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Quality Markers of Functional Tomato Juice With Added Apple Phenolic Antioxidants

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Quality markers of functional tomato juice with added apple phenolic antioxidants

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Abstract

The natural approach to food stabilisation is at the forefront of research on plant compounds for food use. Matrix interactions and stability studies of the incorporated foods are necessary prior to further food processing. In this study, apple peel phenolic fractionated based on acidity (flavonoids and phenolic acids) were added to a commercial bottled tomato juice. The juice was left open i.e. air in the headspace at 4°C for 4 days to assess its physical-chemical quality, and for 10 days for the microbiological analysis. Quality markers were analysed (colour and turbidity) and showed that apple peel phenolics, particularly procyanidins, could form insoluble complexes with colloidal pectins thus affecting the turbidity; this led to brighter juice colours with enhanced yellowness due to added flavonol glycosides. The presence of other natural antioxidants (i.e. ascorbic acid and carotenoids) in the tomato juice was not affected by the presence of peel phenolics. Ascorbic acid was partially reduced during storage in all the juice samples. However, such decrease was counterbalanced by the presence of the added peel phenolics whose amount remained constant over time, thus contributing to a higher radical scavenging capacity compared to the control. The microbiological spoilage of the opened

tomato juice was also delayed by 2-3 days in the presence of apple peel phenolics compared to the control. As a result, the open-package shelf-life of the bottled tomato juice was extended. The antimicrobial capacity was possibly due to the acidity of phenolic acids and the presence of apple flavonoids such as flavan-3-ols and flavonols that are likely to affect the transportation of nutrients across the cell membranes of the spoilage microflora.

Keywords: Apple peels; Polyphenols; Tomato juice; Physical-chemical quality; Microbiological quality.

1. INTRODUCTION

Apple waste lines have been widely investigated for the recovery of functional ingredients and nutraceuticals (Moure et al., 2001; O'Shea, Arendt & Gallagher, 2012; Rabetafika, Bchir, Blecker & Richel, 2014). The combined presence of dietary fibre and phenolics in the apple fruit supplies multiple beneficial effects to the human body (Boyer and Liu, 2004). The recovery of phenolics from cooking apple varieties, such as cv Bramley's Seedling which is grown in the British Isles, was previously reported (Massini, Martin Diana, Barry-Ryan & Rico, 2010); the peels are normally discarded from processing lines for non-valuable applications, i.e. land fertilising or as cattle feed. The recovery of plant waste-derived ingredients (i.e. botanicals, herbs and their essential oils) is regarded as an opportunity for the development of functional foods and dietary supplements (Eussen *et al.*, 2011); botanicals were also added to foods and beverages in order to enhance taste or colour, especially herbs and spices (and their essential oils). Products already marketed with added botanicals and with a "healthy" image include smoothies, functional drinks, and yoghurts with green tea, grape seed, lemon balm, and Aloe Vera; these products are used mostly for well-being purpose, or for energy performance (Berdahl, Nahas &

Barren, 2010). Over the last few years, the market of natural antioxidants has grown significantly compared to that of synthetic additives (Decker, Elias & McClements, 2010). This trend is the result of a more natural approach to food stabilisation for the protection of essential fatty acids such as *n*-3 PUFAs (polyunsaturated) (Medina, González, Pazos, Della Medaglia, Sacchi & Gallardo, 2003); the natural approach is welcomed by a final consumer who seeks for healthy foods that are also nutritious, tasty, and can remain as “natural” as possible (Bech-Larsen & Scholderer, 2007); the trend towards “naturalness” for food additives and preservatives also reflects the usage restrictions for synthetic food additives i.e. antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ), and preservatives such as sulphites based on health grounds (Balasundram, Sundram & Samman, 2006; Berdahl et al., 2010; Lück & Jager, 1997). As a consequence, the food research and development is nowadays focused on the understanding of the underlying chemistry of the oxidation and biochemistry of natural plant substances in complex food matrices, and the development of more powerful and targeted, naturally derived additives (Pokorný, 2007). The applications of botanicals in beverages rather than in bulk oils or oil-in-water emulsions represents a growing market (Gruenwald, 2009). Functional beverages and smoothies, especially from vegetables i.e. low calorie and with added fibre, are products with a healthy image that primarily quench thirst but also provide health benefits (Harbourne, Marete, Jacquier & O’Riordan, 2013). The present study introduces preliminary results on the quality markers and microbiological stability of a vegetable juice from tomato that was added with apple peel-waste derived phenolics. It aims at studying the interaction of the added natural antioxidants with other matrix constituents (carotenoids, ascorbic acid) and with the physico-chemical environment (pH, turbidity, colour) under critical storage conditions i.e. during an open-package storage at refrigerated temperatures.

