Research Article

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Natural color pigments: Oxidative stability and degradation kinetics during storage in thermally pasteurized vegetable purees

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Abstract

BACKGROUND: Package oxygen transmission rate (OTR) can affect the stability of natural color pigments such as anthocyanins, betalains and chlorophylls in foods during storage. In the present study, we investigated the oxygen sensitivity of selected pigments in thermally pasteurized vegetable purees held at a refrigeration temperature. We modulated the oxygen ingress in packaging using multilayer films with OTRs of 1, 30 and 81 $cm^3\ m^{-2}\ day^{-1}$. Red cabbage, beetroot and pea purees were vacuum packed, pasteurized to achieve a cumulative lethality of $P^{10^\circ C}_{90^\circ C} = 12.8–13.4$ min and stored at 7 °C for 80 days.

RESULTS: Anthocyanins were relatively stable (< 4% losses), regardless of the film OTR. Betalains showed the highest sensitivity to different OTRs, with total losses varying from 4% to 49% at the end of storage and showing significant differences ($P < 0.05$) among the three films. Chlorophylls showed no significant difference ($P > 0.05$) in sensitivity to film OTRs. However, continuous degradation of chlorophylls was observed for all film types, with total chlorophyll losses ranging from 33% to 35%. Overall color differences (ΔE) at the end of storage for cabbage, beet and pea puree were between 0.50–1.70, 1.00–4.55 and 7.41–8.08, respectively. Betalains and chlorophylls degradation followed first-order and fractional conversion kinetics, whereas ΔE followed zero-order and fractional conversion kinetics during storage.

CONCLUSION: All three pigments behaved differently to oxygen ingress during storage. Low to medium barrier films are suitable for products containing red cabbage anthocyanins. High barrier films are must for betalains, whereas medium to high barrier films are suitable for chlorophyll-containing products.

Keywords: packaging; anthocyanins; betalains; chlorophylls; pasteurization; kinetics

INTRODUCTION

Color is one of the most important physical attributes of raw and processed food products and is a natural indicator of food quality that affects consumer acceptance. Natural color pigments such as anthocyanins, betalains and chlorophylls are responsible for the distinct hues of fruits and vegetables. Although processed food products are often colored with synthetic, inorganic, nature-identical and natural food colorants, the increasing consumer demand for 'all natural' and 'clean label' products can make these natural color compounds a potential replacement for artificial colorants in processed food products. Anthocyanins and betalains also have health benefits in addition to their color properties. The health benefits of anthocyanins include antioxidative effects, antiangiogenesis, prevention of cardiovascular disease, anticancer effects, antidiabetes effects, improved visual health, anti-obesity effects, antimicrobial effects and neuroprotection.[1] Betalains have shown potent antiradical scavenging activity,[2] as well as antioxidant, antiproliferative, cardioprotective, anti-inflammatory and antimicrobial activities.[3]

Anthocyanins are phenolic compounds in the forms of anthocyanidin glycosides and acylated anthocyanins, whereas anthocyanidins are grouped into 3-hydroxyanthocyanidins, 3-deoxyanthocyanidins and O-methylated anthocyanidins.[1] Anthocyanins are widely distributed in flowers, fruits and vegetables, giving them a red, blue or purple appearance. Betalains are water-soluble nitrogenous pigments that are derivatives of betalamic acid, consisting of red betacyanins and yellow betaxanthins.[4] Betalains can be found in fruits, flowers and roots, although the main commercial source is red beetroot.[2] Chlorophylls are major pigments in green plants and vegetables, comprising magnesium complexes derived from porphin, which has completely unsaturated structure and contains four pyrrole rings.[5] All three pigments are sensitive to various intrinsic and extrinsic factors, including temperature, pH, light, oxygen, metal ions, enzymes and sugars.

Thermally processed red cabbage, beetroot and pea purees can be very good sources of anthocyanins, betalains and chlorophylls,

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respectively. Thermal processing increases shelf life by inactivating enzymes, as well as pathogenic and spoilage micro-organisms. Polymeric packaging is often used to maintain the chemical, nutritive and microbiological quality of products under specific storage conditions. However, as a result of wide variety of commercially available packaging materials, the selection of suitable packaging material is complex. The oxygen transmission rate (OTR) of the packaging films influences the amount of oxygen ingress into polymeric food packages. This can affect the stability of oxygen-sensitive components of food products. Thus, the gas barrier properties of packaging, specifically the OTR and water vapor transmission rate (WVTR), can affect shelf life. Oxygen permeating through the packaging film can affect the stability of natural color pigments because they are sensitive towards molecular oxygen as a result of their unsaturated structure.[4,5] To our knowledge, the available information about the sensitivity of these pigments to the OTR of packaging films is very limited.

In the present study, we examined the sensitivity of natural color pigments to oxygen in thermally pasteurized vegetable purees. The purees were packed in polymeric pouches with varied OTRs when stored at refrigeration temperature. We used mesophilic and psychrophilic plate count, weight loss, pH, instrumental color and pigment content as quality parameters. We analyzed the obtained data using reaction kinetic models. The findings obtained demonstrate the sensitivity of natural color pigments to the OTRs of packaging films and may be used to guide food industry/processors in the selection of suitable packaging material for thermally pasteurized vegetable-based products.

MATERIALS AND METHODS

Materials

Fresh red cabbage, fresh beets (Mosby Bros Farms, Green Valley, WA, USA) and frozen green peas (Great Value) were purchased from a local supermarket. Potassium chloride (United States Pharmacopeia grade), sodium acetate (American Chemical Society grade) and hydrochloric acid (American Chemical Society grade) were purchased from Fisher Scientific Co. (Fair Lawn, NJ, USA). Methanol (high-performance liquid chromatography grade) and acetone (American Chemical Society grade) were obtained from VWR International (Radnor, PA, USA).

Three types of polymeric films with different OTRs were used to form 110 × 150 mm (width × height) dimension pouches using a Koch Easy Pack vacuum sealer (Ultrasource LLC, Kansas City, MO, USA). These films/pouches were designated as F-1, F-30 and F-81 based on their OTRs of 0.99 ± 0.05, 29.8 ± 1.38 and 80.9 ± 2.15 $cm^3\ m^{-2}\ day^{-1}$, respectively, at 23 °C, 55 ± 1% relative humidity and 1 atm. The F-1 film had an eight-layer [polyethylene terephthalate (PET)/linear low-density polyethylene (LLDPE)/low-density polyethylene (LDPE)/Tie/Nylon66/Tie/LLDPE/LDPE] structure with a thickness of 102 ± 2.73 µm. The F-30 film had five-layer (LDPE/Tie/Nylon/Tie/LDPE) structure with a thickness of 86.4 ± 1.36 µm. The F-81 film had PET-polyethylene based structure with a thickness of 70.0 ± 1.26 µm. The F-1, F-30 and F-81 films had WVTRs of 3.94 ± 0.03, 4.18 ± 0.06 and 6.59 ± 0.03 $g\ m^{-2} day^{-1}$, respectively, at 38 °C and 100% relative humidity.

Puree preparation

Beets and cabbage were sanitized with hypochlorite solution (100 ppm) for 1 min and then rinsed with potable water for 1 min at room temperature. Beets were peeled and cut into 5 mm slices. Cabbage was cut into four halves followed by removal of the central core and the separation of leaves. Green peas were directly blanched without a sanitizing treatment. Blanching was conducted with food grade steam at 98 °C for 3, 5 and 10 min for peas, cabbage and beets, respectively, to inactivate enzymes and soften the texture. The blanched vegetables were cooled immediately by rinsing in chilled water (4 °C) for 1 min. The puree was prepared by blending 200 g of blanched peas, cabbage and beets with 75, 100 and 100 g, respectively, of water for 1 min using a kitchen blender. The puree was prepared in small batches of 450 g each and mixed together for another 5 min with a mixer after every ten batches. Portions of puree (200 g) were filled into each pouch and vacuum sealed at 0.8 bar pressure using a Ultravac vacuum sealer (Ultrasource LLC., Kansas City, MO, USA). Headspace in the vacuum-packed pouches ($n = 9$) was measured using the water displacement method and found to be 0.7 ± 0.2 cm^3.

