioxidant capacity, thus attracting more potential applications as localizing functional effect at the food surface.

The scientific objectives of this study were to functionalize HPMC films with natural red color compound to give them additional properties and to investigate the impact of aging on color stability, light transmission, antioxidant capacity and HPMC oxidation.

2. Materials and methods

2.1. Materials

Hydroxypropyl methylcellulose (Fluka-Biochemika, Japan) is a biochemical product containing 9% hydroxylpropoxyl and 28% methyl radicals. It had a viscosity of 15 mPas and a water solubility of 2% at 25 °C. Ethanol 96.2% (Pharmaceutics Carlo Erba) was used to improve HPMC solublisation, reduce air bubbles in film forming solution (FFS) and accelerate film drying. Petri-dishes (optilux) were provided by Nunclon™ Fisher (DK-4000 Roskilde, Denmark). Height and diameter of Petri-dishes were 1 cm and 8.5 cm respectively. A red liquid "natural color blend" of beetroot juice (E162) and Purple Carrot Extract (E163) containing about 20% glycerin was obtained from ColorMaker, CA, USA. It was used as an active coloring agent to investigate the improvement of antioxidant and color properties of HPMC films. HPLC grade reagents and solvents (ethanol & acetonitrile) were purchased from Pharmaceutics Carlo Erba (France).

2.2. Methods

2.2.1. HPLC analysis

The HPLC equipment was a Shimadzu (Tokyo, Japan) with auto sampler (SIL-20AC), communication bus module (CBM-20A), pump (LC-20AD), column-oven (CTO-20AC) with ULFC (Shimadzu) cooling module in series with a diode array detector (SPD-M20A). Optimum separation of anthocyanins and betalains was achieved on an analytical scale (250 mm × 4.6 mm i.d.) Agilent C18 (5 μM) reversed phase column with a particle size of 5 μm (Phenomenex, Torrance, CA), fitted with a security guard C18 ODS (4 mm × 3.0 mm i.d.) at a flow rate of 0.5 mL/min and a constant temperature of 25 °C. Eluent A was 5% formic acid and B was MeCN/H_2O (60/40, v/v). Separation was accomplished starting with 3% B, followed by a linear gradient to 20% B for 30 min and then to 50% B for 40 min. Maximum absorption of betalains tended to be higher than those of the anthocyanins. Therefore, an intermediate monitoring wavelength of 530 nm was chosen for both pigment groups. Aliquots mixed samples of 20 μL were injected for analyzes. Duplicate determinations were performed throughout.

2.2.2. Mass spectrometric conditions

The LC–MS equipment includes a binary solvent delivery pump and a linear ion trap mass spectrometer (LTQ-MS, Thermo Finnigan, San Jose, CA, USA). LC analysis parameters were the same as described above except use of a specific LCMS C18 column (150 mm × 2.1 mm and 5 μm – Alltima, Alltech, France) at a smaller flow rate of 0.2 mL/min. LTQ equipped with an atmospheric pressure ionization interface operating in electro spray positive mode (ESI positive). Data were processed using Xcalibur 2.1 software. The operational parameters of mass spectrometer were as follows. Spray voltage was 4.20 kV and the temperature of heated capillary was set at 300 °C. Flow rates of sheath gas, auxiliary gas, and sweep gas were set (in arbitrary units min^{-1}) to 35, 10, and 10, respectively. Capillary voltage was −48 V, tube lens was −13 V, split lens was −38 V and the front lens was −4.25 V. All parameters were optimized by using a standard rutin solution as representative glycosylated flavonoid (0.1 g/L) in mobile phase (A/B: 50/50) at a flow rate of 5 μL/min. The compounds of interest were monitored through specific MS2 scans in addition of MS full scan (50–1000 m/z): MS2 (743), MS2 (581), MS2 (949), MS2 (919), and MS2 (889) for the screening of anthocyanins compounds and MS2 (551), MS2 (507), MS2 (389), and MS2 (549) for screening of betanin compounds.

2.2.3. Preparation of film forming solution and films casting

Film forming solutions were prepared according to Akhtar et al. (2010) by dissolving 6 g of HPMC in a 35% ethanol solution for 40 min at 65 °C using a heating magnetic stirrer (Fisher Bio-block Scientific). For better dissolving and avoiding heat oxidation, NRC was dissolved separately in 35% ethanol solution at 20 °C. Both, HPMC and NRC solutions were then mixed and stirred for 30 min at 20 °C to obtain homogeneous solution. NRC solutions pH was adjusted at 3.17 ± 0.1 with HCl (0.1 M). After stirring, the solutions were degassed at room temperature under vacuum "Handy Aspirator WP-15 (Yamato®)" for 30 min. Films were made by pouring 6 g of each film forming solution (FFS) in the lids of the Petri-dishes. Films were then left in a dark room (pre-equilibrated at 20 °C, 50% RH) for drying on a levelled surface for 48 h. Composition of HPMC films, glycerin and NRC concentrations are shown in Table 2.

2.2.4. Film thickness measurement

Film thickness was measured according to the standard NF Q 03-016 with a manual micrometer (Messmer, London, England) equipped with a measuring head of 1 cm in diameter and a sensitivity of 2 μm. Thicknesses were measured in 10 randomly selected points on each film and an average value was calculated.

2.2.5. Film aging

For photo-aging, the films were conditioned under the fluorescent light (OSRAM L36W/640) or darkness for 20 days in an experimental chamber with controlled conditions of temperature (20 °C) and relative humidity (50%). The distance of fluorescent tube from the films was 14 cm.