2. MATERIALS AND METHODS

2.1. EXPERIMENTAL DESIGN

A commercial tomato juice was used as the food model to test the capacity of added peel phenolic antioxidants during a post-opening storage study. The use of bottled tomato juice ensured that the food matrix was standardised across the replicated experiments; the experiment was duplicated using two independent batches of samples. The bottles of tomato juice were purchased in 2010 from a health shop in Dublin; according to the label information, the juice was organic certified, free from added preservatives, with less than 1% of lemon juice. Apple peel phenolics were solubilised from dried apple peels using a conventional solvent extraction method with 80% ethanol (v/v) followed by clean-up with petroleum ether and final partitioning with ethyl acetate at pH 7 and then at pH 2. The juice was added with 10% of apple peel phenolics (i.e. final concentration: 200 mg GAE/L or gallic acid equivalents, as total phenolics) and then kept in glass jars at refrigerated temperatures of 4°C for 4 days; a control with distilled water instead of sample was also prepared. The physical-chemical analysis of the enriched tomato juice and control was tested at time 0 (before storage) and at time 4 (days from opening). The storage time was selected based on the indications of the juice manufacturers which recommended the consumption of the tomato juice within 4 days from opening at temperatures equal to or below 5°C. The quality of the tomato juice at time 4 was selected as the threshold for the quality acceptability. In order to assess the preservative capacity of the phenolic extracts, the microbiological quality of the tomato juice was also evaluated. The microbial load of the samples was tested every 2-3 days over a 10-day storage under the same open-package conditions, and the microbial load detected in the control at time 4 days was used as the threshold of acceptability for the microbial quality of the tomato juice.

2.2. APPLE PEELS

Peel samples were obtained from apples (*Malus domestica* Borkh.) cv Bramley's Seedling that were purchased from a local store (Dublin, Ireland) in 2009-2010; the apples were stored at 4°C in

polyethylene bags until further processing. From each batch of apples (between 3 and 5 kg), three independent samples were prepared by randomly pooling fruits from different trays. After the fruits were washed under tap water, the peels were removed manually with a hand peeler and dried using stainless steel trays at $60 \pm 2^\circ\text{C}$ in a convection oven with forced air ventilation (BS Oven 250, Weiss Gallenkamp, Loughborough, UK) until a constant weight was achieved. The dried peels were pulverised using a coffee grinder and the powders stored in glass jars at -20°C until further analysis.

2.3. EXTRACTION OF PHENOLIC COMPOUNDS

Apple peel phenolics were solubilised from dried apple peels using a conventional solvent extraction method using 80% aqueous ethanol (v/v). Crude extracts were obtained through homogenisation of the peel powder with the chilled solvent followed by filtration (Massini, Rico, Martin-Diana & Barry-Ryan, 2013). The filtrates were pooled and the organic solvent was removed at 40°C in a rotary evaporator (Büchi Labortechnik, Flawil, Switzerland) until a final concentration of 10-20% (v/v). Crude extracts were washed by partitioning twice with petroleum ether (1:1); the organic layers were discarded, while the aqueous phase was retained and extracted three times with ethyl acetate at pH 7.0. This fractionation based on acidity was required to isolate phenolics such as flavonoids and the obtained extract was referred to as APN. The pH of the aqueous phase was then shifted to pH 2.0 and extracted with ethyl acetate three times in order to isolate phenolic acids, as previously reported (Delage, Bohuon, Baron & Drilleau, 1991). The acidic extract was referred to as APA. The extracts were pooled and the organic solvent was evaporated off under vacuum. The residues were dissolved in distilled water. The aqueous extracts of peel flavonoids were finally filtered using a $0.20 \mu\text{m}$ PES (Polyether Sulfone) membrane disc filters (Sarstedt, Nümbrecht, Germany) and kept at -20°C in the dark until further analysis. The amount of extracted phenolics in APN and APA was measured as total

phenolics and expressed as mg GAE/L or gallic acid equivalents using Folin-Ciocalteu assay as previously reported (Massini et al., 2013).

2.4. DETERMINATION OF PHENOLIC CONTENT

The identification and quantification of phenolic compounds was carried out with a RP-HPLC-PDA method previously described, with minor modifications (Schieber, Keller & Carle, 2001). A volume (0.02 mL) of phenolic extract in LC methanol was filtered through 0.45 µm PTFE membrane disc filter (Acrodisc, Pall, Portsmouth, UK) and then injected into e2695 Separation Module (Waters Alliance, Dublin, Ireland). The HPLC system was equipped with a quaternary solvent system pump, an inline vacuum degasser, a photodiode array detector (PDA 2998). Empower 2 Chromatographic Software was used to acquire and analyse the chromatograms and UV-Vis spectra (Waters Alliance, Ireland). The analysis was carried out on Nucleosil C₁₈ column (250 x 4.6 mm I.D., 5 µm packing) (Varian, JVA Analytical, Dublin, Ireland) at a controlled temperature of 25.0°C ± 0.2 and at a flow rate of 0.9 mL/min. The gradient consisted of a mixture of 0.33 mol/L acetic acid in water (solvent A) and 0.083 mol/L acetic acid in water:acetonitrile (50:50) (solvent B); it was increased linearly from 10% to 55% B in 45 min; from 55% to 57% B in 5 min; from 57% to 70% B in 10 min; from 70% to 100% B in 5 min; and from 100% to 10% in 1 min. The elution of target groups of phenolics was simultaneously monitored at 280 nm (flavan-3-ols; dihydrochalcones and derivatives); 320 nm (hydroxycinnamic acid and derivatives), and 370 nm (flavonols and flavonol glycosides). The identification of phenolic components was carried out through comparison of their retention times with commercial standards; the latter were of compatible purity for LC analysis and included: (+)-catechin; phloretin-2'-*O*-glucoside (phloridzin); quercetin; quercetin-3-*O*-rutinoside (rutin); gallic acid; caffeic acid; *p*-coumaric acid; 5'-caffeoylquinic acid (chlorogenic acid) (Sigma-Aldrich, Ireland). Procyanidin B₂; (-)-epicatechin; quercetin-3-*O*-galactoside (hyperoside); and quercetin-3-*O*-glucoside (isoquercitrin) were from