Pasteurization and storage

Pre-packed puree pouches were pasteurized using hot water in a steam-jacketed kettle maintained at water temperature of 92 ± 1 °C. Preliminary experiments were conducted to determine the processing time to achieve a 6-log reduction of psychrotrophic, non-proteolytic *Clostridium botulinum* type E (i.e. $P^{10°C}_{90°C}$=10 min) at the cold spot of the package to achieve an expected shelf life of up to 6 weeks at 5 °C.[6] The total heating time was 29, 30 and 33 min for cabbage, beets and peas with a cumulative lethality ($n = 2$) of 12.8 ± 0.2, 12.8 ± 2.3 and 13.4 ± 0.8 min, respectively. The pouches were cooled with chilled water at 4 °C for 20 min and transferred to the storage area at 7 ± 0.5 °C. The storage temperature of 7 °C was selected to simulate the storage in home refrigerators and the mild temperature abuse scenario during transport and handling. In total, 21 pouches for each type of puree was prepared. Pouches were stored for 80 days under dark conditions in a laboratory incubator (MIR-254; Panasonic Healthcare Corporation of North America, Wood Dale, IL, USA) (238 L). The storage period was selected considering the shelf life of commercially available refrigerated products. The pouches were drawn in triplicate for quality analysis on day 0, 7, 15, 31, 45, 60 and 80 of storage. At each time interval, the pouch weight was measured to determine weight loss as a result of water migration from the inside of the pouch to the storage environment.

Total plate count

For microbial analyses, 10 g of puree was homogenized with 90 mL of 0.1% peptone water in a stomacher bag using a stomacher (400 Circulator; Seward, Worthing, UK) at 230 r.p.m. for 30 s. Subsequent dilutions were prepared by transferring 1 mL of higher dilution to 9 mL of 0.1% peptone water in test tubes. Aliquots (1 mL) were pour plated in duplicate on tryptic soy agar (BD and Company, Sparks, MD, USA) and incubated, both aerobically and anaerobically, at 35 °C for 48 h for the mesophilic (microorganisms that grow best between 20 and 45 °C) count and 7 °C for 10 days for the psychrophilic (microorganisms that grow best below 15 °C) count. Microbial colonies were counted and reported as log colony-forming units (CFU) g^{-1} ($n = 6$). The same pouches were then used for further analysis.

pH

The pH of the 10% aqueous suspension by weight was measured by immersing the electrode of the pH meter (Seven Go SG2;

Mettler Toledo, Schwerzenbach, Switzerland) into the sample. The pH was recorded until the value was stabilized.

Instrumental color measurement

Instrumental color was measured using a CM-5 spectrophotometer (Konica Minolta, Ramsey, NJ, USA) with the specular component excluded, an observer angle of 10°, an 8-mm measurement area and the illuminant D65 (daylight, color temperature 6504 K). The puree was placed in a plastic Petri dish (60 × 15 mm) and the color was measured at three locations for each sample. CIE L^* a^* b^* color values ($n = 9$) were recorded, where L^* is lightness, a^* is red/green chromacity and b^* is yellow/blue chromacity. This was used to calculate the total color difference using:

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (1)$$

where L_0^*, a_0^* and b_0^* stand for the initial values of L^*, a^* and b^* immediately after processing. Visual color changes during storage were monitored using an SLR camera system (EOS 60D; Canon Inc., Melville, NY, USA).

The following scale of the value of ΔE indicates the difference between the two colors[7]: $\Delta E < 0.2$: no perceptible difference; $0.2 < \Delta E < 0.5$: very small difference; $0.5 < \Delta E < 2$: small difference; $2 < \Delta E < 3$: fairly perceptible difference; $3 < \Delta E < 6$: perceptible difference; $6 < \Delta E < 12$: strong difference; $\Delta E > 12$: different color.

Quantification of pigment content

Anthocyanins

Five grams of puree was homogenized with 10 mL acidified methanol (0.1% HCl in methanol) using a Polytron PT 2500E homogenizer (Kinematica, Bohemia, NY, USA) in a 50-mL tube for 5 min at 7000 r.p.m. The homogenate was vacuum filtered through filter paper (Whatman No. 1; GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) and the remaining solids were homogenized twice in 10 mL of solvent for 5 min. The extract from three extractions was combined, diluted to 50 mL in a volumetric flask, and used for anthocyanins quantification using a spectrometer (Ultraspec 4000; Pharmacia Biotech Inc., Piscataway, NJ, USA) in accordance with the pH differential method of Giusti and Wrolstad.[8] The extract was diluted by a factor of 3 using 0.025 mol L^{-1} potassium chloride buffer (pH 1.0) and 0.4 mol L^{-1} sodium acetate buffer (pH 4.5). The mixtures were kept in the dark at 23 °C for 15 min and absorbance was measured at 520 and 700 nm. The total monomeric anthocyanin content was quantified using:

$$\text{Total monomeric anthocyanin content (mg L}^{-1}) = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l} \quad (2)$$

where $A = (A_{520} - A_{700})_{\text{pH 1.0}} - (A_{520} - A_{700})_{\text{pH 4.5}}$, MW is the molecular weight, DF is the dilution factor; l is path length (cm) and ε is the molar extinction coefficient. The total anthocyanin content in red cabbage was quantified by considering cyanidin-3-glucoside as major anthocyanin, with a molecular weight of 449.2 g mol^{-1} and a molar extinction coefficient of 26 900 L cm^{-1} mol^{-1}.[9]

Betalains

The betalain content of beet puree was quantified using the method of Wruss *et al.*[10] with some modifications. Puree (5 g) was homogenized with 15 mL of deionized water using a homogenizer in a 50-mL tube for 3 min at 7000 r.p.m. The homogenate was vacuum filtered through Whatman No. 1 filter paper, and the remaining solids were homogenized again in 10 mL of solvent for 1 min. The homogenate was again filtered, combined, diluted to 100 mL in a volumetric flask and used for betalain quantification using a spectrometer. The betalain content was quantified using:

$$\text{Betacyanin/betaxanthin content (mg L}^{-1}) = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l} \quad (3)$$

where $A = A_{536} - A_{650}$ (betacyanins) or $A_{485} - A_{650}$ (betaxanthins), MW is the molecular weight, DF is the dilution factor, l is path length (cm) and ε is the molar extinction coefficient. The molecular weight and molar extinction coefficients of betacyanins and betaxanthins in water are 550 and 339 g mol^{-1} and 60 000 and 48 000 L mol^{-1} cm^{-1}, respectively.

Chlorophylls

The chlorophyll content of pea puree was quantified using the method of Lichtenthaler[11] with some modifications. Puree (3 g) was homogenized with 25 mL of 80% acetone in deionized water using a homogenizer in a 50-mL tube for 5 min at 7000 r.p.m. Tubes were then kept in shaker for 30 min at 300 r.p.m. at room temperature for extraction. Next, tubes were centrifuged at 2968 × g (AccuSpin 400; Fisher Scientific, Pittsburgh, PA, USA) for 6 min at room temperature. The supernatant was collected in a 25-mL volumetric flask, made to the volume and used for chlorophylls quantification using a spectrometer. The chlorophyll content was quantified using:

$$\text{Chlorophyll } a = 12.25\, A_{663} - 2.79\, A_{647} \left(\frac{\mu g}{\text{mL}} \text{of extract}\right) \quad (4)$$

$$\text{Chlorophyll } b = 21.50\, A_{647} - 5.10\, A_{663} \left(\frac{\mu g}{\text{mL}} \text{of extract}\right) \quad (5)$$

$$\text{Total chlorophylls} = 7.15\, A_{663} + 18.71\, A_{647} \left(\frac{\mu g}{\text{mL}} \text{of extract}\right) \quad (6)$$

Data analysis

Kinetic data was analyzed using zero-order, first-order or fractional conversion model to determine the reaction rates for a given quality parameter using Eqns (7), (8) and (9), respectively[12]::

$$C_t = C_0 - kt \quad (7)$$

$$C_t = C_0 e^{-kt} \quad (8)$$

$$\frac{C_t - C_f}{C_0 - C_f} = e^{-kt} \quad (9)$$

where C_t is the quality parameter at given time t, C_0 is the initial concentration, k is the reaction rate constant and C_f is the final equilibrium value.