2.2.6. Color measurements

Color measurements were carried out with a Minolta CM, CR-210 colorimeter (Minolta, Colombes, France) using the Hunter and CIE scale. A black standard color plate (L^* = 24.60, a^* = 0.16, b^* = −0.28) was used as a background for color measurements. Value L^* describes lightness (0 = black to 100 = white). Value a^* describes the amount of redness (positive) or greenness (negative) present in the specimen, while value b^* describes the amount of yellowness (positive) or blueness (negative) present in the specimen. Combined values a^* and b^* define the hue and intensity (saturation) of the color (Moslemi, 1967). The L, a, and b values of each film were taken as the average of at least five points. Color difference (ΔE) is the magnitude of the resultant vector of three component

Table 1
Peak assignments for betalains and anthocyanins of natural red colour (NRC).

	Compound name	Rt (min)	UV–vis$_{max}$ (nm)	m/z $[M+H]^+$	m/z MS^2 of $[M+H]^+$	(%) Area at 530 nm
1	Betd 5-glc (betanin)	7.69	271.9/293.6/538.9	551.16	389.12	48.78
2	Isobetd 5-glc (iso-betanin)	11.95	271.9/293.6/538.9	551.16	389.13	47.91
3	Cyd 3-xyl-glc-gal	22.53	283.7/514.3	743.1	287.0	0.23
4	Cyd 3-xyl-gal	24.38	283.7/514.3	581.1	287.0	1.11
5	Cyd 3-xyl-glc-gal-sin	27.81	287.8/335.5/523.8	949.2	287.0	0.29
6	Cyd 3-xyl-glc-gal-fer	29.58	287.8/334.3/519.0	919.2	581.1/287.0	1.19
7	Cyd 3-xyl-glc-gal-coum	30.88	287.8/319.0/519.0	889.2	287.0	0.49

Aglycons: Betd, betanidin; Cyd, cyanidin; Xyl, xylose; Glc, glucose; Gal, galactose; Fer, ferulic acid; Sin, sinapic acid; Coum, p-coumaric acid.

differences. Total color difference (ΔEab), was calculated by following equation:

$$\Delta Eab = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2} \quad (1)$$

where $\Delta a = a_i - a_0$, $\Delta b = b_i - b_0$ and $\Delta L = L_i - L_0$. The index i, indicates the values observed after storage period and index 0, indicates initial values observed before samples storage (Jutaporn, Suphitchaya, & Thawien, 2011).

2.2.7. Light transmission

The barrier properties of HPMC films against ultraviolet (UV) and visible light were measured at selected wavelengths between 200 and 900 nm, using UV–visible recording spectrophotometer (Ultrospec 4000 UV/visible, Pharmacia Biotech, Orsay, France) according to Fang, Tung, Britt, Yada, and Dalgleish (2002).

2.2.8. ABTS radicals scavenging activity

The evaluation of 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\bullet+}$) radical scavenging activity was based on the ability of antioxidants to inhibit the long-life ABTS radical cation (Sigma, Germany), a blue/green chromophore with characteristic absorption at 734 nm, in comparison with that of Trolox. ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in darkness, at room temperature, for 12–16 h before use. To study antiradical activity of NRC, $ABTS^{\bullet+}$ solution was diluted with ethanol at 30 °C, to obtain an absorbance of 0.70 ± 0.02 at 734 nm. After addition of 1.0 mL of diluted $ABTS^{\bullet+}$ solution to 10 μL of sample or standard Trolox in ethanol (concentration between 0 and 16 μM), the absorbance was measured at 30 °C exactly 6 min after initial mixing. Appropriate solvent blanks were run in each assay. All experiments were performed in triplicate. A standard curve was obtained by using Trolox standard solution at various concentrations. The absorbance of reaction samples was compared to that of Trolox standard and results were expressed in terms of Trolox equivalents (Re et al., 1999). TEAC value is defined as the concentration of standard Trolox with the same antioxidant capacity as a 1 mM concentration or 1 mg/mL of the antioxidant compound under investigation (Maisuthisakul, Pongsawatmanit, & Gordon, 2007).

2.2.9. FTIR analysis of HPMC films

Changes in structure of HPMC composite films after 20 days of continuous light exposure were followed by Fourier transform infrared spectroscopy in total attenuated reflection mode (ATR-FTIR). Measurements were performed at 25 °C with a Tensor 27 mid-FTIR Bruker spectrometer (Bruker, Karlsruhe, Germany) equipped with a Platinum ATR optical cell and an RT-Dla TGS detector (Bruker, Karlsruhe, Germany). The diaphragm was set at 4 mm. The scanning rate was 10 kHz, and 80 scans were performed both for the reference and the sample from 4000 to 800 cm^{-1} with 4 cm^{-1} of resolution. All data treatments were carried out using OPUS software (Bruker, Karlsruhe, Germany). Raw absorbance spectra were smoothed using a nine-point Savitsky-Golay smoothing functions. Elastic baseline correction was applied to spectra, which were further cut between 1800 and 800 cm^{-1}, centered and normalized.

The stability of NRC compounds was calculated by spectral deconvolution using second derivative resolution enhancement and the curve-fitting procedure of 1800–1500 cm^{-1} region. Second derivative spectra were calculated on centered and normalized data with an additional nine-points Savitsky-Golay smoothing function. The second derivative spectra were used only for identifying individual peak positions. The spectra were then deconvoluted by a non linear regression curve fitting program of Gaussian peaks to the original spectra (Opus Software). Optimal Fits were supported by favorable RMS (root mean square) values on the order of 10^{-5}, which were less than baseline noise. The resulting curves fitted were analyzed and percentage of each covalent bond was quantified.

