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Stability of antioxidants in an apple polyphenol–milk model system

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Abstract

The stability of antioxidants in an apple polyphenol–milk model system was examined. The model system consisted of skim milk fortified with pH-neutralised apple polyphenols (AP, 0–200 mg per 100 ml milk), with or without ascorbic acid (100 mg per 100 ml milk). Physical and chemical changes were evaluated after thermal treatment (120 °C, 5 min) and oxidative storage (20 °C and 38 °C, up to 12 weeks). Antioxidant capacity was determined using both oxygen radical absorbance capacity (ORAC) assay and ferric reducing antioxidant power (FRAP) assay. Significant antioxidant capacity was detected in the presence of milk. Antioxidant capacity was retained during thermal treatment but decreased slowly during storage.

The concentration of ascorbic acid decreased rapidly, and was close to zero after 2-week storage at 38 °C or 10-week storage at 20 °C. The brownness of the polyphenol–milk system increased over storage duration of 0–12 weeks; this effect was retarded by the addition of ascorbic acid. This high polyphenol–milk has demonstrated good physical stability.

Keywords: Apple polyphenol–milk; ORAC assay; FRAP assay; Physical stability; Ascorbic acid

1. Introduction

Novel functional foods containing different food components may give health benefits beyond those arising from individual food components. However, it is important to assess whether the claimed health benefits are maintained within food mixtures during shelf life. Fruit and milk is one such mixture in which the antioxidant capacity of fruit constituents can be delivered in combination with the health benefits of milk.

The well-known health benefits of fruits have been attributed in part to their antioxidant content, as well as other bioactive components such as dietary fibre. Apples contain various antioxidative phenolics, such as procyanidin, catechin, epicatechin, chlorogenic acid, phloridzin, quercetin and their conjugates (Boyer & Liu, 2004). The polyphenols are the main compounds responsible for the total antioxidant capacity of apples (Gardner, White, McPhail, & Duthie, 2000; Lee, Kim, Kim, Lee, & Lee, 2003). Milk also contains substances with antioxidant capacity, such as urate, proteins, carotenoids and vitamins (Calligaris, Lara Manzocco, Monica Anese, & Cristina Nicoli, 2004; Chen, Lindmark-Månsson, Gorton, & Åkesson, 2003; Lindmark-Månsson & Åkesson, 2000). Therefore, a convenient food format to deliver the goodness from both apples and milk is possible, through fortifying apple polyphenol extracts into milk drinks.

Limited work has been done on oxidation of apple polyphenols during storage, in relation to browning and loss of antioxidant capacity. The development of browning in apple juices stored at 35 °C occurred when the flavonoid content was relatively high (Spanos, Wrolstad, & Heatherbell, 1990). Accelerated aging of apple juice enriched with polyphenols resulted in a 20–40% reduction in antioxidant capacity (van der Sluis, Dekker, & van Boekel, 2005). Bradshaw, Cheynier, Scollary, and Prenzler, (2003) reported the initial anti-browning function of ascorbic acid and the subsequent acceleration of the browning of phenolic intermediates in a model wine.

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Interactions between polyphenols and proteins, which may mask or enhance antioxidant capacity, have been examined (Arts et al., 2002; Perez-Jimenez & Saura-Calixto, 2006; Rohn, Rawel, & Kroll, 2004). Individual polyphenols and dairy components (including caseins and whey proteins) may form polyphenol-protein complexes (Haslam, 1998; Rawel, Kroll, & Hohl, 2001). Studies on tea polyphenol–milk drinks have shown that tea extracts containing 60–70% polyphenols could stabilise milk on heating (O'Connell, Fox, Tan-Kintia, & Fox, 1998). Synergistic effects were found in the antioxidant capacity of milk products mixed with berry extracts (Skrede, Larsen, Aa by, Jorgensen, & Birkeland, 2004).

This study investigates the physical and antioxidant stability of apple polyphenol–milk model systems (containing high antioxidant content) during oxidative storage, as a function of polyphenol concentration, addition of absorbic acid, storage temperature and duration. Colour, solution pH and protein precipitation of the fortified milk were measured as indices for product stability and the changes in ascorbic acid concentration and total antioxidant capacity were monitored.

2. Materials and methods

2.1. Materials and chemicals

Skim milk powder (Material No. 103880) was obtained from New Zealand Milk Products, Palmerston North, New Zealand. It contained 54% lactose, 33% protein, 0.8% fat, 7.9% minerals and 3.8% moisture. Filtered tap water was used for milk powder preparation (Manufacturer information).