The SAS University Edition was used for statistical analysis of data using Tukey's honestly significant difference at $\alpha = 0.05$. The general mixed model was employed using the film OTR and the time as independent variables. The effect of thermal treatment on quality parameters was analyzed using one-way analysis of variance.

Table 1. Effect of thermal processing and packaging film on color and pigment content

Attribute		[1]Before pasteurization			After pasteurization		
		F-1	F-30	F-81	F-1	F-30	F-81
Red cabbage	MAC (mg g^{-1} db)	5433 ± 6 a	5970 ± 18 a	5898 ± 11 a	5523 ± 151 a	6003 ± 66 a	5972 ± 107 a
	L^*	20.4 ± 0.13 a	19.6 ± 0.04 a	18.5 ± 0.13 a	22.0 ± 0.45 b	20.7 ± 0.40 a	19.8 ± 0.17 b
	a^*	−0.69 ± 0.03 a	−1.11 ± 0.03 a	1.16 ± 0.02 a	−1.62 ± 0.04 b	−1.38 ± 0.02 b	−0.95 ± 0.04 b
	b^*	−12.4 ± 0.08 a	−13.0 ± 0.05 a	−14.1 ± 0.04 a	−10.1 ± 0.16 b	−10.5 ± 0.03 b	−11.5 ± 0.03 b
	[2]ΔE				2.95 ± 0.14 A	2.81 ± 0.03 A	3.59 ± 0.03 B
Beetroot	BC (mg g^{-1} db)	4747 ± 54 a	4496 ± 72 a	4600 ± 99 a	4137 ± 94 b	3893 ± 156 b	3960 ± 91 b
	BX (mg g^{-1} db)	3003 ± 23 a	2835 ± 25 a	2819 ± 64 a	2699 ± 40 b	2532 ± 168 a	2507 ± 60 b
	L^*	9.41 ± 0.06 a	9.89 ± 0.02 a	9.44 ± 0.05 a	10.2 ± 0.07 b	10.9 ± 0.19 b	10.6 ± 0.24 b
	a^*	13.9 ± 0.04 a	14.1 ± 0.02 a	14.0 ± 0.06 a	12.9 ± 0.36 a	13.1 ± 0.48 a	13.2 ± 0.37 a
	b^*	2.83 ± 0.04 a	2.89 ± 0.02 a	2.79 ± 0.06 a	3.71 ± 0.19 b	3.60 ± 0.18 b	3.66 ± 0.13 b
	[2]ΔE				2.44 ± 0.06 A	2.45 ± 0.10 A	2.89 ± 0.12 B
Peas	Chl. a (μg g^{-1} db)	225 ± 0.7 a	229 ± 1.2 a	225 ± 1.2 a	169 ± 5.0 b	165 ± 4.0 b	165 ± 1.5 b
	Chl. b (μg g^{-1} db)	108 ± 1.2 a	106 ± 2.1 a	107 ± 0.6 a	90 ± 5.8 b	88 ± 3.6 b	87 ± 0.7 b
	L^*	51.8 ± 0.04 a	52.0 ± 0.26 a	52.0 ± 0.22 a	52.0 ± 0.11 a	52.3 ± 0.10 a	52.5 ± 0.14 a
	a^*	−16.0 ± 0.07 a	−15.9 ± 0.04 a	−16.0 ± 0.12 a	−8.48 ± 0.08 b	−8.13 ± 0.04 b	−7.93 ± 0.10 b
	b^*	38.7 ± 0.41 a	38.7 ± 0.39 a	39.1 ± 0.02 a	37.5 ± 0.06 a	37.6 ± 0.26 a	37.6 ± 0.02 b
	[2]ΔE				7.64 ± 0.13 A	7.84 ± 0.00 A	8.17 ± 0.13 B

MAC, monomeric anthocyanin content; BC, betacyanins; BX, betaxanthins; Chl. a, chlorophyll *a*; Chl. b, chlorophyll *b*.
1: $n = 2$ for pigments and $n = 6$ for color, 2: calculated using Eqn (1).
Values with different superscript letters are significantly different ($P < 0.05$).

RESULTS AND DISCUSSION

Processing effect on instrumental color and pigment content

Table 1 demonstrates the variable effect of thermal pasteurization on the color parameters and pigment content of three types of puree depending upon the pouch type.

Cabbage puree

All three color attributes in cabbage puree were significantly affected ($P < 0.05$), regardless of pouch OTR, with an increase in L^* (lightness) (except F-30) and a^* (greenness) and a decrease in b^* (blueness). Jiang *et al.*[13] recently reported a similar trend during pasteurization treatments of purple sweet potato extract in different buffer solutions.

Pasteurization had no significant ($P > 0.05$) effect on the anthocyanin content of red cabbage puree. Dyrby *et al.*[14] reported excellent heat stability of anthocyanins from red cabbage extract in a soft drink model system compared to blackcurrant, grape skin and elderberry anthocyanins when heated at 25–80 °C for 15–360 min. This was attributed to the higher degree of acylation, with most of the anthocyanins being diacylated to number of organic acids. Acylation protects anthocyanins from hydration and improves their stability.[15] Ahmadini *et al.*[9] and Charron *et al.*[16] reported 19.6% and 21.3%, respectively, of nonacylated anthocyanins in red cabbage among all of the major anthocyanins. Similarly, Kirca *et al.*[17] attributed the stability of black carrot anthocyanins heated at 70–90 °C to diacylation in addition to solid content and pH. However, Volden *et al.*[18] found a 59%, 41% and 49% reduction in anthocyanins following blanching, boiling and steaming of red cabbage, respectively.

Beet puree

For beet puree packed in all three different OTR pouches, L^* and b^* (yellowness) were significantly ($P < 0.05$) increased, whereas a^* (redness) was not significantly ($P > 0.05$) reduced. Chandran *et al.*[19] also observed a similar trend during thermal degradation kinetics of beet color over a temperature range of 50–120 °C. According to Chandran *et al.*,[19] betacyanin degradation during heating results in the change from a deep violet–red color to yellowish–brown color, increasing the b^* value. However, Kathiravan *et al.*[20] reported a significant ($P < 0.05$) increase in a^* along with other two parameters during batch pasteurization of ready-to-drink beetroot juice.

Pasteurization significantly ($P < 0.05$) affected the betalain content of beet puree with a 12.8–13.9% and 10.1–11.1% reduction of betacyanins and betaxanthins, respectively depending upon the film type, with larger losses in the high OTR film. Other studies have reported losses of between 6–81% and 13–73% in betacyanins and betaxanthins, respectively, during thermal treatments of red beets and beet products.[20–23] According to Herbach *et al.*,[4] betaxanthins are more sensitive to thermal treatment than betacyanins. However, in the present study, betaxanthins showed higher retention than betacyanins. Jiratanan & Liu[21] observed a reduction of 24%, 62% and 81% in betacyanins, as well as 13%, 60% and 73% in betaxanthins, after treatment at 105, 115 and 125 °C for 30 min, respectively. During thermal treatment, betanin is degraded by isomerization to isobetanin, decarboxylation or aldimine bond cleavage, reducing the red color, as well as dehydrogenation to form neobetanin, imparting a yellow shift.[2,24]

Pea puree

In the case of pea puree, a^* (greenness) decreased significantly ($P < 0.05$) in all pouch types, with a maximum reduction in the F-81 pouch. However, there was no significant ($P > 0.05$) increase in L^* for all pouch types, and no significant ($P > 0.05$) reduction in the b^* (yellowness) value, with the exception of the F-81 pouch. Paciulli *et al.*[25] have reviewed the numerous studies reporting the loss of green color as a result of various thermal treatments in green fruits and vegetable.