2.2.10. Statistical analysis

A factorial design was used to characterize the composite films. Experimental values were given as means ± standard deviation (SD). Analysis of variance (ANOVA) was used to compare mean differences of the samples. If the differences in mean existed, multiple pairwise comparisons were performed using XL STAT software. Differences at $P < 0.05$ were considered to be significant.

3. Results and discussion

3.1. HPLC–MS/MS characterization of NRC

Various analytical methods have been reported to differentiate betalains and betacyanins (Charron et al., 2009; Nielson & Harley, 1996). An official HPLC method providing fingerprints of common fruit juices to characterize betacyanins has been published (IFU, 1998). In the present study, newly established HPLC-DAD–MS/MS method (Stintzing et al., 2005) was used allowing simultaneous determination of betacyanins. Betanin and iso-betanin were previously detected in prickly pear (*Opuntia* spp.) by Castellanos-Santiago and Elhadi (2008) and anthocyanin in purple carrots by Kurilich, Clevidence, Britz, Simon, and Novotny (2005). Based on comparison with the standards and bibliographical data (Stintzing et al., 2005), betalains and anthocyanin were readily identified by their retention time order, spectral and mass characteristics including daughter ion and neutral loss scanning (Table 1). As previously reported (IFU, 1998), betacyanins were generally more polar than anthocyanins therefore betanin (betanidin-5-O-β-glucoside) and iso-betanin (isobetanidin-5-O-β-glucoside) eluted considerably earlier than the minor components such as anthocyanins (Fig. 2a).

3.1.1. Identification of major components (betanin & isobetanin)

Major components of NRC, betanin (1) and its C_{15} epimer iso-betanin (2) amounted to 96.69% together were identified on UV–visible chromatogram and single ion chromatogram at

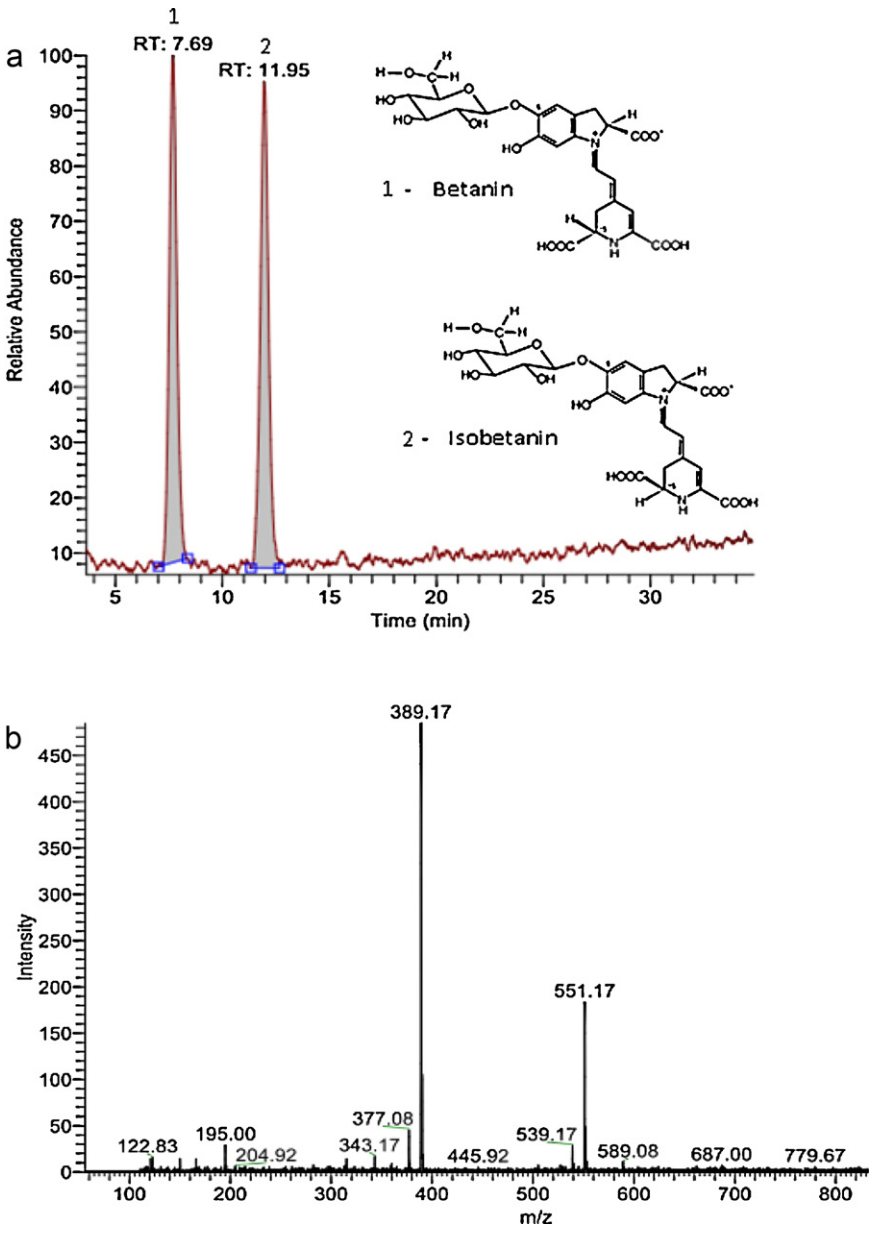

Fig. 1. Identification of major components (betanin & isobetanin) of natural red colour (NRC). Peak assignment is given in Table 1. (a) Single ion chromatogram (SIC) of NRC at $m/z = 551$. Betanin & isobetanin structures were adopted from Herbach et al. (2006a,b). (b) NRC Full MS spectrum at 7.69 min (same at 11.95 min) with parent ion $m/z = 551$ $[M+H]^+$ and source fragmentation ion $m/z = 389$.

retention time of 7.69 min and 11.95 min, respectively (Fig. 1a). They were further confirmed with MS and MS^2 steps.

