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Plum (*Prunus salicina*) peel and pulp microparticles as natural antioxidant additives in breast chicken patties

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ABSTRACT

Fiber microparticles (MPCs) separately obtained from peel and pulp of Japanese plum residues contained co-extracted β-carotene, lutein, and α- and γ-tocopherols, as well as polyphenols (cyanidins, quercetin derivatives, pentameric proanthocyanidins). Peel and pulp MPCs were then separately evaluated as natural antioxidant additives (2.0% w/w level) in raw breast chicken patty, susceptible to oxidation. Their effect on technological properties was also analyzed. MPCs reduced in 50% the formation of thiobarbituric acid reactive substances (TBARS) in raw patties during 10-days storage at 4.0ºC. Ferric reducing power (FRAP) was 77-157% higher in MPCs-added patties, especially with peel MPCs, being then attributed to the antioxidants supplied by these MPCs. It can be also associated to the highest α- and γ-tocopherol levels found in the peel MPCs-added patties, which remained high after cooking as well. Also, higher pectin and low lignin contents of pulp MPCs determined greater hydration, stabilized the cyanidins and, hence, the red color transferred to raw patties, and increased springiness of cooked patties. Plum peel and pulp MPCs are efficient additives for chicken meat products.

Keywords: Plum fiber microparticles, natural antioxidant additive, chicken patties, tocopherols, color and texture modifier, hydrated pectins.

Chemical compounds: Beta carotene (PubChem CID: 5280489); Lutein (PubChem CID: 181579); Alpha-tocopherol (PubChem CID: 14985); Gamma-tocopherol (PubChem CID: 92729); Malondialdehyde (MDA, PubChem CID: 10964); Proanthocyanidin (PubChem CID: 21881649); Cyanidin (PubChem CID: 128861); Quercetin (PubChem CID: 5280343); Lignin (PubChem CID: 73555271); Polygalacturonic Acid (PubChem CID: 445929).

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1. Introduction

Food processing (mechanical stresses, heating, irradiation) generates a whole range of radical and non-radical reactive oxygen species (ROS) from lipid and protein oxidation, and from ionization of intracellular water (e.g. aqueous electron, hydroperoxide radicals, $^{\bullet}OH$, H_2O_2) (Latorre, Narvais, Rojas, & Gerschenson, 2010). Lipid oxidation produces the alteration of sensory characteristics and shortens the shelf-life of meat products among other processed foods. Nitrites and phosphates are two additives with antioxidant activity commonly used in meat products. Nitrites prevents from the iron release from the heme prosthetic group of myoglobin. Phosphates chelate ions of pro-oxidant metals, and increase the pH and ionic strength, inducing changes in the myofibrillar protein conformation which partially inhibit reactions with peroxidized lipids. Additionally, the meat industry also uses additives known as synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Synthetic antioxidants are subjected to concerns regarding safety. Therefore, the research about strategies for inhibiting lipid oxidation while making the meat products healthier has increased considerably in recent years. Natural antioxidants have gained ground due to their health and safety advantages linked to its efficiency in reducing lipid oxidation (Miotto Bernardi, Bertol, Bertelli Pflanzer, Sgarbieria, & Rodrigues Pollonio, 2016; Pokorný & Schmidt, 2001).

Annually, 700 million tonnes of plums are sold, with an export value of 650 million Euros (Fresh Plaza report, 2015). Argentina continues being the world's fourth biggest producer of this fruit (Fresh Plaza, 2017). The small misshapen, bruised and/or overriped Japanese plums (*Prunus salicina*) that are discarded, can be an alternative carbon source for chemical commodities (i.e. food additives), adding value to the raw

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materials and contributing to a sustainable development (COST, 2013-2014). Basanta, Marin, De Leo, Gerschenson, Erlejman, Tomás-Barberán, and Rojas (2016) upgraded the discarded Japanese plums as fiber microparticles (MPCs) that were separately obtained from the exocarp (peel or skin) and mesocarp (pulp or flesh) tissues, and then freeze-dried. Despite the ethanolic treatment used for the extraction, plum MPCs retained polyphenolic compounds as pentameric proanthocyanidins, with a similar proportion in peel and pulp MPCs. Simultaneously, the higher content of cyanidins found in peel MPCs can be responsible for the intense fiber's red color. All these compounds provided antioxidant capacity as well as protective effect on human embryonic kidney 293 cells against the oxidative stress induced by *tert*-butylhydroperoxide, together with low citotoxicity (50%-cytotoxic concentration > 100 μg/mL). Therefore, plum MPCs separately obtained from peel and pulp can be proposed as natural antioxidant additives for example for meat products. Anyhow, fiber MPCs should be considered since they can affect the organoleptic quality of the food products where the fibers are added.

Grape seed extracts, a broccoli powder aqueous extract, rosemary and green tea extracts as well as the skin by-product of the peach processing industry were previously assayed as natural antioxidants in meat products, including goat meat nuggets, chicken burgers and ground turkey meat, among others. They were able to significantly inhibit the lipid oxidation, in general determined through the TBARs' test, during storage of meat products under refrigeration (4ºC) or freezing (−18ºC), being also efficient in comparison to synthetic antioxidants such as BHA and BHT (Banerjee, Verma, Das, Rajkumar, Shewalkar, & Narkhede, 2012; Pateiro, Lorenzo, Amado, & Franco, 2014; Zhang, Han, Bridges, and Dawson, 2016; Pires, Munekata, Villanueva, Tonin, Baldin, Rocha, et al., 2017).

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As reported by Miotto Bernardi et al. (2016), studies have also been performed on the use of plum extract, purée and concentrated juice as antioxidants in meat products. Positive results were reported in this sense for cooked sausages, ham, roast beef, lean beef cuts, turkey breast and low fat beef patties during refrigeration or freezing. Simultaneously, negative effects on color, and higher cooking loss (CL) and shear strength were observed in the meat products containing the plum additive than in control samples without the plum ingredient.

Chicken meat is characterized by a high PUFA (polyunsaturated fatty acids) content, which are particularly susceptible to lipid oxidation (Ganesan, Brothersen, & McMahon, 2014; Milićević, Vranić, Mašić, Parunović, Trbović, Nedeljković-Trailović, et al., 2014). Additionally, chicken skin's fats are particularly rich in oleic (≈57%) and palmitoleic (≈11.7%) acids (Méndez-Lagunas, Siles-Alvarado, Rodríguez-Ramírez, & Aquino-González, 2015). Hence, a chicken meat product constitutes an interesting matrix to evaluate the utility of plum peel and pulp MPCs as antioxidant food additives.

Based on the phenolic content and the protective effect against the oxidative stress, together with the low cytotoxicity previously determined by Basanta et al. (2016), the purpose of the present work was to evaluate separately the antioxidant capability of plum peel and pulp MPCs in the preservation of raw breast chicken patties during refrigerated storage at 4.0ºC, in comparison to chicken patties made without plum fiber MPCs (control system). MPCs were assayed in this work at the level of an additive: 2.0 g of peel or pulp MPCs per 100 g of total patty. Unlike previously published works, information was also obtained concerning the contents and stability of α- and γ-tocopherols during refrigerated storage of chicken patties and after cooking,

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associated to the FRAP reducing activity observed. Also, the effect of plum MPCs on the technological properties (cooking loss, expressible moisture), color and textural parameters of cooked breast chicken patties, which were derived from other functional properties associated to the respective biopolymer composition of plum fibers, were analyzed.

2. Materials and methods

2.1. Chemicals

Sodium chloride (NaCl, Dos Anclas, Buenos Aires, Argentina) and sodium tripolyphosphate (STPP, N 15-16 Chemische Fabrik Budenheim R.A Oetker, Budenheim, Germany) were used for chicken patties manufacturing. The other chemicals were from Sigma-Aldrich (Saint Louis, USA) and Merck (Argentina). Deionized water (Milli-Q™, Millipore, USA) was used for chemical analyses.

2.2. Plum fiber microparticles

The MPCs were extracted with ethanol separately from the exocarp (peel) and mesocarp (pulp) tissues of discarded Japanese plums, according to Basanta et al. (2016). They were separately used after freeze-drying (Christ Alpha lyophilizer, Germany; Pfeiffer vacuum pump, Germany) for 24 h at room temperature, of the frozen alcohol insoluble residue and evaluated in the present work as two potential antioxidant additives.

2.3. Physical and hydration properties of fibers

The specific volume (SV) as well as swelling (SC), water holding (WHC) and water retention (WRC) capacities, and the kinetic of spontaneous water absorption were

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separately determined in peel and pulp fiber MPCs, and data were fitted through the following power law corresponding to the Ritger and Peppas' model, as described by Idrovo Encalada, Basanta, Fissore, De'Nobili, and Rojas (2016).

$$q = k \cdot t^{n} \qquad (1)$$

where q (mL/g) is the water absorbed at time t, k is a constant dependent on kinetic features and experimental conditions, and n is the swelling exponent.