Apple extract was purchased from Penglai Marine Biochemicals Ltd., Shandong, China, containing 770 mg oligoproanthocyanidins complex/g dried weight. L-Ascorbic acid was purchased from BASF, New Zealand. Acetic acid and sodium hexametaphosphate were from Ajax Fine Chemicals, Australia. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tris(2-pyridyl)-*s*-triazine, dichlorophenol indophenol and Folin-Ciocalteu phenol reagent were from Sigma-Aldrich (Auckland, New Zealand). Milli-Q^{PLUS} water was used for all reagent preparation.

2.2. Preparation of polyphenol–milk model

Milk stock preparations (15% w/v) were prepared by first dissolving a desired amount of milk powder in water, then homogenising it under high pressure (25,000 psi, 1 pass) in an EmulsiFlex®-C3 High Pressure Homogeniser (Avestin, Ottawa, Canada), and stored at 4 °C.

Apple polyphenol (AP) stock solutions (final concentrations ranging from 100–400 mg polyphenol/100 ml), were prepared by slowly adding a desired amount of apple extract, with or without 400 mg ascorbic acid, in 40 °C water. Dissolution was facilitated by stirring, and the pH of the solution was adjusted to 6.2 using 0.2 M KOH. The final volume was adjusted to 200 ml with water.

Polyphenol–milks were made up by mixing equal volumes of the milk stock preparations and the AP stock solutions, followed by homogenisation. The final polyphenol–milks contained skim milk powder (7.5 g per 100 ml milk), AP (50–200 mg per 100 ml milk), and ascorbic acid (either 0 or 100 mg per 100 ml milk). Milk controls (0 mg AP/100 ml), in the absence or presence of ascorbic acid, were prepared by mixing equal volumes of the milk stock preparations and water (with or without the addition of 400 mg ascorbic acid), followed by homogenisation.

2.3. Thermal treatment

Polyphenol–milks or milk controls were added into polypropylene tubes (15 ml BLUE MAX™ Jr., Becton Dickson, NJ) and these tubes were capped. Polyphenol–milks or milk controls were thermally treated in a pressure cooker at 15 psi for 5 min. During processing, a 2 ml (approximately) airspace in the top of tubes resulted from overflow of liquid. Once heating was completed, lids were tightened while still hot, and tubes were kept upright during production and storage. Three separate batches of both the polyphenol–milks and milk controls were prepared.

2.4. Storage trials, physical stability and pH tests

Polyphenol–milks or milk controls were left at 20 °C for 24 h (referred to as "zero-week" thereafter), followed by storage in the dark at 20 °C or 38 °C.

Two aliquots of the polyphenol–milks and of milk controls were sampled at the 2nd, 4th, 6th, 8th or 12th week, from each of the three batches, for subsequent analyses. After sampling, the pH values of all polyphenol–milks and milk controls were measured. A portion of the sample was immediately centrifuged at 4400*g* for 5 min for precipitation evaluation, with the remaining portion being frozen at −80 °C.

2.5. Analysis of apple extract

Individual phenolic content, total phenolics and tannin content of apple extract were analysed using HPLC (Stevenson, Wibisono, Jensen, Stanley, & Cooney, 2006), Folin-Ciocalteu assay (Singleton, Orthofer, & Lamuela-Raventos, 1997) and the acid–butanol method (Butler, 1982), respectively.

2.6. Colour determination

Sample colour was measured using a Minolta CR-300 colorimeter (Konica Minolta Sensing, Inc., Osaka, Japan) and expressed as Hunter $L^*a^*b^*$ colour values. An aliquot (0.7 ml) of sample was added into 1-ml dimples (diam. 20 mm) in a white ceramic plate. The colorimeter head was masked in black outside the aperture, and positioned

at the centre of the dimple. Readings with variations (in triplicate) were obtained as L^*, 0.06%; a^*, 0.99%; b^*, 0.32%.

2.7. Antioxidant capacity determination

The ferric reducing/antioxidant power (FRAP) assay (Benzie & Strain, 1999) was used with some modifications and performed in duplicate. FRAP reagent was prepared 2 h before each assay at 37 °C. An aliquot (198 μl) of FRAP reagent was incubated with 10 μl sample in a microplate for 20 min at 20 °C before A_{593} measurements. Fresh working Trolox standards (100–1250 μM) were used to establish a calibration curve.

The oxygen radical absorbance capacity (ORAC) assay (Walton, Lentle, Reynolds, Kruger, & McGhie, 2006) was also performed in triplicate to measure the antioxidant capacity.