3.1.2. Identification of minor components (anthocyanins)

NRC showed the minor components such as anthocyanins typically cyanidin derivatives (3–6) along with a couma-royl-derivative (7) as also described by Stintzing et al. (2005) and Glässgen, Seitz, and Metzger (1992). Presence of these anthocyanins was confirmed by comparing their specific retention times (Fig. 2a) with those of a standard anthocyanin mixture chromatogram (Fig. 2b).

3.2. Film thickness measurement

The thickness of edible films is an important parameter because it directly affects the biological properties and the shelf life of the coated food. Thickness is dependent on the type of dry matter and film preparation methods (Sebti, Chollet, Degraeve, Noel, & Peyrol, 2007). HPMC films incorporated with NRC at the level of 1, 2, 3, or 4% (v/v) were compared for their thickness with 3 types of control HPMC films; composition is shown in Table 2. No significant change in film thickness was found by the addition of glycerin (1% or 4%, w/w of film dry matter). Similar results were reported by Imran, El-Fahmy, Revol-Junelles, and Desobry (2010) by the addition of 10% (w/w) plasticizer into HPMC films. A slight increase in film thickness may be associated with the property of glycerin to retain high moisture content at the end of film drying (Chen & Lai, 2008). However, a gradual but non-significant increase in the thickness of films containing NRC may be combined effect of glycerin and betacyanin molecules containing lots of hydrophilic groups (Díaz, López1, Kerstupp1, Ibarra1, & Scheinvar, 2006). A slight increase in thickness of films containing 4% NRC compared to films containing 1% of glycerin confirmed the plasticizing effect of phenolic compounds on film thickness.

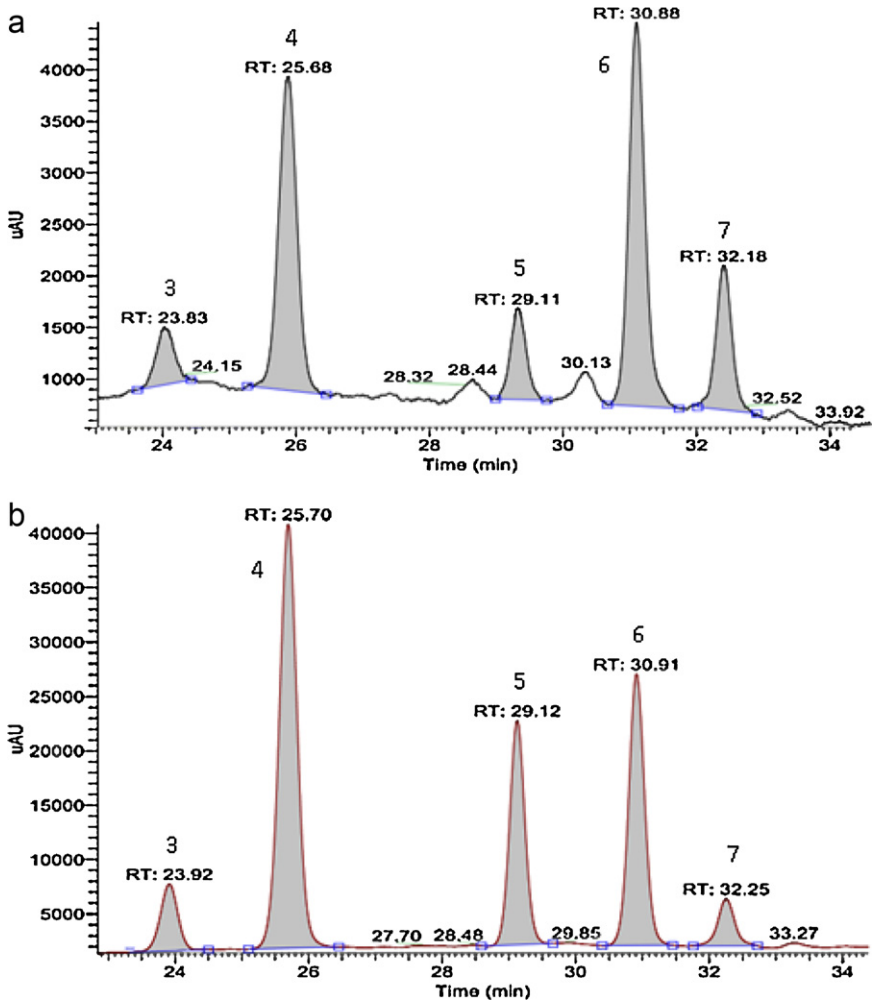

Fig. 2. Identification of NRC minor components (anthocyanins): comparison between NRC UV–visible chromatogram (a) and standard anthocyanins UV–visible chromatogram (b) at 530 nm. Peak assignment is given in Table 1.

3.3. *Optical properties of film: color and transparency*

Optical properties are the first ones detected by human vision and could affect food quality (Fabra, Talens, & Chiralt, 2009).

3.3.1. *Color stability*

Color parameters of control and NRC films were analyzed (Table 3). Control HPMC films without NRC and glycerin appeared clear and transparent which showed complete solublisation of HPMC powder in 35% ethanol solution. Incorporation of NRC into HPMC films modified their appearance in both color and transparency. A decrease in *L* values and an increase in *a* values were observed in NRC films. Hunter *L*, *a* and *b* values were statistically identical for control HPMC films but they were significantly different for the films containing NRC up to 4% (v/v). Significant increase in redness (*a*) of NRC films was observed representing its good coloring ability.