2.4. Preparation of chicken patties

Chicken breasts were separated under strict good manufacturing practice (GMP) conditions from forty-two days old double-breasted male broilers (2600 ± 230 g body weight), raised in indoor confinement (Granja Tres Arroyos S.A., Buenos Aires, Argentina). First, skin and fat were totally removed. Then, skin and meat were refrigerated (1.5 ± 0.5°C). Two chicken patty systems containing either peel MPCs or pulp MPCs as well as the corresponding control patty system without fiber MPCs were prepared in different batches according to the composition shown in **Table 1**. The percentage of water was modified with the addition of fiber (**Table 1**). Meat and skin were separately minced using a 13 mm and a 4 mm plate, respectively, in a Hobart meat grinder (Hobart Corp., Troy, Ohio, USA). During mincing, temperature was monitored using a puncture thermometer (Testo model 230, Sparta, NJ, USA), and it did not overcome 10°C. After mixing meat and skin by hand, STPP (dry powder) and NaCl (previously dissolved in water at 8°C) were incorporated. Then, the mix was again mixed by hand during 5 min. After that, portions of 140 g were formed into patties between grease proof papers using a manual patty press (100 mm diameter, 1.5-cm height). Patties were placed in plastic trays and stored at −20 °C for 24 h. Two smaller patties (50 mm diameter each one) were obtained from each patty using a punch. Patties

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were put on expanded polystyrene trails, covered by polyethylene films (LDPE; oxygen permeability = 5.20×10^{-12} $m^3 \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$), and stored, in the dark, at 4.0 ± 0.5 °C.

Raw patties were evaluated on days 0, 1, 3, 6 and 10. Besides, patties separately taken at 0, 3 and 10-day-storage were placed in aluminum trays and cooked in a grill (George Foreman, USA) at 165-180°C for being evaluated in their α- and γ-tocopherol contents following described, until reaching a temperature of 75°C at the center of the sample (end of cooking). The temperature in the center of each patty was monitored with a T-type flexible thermocouple coated with high temperature resistant ceramic, and data were recorded using a digital multimeter Hydra 2625A Fluke Model Brand (John Fluke Mfg. Co., Inc., Everett, USA). Technological (cooking loss, expressible moisture, color) and textural parameters were measured only in patties cooked at 10-day-refrigerated-storage.

The complete procedure above described was performed in triplicate for each chicken patty system prepared according to **Table 1**.

2.5. Chemical analysis

2.5.1. Lipid oxidation

It was determined in the raw chicken patties by measuring the thiobarbituric acid reactive substances (TBARS), according to Pouzo, Descalzo, Zaritzky, Rossetti, and Pavan (2016). Briefly, triplicate aliquots (10 g) of meat were chopped and processed in a stomacher-type homogenizer for 180 s in bags containing 50 mL trichloroacetic acid solution (10% w/v). Slurries were filtered, an equal volume (10 mL) of 0.02 M 2-thiobarbituric acid was added, and samples were incubated at 25ºC overnight to yield a pink color development. Color intensity was determined at maximum absorption (530 nm), and TBARS concentrations were calculated form a calibration curve using 1,1,3,3-

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tetraethoxypropane as standard within the 0.0-0.5 μM range. Results were expressed as mg of malondialdehyde (MDA) per kg of product.

2.5.2. Antioxidant capacity

It was determined in raw chicken patties as the ferric reducing power (FRAP), according to the technique modified by Pouzo et al. (2016) for meat products tomeasure endogenous ions that could react with TPTZ, and develop blue color (i.e. endogenous Fe^{II}). Briefly, 5 g of chopped meat sample was disrupted for 2 min at 3000 rpm with an Ultraturrax (IKA, Germany) homogenizer in 10 mL potassium phosphate buffer (pH 7.2). Homogenates were centrifuged at 10,000×g (30min), and the supernatant collected. An aliquot of supernatant was added to a FRAP buffer volume (10mM TPTZ, 40 mM HCl, 20 mM Fe_2Cl3 in 300 mM acetate buffer), and incubated for 30 min in a 37ºC-water bath, cooled in an ice water bath and immediately measured at 593 nm (Spectrometer UV–vis-BIO Lambda 20, Perkin Elmer, USA). Results were expressed as Fe^{2+} equivalent in mM per kg of product.

2.5.3. Tocopherols, β-carotene and lutein contents

α-Tocopherol, γ-tocopherol, β-carotene and lutein were extracted from each kind of plum fiber assayed (pulp or peel MPCs) in triplicate as described by Rossetti et al. (2010). Briefly, saponification was performed for 30 min at 70ºC with 10 N KOH. Samples were then extracted twice with n-hexane, evaporated under a nitrogen flow, dissolved in absolute ethanol and filtered through a 0.45 μm pore nylon membrane before injection. Afterwards, all samples and external standards for each compound were analyzed through high performance liquid chromatography (HPLC) by using a quaternary gradient pump (P4000, Thermo Scientific, USA), with a membrane vacuum

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degasser connected to an auto sampler AS2000 (Thermo Separation Products) with an injection loop (10 to 100 μL), and a C18 column (250 × 4.6mmi.d., Alltima, 5 μmparticle size; Alltech, USA) fitted with a guard column (Security GuardAlltima C18, Alltech, USA) and a mobile phase of ethanol:methanol (60:40 v/v) at a flow rate of 1 mL/min. The technique was optimized to determine tocopherols, carotenoids and retinol within the same elution time of 25 min, and detection was carried out through fluorescence (FL3000; Thermo Separation Products, USA) and a diode array detector (UV6000; Thermo Separation Products, USA), as described by Pouzo et al. (2016).

On the other hand, α-tocopherol and γ-tocopherol were the only antioxidant compounds that were also determined in raw (0, 1, 3, 6 and 10 days storage) and cooked (0, 3 and 10-day-storage) chicken patty samples. Their extraction from these meat products and quantification by the HPLC method above described were performed as indicated by Pouzo et al. (2016).

2.6. Color parameters

*L** (lightness), *a** (redness/greenness) and *b** (yellowness/blueness) color parameters were measured in the CIE *L*a*b** system on raw chicken patties at day 0 and day 10 of refrigerated storage, as well as on patties cooked after 10 days of refrigerated storage, with a Minolta chroma meter (model CR400, Konica Minolta, Japan), using D65 standard illuminant and 2° observer angle. Each patty was placed on a white tray and color parameters were measured on four different points on patties surface, located at 0.5 cm from the edge and at 90º to each other.

2.7. Cooking loss (CL)

It was determined by weighing patties (10-days storage) before and after

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cooking. The cooked patties were dried with towel papers to remove the excess of fat and liquid released during heating. CL was calculated as:

$$CL = 100 \cdot \frac{(m_1 - m_2)}{m_1} \qquad (2)$$

where m_1 and m_2 are the weights of raw and cooked patties, respectively.

2.8. Expressible moisture (EM) in cooked samples

Raw patty samples with 10 days of refrigeration were cooked. Cooked patty samples of 1.5 ± 0.2 g were placed in a 50 mL centrifuge tube containing a thimble, which consists in a Munktell 1003 filter paper (6 μm particle retention) folded around with a Munktell 1F filter paper (3 μm particle retention). Then, tubes were centrifuged at 4800×g for 20 min at 4ºC (RC3C centrifuge, Sorvall Instruments, USA). Expressible moisture (EM) was calculated as:

$$EM = 100 \cdot \frac{(m_1 - m_2)}{m_1} \qquad (3)$$

where m_1 and m_2 are the weights of the sample before and after centrifugation, respectively.

2.9. Texture in cooked samples

To perform the texture studies, raw patties with 10 days of refrigeration were cooked. After, they were cooled to room temperature (25°C).

2.9.1. Shear force and work of shearing

For this assay, cooled patties were cut in half to obtain two pieces of 75-mm height from each one, and weighed. The shear force and work of shearing were

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measured using a ten-blade Kramer cell attached to a texture analyzer (Model TA.XT plus, Stable Micro Systems, UK) with a 50 kg load cell. The speed conditions followed were 1 $mm \cdot s^{-1}$ for pre-test and test, and 10 $mm \cdot s^{-1}$ for post-test. Force-deformation curve data were recorded. Force was expressed as $N \cdot g^{-1}$ and work as $J \cdot g^{-1}$.

2.9.2. Texture profile analysis (TPA)

TPA was performed on 10 cylindrical samples (1.5-cm diameter and 1.5-cm height) cut through a cork borer from three cooked and then cooled patties for each treatment studied, equilibrated at room temperature (25ºC). Texture parameters were determined by a double compression test (0.5 $mm \cdot s^{-1}$) using a cylindrical probe (3.5-cm diameter) attached to the texture analyzer (Model TA.XT plus, Stable Micro Systems, UK). Samples were compressed to 50% of its original height. The evaluated parameters, calculated using Texture Exponent 32 software (v 5.1.1.0), were hardness (N), springiness, cohesiveness and chewiness (N).

2.11. Statistical analysis

Results were reported as the average and standard deviation (SD) for the *n* samples measured. Results were analyzed through ANOVA ($p<0.05$) followed by multiple comparisons evaluated through a least square significant difference test (Statgraphic Plus for Windows, version 5.0, 2001, Manugistics Inc., USA), according to Sokal & Rohlf (2012). Nonlinear regressions corresponding to Eq. (1) were performed through the Prism 5 (GraphPad Software Inc., 2007, USA).

3. Results and discussion

3.1. Particle size and hydration properties of the plum MPCs

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Plum MPCs were characterized by the average size distribution shown in **Fig. 1a**. The particles showed mainly average particle sizes of 210 μm (40-50% proportion) and 105 μm (30% proportion).