2.8. Determination of reduced ascorbic acid concentration

Reduced ascorbic acid was determined using the AOAC titration method (AOAC, 1990) with some modifications. Hexametaphosphate stock (1:5, w/v), glacial acetic acid and deionised water were mixed in a volume ratio of 2:1:7 (v/v/v). The resulting solution was used to dilute the polyphenol–milks (containing 1.5 ml milk stock preparations and 5.5 ml of AP stock solutions). Ascorbic acid content was determined via titration using dichlorophenol indophenol. Calibration was performed through titration, using an ascorbic acid standard (1 mg/ml, 5680 μM ascorbic acid).

3. Statistical analysis

Replication in the experiment included three independent batches for each polyphenol–milk or milk control. Polyphenol–milks or milk controls were processed and stored in duplicate tubes ($n = 6$). Data were analysed using Repeated-Measures ANOVA (Minitab 15).

4. Results and discussion

4.1. Composition of the commercial apple polyphenol extract

The composition of the AP extract used in this study is shown in Table 1, suggesting that this AP extract contained phenolic compounds commonly found in apples (Lea, 1978; Vidal et al., 2003). The soluble polyphenols quantified using HPLC accounted for approximately 40% of the extract weight. Epicatechin oligomers present in the tested AP extract appeared to be the largest class present among the individual phenolic compounds detected. Other compounds present included quercetin and phloretin, as well as their respective glycosides, caffeoyl quinates (chlorogenic acid) and epicatechin. Major classes of apple polyphenols found are shown in Fig. 1.

4.2. Physical stability and pH

For all of the polyphenol–milks and milk controls, physical stability, determined as the pellet remaining after centrifugation, was maintained during production and throughout storage (data not shown). No detectable difference in the amounts of precipitate was observed between the polyphenol–milks and milk controls.

The pH of the polyphenol–milks and milk controls decreased with time in storage. For a specific concentration of polyphenols, the pH values of the polyphenol–milks and milk controls were lower after 2-week or 12-week storage. Furthermore, the pH values of polyphenol–milks and milk controls in the presence of ascorbic acid decreased to a larger extent than those of the polyphenol–milks and milk controls in the absence of ascorbic acid after storage. By the 12th week (at 38 °C), there was little difference in the pH values between individual polyphenol–milks and milk controls at different concentrations of AP, either in the presence or absence of ascorbic acid, respectively. The pH of polyphenol–milks and milk controls decreased during storage, but the decrease was independent of AP concentration (Table 2). Therefore, addition of AP may not interfere with the pH of the polyphenol–milks, although the pH of milk was reported to be dependent on the partition of calcium phosphate salts between soluble and colloidal fractions (Fox & McSweeney, 1998).

The pH values are associated with the negative logarithm of hydrogen ion concentration. In an aqueous environment, a pH drop indicates a significant increase in H^+ ion concentration. The changes in pH of polyphenol–milks and milk controls after storage may be due to a number of reasons. Slow chemical reactions may have gradually caused deterioration of these milk samples over time and

Table 1
Composition of the commercial apple polyphenol extract used in this study

Total phenolics[a] (mg/g)	Tannin content[b] (mg/g)	Total soluble polyphenols[c] (mg/g)	Individual polyphenol classes[c] (μM)						
			Caffeoyl quinates	*p*-Coumaroyl quinate	Quercetin (glycosides)	Phloretin glycosides	Epicatechin	Procyanidin dimers	Epicatechin oligomers[d]
770	510	382	52.2	18.6	39.2	75.6	35.5	16.6	453

[a] Analysed by Folin-Ciocalteu as catechin equivalents.
[b] Analysed by the acid–butanol method.
[c] Analysed by HPLC (not including oligomeric procyanidin content).
[d] Analysed by HPLC.