Similar results were observed by Park and Zhao (2006) by the addition of Cranberry Pomace Extracts in low methoxyl pectin (LMP) and high methoxyl pectin (HMP) films.

Color stability was studied by conditioning the films under fluorescent light at 20 °C for 20 days. The control samples, HPMC films alone or containing 1 or 4% (w/w) of glycerin exhibited non significant color difference over 20 days of light storage. A significant decrease in *a* values was observed for all NRC films causing fading of the red surface color (Table 3). The visual color changes observed

Table 2
Thickness and composition of HPMC–NRC–plasticizer composite films and pH values of FFS (mean and standard deviation of triplicate analysis).

Film type	Film composition glycerin, G% (w/w) of dry matter	pH of film forming solutions (FFS)	Film thickness (μm)
HPMC	0.00	7.61 ± 0.50	48.25 ± 3.48[a]
HPMC + G1%	1.00	7.30 ± 0.50	49.03 ± 4.15[a]
HPMC + G4%	4.00	7.35 ± 0.50	49.50 ± 5.03[a]
HPMC + NRC1%	0.20	3.17 ± 0.50	49.38 ± 3.39[a]
HPMC + NRC2%	0.40	3.17 ± 0.50	52.29 ± 3.92[a]
HPMC + NRC3%	0.60	3.17 ± 0.50	53.09 ± 4.85[a]
HPMC + NRC4%	0.80	3.17 ± 0.50	54.29 ± 5.62[a]

Test conditions (temperature 20 ± 2 °C; RH, 50 ± 2%). NRC, natural red colour; HPMC, hydroxypropyl methylcellulose. Same letters within the column (film thickness) indicate non-significant difference at $P < 0.05$.

Table 3
Colour parameters (L^*, a^*, b^*) of edible HPMC films as a function of NRC concentration and 20 days of aging under fluorescent light (mean values of triplicate analysis).

Films types	L^* (lightness)		a^* (redness/greenness)		b^* (yellowness/blueness)		ΔEab^* (0–20 d)
	0 d (P.A.)	20 d (P.A.)	0 d (P.A.)	20 d (P.A.)	0 d (P.A.)	20 d (P.A.)	
HPMC	32.18 ± 0.05^{Aa}	32.16 ± 0.09^{Aa}	-0.067 ± 0.006^{Ae}	-0.10 ± 0.01^{Be}	-0.48 ± 0.04^{Ad}	-0.46 ± 0.03^{Ae}	0.06 ± 0.02^{b}
HPMC + G1%	32.17 ± 0.02^{Ba}	32.25 ± 0.02^{Aa}	-0.097 ± 0.021^{Ae}	-0.11 ± 0.01^{Ae}	-0.44 ± 0.01^{Ad}	-0.50 ± 0.01^{Be}	0.10 ± 0.03^{b}
HPMC + G4%	32.16 ± 0.03^{Aa}	32.17 ± 0.22^{Aa}	-0.093 ± 0.006^{Ae}	-0.10 ± 0.02^{Ae}	-0.44 ± 0.02^{Ad}	-0.52 ± 0.04^{Be}	0.08 ± 0.03^{b}
HPMC + NRC1%	31.78 ± 0.16^{Ab}	31.91 ± 0.04^{Aa}	0.997 ± 0.085^{Ad}	0.66 ± 0.04^{Bd}	2.34 ± 0.19^{Ac}	1.99 ± 0.20^{Ad}	0.56 ± 0.32^{b}
HPMC + NRC2%	29.39 ± 0.19^{Bc}	30.40 ± 0.08^{Ab}	5.013 ± 0.267^{Ac}	3.27 ± 0.11^{Bc}	5.99 ± 0.18^{Ab}	5.77 ± 0.15^{Ac}	2.03 ± 0.27^{a}
HPMC + NRC3%	28.87 ± 0.16^{Bd}	30.03 ± 0.15^{Ac}	5.930 ± 0.267^{Ab}	4.18 ± 0.29^{Bb}	6.35 ± 0.05^{Aa}	6.52 ± 0.08^{Ab}	2.11 ± 0.27^{a}
HPMC + NRC4%	27.96 ± 0.02^{Be}	28.88 ± 0.17^{Ad}	6.943 ± 0.051^{Aa}	5.86 ± 0.24^{Ba}	6.31 ± 0.03^{Aa}	6.95 ± 0.04^{Ba}	1.56 ± 0.22^{a}

0 d (P.A.), 0 day photo-aging; 20 d (P.A.), 20 days photo-aging; ΔEab^* (0–20 d), total colour change during 20 days of photo-aging. Different small letters within each column and different capital letters within each row indicate significant differences among the values of the same colour property at $P < 0.05$.

for light stored films appeared in L values, significantly increased by photo-aging. The lightening of initial red surface color of films was due to photo-degradation of betacyanins (Herbach, Stintzing, & Carle, 2006).

The changes in L, a and b values were summarized by calculating total color difference (ΔEab). There was an increase in ΔE values of NRC added films under fluorescent light conditions (Table 3). The increase in ΔE values was higher for the films incorporated with NRC 2, 3 or 4% over 20 days of photo-aging. This increase in ΔE resulted from a decrease in a values and increase in L values. The ΔE value of 1.0 is the smallest color difference a normal human eye can detect so any ΔE less than 1.0 is imperceptible (Jonathan Sachs, 2001–2002). Some color differences even greater than 1 are perfectly acceptable, may be even unnoticeable, depending on the color, shade and density. For example, the ΔE color difference between two reds may be the same but may not look like same difference to the human eye (Jonathan Sachs, 2001–2002). Keeping in view this statement, the films other than those containing 2, 3 and 4% NRC were stable for their color properties after 20 days of photo-aging.