The rheological performance of vegetable fibers is associated to their hydration properties, which are related to the polysaccharide composition (de Escalada Pla, Rojas, & Gerschenson, 2013; Idrovo Encalada et al., 2016). SC and WRC values determined in plum fiber MPCs were significantly (p<0.05) higher for pulp MPCs, while WHC values had no significant differences (**Fig. 1b**). The fitted kinetic curve (continuous line; Eq. 1) of water absorption obtained from pulp MPCs was well above that recorded from peel MPCs (**Fig. 1c**), showing higher capacity (up to ≈ 9 mL/g in 170 min), and velocity (5.0±0.2 $mL \cdot g^{-1} \cdot min^{-0.116}$) of water absorption than peel MPCs (2.2±0.3 $mL \cdot g^{-1} \cdot min^{-0.26}$). The drying process affects the apparent density and SV of powders because it contributes to determine their porosity (Idrovo Encalada et al., 2016). The SV was significantly (p<0.05) higher for peel MPCs (lower apparent density) (**Fig. 1b**). As previously determined by Basanta et al. (2016), peel and pulp MPCs consisted of cell wall biopolymers, i.e. non cellulosic carbohydrates (hemicelluloses and uronic acids of pectins), cellulose, lignin (Table 2) and proteins (≈12%). Since both kinds of plum MPCs showed similar average particle size distribution (**Fig. 1a**) and were obtained using the same extractive and freeze-drying procedures, higher hydrophilicity and water absorption capacity can be mainly attributed to the significant higher content of pectins (uronic acids) found in pulp MPCs, together with its lower lignin content (Table 2) (Basanta et al., 2016). Lignin, the secondary cell wall component, replaces water into the cell wall network transforming the hydrophilic, hydrated pectin-gel of the primary cell wall matrix into a hydrophobic, impermeable environment (Brett & Waldron, 1996).

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3.2. Antioxidants in plum peel and pulp MPCs

An important content of co-extracted antioxidant compounds constituted by phenolics (Basanta et al., 2016), as well as carotenoids and tocopherols were found in the plum MPCs (**Table 2**). These contents were very different between pulp and peel MPCs (**Table 2**). Therefore, at the same level assayed as food additives (2.0 g of peel or pulp MPCs per 100 g of total patty), different antioxidant effects could be expected in this study. Phenolics were mainly pentameric proanthocyanidins, but also minor quantities of cyanidins , and flavonols like quercetin derivatives (Table 2). The contents of the phenolic compounds were higher (p<0.05) in peel than in pulp MPCs.

In the present work, other antioxidants such as carotenoids (β-carotene, lutein) and tocopherols (α- and γ-isomers) were determined in the plum MPCs. The contents of these antioxidant compounds were also significantly (p<0.05) higher in peel than in pulp MPCs (**Table 2**), as occurred with the phenolics. The α-isomeric form of tocopherol was notably more abundant than the γ-isomer (**Table 2**).

Lutein is a carotenoid abundant in plants which can be used as a tracer of pasture feeding, as animals are not able to synthesize this molecule and it is stored in the animal's fat after absorption and thus found in milk and meat (Descalzo, Rossetti, Páez, Grigioni, García, Costabel, et al. 2012). The α- and γ-tocopherols as well as β-carotene were found in yellow plums (*Prunus domestica* L.), being their contents dependent on the agronomic procedure utilized for plum cultivation (Lombardi-Boccia, Lucarini, Lanzi, Aguzzi, & Cappelloni, 2004). In this context, the α- and γ-tocopherol levels varied into 411-585 µg/100 g (fresh weight) and 7.2-11.0 µg/100 g concentration ranges, respectively. The β-carotene content varied beween 68 and 117 µg/100 g (fresh weight) in yellow plums. Considering that the edible proportion of this kind of plums

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(pulp and peel) was 96.9% and the water content was 88.7% (fresh weight), the proportion of solids was 8.2% in average. Assuming that all these liposoluble compounds were retained by the solid fraction of the yellow plums, the results shown in Table 2 for pulp MPCs were in the same order of magnitude as those reported by Lombardi-Boccia et al. (2004).

3.3. Antioxidant preservation of breast chicken patties by plum MPCs

Based on the contents of phenolic compounds as well as of β-carotene and lutein, α- and γ-tocopherols (**Table 2**), peel and pulp MPCs separately obtained from residues of plum harvesting were proposed as food antioxidant additives. Peel or pulp MPCs were then assayed at the concentration of an additive (2.0% w/w). In view of its healthier composition and vulnerability to oxidation as above reported, breast chicken meat was then selected for processing as food matrix to evaluate the utility of plum peel and pulp MPCs.

TBARS test is commonly applied as an objective method of detecting lipid oxidation in meat products (Banerjee et al., 2012; Brannan, 2008; Gordon, 2001; Pateiro et al., 2014; Pires et al., 2017; Yıldız-Turp & Serdaroglu, 2010). Raw patties containing either peel or pulp MPCs had significant (p<0.05) lower values of TBARS in relation to their control during all the storage period, and differences were more marked between groups especially at day 10 of refrigeration (**Fig. 2a**). The maximum value of TBARS reached by the raw control patties was 9.4 mg MDA/kg, even with the presence of the iron chelator STPP, whereas this value was reduced around 50% at day 10 in raw patties containing pulp or peel MPCs (**Fig. 2a**).

The antioxidant capacity determined as the reducing (FRAP) activity was also analyzed during refrigeration of raw patties (**Fig. 2b**). The lowest (p<0.05) FRAP values

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were in general observed in control patties (3-4 mM Fe^{2+}/kg) during the storage period, while raw patties containing peel MPCs exhibited the highest (p<0.05) FRAP values (9-12 mM Fe^{2+}/kg) during the whole storage period (**Fig. 2b**). The increment of the antioxidant capacity observed in patties containing fiber MPCs, especially peel MPCs, can be associated to the content of antioxidants such as tocopherols, β-carotene, lutein, cyanidins (anthocyanins) and flavonoids found in fiber MPCs (Pokorný & Schmidt, 2001), together with the important contents of proanthocyanidins (**Table 2**). It is interesting to remark that just at time 0 of storage, raw chicken patties containing plum MPCs showed significantly (p<0.05) higher FRAP values than control patties, without MPCs (**Fig. 2b**), demonstrating that the excess of reducing (FRAP) activity exhibited by patties containing plum MPCs was due to the antioxidant compounds contributed by these fibers (**Table 2**).

In this study, the α- and γ-tocopherol contents that contribute to the antioxidant activity, were also measured in raw breast chicken patties during refrigeration. Results are reported in **Fig. 3 (a,b,e,f)**. This determination is not previously reported in the literature. In addition, the tocopherol contents were also determined after cooking of those patties that were previously stored under refrigeration for 0, 3 and 10 days (**Fig. 3c,d,g,h**). Just from time 0 of storage, the α- and γ-tocopherol contents were higher (p<0.05) in raw (**Fig. 3a, b**) as well as in the corresponding cooked (**Fig. 3c, d**) patties containing peel MPCs than in their respective control systems, or than in the patties containing pulp MPCs (**Fig. 3e, f**). This result is coherent with the highest contents of α- and γ-tocopherol found in the peel MPCs (**Table 2**). Also, it can be observed that cooking almost did not affect the tocopherol contents of peel MPCs-loaded patties (**Fig. 3c, d**).

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3.4. Effect of plum MPCs on the characteristics of raw and cooked patties

In addition to their antioxidant activity, some phenolics like cyanidins can modify the color of the meat products where the fiber is added as an additive. Furthermore, fibers are mainly constituted by polysaccharides, which are the components of the cell walls. After hydration, these polymers have functional properties which include thickening and/or gelling effects, or can act as bulking agents in food formulation. In the present work, the highest proportion of pectins found in pulp MPCs and of lignin in peel MPCs (Table 2) produced different hydration properties (**Fig. 1b**) in these fibers and, hence, it was suggested that they can provide different characteristics to the meat products, apart from the antioxidant preservation associated to the co-extracted phenolics, tocopherols and carotenoids. Therefore, information was also obtained concerning the effect of plum MPCs on binding (cooking loss, expressible moisture), color and textural properties of cooked breast chicken patties.

3.4.1. Effect of plum MPCs on color parameters of raw and cooked patties

The CIE *L*a*b** color parameters were determined on raw chicken patties at day 0 and day 10 of refrigerated storage, and on patties cooked after 10 days of refrigerated storage. Since a notably effect was observed with the addition of plum MPCs particularly on the *a** color parameter of patties, the corresponding results were the only one reported in **Fig. 4**.

The *a** values measured in patties were all above zero, meaning that patty's color was in the red side. Regarding raw patties with pulp MPCs, the *a** values were significantly (p<0.05) higher than those determined for control patties at day 0 as well as after 10 days of refrigerated storage (**Fig. 4a**). It proved that the pulp MPCs colored the raw chicken patty just from day 0, due mainly to the cyanidins (Table 2). The

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refrigerated storage of raw patties significantly (p<0.05) increased this parameter for both control and, mainly, patties with pulp MPCs. The a* parameter significantly (p<0.05) decreased after cooking, especially for control-patties, when compared to the a* value shown by the respective sample of raw patty at day 10 of refrigeration (**Fig. 4a**). Significantly (p<0.05) higher a* values were observed in patties with pulp MPCs after cooking (**Fig. 4a**) than in cooked patties containing peel MPCs (**Fig. 4b**).

At day 0 of refrigeration, raw patties with peel MPCs had significantly (p<0.05) higher a* values than control ones (**Fig. 4b**). The control chicken patties used for the study whose results are shown in **Fig. 4b** were just characterized at day 0 by a redness (a* value) higher than the control chicken patties used in **Fig. 4a**. At day 0, the a* value shown by peel-MPCs-loaded patties was 1.5 times above the respective control patties, whereas the a* value shown by pulp-MPCs-loaded patties doubled that determined in the respective control patties (**Fig. 4a**). Beyond this fact, at day 10 of refrigeration no differences were observed between the a* values of raw patties with peel MPCs and the respective control, and these values were both lower than the ones measured at day 0 (**Fig. 4b**). After cooking, patties with peel-MPCs and control ones had significantly (p<0.05) lower a* values than the corresponding raw patties refrigerated for 10 days. Non-significant differences between the a* values of cooked patties' samples were observed (**Fig. 4b**).