Extrasynthèse (Cedex, France). Provisional identification of unknown compounds for which commercial standards were not available was carried out based on the comparison of their UV-Vis spectral characteristics with known standards using the matching angle software of the Empower 2 chemical station (Waters Alliance, Ireland). The quantification study was carried out with calibration curves of standard phenolic compounds (20-200 mg/L): (+)-catechin for flavan-3-ols (280 nm); quercetin for flavonols and flavonol glycosides (370 nm); phloridzin for dihydrochalcones and derivatives (280 nm); chlorogenic acid for hydroxycinnamic acids and derivatives (320 nm).

2.5. PHYSICAL-CHEMICAL ANALYSIS

The quality of the enriched tomato and control juices in the post-opening storage was analysed with various markers of physical-chemical quality and antioxidant capacity.

2.5.1. pH

The pH was measured with a pH-meter (Orion-2 Star, Thermoscientific, Essex, UK) that was calibrated daily with standard buffered solutions at pH 4 and pH 7

2.5.2. Turbidity

The measurements were duplicated. The turbidity was measured with a turbidimeter (2100Qis, Hach-Lange, Dublin, Ireland); the juices (1 mL) were diluted up to 25 mL with de-aerated distilled water in order to adjust their readings within the measuring range of the instrument (between 0 and 1000 FNU, Formazin Nephelometric Unit). The instrument was calibrated with a set of primary standards (20-1000 FNU) using StablCal® Stabilised Standards (Hach-Lange, Dublin, Ireland) on a 5-point scale. The turbidity was expressed as FNU. The measurements were repeated in triplicate.

2.5.3. Total phenolic content

The total phenolic content (TPC) of enriched and control tomato juices was assessed before and after 4-day storage using Folin-Ciocalteu assay as previously described (Massini et al., 2013). The TPC was expressed as mg GAE/100 mL juice. The measurements were carried out in triplicate.

2.5.4. Colour

A colorimeter (ColorFlex 45/0, Hunter Lab, Reston, USA) was used for the analysis of the colour of the tomato juices. The instrument was calibrated with black and white tiles before use. A glass cell with a white ceramic lid on the top was used for holding the samples (~20 mL). The colour of the samples was measured in reflectance mode, using the CIELAB* coordinate system (light source: D65; 10° observer). The colour difference between the enriched sample and the control was also calculated as:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad \text{Eq. 1}$$

Where:

$$\Delta L^* = (L^*_{\text{sample}} - L^*_{\text{control}})$$

$$\Delta a^* = (a^*_{\text{sample}} - a^*_{\text{control}})$$

$$\Delta b^* = (b^*_{\text{sample}} - b^*_{\text{control}})$$

The level of saturation indicated by the Chroma value was also calculated as:

$$\text{Chroma} = \sqrt{a^{*2} + b^{*2}} \quad \text{Eq. 2}$$

2.5.5. Ascorbic acid content

The extraction and analysis of ascorbic acid was carried out with a HPLC chromatographic technique as previously reported, with some modifications (Hernández, Lobo & González, 2006). The juice (2 mL) was mixed with 1 mL of chilled extractant solution (3% metaphosphoric acid, w/v), homogenised for 1 min (in ice and darkness) at 9,500-13,500 min⁻¹ (Ultra-Turrax T25, IKA-Werke, Staufen,

Germany) and then centrifuged at refrigerated temperatures for 15 minutes. The extraction was repeated in duplicate. The samples were filtered through 0.2 µm PES membrane disc filters (Sarstedt, Nümbrecht, Germany). The ascorbic acid content was analysed using an e2695 Separation Module (Waters Alliance, Dublin, Ireland). The HPLC system that consisted of an auto sampler, a column heater, a quaternary solvent system pump, an inline vacuum degasser, a photodiode array detector (PDA 2998), and was equipped with Empower 2 Chromatographic Software (Waters Alliance, Ireland). The organic acids were separated on a hydrophilic C₁₈ stationary phase (YMC-Pack ODS-AQ column, 5 µm particle size, 250 x 4.6 mm I.D.) (Apex Scientific, Ireland). The injection volume of the sample was 20 µL, the solvent system consisted of 50 mmol/L phosphate buffer (pH 2.8); the separation was carried out in isocratic mode (flow rate: 1 mL/min) for 15 minutes at 25°C. The ascorbic acid content was quantified at $\lambda_{\text{max}} = 245$ nm by comparison with a calibration curve with standard L-ascorbic acid; the content was expressed as mg AA/100 mL juice. The measurements were carried out in duplicate.