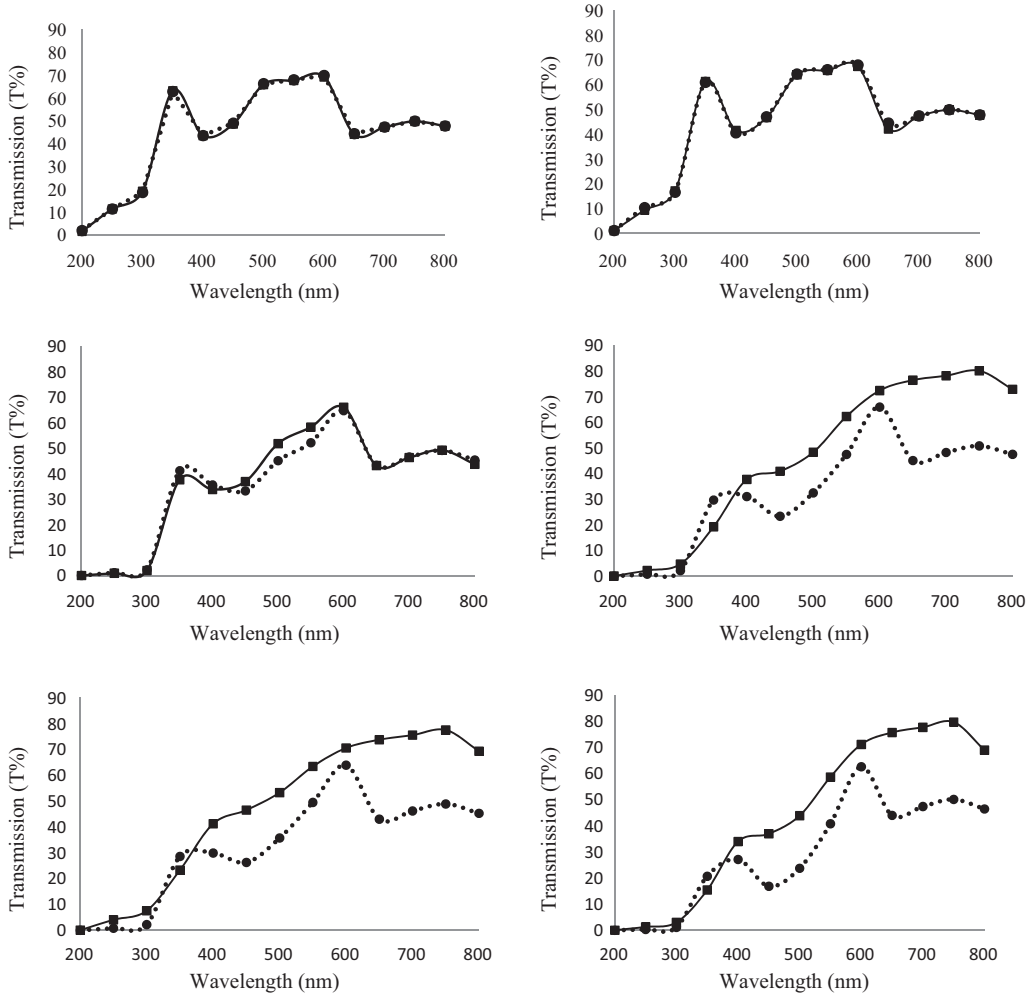

Fig. 3. Light transmission (T%) of UV–visible for HPMC–NRC–plasticizer composite films before and after 20 days of photo-aging. (a) HPMC 0 d (●), HPMC 20 d (■). (b) HPMC + G1% 0 d (●), HPMC + G1% 20 d (■). (c) HPMC + NRC1% 0 d (●), HPMC + NRC1% 20 d (■). (d) HPMC + NRC2% 0 d (●), HPMC + NRC2% 20 d (■). (e) HPMC + NRC3% 0 d (●), HPMC + NRC3% 20 d (■). (f) HPMC + NRC4% 0 d (●), HPMC + NRC4% 20 d (■).

3.3.2. Light transmission

For use as packaging materials, transparency of HPMC films is required to fulfill consumer eagerness to see food through packaging. Comparing the effect of each mixture component on % transmission, NRC concentration was the main factor reducing film transparency. The lowest transmission through films was noticed for the greatest concentration of NRC. Fig. 3 shows transmission of UV and visible light, at selected wavelength between 200 and 700 nm, through films before and after 20 days of light exposure.

Increase in NRC contents of HPMC films showed a decrease in light transmission of films for both UV and visible regions. This result was in accordance with Jutaporn, Suphitchaya, and Thawien (2011) who observed that HPMC films became less transparent with the increase of phayom wood extract contents. No change in transmission of HPMC films alone and with glycerin 1% was noticed after photo-aging as shown in Fig. 3a and b. It is clear that NRC films became more transparent after light exposure due to color degradation. Increase in light transmission after photo-aging was more pronounced in films containing high concentration of NRC and was confirmed by total change in color (ΔE). Indeed, the reflected and transmitted spectrum of a colored layer was based on a material dependent scattering and absorption of light in visible spectra (Hutchings, 1999).

