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Efficacy of Natureseal® AS1 browning inhibitor in fresh-cut fruit salads applications, with emphasis on apple wedges

By C. RÖßLE[1*], T. R. GORMLEY[1] and F. BUTLER[2]
[1]Ashtown Food Research Centre (Teagasc), Ashtown, Dublin 15, Ireland
[2]Biosystems Engineering, School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Dublin 4, Ireland
(e-mail: christian.roessle@teagasc.ie) *(Accepted 31 August 2009)*

SUMMARY
Prevention of browning in fresh-cut fruit is of major commercial significance as fresh-cut fruit salads are major players in the international marketplace. Natureseal® AS1 and AS5 browning inhibitors out-performed ascorbic acid and citric acid in tests with fresh-cut wedges of 'Bramley' apple. A dipping regime of 2 min in 6% (w/v) AS1 solution was found to be optimal, as longer dip-times and higher concentrations led to significant residues of AS1 on the fresh-cut 'Bramley' wedges. Tests on fresh-cut wedges from ten apple cultivars indicated that AS1 was significantly beneficial ($P < 0.001$) to maintain wedge colour during chill-storage in all cultivars, with the exception of 'Shampion', which had a low browning tendency and performed equally well with or without AS1. Tests on the anti-oxidant status of a generic fruit salad, which was not treated with AS1 and stored for 15 d, indicated a significant reduction in anti-oxidant status, due largely to the depletion of vitamin C (from 137 to 97 mg 100 g^{-1} DW). Total phenolics contents remained relatively constant over the 15-d storage period. The outcomes from these trials have been presented to commercial food companies with a view to their uptake.

The market for fresh-cut fruit salads is expanding rapidly as a consequence of increasing consumer demand for healthy eating and convenience (Buckley *et al*., 2007; Gorny, 2003). Slowing or preventing the enzymatic browning of sliced fruit is a continuing problem for the processors of fresh-cut fruit. Browning is due to many factors, including: cell disruption and the release of polyphenol oxidases (PPOs; Shapton and Shapton, 1998; Garcia and Barret, 2002; Martìn-Belloso *et al*., 2006); the stage of ripeness; the activity of oxidative enzymes; oxygen availability; and/or the compartmentalisation of enzymes and substrates (Nicoli *et al*., 1994; Rocha *et al*., 1998). There is an extensive literature on inhibitors of browning in fresh-cut fruits, with trials using ascorbic acid (Sapers *et al*., 1989; Gorny *et al*., 2002), citric acid (Jiang *et al*., 2004), or their derivatives (Moline *et al*., 1999), or other compounds (Pilizota and Sapers, 2004; Tortoe *et al*., 2007; Lu *et al*., 2007, Cortez-Vega *et al*., 2008; Pristijono *et al*., 2008). Several studies have shown that Natureseal® products can reduce browning in fresh-cut fruit slices (Abbott *et al*., 2004; Bhagwat *et al*. 2004; Rupasinghe *et al*., 2005; Toivonen, 2008).

The aim of the current study [four Trials (1 – 4)], conducted as part of the ISAFRUIT Integrated Project, was to evaluate the efficacy of two specific Natureseal® products (AS1 and AS5) in maintaining colour in wedges of 'Bramley' apple, as they have a strong browning tendency and are therefore a good candidate for such browning inhibitor trials (Trial 1 and Trial 2). Natureseal® AS1 was also tested in other cultivars over two growing seasons (2007 and 2008), with a view to recommending its use in fresh-cut fruit applications (Trial 3). Natureseal® AS1 is already used commercially in Europe and elsewhere, thus adding practical value to the current trials. The anti-oxidant status of a 'generic' fruit salad stored at 2° – 4°C for 15 d was also evaluated, as any significant loss in its inherent anti-oxidant status would be of concern to consumers, if in fact they were aware of it (Trial 4). AS1 was not used in Trial 4 as it has a high anti-oxidant capacity which would dwarf the anti-oxidant status of the fruit components in a fruit salad.

*Author for correspondence.

MATERIALS AND METHODS

Trial 1: Evaluation of browning inhibitors

Tests were conducted on two Natureseal® browning inhibitors (AS1 and AS5), in comparison with ascorbic acid and citric acid, using 'Bramley' apple wedges.