2.5.6. Total carotenoid content

Carotenoids were extracted from the juices as previously reported, with minor modifications (Llorach, Espín, Tomás-Barberán & Ferreres, 2002). Samples were finally quantified as total carotenoids with a spectrophotometric method (Biehler, Mayer, Hoffmann, Krause & Bohn, 2010). A volume of 0.3 mL of juice was added with 0.3 mL of distilled water and 0.6 mL of extractant solution (acetone/methanol, 70:30) by shaking for 10 minutes in an ice box (Gyrotory Shaker G-2, Mason Technology, Ireland), and then centrifuged at 8,000 rpm for 10 minutes. The supernatant was recovered, and the pellet was mixed with 0.5 mL of extractant solution, then sonicated for other 5 minutes and centrifuged again until discolouration. The supernatants were pooled and partitioned with petroleum ether (1:1) at least two times by adding saturated sodium chloride in a separatory funnel. The organic layers were collected,

pooled and then weighed. An aliquot (1 mL) was evaporated in a rotary evaporator under vacuum. The remaining solids were resuspended in the same volume of acetone and their absorbance measured in a spectrophotometer at $\lambda = 450$ nm. The measurements were repeated in triplicate. The amount of total carotenoids (TCAR) was calculated according to Biehler et al. (2010):

$$\text{TCAR (mg/100 mL juice)} = \frac{\text{Abs}_{450}}{135310} * \text{MW} * \text{df} * 10^2 \quad (\text{d} = 1 \text{ cm}) \quad \text{Eq. 2}$$

Where:

df: dilution factor; it includes the volume adjustments for extracting, drying and reconstituting the sample in acetone;

MW: average molar mass for total carotenoids (548 g/mol)

2.5.7. Antioxidant capacity equivalent to ascorbic acid (AEAC)

The antioxidant capacity equivalent to ascorbic acid (AEAC) of enriched and control juices was assessed as a sum of their hydrophilic (e.g. water-soluble) and lipophilic (e.g. fat-soluble) antioxidant components (Larrosa, Llorach, Espín & Tomás-Barberán, 2002). The antioxidant capacity was calculated using FRAP and DPPH assays, as previously described {Massini, 2013 #59}. The values were expressed as mg AAE or ascorbic acid equivalents/100 mL of juice. The measurements were carried out in duplicate.

2.6. MICROBIOLOGICAL ANALYSIS

The microbial load of the enriched tomato and control juices was assessed before (t_0) and throughout storage, after 1, 2, 4, 6, and 9 days from opening (referred to as t_1 , t_3 , t_5 , t_7 , and t_{10} respectively). The samples were serially diluted (1:10) with sterile buffered peptone water (Biokar diagnostics, Beauvais, France) and then 0.1 mL of sterile solution was spread plated on Plate Count Agar (PCA) solid medium (Scharlau Chemie, Barcelona, Spain) for the determination of the total viable count (TVC) (incubation: 30°C for 2 days) (CCFRA, 2003). At the end of storage, the samples were assessed for their yeasts and moulds counts, using Potato Dextrose Agar (PDA) solid medium (Scharlau Chemie, Barcelona, Spain). For each serial dilution, a minimum of two plates were assayed. The experiment was duplicated. The average number of colony forming units per mL of juice (CFU/mL) was calculated using the first two consecutive serial dilutions with a number of colonies between 30 and 300 (Adams & Moss, 2000).

2.7. STATISTICAL ANALYSIS

The variability of the physical-chemical analysis of various samples of tomato i.e. enriched vs. control, at different storage times (before and after 4-day storage), was subjected to the analysis of ANOVA using Statgraphics Centurion XV (Statpoint, Warrenton, USA).

3. RESULTS AND DISCUSSION

3.1. COMPOSITION OF APPLE PEEL PHENOLICS

The neutral phenolics comprised mostly of flavan-3-ols including catechins and dimers, but also proanthocyanidins, followed by flavonol glycosides and dihydrochalcone derivatives including phloridzin and another phloretin derivative; the acidic phenolics mostly comprised of hydroxycinnamic acids derivatives, mainly of caffeic acid such as 5'-caffeoylquinic acid or chlorogenic acid i.e. typically of the apple fruit, followed by dihydrochalcone derivatives and to a lesser extent procyanidins e.g. trimers/tetramers that were not completely extracted at pH 7 (Figure 1).