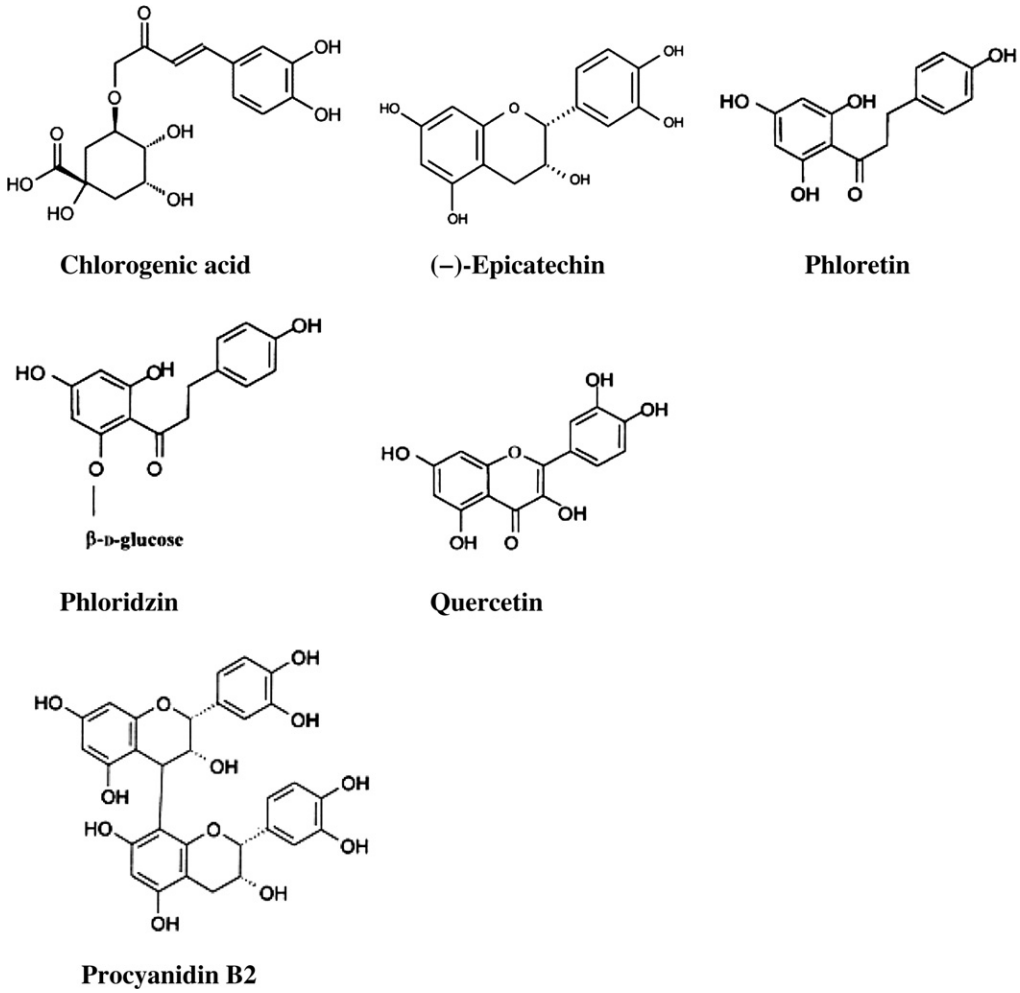

Fig. 1. Chemical structure of major classes of apple polyphenols.

Table 2
Effect of storage at 20 °C or 38 °C on the pH of polyphenol-milks and milk controls

Milk sample	pH				
	No storage	Storage at 20 °C		Storage at 38 °C	
	0-Week	2-Week	12-Week	2-Week	12-Week
0 mg AP/100 ml milk					
Without AA	6.73 ± 0.04	6.70 ± 0.02	6.69 ± 0.01	6.73 ± 0.02	6.50 ± 0.00[a]
With AA	6.56 ± 0.04[b]	6.56 ± 0.01[b]	6.47 ± 0.01[a,b]	6.40 ± 0.03[a,b]	6.24 ± 0.02[a,b]
50 mg AP/100 ml milk					
Without AA	6.73 ± 0.05	6.72 ± 0.02	6.69 ± 0.02	6.67 ± 0.01	6.47 ± 0.00[a]
With AA	6.65 ± 0.01	6.59 ± 0.01[a]	6.43 ± 0.01[a,b]	6.42 ± 0.02[a,b]	6.24 ± 0.01[a,b]
100 mg AP/100 ml milk					
Without AA	6.68 ± 0.04	6.72 ± 0.01	6.67 ± 0.01	6.65 ± 0.02	6.50 ± 0.00[a]
With AA	6.69 ± 0.02	6.61 ± 0.02[a]	6.41 ± 0.02[a,b]	6.40 ± 0.03[a,b]	6.24 ± 0.02[a,b]
200 mg AP/100 ml milk					
Without AA	6.68 ± 0.03	6.64 ± 0.02	6.65 ± 0.01	6.63 ± 0.03[a]	6.49 ± 0.01[a]
With AA	6.69 ± 0.03	6.65 ± 0.02	6.37 ± 0.02[a,b]	6.40 ± 0.02[a,b]	6.19 ± 0.02[a,b]

[a] Significant difference ($p < 0.05$) from week 0 samples at the same AP (apple polyphenol) concentration.
[b] Significant difference ($p < 0.05$) from those samples without ascorbic acid (AA) at the same AP concentration. Statistical comparisons were made between samples using *t*-test.

were naturally accelerated by heat. Degradation of polymeric compounds could be possible over the storage time, due to exposure to heat. O_2 that has permeated through the packaging, and/or other chemicals may also contribute.