Sample preparation and dipping: 'Bramley' apples were washed in water, cored using a 20-mm diameter stainless steel cork borer, and cut with a stainless-steel knife into wedges (each *ca*. 10 g). Three skin-on wedges from each of five apples (chosen at random) were used for each treatment. Wedges were dipped for 2 min in 6% (w/v) solutions [at a 3:1(v/w) solution:wedge ratio] of the browning inhibitors Natureseal® AS1, Natureseal® AS5 (AgriCoat Ltd., Great Shelford, UK), ascorbic acid (AA), or citric acid (CA). All wedges were then drained for 2 min, packed in clear trays (15 cm × 10.5 cm × 3 cm; Versatile Packaging, Silverstream, Ireland), covered (heat-sealed) with a breathing film (O_2 transmission < 2 ml 24 h^{-1} at 23°C; water vapour transmission < 6 g 24 h^{-1} at 38°C) and stored at 2° – 4°C for 14 d. 'Bramley' wedges dipped in water were used as controls.

Test procedures and statistical design: All apple wedges were tested for their colour surface and texture after 0, 3, 7, or 14 d of storage at 2° – 4°C. The colour of each apple wedge surface was measured using a HunterLab D25A DP-9000 colour meter (HunterLab, Reston, VA, USA). The colour of five wedges per replicate was measured and expressed as a three dimensional *L*, *a*, and *b* colour solid. The texture (shear force) of the apple wedges was measured using a T-2000 Texture System (Food Technology Corporation, Sterling, VA, USA) which was calibrated before use every time. The Kramer standard test cell (Model CS-1) was loaded with 100 g of apple wedges without cores. The results were expressed as kN 100 g^{-1} FW of sample.

The statistical design was five dip treatments (control, AS1, AS5, AA, or CA), four sampling dates from storage (0, 3, 7 or 14 d) with three replicates, with 59 degrees of freedom (df), followed by ANOVA (Genstat 5 Version 3.2; Lawes Agricultural Trust, Rothamsted, Harpenden, UK).

Trial 2: Effect of dip concentration and duration on the efficacy of Natureseal® AS1 browning inhibitor in 'Bramley' apple wedges and on AS1 residues in the product

This Trial involved dipping 'Bramley' apple wedges in different concentrations of Natureseal® AS1 for various times, as this browning inhibitor had performed best in Trial 1.

Sample preparation and dipping: Preparation of the 'Bramley' apple wedges was done as in Trial 1. Natureseal® AS1 was applied as a dip to skin-on wedges at three concentrations [1.5, 3.0, or 6.0% (w/v)], each for three dipping times (2, 60, or 120 min). All wedges were then packed, as described for Trial 1, stored at 2° – 4°C and tested after 7 d. The recommended treatment for commercial use by the manufacturers of apple wedges is to dip in 6% (w/v) Natureseal® AS1 solution for 2 min. However, certain applications, such as the osmotic treatment of fruit wedges, may require longer soak times; hence, the dips for 60 min and 120 min in this Trial. The results of apple wedges treated with AS1 were compared with the corresponding values for untreated fresh-cut samples.

Test procedures and statistical design: The colour and texture of the apple wedges after dipping were measured as described for Trial 1. The gain in fresh weight (FW) of 15 wedges, post-dipping, was also recorded.

Soluble solids contents (SSC) were measured using an Abbe refractometer (2WAJ; Guru Nanak Instruments, New Delhi, India) and approx. 5 g of homogenous and filtered apple pulp.

Titratable acidity (TA), expressed as meq 100 g^{-1}, was determined by titration with 0.1 M NaOH solution to the end point of phenolphthalein.

pH was measured on homogenous apple pulp using an Orion pH meter (420A; Thermo Fisher Scientific Inc., Waltham, MA, USA) which was calibrated prior each measurement with phosphate buffers at pH 4.005 and 7.000

Dry matter (DM) contents were measured using 10 g of apple pulp. Samples were dried in a vacuum oven (OVA031; Gallenkamp, Loughborough, UK) to constant weight at 70°C and 58 kPa overnight (approx. 18 h). The moisture content was determined by weight difference, and DM was expressed as a percentage of the initial sample weight (AOAC, 1990).

The calcium contents were analysed by atomic absorption spectrometry in previously dry-ashed samples, as detailed in Analytical Methods for Atomic Absorption Spectrometry (Anon, 1994). Analyses were performed using an Atomic Absorption Spectrometer (Model 3110; Perkin Elmer, Norwalk, CT, USA) under standard conditions for calcium, as described by the instrument manufacturer (Anon, 1994). Natureseal® AS1 was also measured, and the latter facilitated calculation of the AS1 content of the treated wedges.