3.4. ABTS radical scavenging activity

The ABTS radical scavenging activity method is based on the ability of molecules to scavenge the ABTS radical cation, in comparison with that of Trolox. The ABTS assay was calibrated with the water soluble alpha-tocopherol analog, Trolox. Antioxidant stability of NRC was investigated for different stages of film formation and light-aging (Fig. 4). All the samples containing NRC displayed antioxidant activities as they were able to scavenge $ABTS^{\bullet+}$ radical cation. They were shown to be antiradical agents compared to the Trolox. FFS, fresh films, films stored under darkness or under light, displayed less free radical scavenging activities than pure NRC solution (TEAC = 0.0133 ± 0.0005). No significant change in TEAC value was observed for FFS (0.0123 ± 0.0004), fresh film (0.0121 ± 0.0005) and those stored under darkness (0.012 ± 0.0004) as compared to pure NRC (0.0133 ± 0.0005), showing their antioxidant stability. The film samples stored under light were significantly different from pure NRC samples for their antioxidant activity (Fig. 4). These results suggested that Trolox equivalent antioxidant activity of NRC was slightly decreased during FFS preparation but no significant change was observed during film casting and film aging after darkness or light exposure. This phenomenon indicated that NRC compound was slightly modified or degraded during aging process. However, the degradation products have conserved some antioxidant capacity. Moreover, NRC was more stable in FFS, fresh films and films stored in darkness. These results were in accordance with those of Díaz et al. (2006) who studied the effect of light and darkness on betalains stability.

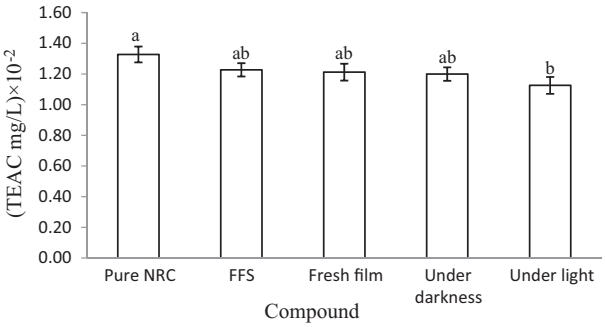

Fig. 4. Antioxidant activities of pure NRC solution, film forming solution (FFS) and NRC incorporated films against $ABTS^{\bullet+}$ radical, expressed as TEAC values. Means within the same column with different letters are significantly different at $P < 0.05$.

3.5. FTIR analysis of HPMC films

FTIR spectroscopy is a rapid technique with minimum samples preparation requirements. It allows qualitative and quantitative determination of organic compounds in samples because intensities of spectrum bands are proportional to concentration (Vlachos et al., 2006). Control and NRC composite films were analyzed by FTIR spectroscopy to characterize new phenolic polymeric structures and light storage effect. FTIR spectra for control and HPMC films colored with NRC (betacyanins), ranging between 1800 and 800 cm^{-1} are shown in Fig. 5. All samples had very strong absorption bands at 1060 cm^{-1} related with a pronounced shoulder at 1115 cm^{-1} attributed to a combination band of C–O stretches and secondary hydroxyl group (O–H).

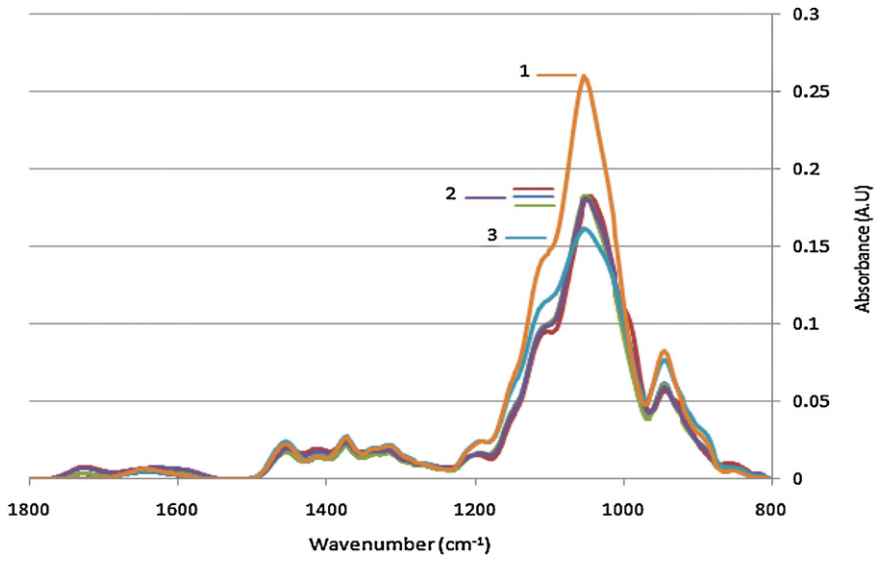

Fig. 5. FTIR spectra of control HPMC films; without light exposure (3), after light exposure (1) and films containing NRC 1% or 4% before and after 20 days of photo-aging (2).

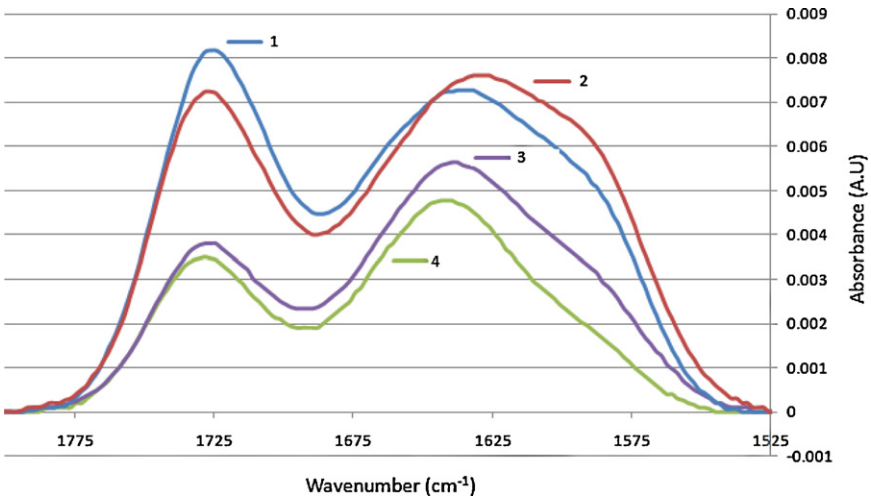

Fig. 6. FTIR spectral region (1525–1775 cm^{-1}) of HPMC films containing 1% NRC; without light exposure (4), after light-ageing (3) and with 4% NRC; without light exposure (1) and after light-ageing (2).