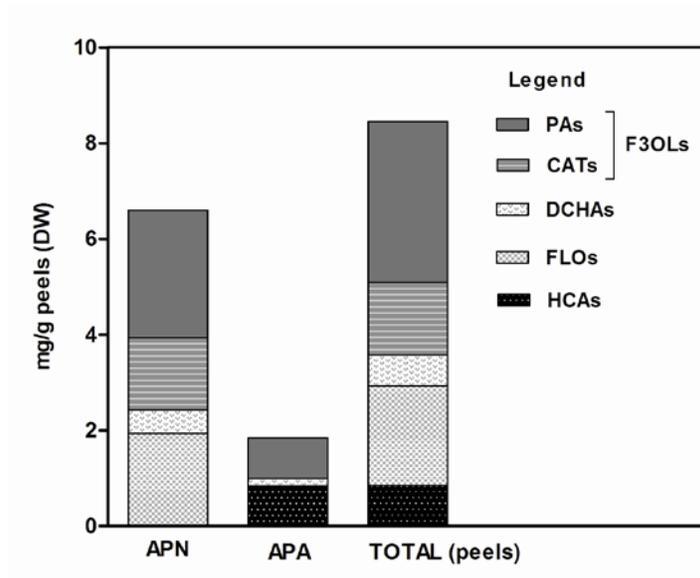


Figure 1 Phenolic composition of neutral (APN) and acidic (APA) phenolic extracts from apple peels. CATs: catechins; PAs: proanthocyanidins (oligomers, up to 4 units); F3OLs: flavan-3-ols; DCHAs: dihydrochalcone derivatives; FLOs: flavonol glycosides; HCAs: hydroxycinnamic acids and derivatives.

1 **Table 1** Quality markers of tomato juice with added phenolic extracts vs. control before (t₀) and after storage (t₄)
 2

Juice	Time (days)	pH	Turbidity (FNU)	Colour				
				L*	a*	b*	Chroma	ΔE
Control	0	4.10 ± 0.01 ^B	232.87 ± 4.06 ^A	26.77 ± 0.13 ^A	31.04 ± 0.18 ^A	30.36 ± 0.30 ^A	43.79 ± 0.09 ^A	-
	4	4.19 ± 0.01 ^A	242.08 ± 3.87 ^A	26.52 ± 0.02 ^A	30.51 ± 0.08 ^B	30.89 ± 0.06 ^A	43.03 ± 0.16 ^B	-
Enriched (APA)	0	4.07 ± 0.01 ^B	268.76 ± 25.96 ^A	26.95 ± 0.00 ^A	31.02 ± 0.02 ^A	30.37 ± 0.08 ^A	43.92 ± 0.09 ^A	0.28 ± 0.07 ^A
	4	4.14 ± 0.01 ^A	255.65 ± 30.70 ^A	26.85 ± 0.05 ^A	30.28 ± 0.04 ^B	31.08 ± 0.14 ^A	42.89 ± 0.09 ^B	0.44 ± 0.12 ^A
Enriched (APN)	0	4.10 ± 0.01 ^B	256.28 ± 29.79 ^A	27.29 ± 0.01 ^A	30.88 ± 0.01 ^A	30.41 ± 0.07 ^B	44.01 ± 0.08 ^A	0.74 ± 0.12 ^A
	4	4.18 ± 0.01 ^A	250.44 ± 17.95 ^A	27.19 ± 0.01 ^A	30.19 ± 0.06 ^B	31.36 ± 0.11 ^A	42.85 ± 0.06 ^B	0.79 ± 0.06 ^A
Mean								
Control		4.14 ^a	237.48 ^a	26.64 ^c	30.77 ^a	30.62 ^a	43.39 ^a	-
Enriched (APA)		4.10 ^b	240.13 ^a	26.90 ^b	30.65 ^{ab}	30.73 ^a	43.40 ^a	0.34 ^b
Enriched (APN)		4.14 ^a	253.36 ^a	27.24 ^a	30.53 ^b	30.88 ^a	43.42 ^a	0.76 ^a
F-test		***	NS	**	**	NS	NS	*
LSD _{0.05}		0.01	-	0.25	0.13	-	-	0.22
Storage time		***	NS	NS	***	*	***	NS
Storage time x Sample		NS	NS	NS	NS	NS	NS	NS

3 *, **, ***: significant (p<0.05), highly significant (p<0.01), very highly significant (p<0.001); NS: non-significant (p>0.05) Values are expressed as mean ± SD (n =
 4 4). Different small case letters indicate significant differences between juice samples; different capital letters within the same type of juice indicate significant
 5 difference between storage times. FNU: formazin nephelometric units.
 6
 7

3.2. QUALITY MARKERS

The physical-chemical analysis of enriched tomato juice vs. control before and after 4 day-storage is shown in Table 1.

There was a colour difference i.e. measured as ΔE between enriched vs. control samples; chroma values were reduced in a similar trend from time 0 to 4 days, thus indicating a loss of colour saturation during storage. The redness (+a*) was the key colour component during the analysis. The main pigment in tomato juice i.e. lycopene is very unstable in the presence of light and air (i.e. as in the open-package storage) and tends to degrade during storage (García-Alonso, Bravo, Casas, Pérez-Conesa, Jacob & Periago, 2009). In the presence of natural phenolics with protein binding capacity such as proanthocyanidins, the juice with added APN reduced its redness but increased significantly ($p < 0.05$) its luminosity; this was explained as due to the abundance of polymeric phenolics (flavan-3-ols) that could lead to precipitation of colloids, thus giving a brighter colour. A similar trend was observed in the enriched juice with APA possibly due to the presence of residual tannins that were not completely extracted at pH 7. The yellowness (+b*) slightly increased after storage but such increase was significant ($p < 0.05$) only for APN and this was explained as due to the abundance of yellow pigments (flavonol glycosides). Natural flavonoids present in the tomato juice also absorb in the same UV-Vis region as apple phenolics, thus reducing the effect of addition on the yellowness of the extracts. The levels of turbidity of the enriched juice vs. control were not significantly different ($p > 0.05$), however, a slight increase in the turbidity was observed for the juices with added neutral extract (APN). The turbidity of juices is associated to the light scattering of the pectins surrounding the fragments of cell walls (pulp) in a colloidal serum of macromolecules, e.g. proteins, sugars, organic acids {Hsieh, 2008 #109}. The presence of phenolic compounds, especially procyanidins with high molecular weight and reduced solubility in water, can lead to complexes with proteins and