The anti-oxidant status of the Natureseal® AS1 powder was measured by the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, as described by Wijngaard *et al.* (2009). The results were expressed as IC_{50} (in mg ml^{-1}; i.e., the extract concentration required to cause a 50% inhibition).

The statistical design was three dip concentrations [1.5, 3.0, or 6.0% (w/v) AS1], by three dipping times (2, 60, or 120 min), with three replicates (26 df). ANOVA was done as in Trial 1.

Trial 3: Browning tendency in wedges from ten apple cultivars with or without Natureseal® AS1 treatment

The browning tendencies of wedges from ten apple cultivars, from two growing seasons (2007 and 2008), with or without Natureseal® AS1 treatment, were measured to investigate its potential for fresh-cut fruit salad application. The cultivars used were: 'Shampion', 'Jonica', 'Gloster', 'Topaz', 'Ariwa', 'Rajka', 'Idared', 'Cortland', and 'Alwa' (all sourced from an ISAFRUIT Project partner in Poland), and 'Braeburn' (purchased in Ireland).

Sample preparation, dipping, and testing: Preparation of apple wedges from all ten cultivars was done as described in Trial 1. Natureseal® AS1 was applied for 2 min as a 6% (w/v) dip solution to one set of samples. The second set was dipped in water as controls. The wedges were then packed (as in Trial 1) and tested for colour and texture (as for Trial 1) after 5 d storage at 2° – 4°C. Colour difference (ΔE) values between day-0 and day-5 were calculated from the Hunter *L*, *a*, *b* values as .

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

The statistical design was ten cultivars by two dips (water or Natureseal® AS1) by two years harvest (2007 or 2008) with two replicates (79 df). ANOVA was conducted as in Trial 1.

Trial 4: Anti-oxidant status of a generic fruit salad during storage at 2° – 4°C for 15 d

Most chilled, pre-prepared fresh fruit salads have a shelf-life of > 10 d and are regarded as 'fresh' by consumers, even though they may be several days old. While their fresh appearance is retained, hidden quality attributes (e.g., anti-oxidant levels, cannot be seen). The anti-oxidant status of a 'generic' fruit salad stored at 2° – 4°C for 15 d was therefore measured. Natureseal® AS1 was not used in this Trial as its anti-oxidant capacity outweighed that of the fruit used in the fruit salad, so AS1 would mask the results.

Preparation, packing, and testing of the fruit salad: A mini-survey was conducted on the makeup of fresh fruit salads in supermarkets in Dublin. Based on this, a model (typical) fruit mix consisting of orange segments (40), diced pineapple (20), 'Braeburn' (17.5) and 'Granny Smith' (17.5) apple slices, and red grapes [5% (w/w)] was used for the Trial. Samples (200 g) were packed in clear trays as described in Trial 1 and samples were stored at 2° – 4°C. Fruit extracts for the determination of the anti-oxidant status of the various fruits were prepared and measured by the DPPH assay on days-0, -5, -10, and -15 as described by Wijngaard *et al*. (2009). Total phenolics contents (TPC) were measured by the Folin-Ciocalteau reagent (FCR) procedure and expressed as gallic acid equivalents (GAE) 100 g^{-1} FW (Singelton *et al*., 1999) with a correction for vitamin C content (2, 6-dichlorophenol-indophenol procedure; AOAC Official Methods, 1995).

The statistical design was four test dates by four replicates (15 df). ANOVA was conducted as described in Trial 1.