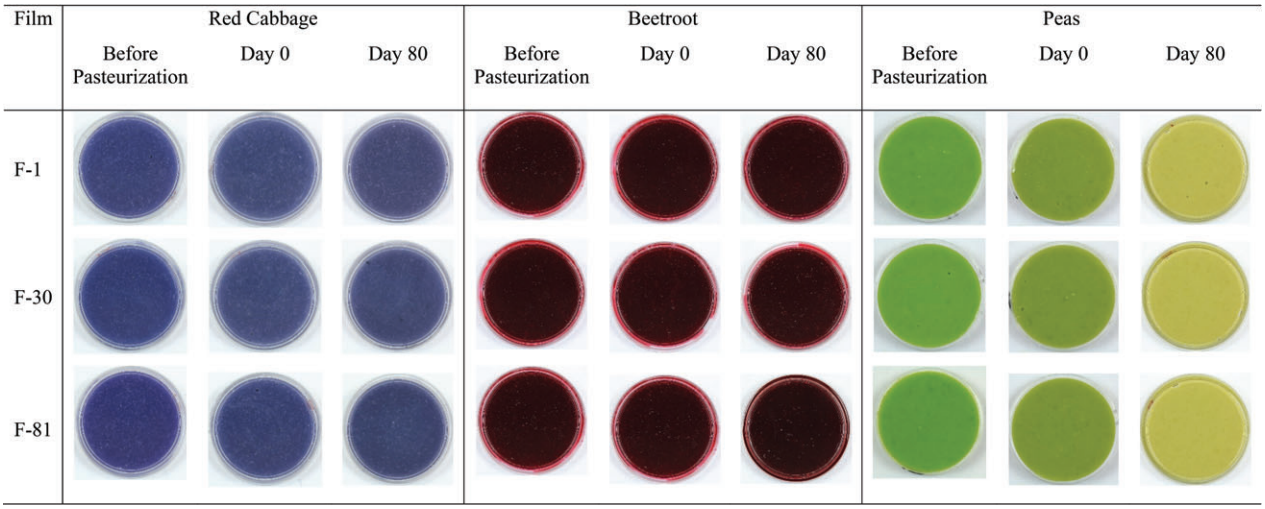

Figure 1. Visual color changes in purees after processing and during storage at 7 °C.

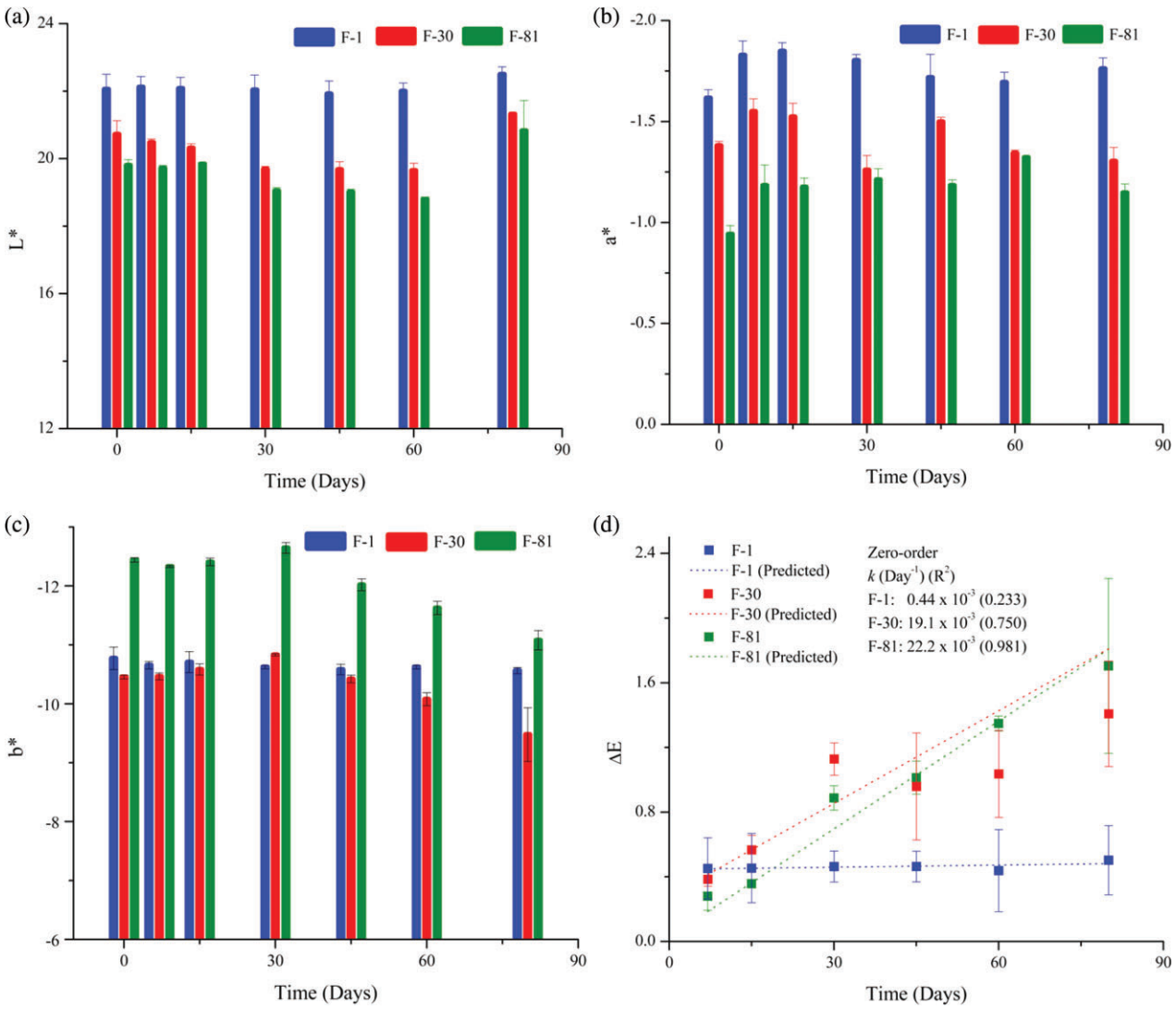

Figure 2. Change in color values for red cabbage puree packed in different packaging films (a) lightness, (b) redness, (c) yellowness and (d) overall color difference during storage at 7 °C.

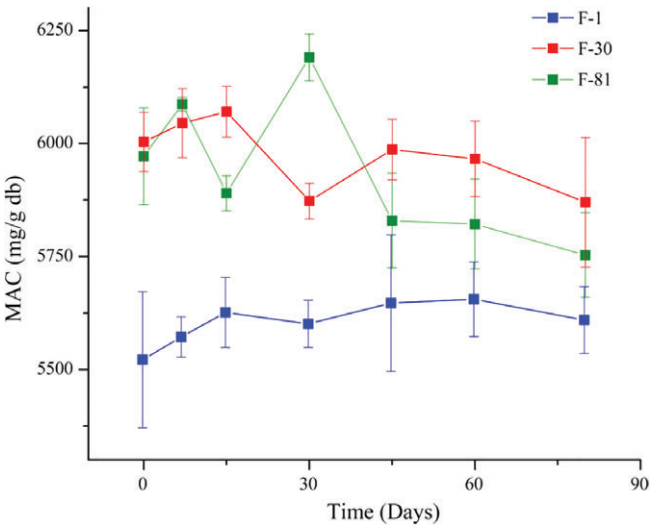

Figure 3. Monomeric anthocyanins content of red cabbage puree packed in different packaging films during storage at 7 °C.