Table 4
Peak assignment of deconvolution FTIR spectra of NRC incorporated HPMC films (HPMC + NRC1% & HPMC + NRC4%) before and after 20 days of photo-aging.

Resonant frequency(cm^{-1})	HPMC + NRC1%			HPMC + NRC4%			Bond types
	% age of covalent bond			% age of covalent bond			
	0 day aging	20 days aging	Variations	0 day aging	20 days aging	Variations	
1581.140	5.38 ± 0.22[b]	9.64 ± 0.39[a]	4.260	9.465 ± 1.51[a]	13.07 ± 0.18[b]	3.603	NH
1609.688	16.51 ± 0.18[b]	20.34 ± 0.15[a]	3.829	16.84 ± 2.75[a]	19.80 ± 0.17[a]	2.967	COO^-
1636.439	9.48 ± 0.110[a]	7.39 ± 0.390[b]	−2.086	2.213 ± 0.59[b]	3.344 ± 0.11[a]	1.130	COO^-
1654.688	36.50 ± 0.19[a]	35.60 ± 0.45[b]	−0.903	37.33 ± 0.34[a]	33.11 ± 0.05[b]	−4.220	NH_2
1699.986	0.000 ± 0.00[b]	0.08 ± 0.027[a]	0.081	1.115 ± 0.76[a]	0.000 ± 0.00[a]	−1.115	C=O
1726.441	32.13 ± 0.41[a]	26.95 ± 0.48[b]	−5.181	33.036 ± 4.6[a]	30.67 ± 0.43[a]	−2.365	C=O

Different letters within each row indicate significant differences among the values of the same NRC percentage at $P < 0.05$.

3.5.1. Changes in absorption band at 1060 cm^{-1}

Absorption band at 1060 cm^{-1} is associated with the hydroxyl group indicating formation of intermolecular hydrogen bonds. In case of pure HPMC films after 20 days of photo-aging, the absorption of this band was increased due to OH groups formation. Increase in peak area in this region was due to the availability of more OH groups of glycerin interacting with cellulosic OH groups. The stretching vibration at 1060 cm^{-1} causing an increase in peak surface indicated light degradation of pure HPMC films after photo-aging (Fig. 5). The increasing slopes of the curves were due to availability of OH groups of phenolic compounds present in NRC for cross-linking with cellulosic OH groups. Same results were observed by Kim, López, Güebitz, and Cavaco-Paulo (2008) from coloration of flax fabrics with flavonoids.

NRC Films were compared with control HPMC films for their FTIR spectra. Absorption band at 1060 cm^{-1} for films incorporated with NRC was stable over 20 days of fluorescent light exposure, which indicated that NRC had an ability to protect pure HPMC films against photo-degradation.

3.5.2. Changes in the region between 1525 cm^{-1} and 1775 cm^{-1}

The comparison between the spectra showed an additional peak around 1726 cm^{-1} for NRC films (Fig. 6). This band was attributed to C=O stretching vibrations and indicated the presence of phenolic compounds (betacyanins) on treated HPMC films. The 1726 cm^{-1} band was attributed to C=O ester absorption and could be generated by flavonoids oxidation after 20 days of light exposure. NRC compounds stability was calculated by spectral deconvolution using second derivative resolution enhancement and curve-fitting procedure of 1800–1500 cm^{-1} region. The resulting curves were then analyzed and percentage of each covalent bond was quantified.

Fitting the bands to a variable number of individual contributing vibration modes was most successful using six peaks (Table 4). Stretching vibration of NH bond was reported at 1581 cm^{-1} and increased after light exposure due to NH_2 oxidative breakdown. The increase % in NH groups was greater in films containing 1% NRC (4.26%) as compared to those containing 4% NRC (3.60%) after light exposure showing NRC oxidation. Similarly, stretching vibration of COO^- was reported at 1609 cm^{-1} and increased after light exposure due to C=O oxidative breakdown. The increase in COO^- groups was greater in films containing 1% NRC (3.82) as compared to those containing 4% NRC (2.96%) after light exposure which indicated NRC oxidation. It could be concluded from Table 4 that NRC was slightly oxidized after 20 days of light exposure.

4. Conclusion

This study demonstrated that natural plant extracts could be used to functionalize edible films with additional benefits. Such films provide unique fruit flavor, color and antioxidant capacity, which would significantly enhance its potential applications in both food and nonfood industries. Miscibility of HPMC and NRC in composite films was confirmed by infrared spectroscopy analysis. Absorption bands in FTIR spectra suggested interactions through hydrogen bonding between components. The additional peak observed for NRC films was due to compounds OH groups interaction with cellulosic OH groups. Increased peak area in this region was directly proportional to NRC concentration making films more hydrophilic. NRC antioxidant capacity during the steps of film

preparation was stable. Color of edible films became darker and redder as NRC increased, while an increase effect of light exposure was noticed on color stability. Results pointed that NRC films has good potential for food applications due to their color, plasticizing property, good antioxidant stability and ability to protect HPMC from photo-degradation. Nevertheless, films stability in respect to color, transparency, microbial growth, and flavor retention needs further studies.

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