RESULTS AND DISCUSSION

Trial 1: Evaluation of browning inhibitors

Natureseal® AS1 performed best ($P < 0.001$) of the four inhibitors tested, as indicated by the Hunter *L* values for the apple wedges (Figure 1). *L* values increased up to day-7, showing a whitening effect. Natureseal® AS5 (67.9) performed similarly to AS1 (69.5), and both gave higher Hunter *L* values on day-14 than AA (54.68), CA (55.2), or the water-dipped controls (54.5). Hunter *L*/*b* (white:yellow) showed a similar pattern, with day-14 *L*/*b* ratios of 4.33 (AS1), 4.12 (AS5), 2.86 (CA), 2.81 (AA), and 2.50 (water-dipped control). Hunter *a* values for the water-dipped controls showed a sharp increase from –2.74 to 4.33 over the 14 d storage period, as did the AA-dipped (–2.62 to 3.59) and CA dipped (–2.99 to 2.92) values, indicating increased redness of the fruit wedges. Hunter *a* values for the Natureseal®-treated products remained negative throughout: AS1 (from –2.55 to –3.14) and AS5 (from –2.65 to –3.03), indicating no development of redness. The anti-browning performances of AS1 and AS5 agreed with findings on other Natureseal® inhibitors (Rupasinghe *et al*., 2005; Toivonen, 2008). The inferior performance of AA and CA, relative to AS1 and AS5, was expected, as AA is often most effective in combination with other compounds (Rocha *et al*., 1998; Cortez-Vega *et al*., 2008; Zheng *et al*., 2008). Citric acid has also been studied extensively as an inhibitor of browning (Pizzocaro *et al*., 1993; Rojas-Graü *et al*., 2008), but it performed less well than AS1 and AS5 in our Trial. Relatively new anti-browning agents for apple such as furanoflavone (Zheng *et al*., 2008), 4-hexylresorcinol, N-acetylcysteine, and glutathione (Rojas-Graü *et al*., 2008) may have potential, but it is not clear whether these are being applied commercially or are still under test at the laboratory level.

Shear values for apple wedges treated with AS1 or AS5 increased over the 14-d storage period. AS1 had a greater firming effect ($P < 0.001$) than AS5 (Figure 2). This was due to cross-linking of both cell wall and middle-lamella pectin by calcium ions (Rico *et al*., 2007). AS1 had a calcium content of 90 mg 100 g^{-1}. The firming effects of AS1 and AS5 are desirable as they maintain the crispness of the wedges. Wedges treated with AA or CA, and those dipped in water, lost their firmness during 14 d of storage (Figure 2; $P < 0.001$).

Trial 2: Effect of dip concentration and duration on the efficacy of Natureseal® AS1 browning inhibitor in 'Bramley' apple wedges, and on AS1 residues in the product

Increasing concentrations of AS1 in the dip solution resulted in higher Hunter *L*/*b* ratios in 'Bramley' wedges dipped for 2 or 60 min. However, the different concentrations had an equal effect in the 120 min dips (Figure 3). The AS1 content of these wedges was high, especially after a 120 min dip at a high dip concentration (Figure 4). This suggests that the concentration [6% (w/v)] and dip time (2 min) recommended by the

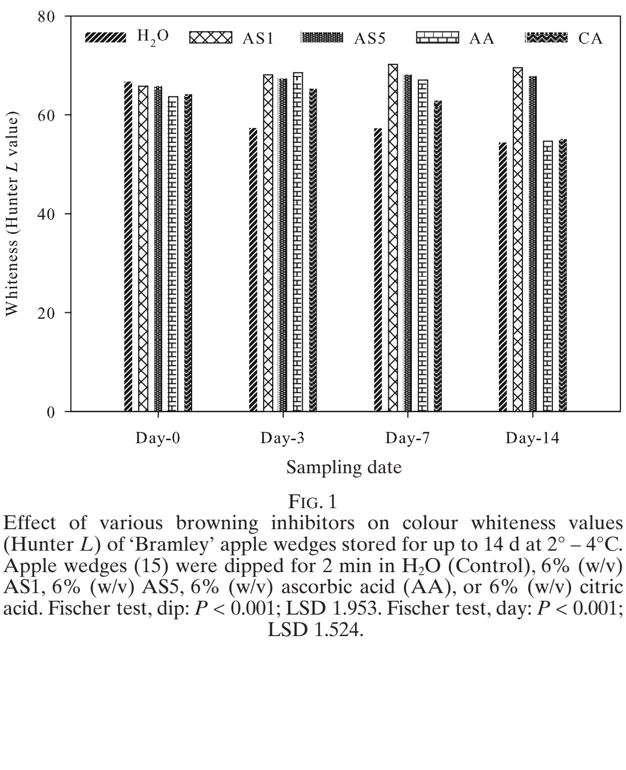

FIG. 1
Effect of various browning inhibitors on colour whiteness values (Hunter *L*) of 'Bramley' apple wedges stored for up to 14 d at 2° – 4°C. Apple wedges (15) were dipped for 2 min in H_2O (Control), 6% (w/v) AS1, 6% (w/v) AS5, 6% (w/v) ascorbic acid (AA), or 6% (w/v) citric acid. Fischer test, dip: $P < 0.001$; LSD 1.953. Fischer test, day: $P < 0.001$; LSD 1.524.