In the present study, pasteurization had a significant ($P < 0.05$) effect on the chlorophyll content of pea puree, with 24.8–27.8% and 16.8–18.1% reductions in chlorophyll *a* and *b*, respectively, depending upon the film type. Larger losses occurred in high OTR film. Paciulli *et al.*[25] reviewed the chlorophyll content and color changes in cooked vegetables, finding a variable degree of losses ranging from 3% to 100% for chlorophyll *a* and from 3% to 90% for chlorophyll *b* after boiling, blanching, steaming and microwaving treatments. Buckle and Edwards[26] reported 11–92% and 8–81% conversion of chlorophyll *a* and *b*, respectively, in high temperature-short time processed pea puree (pH–6.95) at 115.6–148.9 °C. Upon prolonged heating, chlorophyll is degraded into olive–brown pheophytin by the replacement of a Mg^{2+} atom by two H^{+} atoms in the centre of the porphyrin ring of chlorophyll, followed by the degradation of pheophytin to pyropheophytin via decarboxylation.[5] Chlorophyll *a* is more sensitive to pheophytin formation than chlorophyll *b* during heating, with the higher stability of chlorophyll *b* being attributed to the electron-withdrawing effect of its C-3 formyl group.[5]

In the present study, the overall color difference as a result of thermal processing in cabbage, beet and pea puree packed in three types of films with different OTRs was between 2.81 and 3.59, 2.44 and 2.89, and 7.64 and 8.17, respectively. There was a significantly higher color difference ($P < 0.05$) in F-81 pouches for all three purees. This suggests that a higher film OTR influences quality parameters during processing. In a previous study, we found a significantly higher reduction of color parameters and ascorbic acid in the highest OTR film among those selected during pasteurization of carrot puree.[27]

Storage effect on instrumental color and pigment content

Storage had a significant effect ($P < 0.05$) on color parameters and pigment content, depending upon film type in all three purees. Figure 1 illustrates the overall visual color of purees during storage and correlates with instrumental color analysis. Changes in color parameters during storage for all three purees were associated with changes in respective color pigments, as described below.

Cabbage puree

In cabbage puree, L^* was not significantly affected ($P > 0.05$), regardless of pouch type. However, a^* (greenness) increased and b^* (blueness) decreased over the storage period, showing significant ($P < 0.05$) changes in the F-81 pouch only (Fig. 2). The overall color difference at the end of storage was significantly ($P < 0.05$) lower for puree in the F-1 pouch than for the other two pouches, ranging between 0.50 and 1.70. Although the differences were significant, the values were less than the visually perceptible color difference value of 3 (Fig. 1). Walkowiak-Tomczak & Czapski[28] reported a decline in the color intensity of pasteurized (85 °C for 15 min) red cabbage colorant preparation (pH: 3–5) stored at 10–30 °C for 0–30 days. They also observed higher colorant losses in samples stored under aerobic conditions compared to under relatively anaerobic ones.

Anthocyanins remained stable, with no significant ($P > 0.05$) reductions during storage, irrespective of film OTR (Fig. 3). Puree packed in F-1 pouch retained anthocyanins during storage, whereas losses of only 2.3% and 3.8% were observed in the puree packed in F-30 and F-81 pouches, respectively. To our knowledge, there are no published studies on the effect of packaging films on thermally pasteurized red cabbage/products. Although anthocyanins are sensitive to oxygen, anthocyanins from red cabbage demonstrate excellent stability during storage, even for higher OTR films. Generally, anthocyanins from red cabbage show the best stability compared to anthocyanins from other fruits and vegetables as a result of the presence of diacylated anthocyanins, which show a higher stability than monoacylated ones during storage.[8] Kirca *et al.*[29] found that storage temperature strongly influenced the degradation of black carrot anthocyanins in black carrot colored juices and nectars with higher Q_{10} values. They found a much lower degradation of anthocyanins at 4 °C over 180 days storage compared to 30 days storage at 37 °C. In the present study, refrigerated storage for comparatively less time would have added in the stability of anthocyanins during storage.

Anthocyanin stability during storage can be correlated with color parameters. Overall color difference for puree packed in the F-1 pouch remained stable throughout storage, whereas there was gradual increase in color difference for puree packed in F-30 and F-81 pouches. Walkowiak-Tomczak & Czapski[28] have reported a positive correlation between color parameters and anthocyanin content in pasteurized red cabbage colorant preparation. They observed a drop in anthocyanin content with an increase in pH, ascorbic acid concentration, storage temperature and time.

Beet puree

The lightness of the beet puree decreased marginally during storage; however, no significant ($P > 0.05$) differences were observed between the three pouches. Similarly, a^* and b^* decreased during storage in all pouch types. Significant ($P < 0.05$) reductions were observed in b^*, irrespective of pouch type, although a^* was significantly ($P < 0.05$) affected only in the F-81 pouch (Fig. 4). The overall color difference at the end of the storage period differed significantly ($P < 0.05$) between the three films, with values of 1.00, 2.08 and 4.55 for puree packed in F-1, F-30 and F-81 pouches, respectively. For the puree packed in the F-1 pouch, the color difference increased during the first 2 weeks of storage because of drop in b^* value (Fig. 4c) and remained stable throughout storage (Fig. 4d). Herbach *et al.*[30] reported a decrease in chroma (C^*) and an increase in L^* during the storage of pasteurized (95 °C for 5 min) purple pitaya juice (pH–4.0) stored in glass vials at 20 °C for 6 months. By