carbohydrates of the cell walls {Alonso-Salces, 2004 #29}. However, this phenomenon could take some time (i.e. weeks) before becoming evident, as it is the case with apple ciders. Therefore, the storage time in the present study was probably insufficient to observe such a phenomenon; nevertheless, turbidity is a quality marker to be considered in the presence of mixtures of procyanidins. The addition of acidic phenolics to the juices significantly lowered their pH in comparison to the control; as it was expected, the same effect was not observed with the mixture of neutral phenolics. The pH of all samples increased after storage; this was explained as due to the reduction of ascorbic acid and the onset of microbial spoilage.

3.3. ASCORBIC ACID AND TOTAL CAROTENOIDS

There was no significant difference between the ascorbic acid content of the enriched tomato juices vs. control during the 4 day-storage under open-package conditions (Table 2).

However, it was observed over time and in all the samples that the content of ascorbic acid reduced significantly (~20%) at the end of the storage. It is known that ascorbic acid is particularly sensitive to the presence of oxygen (i.e. air in the headspace of the samples), thus it degrades quickly; moreover, in the open-package storage, the sterility is broken, therefore ascorbic acid can be also degraded as a result of the growth of the resident microflora. Under less critical storage conditions and after being pasteurised, a bottled tomato juice stored for 28 days at 4°C was reported losing almost 50% of its original ascorbic acid content (García-Alonso *et al.*, 2009). The ascorbic acid content in tomato juice was in agreement with previous literature data (García-Alonso *et al.*, 2009; Podsędek, Sosnowska & Anders, 2003). The amount of total carotenoids in the juices (control and enriched) was not affected by the storage time ($p > 0.05$); the enriched samples and the control had similar contents. The amount of total carotenoids in the samples was similar to previous results by Podsędek *et al.* (2003) who found a content of total carotenoids in the range between 4.57 and 8.69 mg/100 g of tomato juice.

Table 2 Antioxidant components (AA; TCAR) and Total Antioxidant Capacity (AEAC) of tomato juice vs. control before (t_0) and after storage (t_4)

Juice	Time (days)	AA (mg/100 mL)	TCAR (mg/100 mL)	AEAC (mg AAE/100 mL juice)	
				FRAP	Radical scavenging capacity (DPPH)
Control	0	5.97 ± 0.03 ^A	5.72 ± 1.31 ^A	47.70 ± 0.42 ^A	34.31 ± 1.09 ^A
	4	4.47 ± 0.01 ^B	4.56 ± 0.80 ^A	39.00 ± 0.24 ^B	30.18 ± 0.36 ^B
Enriched (APA)	0	6.06 ± 0.08 ^A	5.21 ± 0.08 ^A	56.15 ± 3.61 ^A	34.85 ± 0.46 ^A
	4	4.62 ± 0.04 ^B	4.67 ± 0.17 ^A	47.71 ± 0.46 ^A	36.19 ± 0.24 ^A
Enriched (APN)	0	5.83 ± 0.02 ^A	5.61 ± 0.51 ^A	86.20 ± 3.54 ^A	48.03 ± 0.25 ^A
	4	4.70 ± 0.01 ^B	5.29 ± 0.45 ^A	65.23 ± 1.78 ^B	47.25 ± 0.49 ^A
Mean					
Control		5.22 ^a	5.12 ^a	43.33 ^c	32.24 ^c
Enriched (APA)		5.27 ^a	5.14 ^a	51.93 ^b	35.52 ^b
Enriched (APN)		5.34 ^a	5.62 ^a	75.69 ^a	47.64 ^a
F-test		NS	NS	***	***
LSD _{0.05}		-	-	6.61	2.38
Storage time		***	NS	***	*
Storage time x Sample		NS	NS	NS	*

*, **, ***: significant (p<0.05), highly significant (p<0.01), very highly significant (p<0.001); NS: non-significant (p>0.05) Values are expressed as mean ± SD (n = 4). Different small case letters indicate significant differences between juice samples; different capital letters within the same type of juice indicate significant difference between storage times. FNU: formazin nephelometric units; AA: ascorbic acid content; TCAR: total carotenoids; AEAC: ascorbic acid equivalent antioxidant capacity.