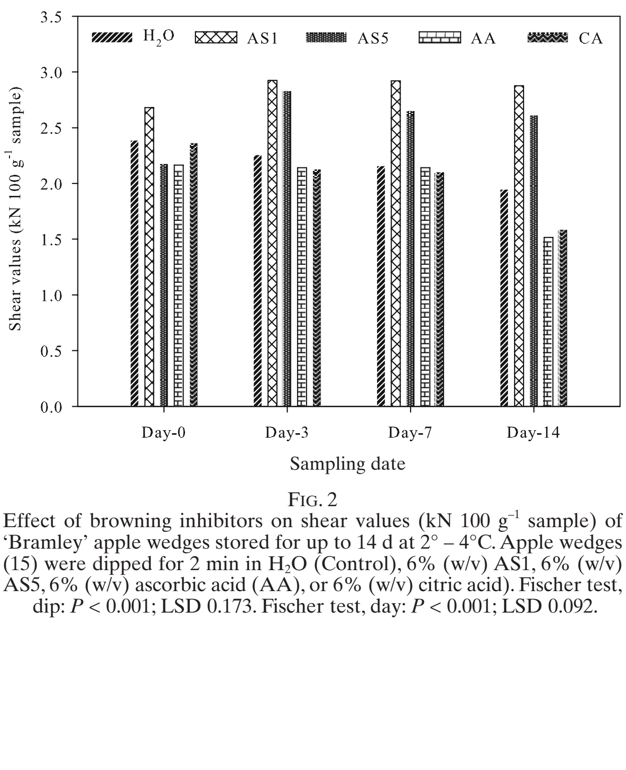

FIG. 2
Effect of browning inhibitors on shear values (kN 100 g^{-1} sample) of 'Bramley' apple wedges stored for up to 14 d at 2° – 4°C. Apple wedges (15) were dipped for 2 min in H_2O (Control), 6% (w/v) AS1, 6% (w/v) AS5, 6% (w/v) ascorbic acid (AA), or 6% (w/v) citric acid). Fischer test, dip: $P < 0.001$; LSD 0.173. Fischer test, day: $P < 0.001$; LSD 0.092.

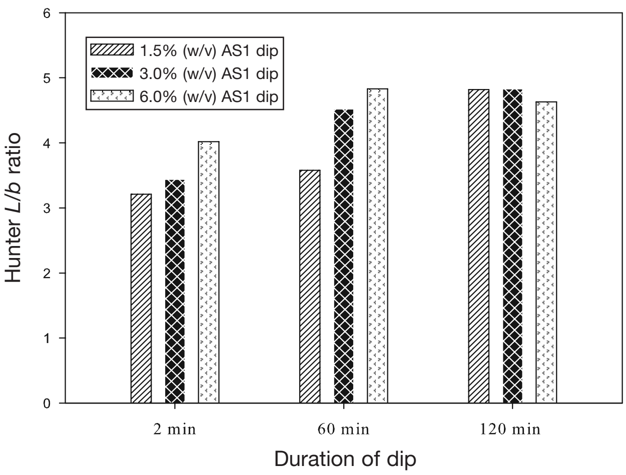

FIG. 3
Effect of various concentrations [1.5%, 3.0% or 6.0% (w/v)] of AS1 and dipping times (2, 60, or 120 min) on Hunter *L*/*b* ratios of 15 'Bramley' apple wedges (tested on day-7 of storage at 2° – 4°C). Fischer test, concentration: $P < 0.05$; LSD 0.484. Fischer test, time; $P < 0.001$; LSD 0.484. Fischer test, interaction: NS; LSD 0.837.

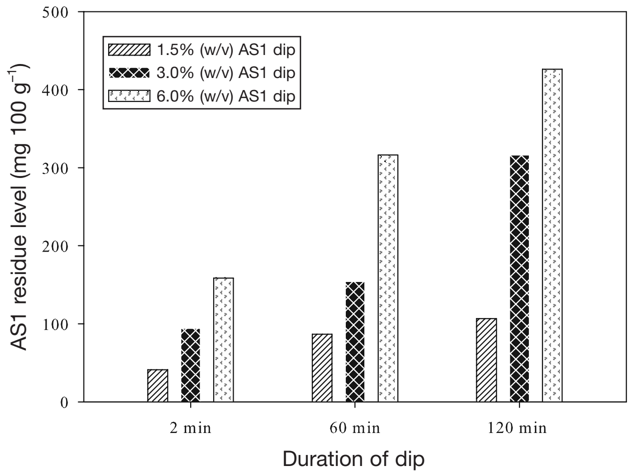

FIG. 4
Effect of various concentrations [1.5%, 3.0% or 6.0% (w/v)] of AS1 and dipping times (2, 60, or 120 min) on AS1 residue levels (mg 100 g^{-1}) in 15 'Bramley' apple wedges tested on day-7 of storage at 2° – 4ºC.