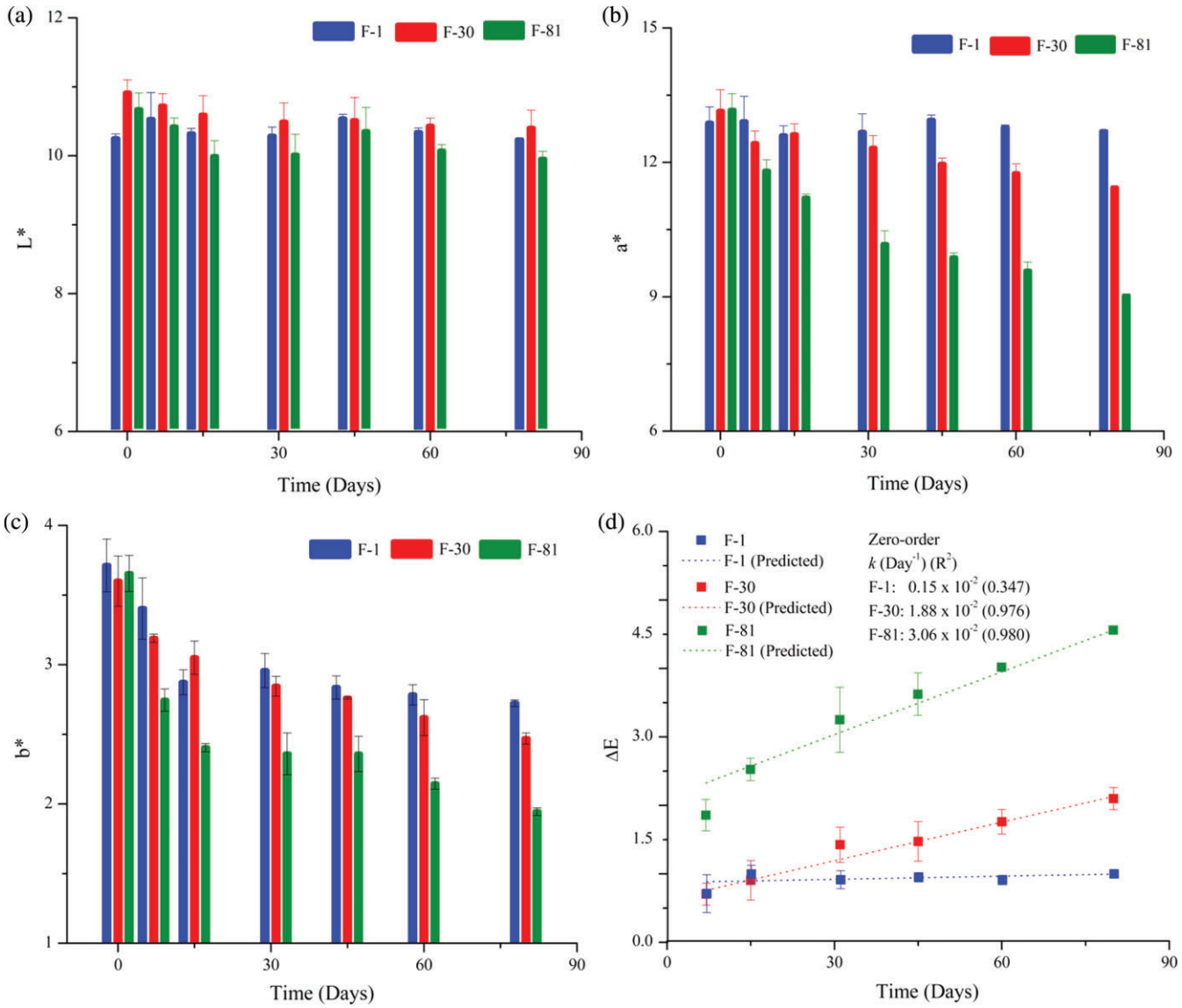

Figure 4. Change in color values for beetroot puree packed in different packaging films (a) lightness, (b) redness, (c) yellowness and (d) overall color difference during storage at 7 °C.

contrast, Kathiravan *et al.*[20] observed an increase in all color parameters during storage of pasteurized ready-to-drink beetroot juice (pH–4.2) packed in Al-foiled pouches at 30 °C for 6 months.

In the present study, betalains exhibited maximum sensitivity to oxygen during storage, with significant differences ($P < 0.05$) among the three different OTR films (Fig. 5). The reduction in total betalains (the sum of betacyanins and betaxanthins) for puree packed in F-1, F-30 and F-81 pouches was 4.51%, 27.0% and 49.3%, respectively, at the end of storage period. Betacyanins were decreased by 0.48%, 27.7% and 54.8%. However, betaxanthins decreased by 10.7%, 25.9% and 40.5% in the puree packed in F-1, F-30 and F-81 pouches, respectively. Betaxanthins decreased linearly during the first month of storage and decreased gradually thereafter. Kathiravan *et al.*[20] observed similar trends for thermally pasteurized acidified beetroot juice packed in PET/aluminium/Nylon/cast polypropylene pouches, with a 64.1% and 67.4% reduction in betacyanins and betaxanthins, respectively, at the end of day 105 day of storage, followed by a gradual decrease thereafter. In the present study, the oxygen in the headspace, dissolved in the matrix, or permeating through packaging material, affected the stability of betalains during storage. This was indicated by the maximum degradation in the puree packed in the F-81 pouches, which had the highest OTR. Few studies have demonstrated the sensitivity of betalains to oxygen.[4,31]

Betalamic acid and cyclodopa-5-*O*-glucoside formed during thermal treatment degrades further in the presence of oxygen to form browning reaction products such as melanoidins.[5] In the present study, this was evident from the browning and higher color difference of puree packed in F-81 pouches (Fig. 1). The loss of betacyanins and betaxanthins during storage correlates well with the loss of color values a^* and b^*, respectively. There was negative correlation between total betalain content and ΔE, with values between −0.06 and −0.97, depending upon the film type. The initial reduction of betaxanthins in the puree packed in the F-1 pouch could be a result of residual oxygen content. This correlates with a decrease in b^* value or an increase in ΔE. Herbach *et al.*[30] have attributed the decrease of chroma to the increased formation of betacyanin degradation products in purple pitaya juice.

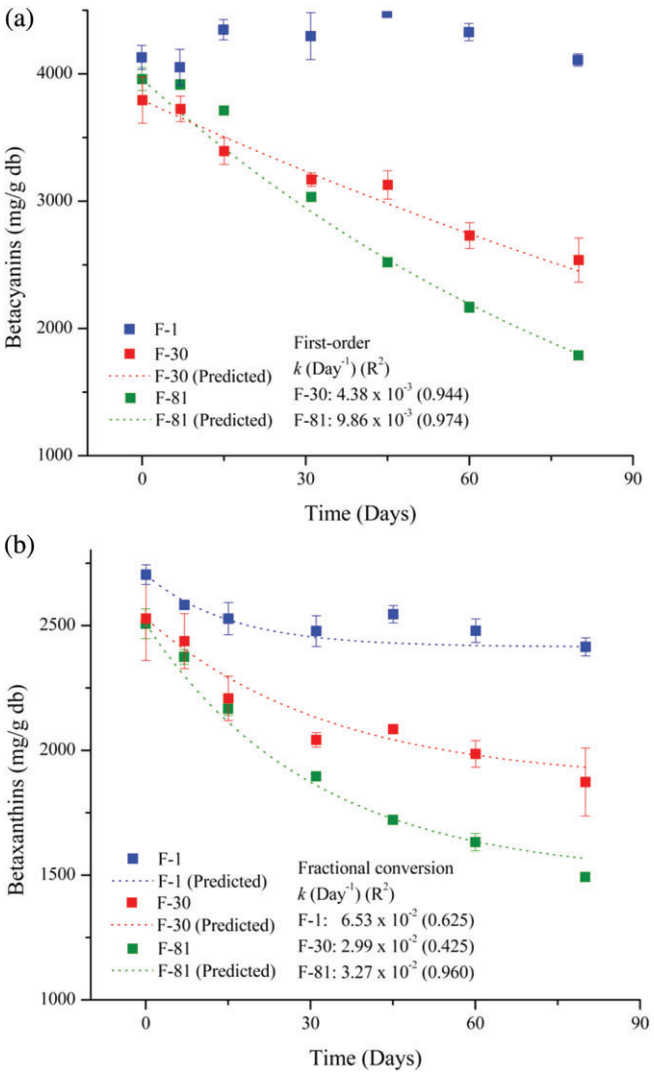

Figure 5. (a) Betacyanin and (b) betaxanthin content of beetroot puree packed in different packaging films during storage at 7 °C.

Pea puree

Pea puree packed in all three pouches exhibited significant ($P < 0.05$) changes during storage, with an increase in L^* and a decrease in a^* (greenness) and b^*, irrespective of film type (Fig. 6). The overall color difference did not differ significantly ($P > 0.05$) among the films, and values ranged between 7.60 and 8.02. There was a rapid loss of color values during the first month of storage, followed by a gradual reduction. Buckle and Edwards[26] reported a similar trend in high temperature-short time pea puree stored at 20 °C for 18 months. Zhao *et al.*[32] observed similar loss of color parameters during storage of pasteurized cucumber juice, with an overall color difference of 9.20 at the end of the storage period.

Chlorophylls were continuously degraded throughout the storage period irrespective of packaging type, with no significant differences ($P > 0.05$) among the three films (Fig. 7). The reduction in total chlorophylls at the end of storage period was 34.9%, 33.9% and 33.1% for the puree packed in F-1, F-30 and F-81 pouches, respectively. Chlorophyll *a* was reduced by 27.3%, 26.5% and 25.9%, whereas chlorophyll *b* decreased by 49.4%, 47.7% and 46.7% in the puree packed in F-1, F-30 and F-81 pouches, respectively. There was a rapid degradation of chlorophylls during the first month of storage. Chlorophyll *a* was relatively stable, whereas chlorophyll *b* degraded gradually after the first month of storage. Zhao *et al.*[32] observed 83% and 77% reductions in chlorophyll *a* and *b* in pasteurized cucumber juice (pH–6.60) stored at 4 °C for 50 days. Benlloch-Tinoco *et al.*[33] reported a similar trend in pasteurized (97 °C, 30 s) kiwifruit puree (pH–3.36) stored at 4–22 °C for 63 days.