It is known that the presence of STPP contributes to maintain the redness of raw patties. According to the SC, WRC (**Fig. 1b**) and kinetic of water absorption (**Fig. 1c**) determined in this work, pulp MPCs can be hydrated and then swollen during mixing with the raw chicken meat as a consequence of their pectin content (**Table 2**). It is herein proposed that pulp MPCs also helped maintain the redness of raw patties and this effect could be due to the stabilization of the cyanidins, which are responsible for this

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color, in the weak gel network formed by the extracted myofibrillar proteins and the pectins of the pulp MPCs (**Table 2**). The redness of peel-MPCs-raw patties, characterized by a high *a** value shown at day 0, diminished notably after 10 days of refrigeration (**Fig. 4b**). After cooking, patties with pulp MPCs retained most of the redness (**Fig. 4a**), and that was no replicated on peel MPCs-patties (**Fig. 4b**), beyond the latter had a higher cyanidin content than pulp-MPCs (**Table 2**). Probably, the different behaviour of pulp and peel MPCs can be ascribed to the lower pectin content together with the highest lignin level of the latter (**Table 2**).

3.4.2. Effect of plum MPCs on binding and textural properties of patties

It was also studied the effect of the addition of peel and pulp MPCs on the binding (CL and EM) and textural properties of patties cooked at day 10 of refrigerated storage at 4.0ºC.

The CL of patties with peel MPCs showed a significant (p<0.05) increase in comparison to control patties. Also, the EM showed a significant (p<0.05) decrease in those samples. However, patties with pulp MPCs had no significant difference with respect to its control (**Table 3**). During heating, myofibrillar proteins changed, and the water content within the meat myofibrils, in the narrow channels between the filaments, changed as meat shrinks within the tissue matrix (Bertola, Bevilacqua, & Zaritzky, 1994), resulting in CL with heating (Murphy & Marks, 2000). Thus, CL directly influences the structural characteristics and thereby the water distribution in the meat (Pearce, Rosenvold, Andersen, & Hopkins, 2011). However, Liu, Arner, Puolanne, and Ertbjerg (2016) indicated that CL involves water and fat as well. The lower EM value correlated with a higher water holding capacity of the meat in the presence of peel MPCs, influencing both meat sensorial quality and economical value (Modzelewska-

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Kapituła, Kwiatkowska, Jankowska, & Dąbrowska, 2015). O'Sullivan, Lynch, Lynch, Buckley, and Kerry (2004) found no differences in CL of chicken nuggets made with NaCl and STPP, with the addition of antioxidants such as sage, rosemary or tea catechins. Naveena, Vaithiyanathan, Muthukumar, Sen, Kumar, Kiran et al. (2013) obtained for chicken patties added with carnosic acid (natural liquid extracts with 8-10% of this component) significantly lower CL than the control samples. The authors concluded that some components in the natural antioxidant extracts, especially carbohydrates, may help in water binding, resulting in higher yield. In the present work, it is interesting to note that pulp MPCs, which contain an important amount of pectins (Table 2), had no influence on the binding properties (CL and EM) or cooking response of patties (**Table 3**), whereas peel MPCs, enriched in lignin (Table 2), had a slight effect on them (**Table 3**).

Regarding the textural properties instrumentally measured by means of the Kramer's cell and reported through the shear force and work of shearing, cooked patties containing peel MPCs had significantly (p<0.05) higher values than its respective control (**Table 3**). However, patties with pulp MPCs had no significant differences in relation to their controls. This result is in accordance with the higher CL (and lower EM) showed by patties with peel MPCs. The decrease in water retention caused a firmer structure, with a lesser gel characteristic, increasing shear force values in patties loaded with peel MPCs.

Table 3 shows the TPA parameters' values obtained from patties cooked at day 10 of refrigeration. Patties with peel MPCs had significant (p<0.05) higher values of hardness, and lower values of springiness and cohesiveness than control patties (**Table 3**). These results may be ascribed to some degree of brittleness in the cooked patties containing peel MPCs. On the other hand, patties containing pulp MPCs only had a

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significant increase in the springiness values, without a change in the rest of the TPA parameters (**Table 3**).

Peel and pulp MPCs not only can behave as antioxidant preservatives in the chicken patties but also, in the case of pulp MPCs, can indirectly contribute to gelation as a non-protein additive (Dong & Holley, 2011). The addition of pulp MPCs, due to its polysaccharide composition, can contribute to hydration and to the gel network formation together with myofibrillar proteins, and did not modify the texture parameters. On the other hand, peel MPCs, as it had a different polysaccharide composition and hydration properties, did not contribute to the water-holding capacity of chicken meat, but modified the textural parameters. Protein-carbohydrate interactions affect the functional properties in foods where proteins are the major ingredients such as meat and fish processed products.

4. Conclusions

Peel and pulp MPCs obtained from the residues of plum harvesting, containing carotenoids (β-carotene, lutein), α- and γ-tocopherols and polyphenolic compounds (pentameric proanthocyanidins, cyanidins and quercetin derivatives), acted as efficient natural antioxidant preservatives at 2.0% w/w concentration for raw breast chicken patties during 10 days of refrigerated storage at 4.0ºC. The highest α- and γ-tocopherol contents probably contributed especially to the highest reducing (FRAP) activity found in raw patties containing peel MPCs, activity that remained high after cooking. These plum MPCs also modified the microstructure's properties and color of chicken patties due to the biopolymeric composition of plum MPCs which affected the hydration properties. Pectins in pulp MPCs increased springiness of cooked chicken patties, while allowed the stabilization of cyanidins and, hence, of the red color transferred to the

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chicken patties by pulp MPCs. Conversely, peel MPCs containing higher lignin level and low pectin content behaved as a bulking agent, which did not stabilize the redness while affecting the CL and EM binding properties together with the texture of patties. It can be concluded that peel and pulp MPCs obtained from discarded plums are useful additives for the preservation of meat products.

Acknowledgements

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Figure captions.

Fig. 1. Plum MPCs: average size distribution (**a**); SV and hydration properties (**b**); kinetic of water absorption (**c**). Bars indicate the standard deviations (n = 3). The same lower case letters for a property indicates non-significant differences (p<0.05).

Fig. 2. Antioxidant capacity of plum MPCs on raw chicken patties: TBARs assay (**a**), reducing power (FRAP) assay (**b**). Bars show the standard deviations (n = 18). The same lower case letters indicates non-significant differences (p < 0.05).

Fig. 3. α- and γ-tocopherol contents determined in raw chicken patties either without (control) or with plum MPCs (**a**, **b, e, f**), as well as in 0-, 3- and 10-days refrigerated and then cooked patties (**c**, **d, g, h**). Bars show the standard deviations (n = 18).

Fig. 4. CIE a^* color parameter evaluated in chicken patties without (control) and with pulp (**a**) or peel (**b**) MPCs. Different lower case (a–c) and capital (A–C) letters mean that values are significantly different (p<0.05) between 0 and 10 days of refrigerated storage of raw patties, and between raw and cooked patties at day 10 of refrigerated storage, respectively.

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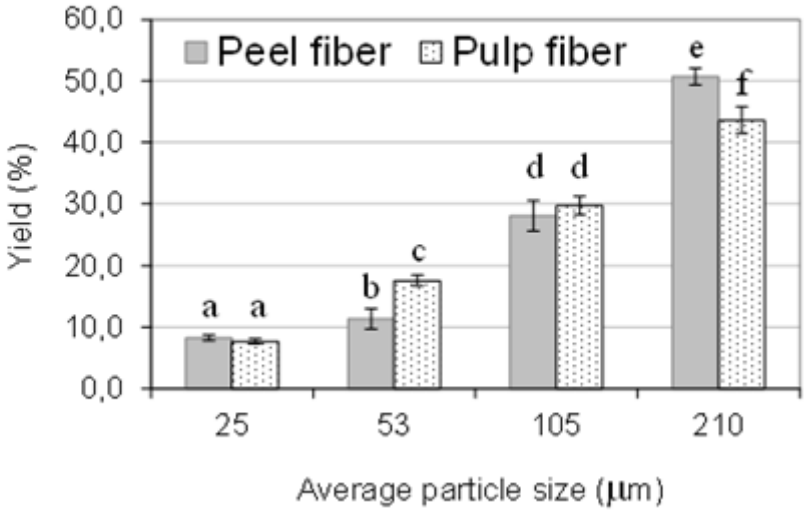
(a)