manufacturer should be adhered to. The levels of AS1 uptake (Figure 4) were calculated from FW gains for wedges during dipping and from their calcium contents based on the calcium content of AS1 being 90 mg 100 g^{-1} with a subtraction for the inherent calcium content of untreated wedges. The bar charts for the dry ash and calcium contents of variously-treated samples showed a similar pattern to Figure 4. Both dip concentration ($P < 0.001$) and time ($P < 0.05$) increased the ash and calcium contents of the wedges. The AS1 concentration recommended by the manufacturer, and used in this trial, was higher than that [0.7% (w/v)] used by Toivonen (2007), but similar to that [6% (w/v) calcium ascorbate (Natureseal®)] used by Rupasinghe *et al*. (2005) for fresh-cut apple slices.

Shear values (mean = 2.61 kN 100 g^{-1} sample) of the wedges were not influenced by AS1 concentration, or by dipping time ($P > 0.05$). This may be due to the short time interval (24 h) between AS1-treatment and testing. In contrast, in Trial 1 where flesh firming was observed, these tests were conducted on day-3, -7 and -14 of storage. The SSC values for apple wedges were unaffected ($P > 0.05$) by AS1 concentration or dipping time (mean = 9.95%), as were pH values (mean = 3.13) and TA (mean = 18.7 meq 100 g^{-1}). DM contents increased with higher concentrations of AS1 and longer dip times but the effects were not significant ($P > 0.05$).

Trial 3: Browning tendency in wedges from ten apple cultivars with or without Natureseal® AS1 treatment

There was a wide variation in the colour difference (ΔE) values recorded over the 5-d storage period at 2° – 4°C for apple wedges between cultivars ($P < 0.001$), and also between dipping in AS1 solution *vs*. water ($P < 0.001$; Table I). However, there was no difference ($P > 0.05$) between 2007 and 2008. Therefore the data for both years were combined in Table I. In the absence of AS1, 'Shampion' wedges had the lowest browning tendency, as indicated by the lowest ΔE values (Table I). This is characteristic for this cultivar (Podsedek *et al*., 2000). 'Alwa' had the highest browning tendency. Treating wedges with 6% (w/v) AS1 for 2 min had a major effect on ΔE values, except for 'Shampion', which performed equally well with or without AS1. ΔE values for 'Jonica', 'Gloster', 'Ariwa', 'Rajka', 'Cortland', and 'Braeburn' showed a greater than two-fold decrease in browning after AS1 treatment. This effect was particularly strong for 'Alwa' (a greater than three-fold decrease) and

TABLE I
Colour differences (ΔE) and shear values (S; in kN 100 g^{-1}sample) for wedges from ten apple cultivars from the 2007 and 2008 growing seasons, pre-dipped in water (–AS1) or in 6% (w/v) Natureseal® AS1 solution (+AS1) for 2 min

Apple cultivar	2007 + 2008		2007		2008	
	ΔE (-AS1)	ΔE (+AS1)	S (–AS1)	S (+AS1)	S(–AS1)	S (+AS1)
'Shampion'	2.79	2.86	1.17	2.04	0.89	2.03
'Jonica'	7.42	3.43	1.29	2.17	1.35	2.40
'Gloster'	7.67	3.60	1.20	2.12	1.59	2.46
'Topaz'	7.74	5.57	1.52	2.20	1.91	2.52
'Ariwa'	8.41	4.02	1.87	2.76	2.39	2.85
'Rajka'	8.46	3.30	1.21	2.26	1.11	1.89
'Braeburn'	10.62	4.51	1.80	2.39	1.96	2.73
'Idared'	11.74	2.85	2.06	2.83	2.36	2.95
'Cortland'	12.39	5.18	1.34	1.91	1.08	1.87
'Alwa'	14.67	4.68	1.64	2.44	1.35	2.19
Fischer - test	Cultivar	$P < 0.001$	Cultivar	$P < 0.001$	Cultivar	$P < 0.001$
LSD	Cultivar	2.579	Cultivar	0.203	Cultivar	0.277
Fischer - test	Dip	$P < 0.001$	Dip	$P < 0.001$	Dip	$P < 0.001$
LSD	Dip	1.153	Dip	0.091	Dip	0.124