A drop in pH during storage may have contributed to chlorophyll degradation. Buckle and Edwards[26] observed a direct relationship between pH changes, pigment content and color during storage. Chlorophyll degradation during storage also correlates well with the loss of greenness (a^*) value and an increase in lightness. There was negative correlation between total chlorophylls content and ΔE, with values between −0.92 and −0.97, depending upon the film type. After chlorophylls are completely converted to pheophytins, they may degrade further to pheophorbides.[34] These pheophorbides may eventually degraded to some colorless compounds through different pathways, possibly affecting lightness of the product.[35]

Kinetics of color change and pigments degradation

Zero-order reaction kinetics describes the best the overall color difference during storage for cabbage (Fig. 2d) and beet puree (Fig. 4d). Several studies have reported a zero-order model for color change during storage, including Patras *et al.*[36] in strawberry jam, Wibowo *et al.*[12] in acidified pasteurized mango juice and Wibowo *et al.*[37] in orange juice. However, fractional conversion model fits well for pea puree color degradation during storage (Fig. 6d). Buve *et al.*[38] reported a fractional conversion model for ΔE in pasteurized (95 °C for 120 s) strawberry juice stored at 20 °C for 32 weeks. Other studies have reported a similar model for ΔE during storage of frozen products, including Gonçalves *et al.*[39] for pumpkin and Martins *et al.*[40] for green beans.

In the present study, first-order model and fractional conversion model gave the best fit for betacyanins (Fig. 5a) and betaxanthins (Fig. 5b) degradation, respectively. The results show that the F-1 pouches preserved betalains during storage, especially betacyanins, yielding a very low regression coefficient value. There is no kinetic information available on betalains degradation during storage as influenced by packaging OTR. However, Attoe & von Elbe[31] examined the degradation kinetics of betanine as a result of oxygen concentration. They concluded that betanin degradation followed pseudo first-order rate when oxygen was present in excess; however, this may differ when oxygen supply is limited. The former could be the case with F-30 and F-81 pouches where a higher OTR may have provided sufficient oxygen supply to follow the first-order rate; the latter could be the case with the F-1 pouch with limited oxygen supply as a result of its high barrier properties.

The fractional conversion model gave the best fit for chlorophyll *a* and *b* degradation during storage; however, regression coefficient values were low for chlorophyll *a* (Fig. 7). To our knowledge, no kinetic information is available for chlorophyll degradation as a function of oxygen concentration. Schwartz and Lorenzo[41] have reported first-order reaction kinetics of chlorophyll *b* degradation in aseptically processed spinach puree packed in aluminium foil pouches stored at 4 °C for 1 month; however, the rate constant for chlorophyll *a* was not calculated as a result of its rapid degradation forming compounds limiting analysis. Benlloch-Tinoco *et al.*[33] have given a second-order model for total chlorophylls and their derivative compounds during storage of pasteurized kiwi puree.

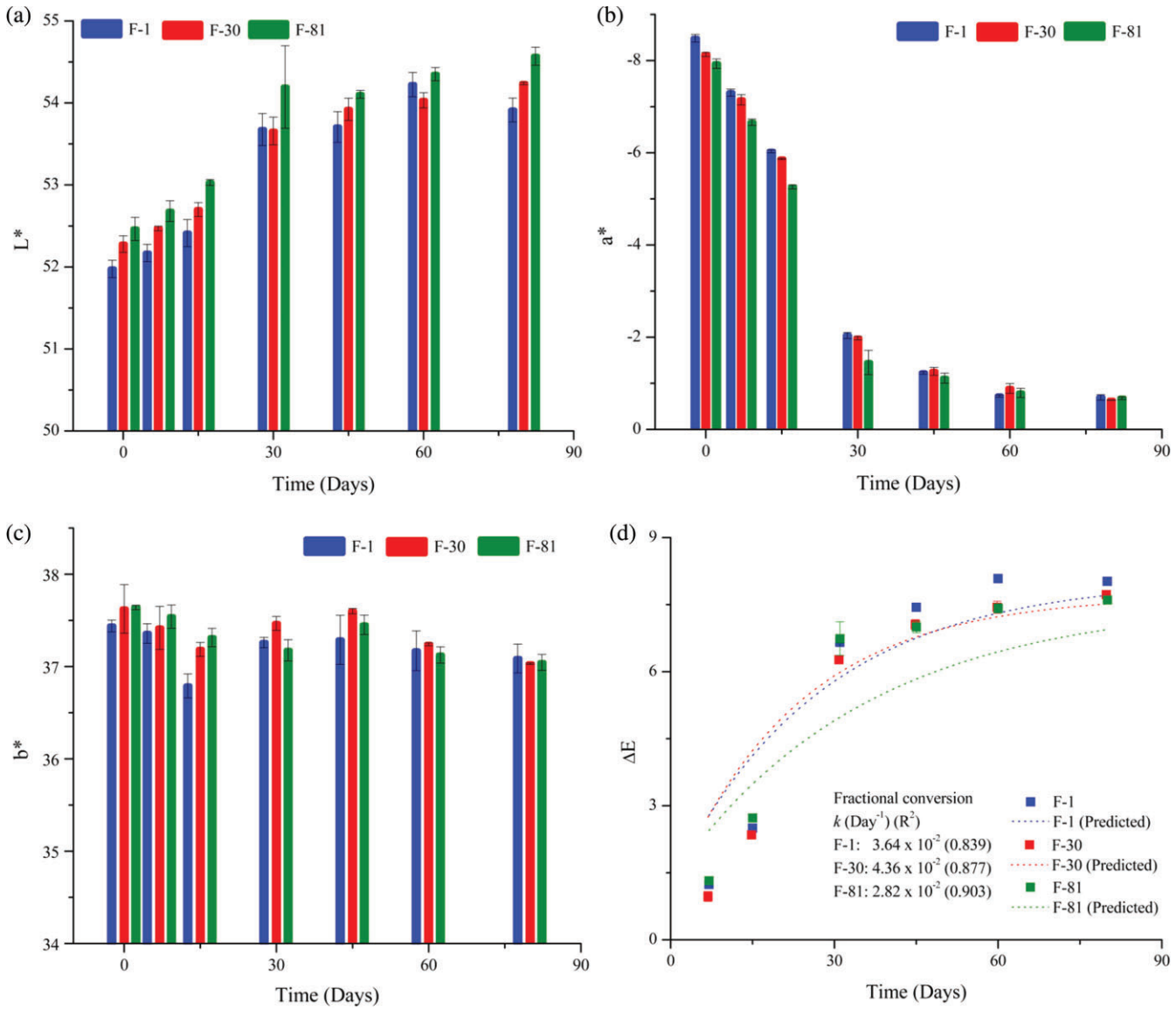

Figure 6. Change in color values for pea puree packed in different packaging films (a) lightness, (b) redness, (c) yellowness and (d) overall color difference during storage at 7 °C.

Total plate count and pH

In the present study, the microbiological quality of cabbage and beet puree was retained at the end of the storage period. However, proliferating growth was observed in pea puree during storage. The anaerobic bacteria count (both mesophilic and psychrophilic) was below the detection limit. The aerobic count increased to 1–3 log CFU g^{-1} from an initial value below the detection limit in the case of cabbage and beet puree at the end of the storage period (Fig. 8). However, for pea puree, the mesophilic and psychrophilic aerobic count, as well as the anaerobic mesophilic count, reached 5–6 log CFU g^{-1} from an initial value of 0–2 log CFU g^{-1}. Additionally, the psychrophilic anaerobic count reached <4 log CFU g^{-1} from an initial level below the detection limit. Although growth was observed during storage of pea puree, no visible spoilage indicators, such as bulging, a putrid smell or discoloration, were observed in any of the pouches. These differences of microbial growth in different types of puree may come from food composition, initial contamination level, disinfection prior to blanching, and the extent of heat treatment during blanching and pasteurization. Carlin *et al.*[42] observed an increase of aerobic and anaerobic count in pasteurised vegetable purees from an initial population of <2 log CFU g^{-1} to 6–8 log CFU g^{-1} during storage of 6 weeks at 4 and 10 °C. They reported that *Bacillus* species was the main spoilage bacteria in commercially pasteurized vegetable purees stored at refrigeration and abuse temperatures. This was a result of the probable survival of spores following pasteurization process designed for non-proteolytic *Clostridium botulinum* type E.