For polyphenol–milks and milk controls, pH drop would result from: (1) Slow hydrolysis and/or degradation of proteins occurring spontaneously; (2) Rise in temperature favouring the production of lactic acid; (3) Interaction between lactose and milk proteins and changes in the solubility of calcium phosphate; (4) Lipolysis and release of free fatty acids and changes in divalent ions' solubility, although skim milk was used in this study. Furthermore, for polyphenol–milks, pH drop might also result from degradation of polymeric phenolics to monomeric phenolic acids with different acid strengths (pK_a), via hydrolytic interactions and oxidative cleavage. Some of these resultant monomeric phenolics would have interacted with milk protein molecules, causing formation of polymer particles. Increased storage temperature would significantly raise the polymerisation rate in such an aqueous environment, and then dilute the concentration of these monomers in solution. Subsequently, the water ratio in the aqueous phase would increase, causing more ionisation of the acidic monomers in solution, and thereby further lowering the pH.

Differential reduction in pH values after storage was observed in the presence and absence of ascorbic acid. This phenomenon may be due to the fact that ascorbic acid quickly oxidised in the milk system, producing peroxide. Degradation products of ascorbic acid such as L-threose (López & Feather, 1992; Shin & Feather, 1990) and L-erythrulose (Simpson & Ortwerth, 2000) might be involved in Maillard reactions with protein derived molecules. Ascorbic acid (or its enediol degradation products) could generate hydroxyl radicals that would have caused scission of polymeric compounds (polyphenols and/or proteins), and thereby increased the amounts of resultant monomers with increased ionisation.

4.3. Ascorbic acid oxidation

The rate of ascorbic acid oxidation in the polyphenol–milks and milk controls with added ascorbic acid at three AP concentrations, at both 20 °C and 38 °C, are shown in Fig. 2. During storage at 20 °C, the concentration of retained ascorbic acid declined linearly for all the polyphenol–milks and milk controls. There was no statistical difference ($p < 0.05$) between the slopes (−813.9 ($r^2 = 0.98$), −825.6 ($r^2 = 0.99$), −857 ($r^2 = 0.97$), respectively), in the cases of the three AP concentrations (0, 50 or 200 mg AP per 100 ml milk). During storage at 38 °C, ascorbic acid oxidised at a much faster rate and completely deteriorated by the 4th week.

In polyphenol–milks and milk controls with added ascorbic acid, oxidation of ascorbic acid during processing was insignificant, whereas the loss of ascorbic acid during the 20 °C-storage occurred at a constant rate, independent of polyphenol concentration. Increased storage temperature (from 20 °C to 38 °C) accelerated ascorbic acid oxidation 6-fold and ascorbic acid was completely oxidised in the polyphenol–milks and milk controls by the 4th week at 38 °C.

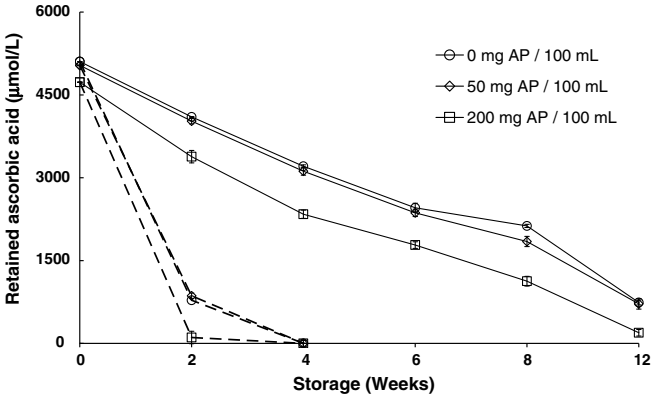

Fig. 2. Effect of storage on the concentration of retained ascorbic acid in polyphenol–milks (50 and 200 mg AP) or milk controls (0 mg AP), with 1 mg/ml ascorbic acid per 100 ml of milk. Solid lines = storage at 20 °C, dotted lines = storage at 38 °C. AP refers to apple polyphenol.

The rate of ascorbic acid oxidation in milks has been shown to be associated with the permeation of oxygen through the packaging (Gliguem & Birlouez-Aragon, 2005; Steskova, Morochovicova, & Leskova, 2006). Oxygen present in the headspace above milk can contribute to the oxidation of milk products (Mestdagh, De Meulenaer, De Clippeleer, Devlieghere, & Huyghebaert, 2005).

4.4. Colour

The colour of the resultant polyphenol–milks and milk controls was measured as Hunter L^* value (lightness), a^* value (red–green) and b^* value (yellow–blue). In this study, an increased a^* value was always accompanied with a decreased L^* value, with b^* value being constant (data not shown). Therefore, only a^* value was used in this paper to indicate the changes in sample colour during storage.