3.4. ANTIOXIDANT CAPACITY

Despite the presence of peel phenolic components with medium-to-high polarity and condensed tannins with medium-to-low polarity, the enriched antioxidant mixtures did not add to the preservation of other hydrophilic (ascorbic acid) or lipophilic (carotenoids) components of tomato juice. However, it was observed that the antioxidant capacity equivalent to ascorbic acid (AEAC) measured with FRAP and DPPH

assays was significantly higher ($p < 0.05$) for the enriched tomato juices compared to the control. During storage, the antioxidant capacity of the control tomato juice decreased significantly ($p < 0.05$); such decrease was not significant in the presence of added phenolic extracts; the highest antioxidant capacity was achieved in the sample of tomato juice with added apple peel flavonoids, mostly because of the presence of catechins and dimers and flavonol glycosides with established radical scavenging capacity. The redox potential (FRAP) depended on the type of phenolic extract added to the juices (APN > APA), and was possibly influenced by the presence of other reducing substances, in particular ascorbic acid. From these findings, it was suggested that the decrease of antioxidant capacity in the enriched and control tomato juices was mainly attributed to the reduction of ascorbic acid during the open-package storage i.e. presence of air in the headspace of the bottles, beginning of microbial growth, increase of pH etc. (Pacheco-palencia, Hawken & Talcott, 2007b). Unlike ascorbic acid, the content of the other hydrophilic antioxidants i.e. polyphenols including those from apple and those naturally present in the tomato juices remained stable during storage (Figure 2).

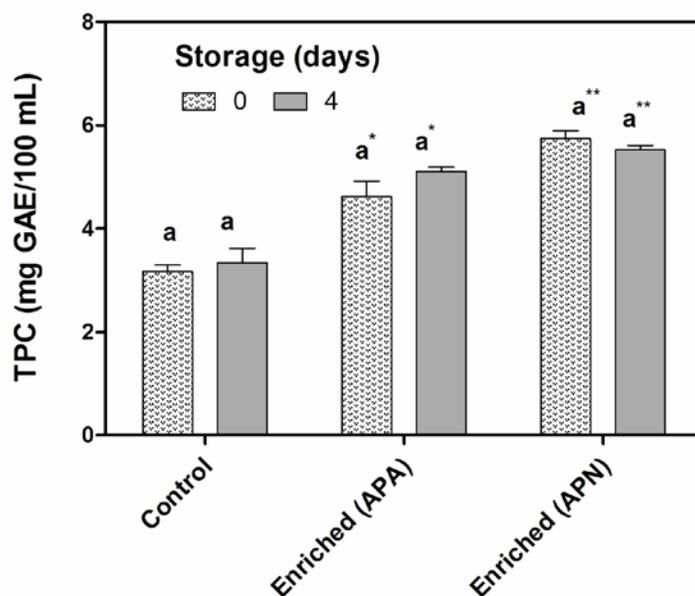


Figure 2 Total phenolic content of tomato juice with added phenolic extracts (neutral APN; or acidic APA) vs. control at time 0 and after 4-day storage. Different letters within the same type of juice indicate significant differences between storage times ($p < 0.05$). TPC: total phenolic content; GAE: gallic acid equivalents.

As a consequence, it was suggested that the maintenance of the antioxidant capacity, in particular radical scavenging capacity, in the enriched juices should be attributed to peel phenolics (Table 2). These findings are supported by previous results which indicate polyphenols as compounds that have higher physical-chemical stability during storage; unlike ascorbic acid, polyphenols can undergo chemical transformations while maintaining their antioxidant capacity, (García-Alonso et al., 2009; Pacheco-palencia, Hawken & Talcott, 2007a). The present findings did not suggest any interaction between the added apple polyphenols and some of the other antioxidant components in the juice (ascorbic acid and carotenoids). However, the present study was preliminary to the identification of quality markers of vegetable juices and was carried out under open-package conditions i.e. short storage time and without further processing of the juice. In literature, synergistic interactions between polyphenols and ascorbic acid were suggested in pasteurised fruit juices in the presence of certain additives (Pernice, Borriello, Ferracane, Borrelli, Cennamo & Ritieni, 2009); the mechanism was not detailed, even though it was reported that citric acid used as an additive could help stabilising ascorbic acid thanks to its metal chelating capacity (Lo Scalzo, 2008). It is known that polyphenols are metal chelators, especially hydroxycinnamic acids with the catechol structure, however such chemical property strongly depends on the environmental conditions and type of solvent medium (i.e. ionization pattern and pH) (Hider, Liu & Khodr, 2001); it is not understood if phenolic chelators could behave as citric acid towards the stabilisation of ascorbic acid. In a study by Yesil-Celiktas *et al.* (2010), the addition of a pine bark extract consisting of catechins and taxifolin to orange juice resulted in a slightly decreased loss of ascorbic acid after 6 months storage compared to the control (-10%). However no statistical significance was reported at the end of the 8-month storage. It has been suggested that lipophilic phenolics (i.e. flavonoids of higher molecular weight such as procyanidins) could interact with lipophilic components, such as tocopherols or carotenoids (García-Alonso et al., 2009). Even though this possibility cannot be excluded, it was not observed in the present study; this was likely due

to the fact that carotenoids were quantified as total instead of being characterised as single components, and because of a short storage time. The mechanisms of interactions between polyphenols and carotenoids are still poorly understood; therefore, the research should focus on developing model systems that allow to understand the interactions of single carotenoids with tannins before introducing them in the complexity of the food matrix and its constituents (Decker et al., 2010).