In the present study, the pre-processing pH ($n=6$) for cabbage, beet and pea puree was 6.86 ± 0.07, 6.40 ± 0.02 and 7.03 ± 0.03, respectively. After pasteurization, the pH dropped marginally in all purees, with values ranging from 6.60 ± 0.00 to 6.69 ± 0.02 for cabbage puree, from 6.31 ± 0.03 to 6.34 ± 0.02 for beet puree and from 6.90 ± 0.01 to 6.95 ± 0.01 for pea puree, depending on the OTR of film. This may be a result of the release of acids during heating of plant tissue.

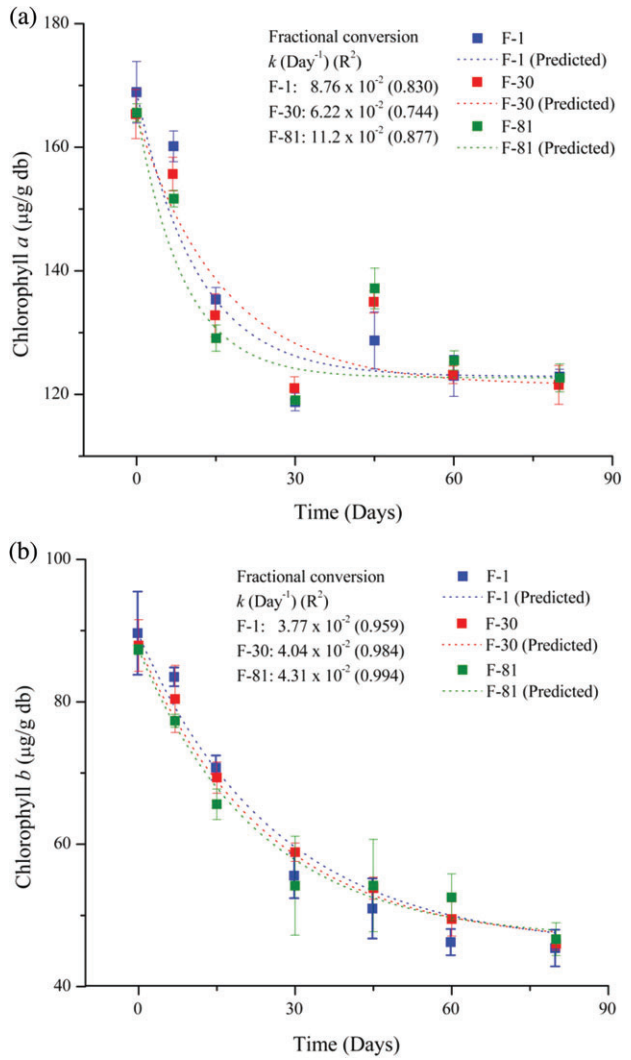

Figure 7. (a) Chlorophyll *a* and (b) chlorophyll *b* content of pea puree packed in different packaging films during storage at 7 °C.

At the end of the storage period, the pH of cabbage and beet puree was relatively stable, ranging from 6.55 ± 0.04 to 6.62 ± 0.03 and from 6.26 ± 0.04 to 6.33 ± 0.03, respectively. However, in the case of pea puree, the pH drop ranged from 5.64 ± 0.03 to 5.70 ± 0.03. Chlorophyll degradation in addition to microbial growth may be responsible for this pH change. Buckle and Edwards[26] reported a pH drop of 0.38–0.86 units in pea puree processed at 115.6–148.9°C and stored at 20 °C for 18 months. Valero *et al.*[43] reported a pH drop from 6.3 to 4 in pasteurized (78.2 °C for 14 min) white asparagus as a result of the outgrowth of lactic acid bacteria and other spoilage organisms. Carlin *et al.*[42] observed a pH drop from an initial average of 6.0–6.5 to 5.5–6.6 in commercially pasteurized (80 °C, 30 min) vegetable purees. Similarly, Zhao *et al.*[32] found a pH drop in pasteurized (85 °C for 15 s) cucumber juice of > 2 pH units and a total aerobic count reaching 5 log CFU g^{-1} at the end of 50 days of storage at 4 °C.

Weight loss

In the present study, the moisture content of cabbage, beet and pea puree after processing was 959 ± 1.0, 909 ± 2.8 and 869 ± 1.9 g water kg^{-1} wet solids, respectively, remaining stable throughout the storage period. The total weight loss at the end of storage

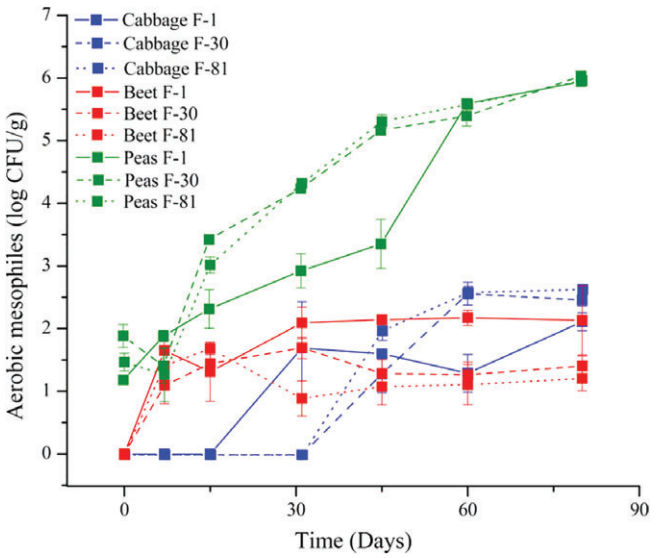

Figure 8. Aerobic mesophilic count of three types of puree packed in different packaging films during storage at 7 °C.

period in all pouch types was between 0.09% and 0.15%, 0.18% and 0.34%, and 0.08% and 0.26% for cabbage, beet and pea puree, respectively. The highest losses occurred in the F-81 pouches. The WVTR of the F-81 film was 6.59 g m^{-2} day^{-1}, which was highest among the selected films. Similar results were observed in pasteurized carrot puree stored at three different temperatures, with a maximum weight loss of 0.42%.[27] Weight loss can be very high for low barrier films; however, low storage temperatures and a relatively short storage period may have contributed to smaller weight losses in the present study.

CONCLUSIONS

The present study has highlighted the oxidative stability of natural color pigments during storage to oxygen permeating through packaging films of varied gas transmission rates. Anthocyanins in red cabbage showed excellent stability, irrespective of film OTR, and the overall quality of cabbage puree was quite stable during storage, indicating that medium to low barrier films are more suitable for processed red cabbage-based products. However, optimum selection should also take in account the stability of other oxygen-sensitive components, such as vitamins and lipids. Red beet puree was highly affected by film type, showing highest losses of betalains in high OTR film. Therefore, high barrier films are necessary to maintain nutritional and visual characteristics of beet-based or betalain-containing products. The results for pea puree were not definitive. The quality deterioration level was similar in pea puree, irrespective of film type. However, based on other quality parameters, high to medium barrier films appear to be optimum for food products containing chlorophyll. Our selected OTR range was representative of those used commercially for in-pack pasteurization. Therefore, our findings may be used to guide optimum packaging selection for pigment-rich products.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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