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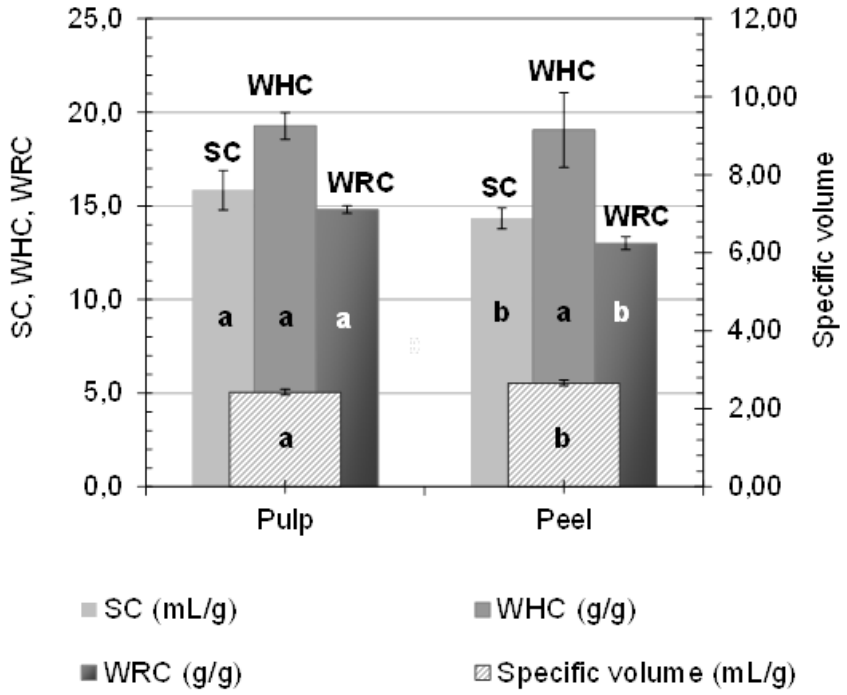

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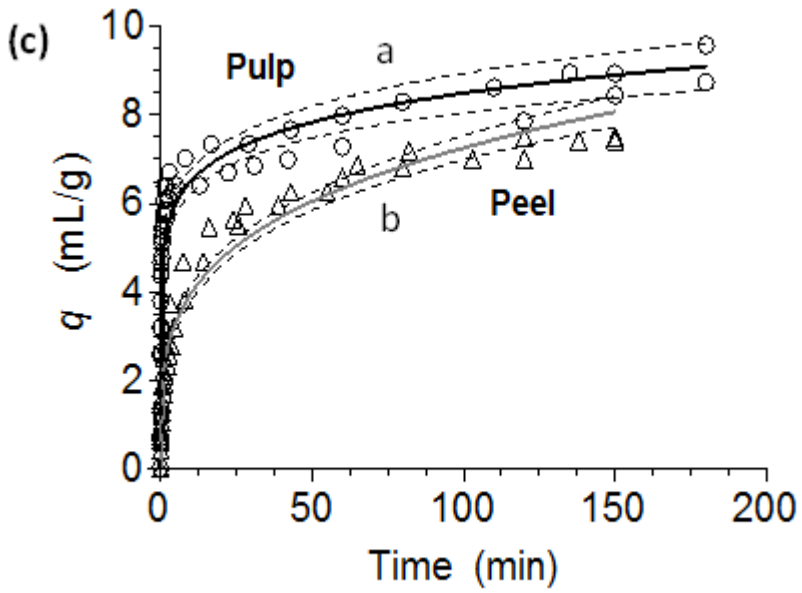

Fig. 1

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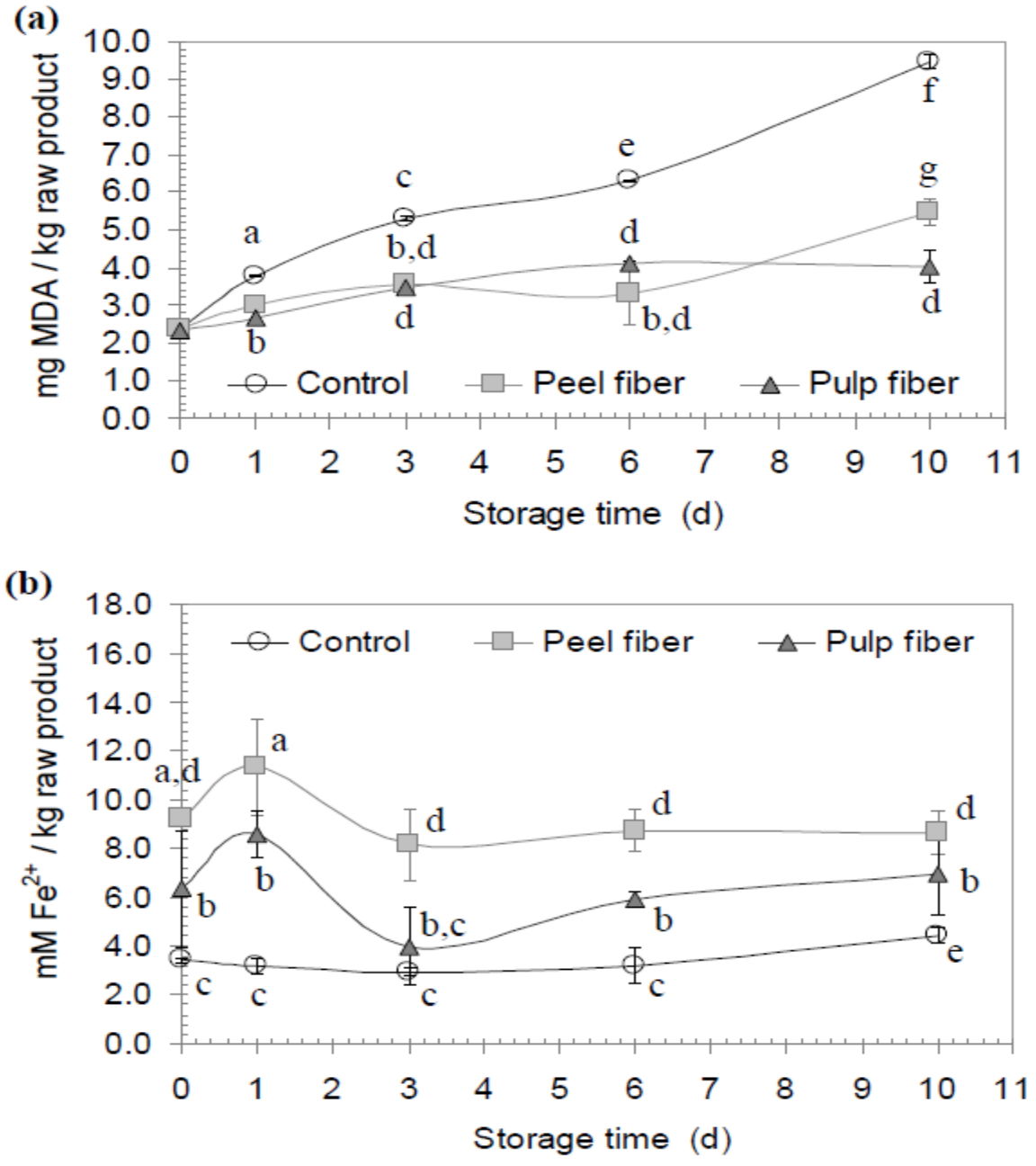

Fig. 2

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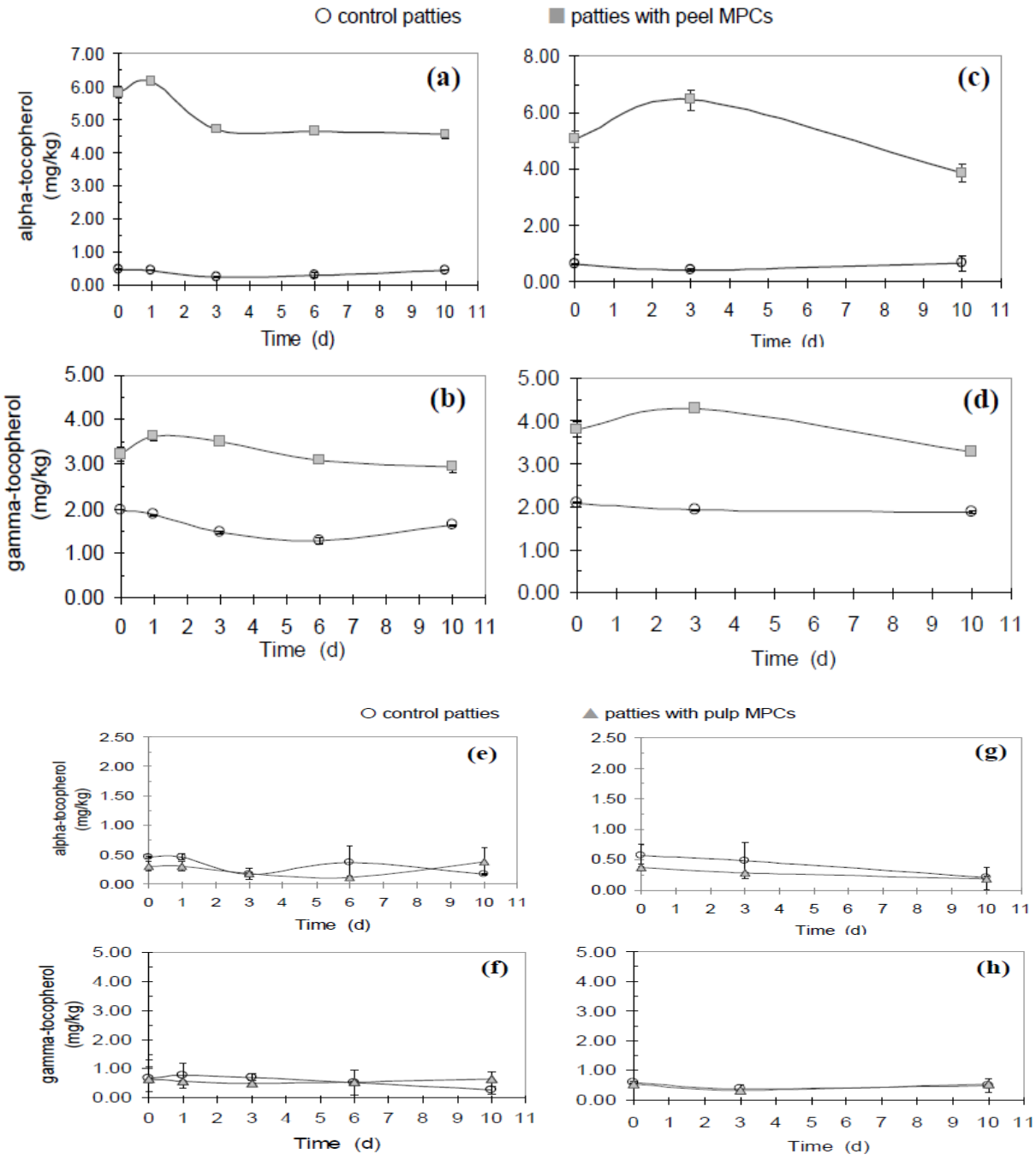