Fig. 3A and B show that the a^* values of polyphenol–milks and milk controls were influenced by AP concentration, storage temperature and storage duration. Addition of apple polyphenols to milk resulted in redness of the polyphenol–milks either in the absence (Fig. 3A) or presence (Fig. 3B) of ascorbic acid. Addition of ascorbic acid suppressed the redness of both polyphenol–milks and milk controls during storage (Fig. 3A and B).

In the absence (Fig. 3A) or presence (Fig. 3B) of ascorbic acid, polyphenol–milks turned redder after 2-week or 12-week storage at 38 °C than they did at 20 °C. Increase in redness was much greater in the absence of ascorbic acid (Fig. 3A) than in the presence of ascorbic acid (Fig. 3B), except that the a^* values became similar (remarkably high) upon the 12th-storage week at 38 °C for polyphenol–milks either in the absence or presence of ascorbic acid. This suggests that the development of redness in polyphenol–milks might be inhibited by added ascorbic acid, until the ascorbic acid was fully oxidised. After 12-week storage at 38 °C, the polyphenol–milks became 2.2-fold redder than those of zero-week storage, in the absence (Fig. 3A) of ascorbic

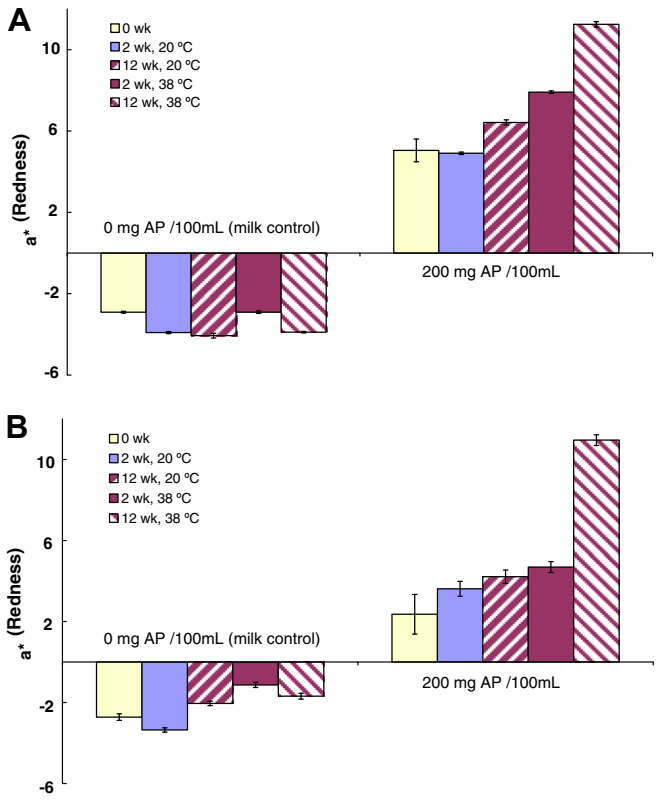

Fig. 3. Effect of storage on the redness (a^*) of polyphenol–milks (200 mg AP per 100 ml of milk) or milk controls (0 mg AP), either without (A) or with (B) ascorbic acid. AP refers to apple polyphenol. Storage weeks (wks): 0, 2 and 12; storage temperature: either 20 or 38 °C.

acid, and 4.6-fold redder than those of zero-week storage, in the presence (Fig. 3B) of ascorbic acid.

Colour development may depend on individual phenolic compounds contained in the mixtures, particularly the concentrations of chlorogenic acid and catechins (Amiot, Fleuriet, Cheynier, & Nicolas, 1997; Lee, 1992). Other coloration mechanisms might also be possible. For example, the resulting colour change in the milk controls could have resulted from the formation of brown Maillard products arising from added ascorbic acid (Champagne, Hinojosa, & Clemetson, 1999). Statistical analysis ($p < 0.05$) supported the association between a lag in the development of redness and the presence of ascorbic acid.

4.5. Antioxidant capacity

The results in Fig. 4 show the antioxidant capacity of the polyphenol–milks and milk controls, determined using FRAP and ORAC assays. No significant difference in antioxidant capacity was detected during processing, including mixing, homogenisation and thermal treatment steps (data not shown). In general, FRAP and ORAC capacity represented the total antioxidant capacity of all the antioxidative compounds present in the polyphenol–milks and milk controls, including those both from milk and AP extracts. Addition of ascorbic acid resulted in a marked elevated FRAP capacity (Fig. 4A) and little change in ORAC capacity (Fig. 4B) of the zero-week polyphenol–milks.