TABLE II
Anti-oxidant status, total phenolics contents, and vitamin C contents of a' typical' fruit salad stored at 2° – 4°C for 15 d (–AS1)

Test day	IC_{50} (mg ml^{-1})	Total phenolics content (mg GAE1 100 g^{-1} DW)	Vitamin C content (mg 100 g^{-1} DW)
0	1.44	162	137
5	1.66	148	130
10	2.09	150	110
15	2.39	177	97
Fischer - test	$P < 0.001$	NS	$P < 0.001$
LSD	0.063	23.2	4.37
Individual fruit			
Orange	0.63	Trace	360
Pineapple	1.17	120	229
'Braeburn' apple	1.09	584	33
'Granny Smith' apple	0.94	575	37
Red grape	2.39	315	16

'Idared' (a greater than four-fold decrease). On the basis of these findings, all ten cultivars were considered suitable for fresh-cut fruit salad applications after dipping in 6% (w/v) AS1 for 2 min, on the basis of their colour retention during storage for 5 d at 2° – 4°C.

Shear values for wedges cut from the ten apple cultivars were different ($P < 0.001$) for 2007 and 2008 (means = 1.91 and 1.99 kN 100 g^{-1} sample, respectively). Both data sets are presented in Table I. Natureseal® AS1 increased shear values for all cultivars. Shear values depend on a number of factors including the inherent texture of a cultivar (i.e., hard, crisp, or soft; King *et al*., 2001), stage of maturity (Zerbini *et al*., 1999; Rojas-Graü *et al*., 2007), duration of storage (Johnston *et al*., 2001; Konopacka and Plocharski, 2004), and post-harvest treatment (e.g., cutting/slicing, AS1 treatment). Wedges from 'Idared', 'Ariwa', and 'Braeburn' (in 2007) were the firmest in the absence of AS1, while 'Rajka', 'Gloster' and 'Shampion' were the softest (Table I). This order was largely maintained in 2008 and, after treatment with AS1, in both 2007 and 2008. On the basis of shear values, 'Idared', 'Ariwa', and 'Braeburn' were most suited for fresh-cut salad applications because of their firm and crisp texture. 'Shampion' had the lowest browning tendency, but was classed as too soft.

Trial 4: Anti-oxidant status of a generic fruit salad during storage at 2° – 4°C for 15 d

Tests on the anti-oxidant capacity of AS1 itself indicated an IC_{50} value of 0.0065 mg ml^{-1}. This far outweighed the endogenous IC_{50} values of the fruit present in the fresh-cut fruits salad, therefore AS1 was not used in this Trial. There was a significant decrease ($P < 0.001$) in anti-oxidant status over 15 d of storage (Table II), but only a small difference ($P > 0.05$) between TPC, despite a wide range between the highest and lowest values. The orange segments, followed by the 'Granny Smith' wedges, had the highest IC_{50} values, while red grapes had the lowest IC_{50} values of the individual fruit (Table II). Orange segments had, by far, the highest vitamin C content, but had only a 'trace' reading for TPC. Moussaid *et al*. (2004) suggested a low TPC in orange tissue, with most studies focussing on the TPC of the skin. The vitamin C value obtained for pineapple was higher than previous literature values (i.e., 229 *vs*. 90 mg GAE 100 g^{-1} DW).

CONCLUSIONS

Natureseal® AS1 and AS5 browning inhibitors out-performed AA and CA in tests on 'Bramley' apple wedges. A 6% (w/v) AS1 solution with a dip time of 2 min was optimal. Higher concentrations and longer dip times resulted in significant levels of AS1 residues in the wedges. Natureseal® AS1 was highly effective in maintaining the fresh-cut colour of apple wedges from ten cultivars, and also increased wedge firmness during storage for 5 d at 2° – 4°C. The anti-oxidant status of a generic fruit salad (not treated with AS1) stored for 15 d at 2° – 4°C, decreased significantly, largely due to the depletion of vitamin C. TPC remained relatively constant over the 15-d storage period. The outcomes from these trials have been presented to food companies with a view to their adoption.

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