Fig. 3

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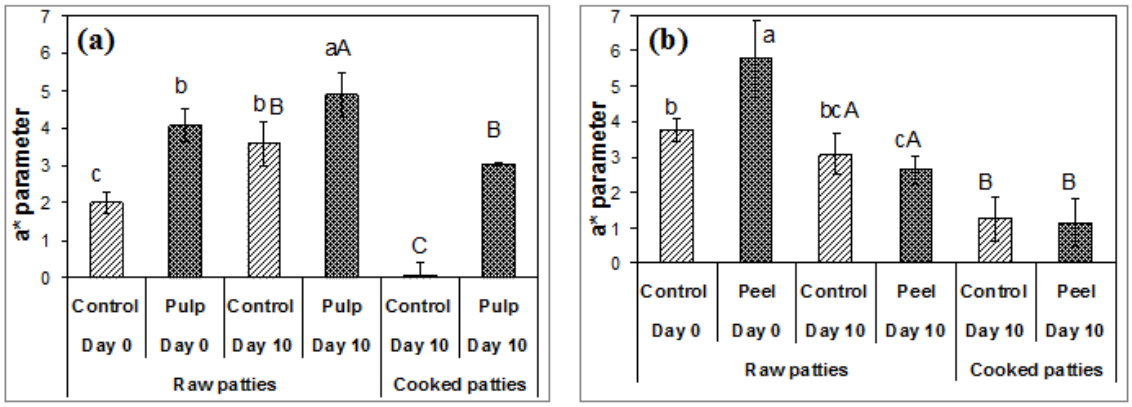

Fig. 4

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Table 1

Formulation used for the manufacture of chicken patties.

	Composition of patties (% w/w)		
	with Peel MPCs	**with Pulp MPCs**	**Control system**
Chicken meat (breast)	80.0	80.0	80.0
Chicken skin	10.0	10.0	10.0
Water	7.3	7.3	8.9
Sodium tripolyphosphate (STPP)	0.1	0.1	0.1
Sodium chloride (NaCl)	1.0	1.0	1.0
Plum peel MPCs	1.6	—	—
Plum pulp MPCs	—	1.6	—

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Table 2

Antioxidant compounds found in the plum fiber microparticles.

Antioxidants	Plum fiber microparticles (mg/kg dm)	
	from Pulp	**from Peel**
β-carotene	2.0 ± 0.2[a]	4.9 ± 0.6[b]
Lutein	0.6 ± 0.1[a]	5.1 ± 0.4[b]
α-tocopherol	35 ± 7[a]	298 ± 15[b]
γ-tocoferol	7 ± 1[a]	47 ± 8[b]
Proanthocyanidins[c,d]	1700 ± 100[a]	2000 ± 200[b]
Cyanidin-3-galactoside and -3-rutinoside[d]	7.7 ± 0.9[a]	121 ± 4[b]
Flavonols (quercetins)[d]	14.4 ± 0.9[a]	84 ± 7[b]
Uronic acids (g/100g MPC)[d]	13.4 ± 0.3[a]	7.6 ± 0.4[b]
Non-cellulosic carbohydrates (g/100g MPC)[d]	46 ± 7[a]	35 ± 4[b]
Cellulose (g/100g MPC)[d]	12 ± 1[a]	18 ± 2[b]
Lignin (g/100g MPC)[d]	5.5 ± 0.6[a]	8.7 ± 0.3[b]

dm: dry mass.
Mean and standard deviation (n=3) are shown.
Mean values with different letters in the same row are significantly different ($p < 0.05$).
[c]Proanthocyanidins were mainly found in a pentameric form.
[d]Basanta et al. (2016).

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Table 3

Kramer shear force and work of shearing, texture profile analysis (TPA) parameters, as well as cooking loss and expressible moisture evaluated in breast chicken patties cooked after 10 days of refrigerated storage.

	Cooking loss (%)	Expressible moisture (%)	Kramer's cell assay		TPA parameters			
			Shear force (N/g)	Work of shearing (J/g)	Hardness (N)	Springiness	Cohesiveness	Chewiness (N)
Control	9.1 ± 0.9^{a}	26 ± 2^{a}	16 ± 2^{a}	46 ± 5^{a}	13 ± 2^{a}	0.82 ± 0.04^{a}	0.62 ± 0.03^{a}	7 ± 1^{a}
Peel fiber	12 ± 2^{b}	23 ± 2^{b}	20 ± 2^{b}	59 ± 6^{b}	16 ± 2^{b}	0.78 ± 0.03^{b}	0.55 ± 0.05^{b}	7 ± 1^{a}
Control	9.5 ± 0.9^{a}	23.98 ± 0.09^{a}	19 ± 1^{a}	54 ± 7^{a}	12 ± 1^{a}	0.86 ± 0.07^{a}	0.60 ± 0.08^{a}	6 ± 1^{a}
Pulp fiber	10 ± 1^{a}	24 ± 1^{a}	19 ± 2^{a}	54 ± 3^{a}	12 ± 1^{a}	1.1 ± 0.3^{b}	0.53 ± 0.05^{a}	7 ± 2^{a}

Mean values with different superscript letters into a column and comparing the fiber-added patty with its corresponding "control" are significantly different ($p < 0.05$). The same lower case letters for a given parameter indicates non-significant differences ($p<0.05$).

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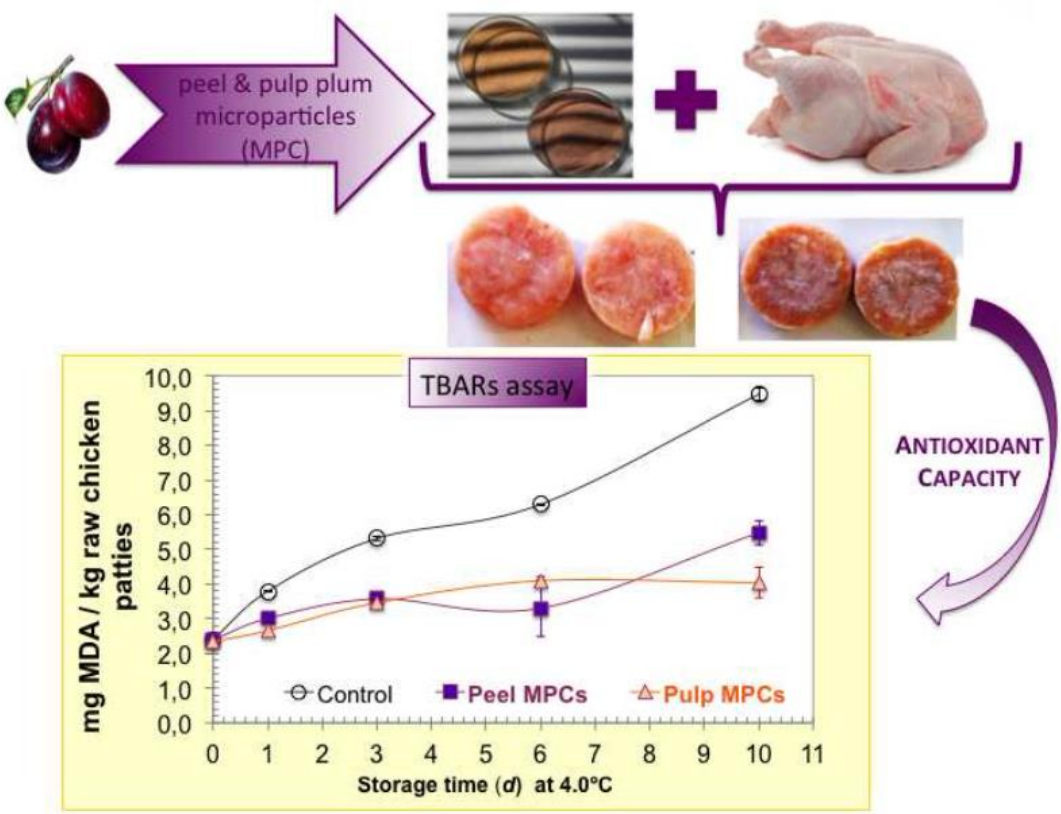

Graphical abstract

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Highlights

- Plum fiber microparticles (MPCs) kept β-carotene, lutein, tocopherols and polyphenols
- Plum peel and pulp MPCs were antioxidants in 10-days refrigerated raw chicken patties
- Higher pectin and low lignin levels in pulp MPCs stabilized a red color in patties
- Pectins from pulp MPCs produced more springy patties after cooking
- Lower pectin and high lignin levels in peel MPCs raised brittleness in cooked patties