3.5. MICROBIOLOGICAL ANALYSIS

The microbial quality of the enriched tomato and control juices was assessed through the evaluation of the total viable counts (TVC) (Figure 3).

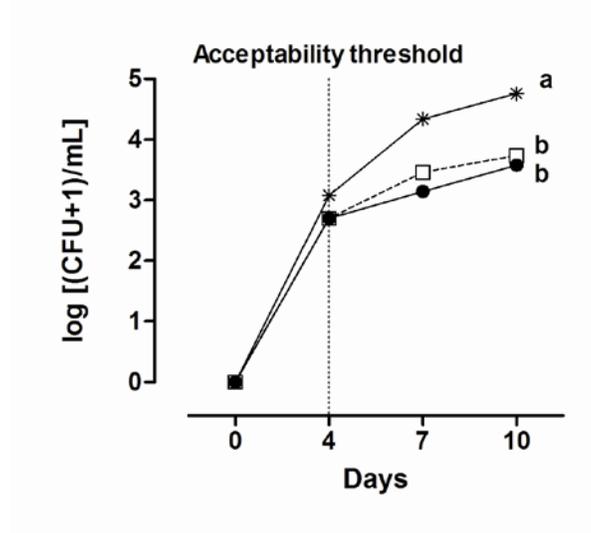


Figure 3 Total viable counts of tomato juices with added phenolic extracts (neutral, APN ●) and (acidic, APA □) vs. control (*) over an extended storage time of 10 days at 4°C. The acceptability threshold at day 4 is indicated with a dotted line. Different letters indicate significant differences ($p < 0.05$) between samples. CFU: colony forming unit.

Results showed that up to day 4, the microbial load of the enriched juices decreased in comparison to the control, with a significant reduction of TVC by ~0.5 log (CFU/mL). The addition of peel phenolics extended the threshold of acceptability of the stored opened bottle from 4 to 6/7 days. These results suggested that peel polyphenols could also act as preservatives against the growth of aerobic and mesophilic microorganisms in tomato juice. In order to establish the magnitude of such preservation, the storage was

extended until day 10. At day 10, the quality of the tomato juice with added phenolic extracts was enhanced in comparison to the control (reduction of ~ 1.1 log CFU/mL). Most of the mesophilic aerobic microorganisms responsible for the spoilage of the tomato juices at day 10 were enumerated as yeasts and moulds on a selective growth medium at 30°C (data not shown). Tomato juice had pH equal to or lower than 4.5, therefore it is considered a high acidity food which is unlikely contaminated by pathogens. In literature, yeasts, lactic acid bacteria and moulds are the main spoilage microorganisms of vegetable juices with high acidity {Ashurst, 2005 #96}. The antimicrobial capacity of the phenolic extracts from apple peels was related to their phenolic composition, the presence of phenolic acids as in APA maintained lower pH compared the control, and this could delay the onset of microbial spoilage. However, it is important to mention that some bacteria could use phenolic acids as substrates for their metabolism, such as coumaric and chlorogenic acid {Rodríguez, 2009 #327}. In APN, and partly in APA, the presence of tannins such as trimers and tetramers were likely to exert an antimicrobial effect. In particular, catechins and oligomeric flavan-3-ols could supply antimicrobial properties because of their ability to interact with both lipid and protein components of biological membranes, thus altering their biochemical properties (i.e. membrane permeation) (Hendrich, 2006). Flavonols and flavan-3-ols with strong membrane affinities could decrease membrane fluidity, thus affecting functions of membrane enzymes and receptors, and the reaction efficacy of membrane components such as transporter proteins (Cushnie & Lamb, 2005). The abundance of flavan-3-ols in the neutral extract (APN) could give to this mixture a better antimicrobial capacity than APA; nevertheless, both extracts supplied a preservative capacity up to day 10 by significantly ($p < 0.05$) reducing the spoilage of tomato juice compared to the control with distilled water instead of sample. These findings are promising towards the applications of natural peel phenolics as natural additives with antioxidant and/or preservative capacity.

4. CONCLUSIONS

The addition of apple peel phenolics to tomato juice had a significant impact on the visual appearance of the enriched juices, with an increased luminosity possibly due to the formation of insoluble pectin-phenolic complexes and increased yellowness due to pigments such as flavonol glycosides. It was also suggested a possible increase of the turbidity levels of the enriched juices due to the presence of procyanidins, which could become more evident over storage. As for the antioxidant capacity, hydrophilic and lipophilic constituents of enriched and control tomato juices followed different degradation patterns under the open-package storage. Ascorbic acid was significantly reduced in control and enriched juices regardless of the added phenolic extract; nevertheless, the addition of phenolics contributed to the maintenance of a higher radical scavenging capacity, thus counterbalancing the loss due to ascorbic acid degradation. Lipophilic components such as carotenoids remained constant during the storage time. Finally, the addition of apple peel phenolics enhanced the microbiological quality of the enriched juices vs. control; such capacity seemed to be promoted in the presence of flavonoids such as flavan-3-ols and flavonols. These findings suggests the recovery of natural apple peel phenolics as novel antioxidants/preservatives for functional applications with vegetable juices.

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Conflict of interest

The authors declare that they have no conflict of interest.

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