Using the FRAP assays, milk controls without ascorbic acid have demonstrated the least antioxidant capacity (Fig. 4A1). FRAP capacity of polyphenol–milks decreased with the length of time in oxidative storage, either at 20 °C or 38 °C. The rate of decrease was greater in the presence of ascorbic acid (Fig. 4A2) than in the absence of ascorbic acid (Fig. 4A1). In the absence of ascorbic acid, only 81% and 62% in FRAP antioxidant capacity of polyphenol–milks were detected after 12-week storage at 20 °C and 38 °C, respectively (Fig. 4A1). In contrast, in the presence of ascorbic acid, considerable losses (42% and 57%) in FRAP antioxidant capacity of polyphenol–milks were detected after 12-week storage at 20 °C and 38 °C, respectively (Fig. 4A2).

Using the ORAC assays, approximately one third of the ORAC value of the zero-week samples was derived from the milk background (Fig. 4B1 and B2). ORAC capacity of the polyphenol–milks decreased with time during oxidative storage at 20 °C and 38 °C. In the absence of ascorbic acid, 80% and 49% ORAC capacity of polyphenol–milks were detected after 12-week storage at 20 °C and 38 °C, respectively (Fig. 4B1). In the presence of ascorbic acid, considerable losses (12% and 43%) in ORAC antioxidant capacity of polyphenol–milks were detected after 12-week storage at 20 °C and 38 °C, respectively (Fig. 4B2).

The ORAC or FRAP capacity value reflects the sum value of antioxidant capacity of all the antioxidative compounds present in a sample. Differences in the behaviour of the polyphenol–milks in FRAP and ORAC assays may be associated with the different mechanisms underlying these two assays. Different types of oxidative intermediates might be produced in the polyphenol–milks during storage, and these intermediates might also be responsible for the obtained antioxidant capacity values. The ORAC assay was conducted in phosphate buffer (with a pH that is close to those of the polyphenol–milks), measuring the ability of antioxidants to donate hydrogen to quench radicals (Schaich, 2006). This assay is sensitive to protein (Perez-Jimenez & Saura-Calixto, 2006). The FRAP assay was conducted under acidic conditions, measuring the ability of the antioxidant to transfer electrons to reduce initiators of oxidation or oxidised metals (Schaich, 2006). This assay is sensitive to compounds that chelate ferric ions (Schaich, 2006) or those compounds with low molecular weight that are present in milk, e.g. urate (Chen et al., 2003).

In this study, there was no obvious correlation between colour change and antioxidant capacity for the polyphenol–milks. Ascorbic acid protects against coloration of samples and its oxidation was independent of polyphenol addition. However, a gradual reduction in antioxidant capacity of the polyphenol–milks was accompanied with an increased redness during the oxidative storage, and greater change in antioxidant capacity was observed after 2-weeks storage at 38 °C than that at 20 °C. This result agrees with Nicoli, Calligaris, and Manzocco (2000), that formation of brown colour in a catechin solution due to