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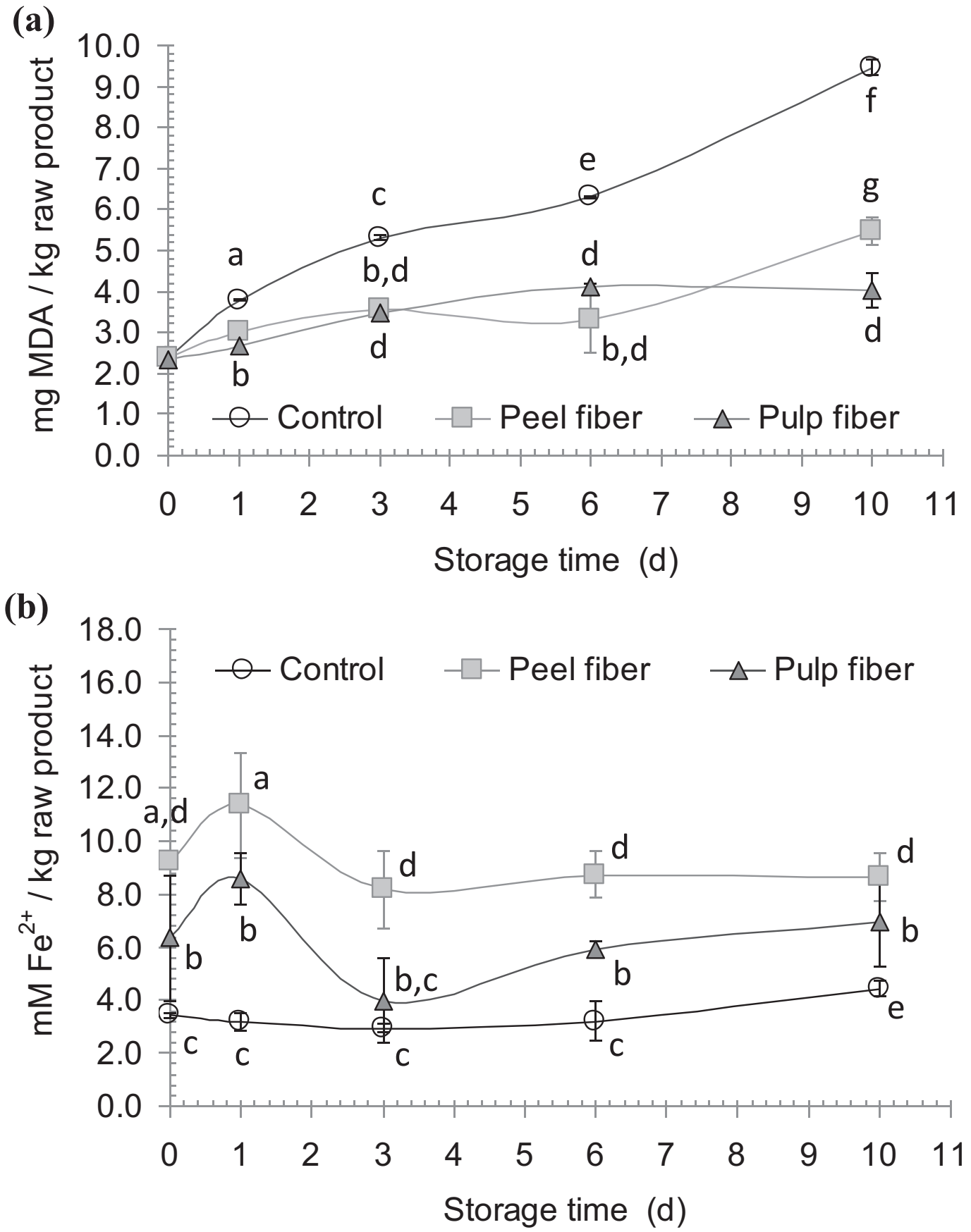

(a)
mg MDA / kg raw product
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
a
b
c
b,d
d
e
d
f
b,d
g
d
Control
Peel fiber
Pulp fiber
0
1
2
3
4
5
6
7
8
9
10
11
Storage time (d)
(b)
mM Fe2+ / kg raw product
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Control
Peel fiber
Pulp fiber
a,d
a
b
b
c
c
d
b,c
c
d
b
c
d
b
e
0
1
2
3
4
5
6
7
8
9
10
11
Storage time (d)

Figure 1

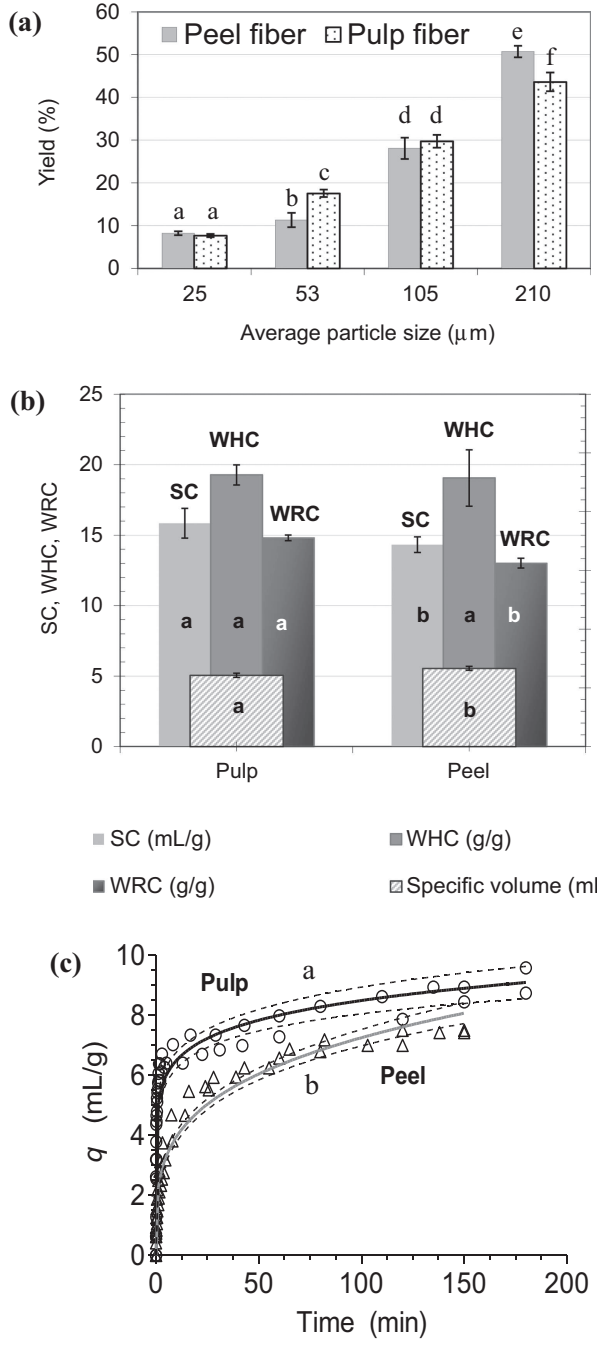

(a)
Peel fiber
Pulp fiber
Yield (%)
Average particle size (μm)
25
53
105
210
(b)
SC, WHC, WRC
Specific volume
WHC
SC
WRC
Pulp
Peel
SC (mL/g)
WHC (g/g)
WRC (g/g)
Specific volume (mL/g)
(c)
Pulp
Peel
q (mL/g)
Time (min)

Figure 2

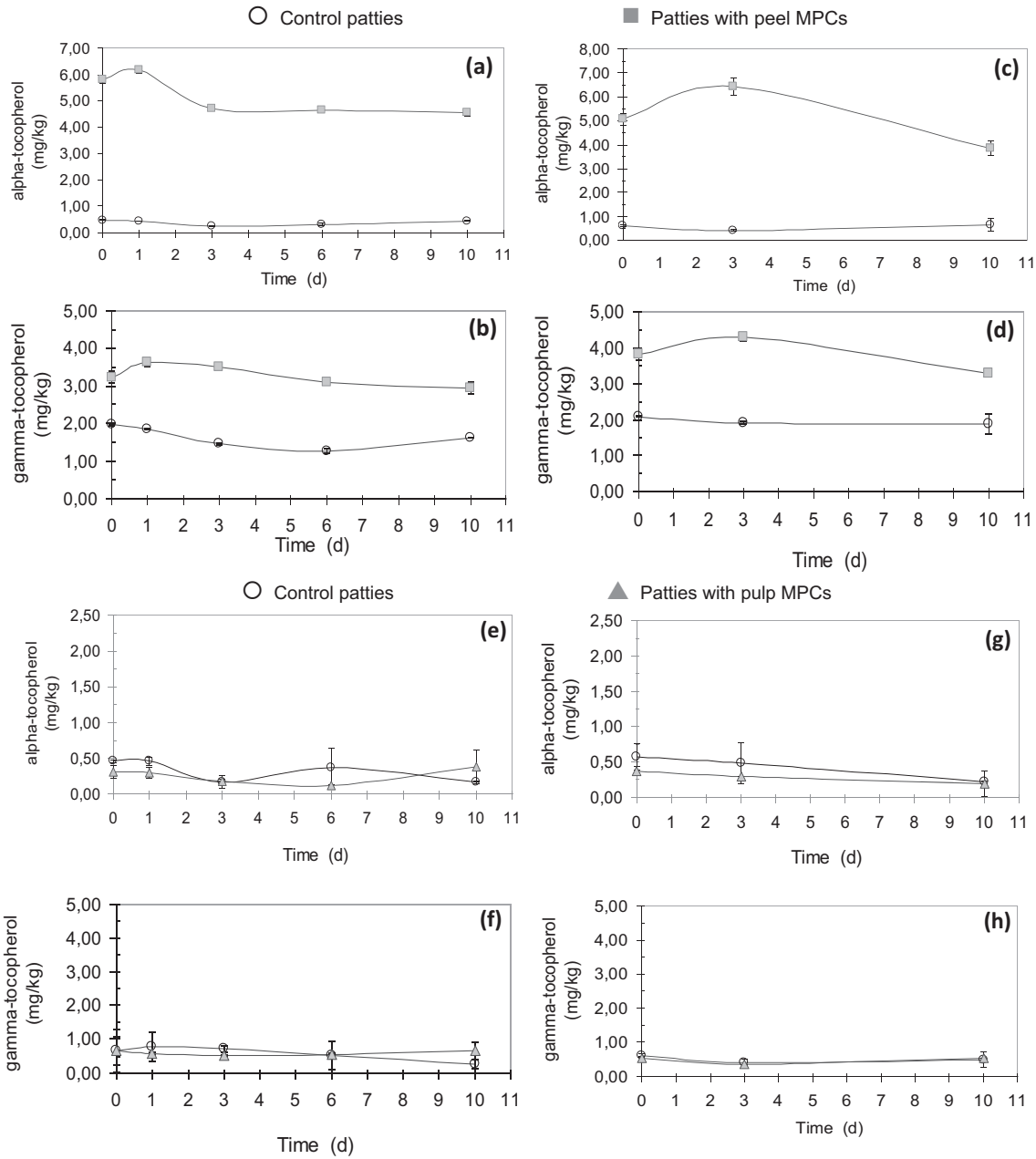
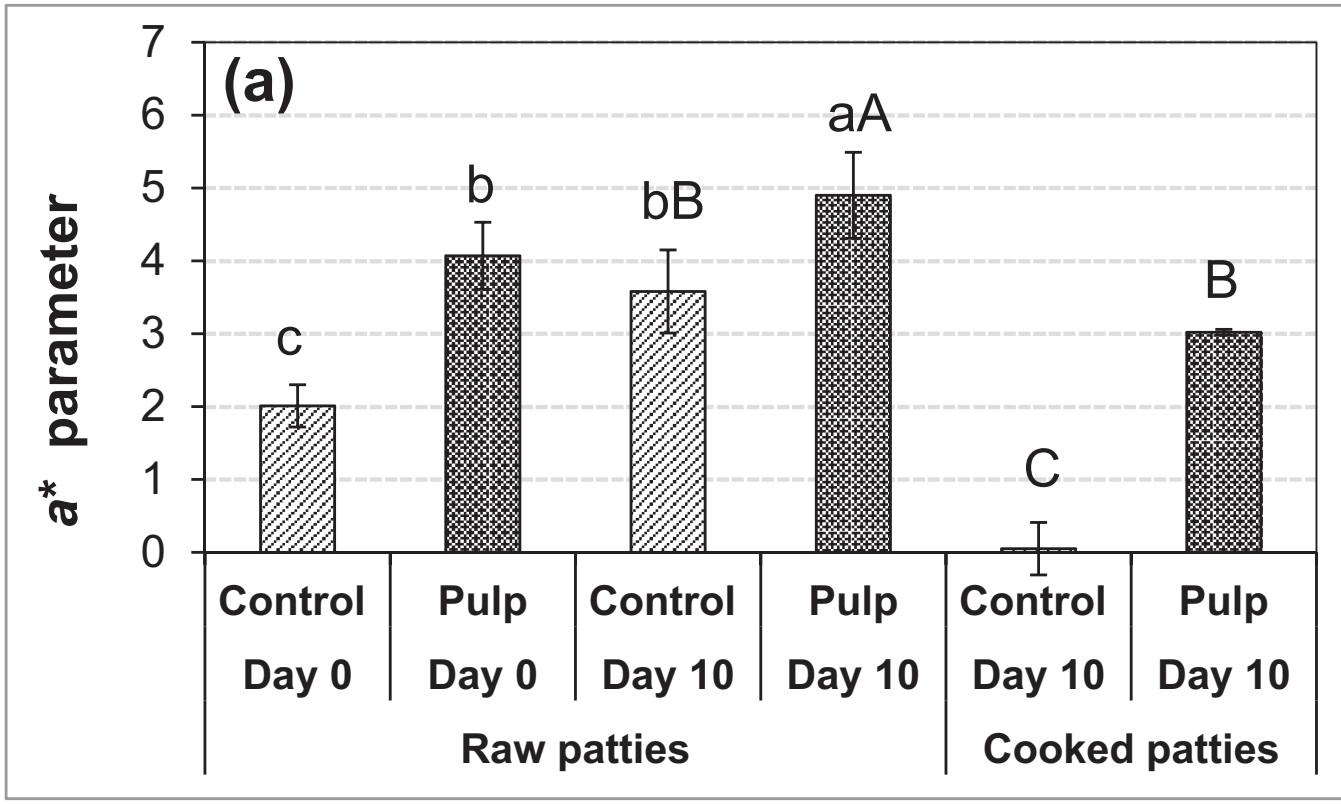

Control patties
Patties with peel MPCs
Control patties
Patties with pulp MPCs
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
alpha-tocopherol (mg/kg)
gamma-tocopherol (mg/kg)
Time (d)

Figure 3


(a)
a* parameter
7
6
5
4
3
2
1
0
c
b
bB
aA
C
B
Control Day 0
Pulp Day 0
Control Day 10
Pulp Day 10
Control Day 10
Pulp Day 10
Raw patties
Cooked patties

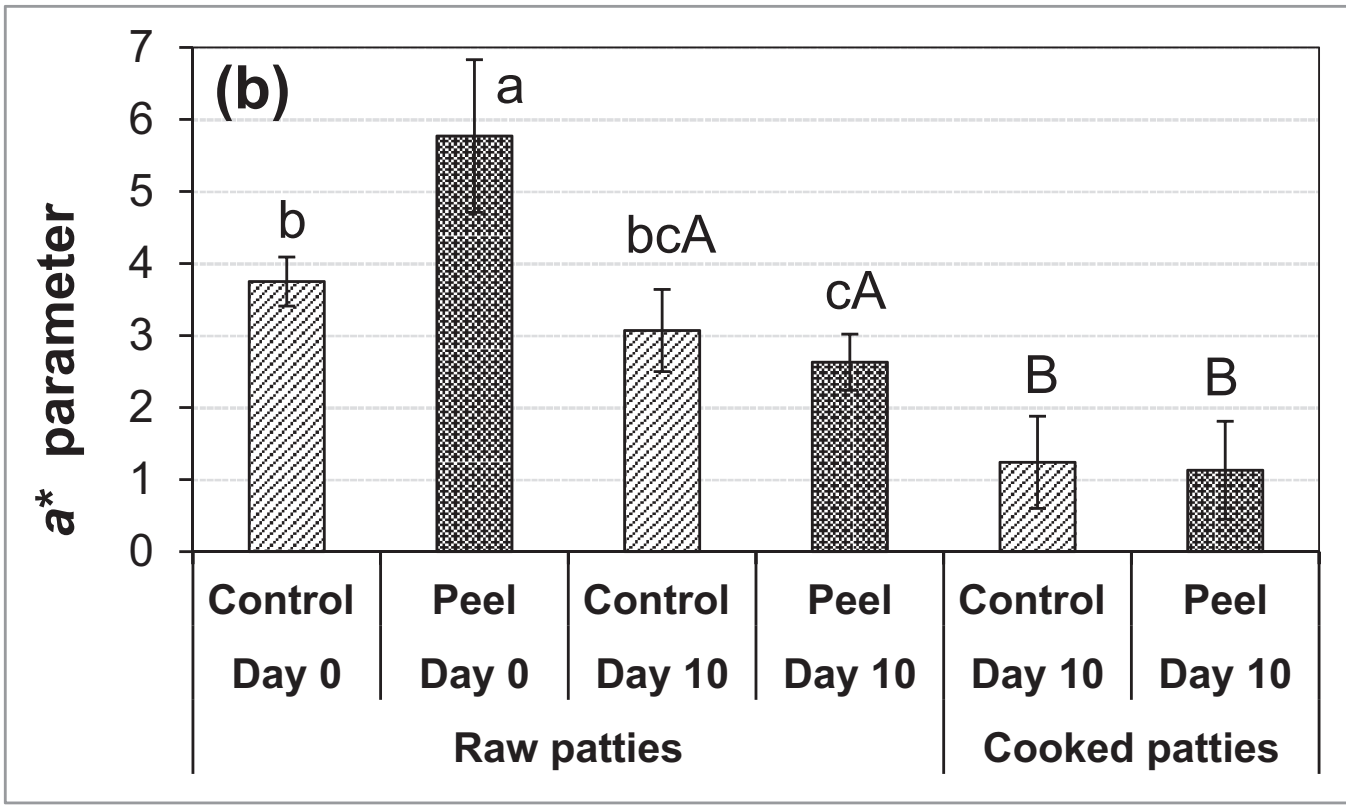

(b)
a* parameter
7
6
5
4
3
2
1
0
b
a
bcA
cA
B
B
Control Day 0
Peel Day 0
Control Day 10
Peel Day 10
Control Day 10
Peel Day 10
Raw patties
Cooked patties

Figure 4