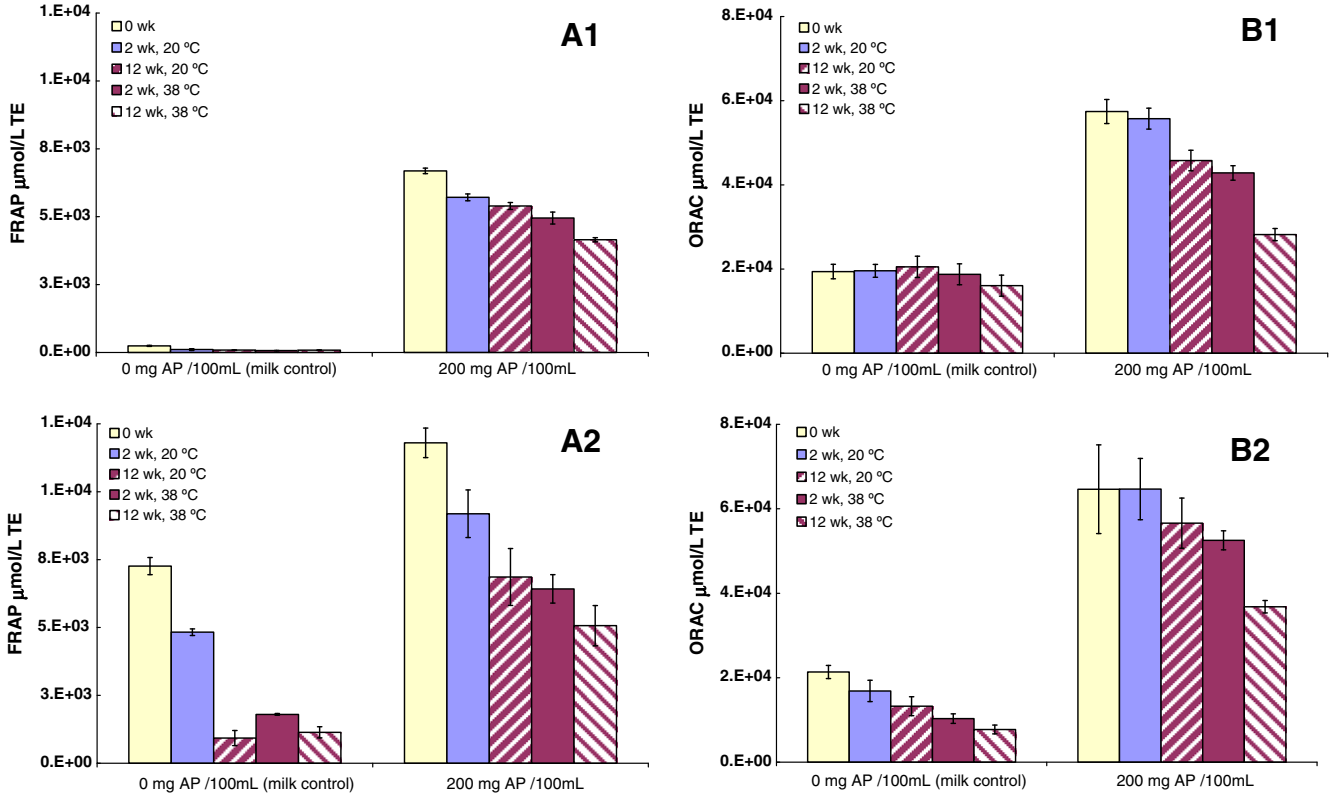

Fig. 4. Effect of storage on FRAP (A1 and A2) and ORAC (B1 and B2) capacity of polyphenol–milks (containing 200 mg AP per 100 ml) or milk controls (0 mg AP) without (A1 and B1) or with (A2 and B2) ascorbic acid. AP refers to apple polyphenol. Storage weeks (wks): 0, 2 and 12; storage temperature: either 20 or 38 °C.

oxidation is associated with a loss of chain-breaking antioxidant ability.

There may be more than one type of mechanism underlying the observed phenomena in this study, functioning individually or simultaneously via physical and chemical effects. Structurally, a range of chemical reactions are possible, each of which can not easily be characterised.

The physical effects are probably associated with the level of molecular complexity, e.g. steric hindrance. Steric hindrance could arise from the high polymerisation of polyphenols with more than four monomer residues (Lu & Yeap Foo, 2000). These physical effects would have affected the ability of antioxidant active sites to approach Fe^{3+}-TPTZ complexes in FRAP assays or the ability of antioxidants to donate hydrogen to quench radicals in ORAC assays (Schaich, 2006).

Possible chemical effects include hydrogen bonding, group-specific chemical reactions and hydrophobic complexation. Hydrogen bonding might involve polyphenols or ascorbic acid and their intermediates, proteins, polysaccharides, among their undissociated carboxyl groups, hydroxyl groups and unshared electron pairs of carbonyl oxygen atoms in the ketone structure (serving as proton acceptors) in the aqueous system. The acidic and phenolic compounds (hydrogen-bonding donors) might contribute to the strength of hydrogen bonding, as a function of the acid strength (pK_a), and the number of carboxyl and hydroxyl groups in a molecule (Nose, 2004). Group-specific reactions might have occurred among specific groups of compounds, e.g. the formation of ester bonds between carboxyl and hydroxyl groups. Hydrophobic complexation might occur between antioxidants and biopolymers, such as proteins and polysaccharides. Hydrophobic regions may have developed and entrapped polyphenols, when the internal hydrogen bonds were formed among hydroxyl groups on the surface of cross-linked water-soluble biopolymers and reduced the number of hydroxyl groups available to interact with water (Ozawa, Lilley, & Haslam, 1987; Sun & O'Connor, 2001). Although these individual hydrogen bonds and hydrophobic interactions are weak, their cumulative effect in very large molecules could theoretically result in quite strong bonding. These interactions could result in positive or negative effects on total antioxidant activity of the mixtures.

5. Conclusion

The results described in this study suggests that polyphenol–milk models are good beverage formats to deliver fruit polyphenols, in which both physical stability and antioxidant capacity can be retained during beverage production and thermal processing. Ascorbic acid provides protection related to antioxidant capacity, as measured by ORAC assay in the polyphenol–milk model beverage.

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