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The Antioxidant Properties of Whey Permeate Treated Fresh-Cut Tomatoes

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Full Title

The antioxidant properties of whey permeate treated fresh-cut tomatoes

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Abstract:

The aim of this research was to analyse the effects of three types of cheese whey permeate treatment on the antioxidant properties of fresh-cut tomatoes. Tomatoes were treated with whey permeate concentrate (PC), delactosed permeate (DP) and delactosed concentrate (DC), stored at 4°C for 10 days and compared to the samples treated with industrial standard, chlorine (120 ppm). Samples were analysed for ascorbic acid, lycopene, total phenols, mineral and trace elements and antioxidant activity (DPPH and FRAP). The samples treated with DP retained significantly ($p<0.05$) higher antioxidant activity (FRAP) and total phenols (TP) when compared with those treated with PC and DC respectively. DP showed significantly ($p<0.05$) higher results than chlorine for DPPH, FRAP and TP. In DPPH assay, all whey permeate-treated samples showed similar antioxidant activity. No significant differences in levels were found among whey permeates and chlorine treatments for naturally present antioxidants such as ascorbic acid and lycopene. The statistical analysis showed a positive significant ($R^2=0.9173$, $p<0.05$) correlation between TP and the antioxidant activity. A good correlation was observed between DPPH and FRAP assay ($R^2=0.8005$, $p<0.05$). Among the three whey permeates, delactosed permeate showed the best results in maintaining the antioxidant properties of tomato. These results suggest that whey permeate could be used to enhance the antioxidant activity of the fresh-cut tomato while retaining the antioxidant components during storage.

Key words: Whey permeate, Ascorbic acid, Lycopene, Antioxidant activity, Total phenols, Minerals.

Introduction

The consumption of a diet rich in fresh fruits and vegetables has been associated with a number of health benefits including the prevention of chronic diseases (WHO, 2003). This beneficial effect is believed to be due, at least partially, to the action of antioxidant compounds, which reduce oxidative damage in the body (Lana & Tijskens, 2006). While the prescription of supplements containing antioxidants has resulted in contradictory results upon human health, the results from epidemiological studies comparing populations with different diets show a clear trend in reduction of chronic diseases when there is an increase in the consumption of fruits and vegetables (Meléndez-Martínez, Fraser, & Bramley, 2010). Campaigns (i.e. 5 a day) to increase the consumption of these products have been launched in many countries.

Tomato is a versatile vegetable that is consumed fresh as well as in the form of processed products. Tomatoes and tomato products are rich in health-related food components as they are good sources of carotenoids (in particular, lycopene), ascorbic acid (vitamin C), vitamin E, folate and flavonoids. In addition, tomato contains essential amino acids, and particularly high amounts of minerals (Fe, Mn, Zn, and Cu) and monounsaturated fatty acids (especially, oleic acid). Regular consumption of tomatoes has been correlated with a reduced risk of various types of cancer and heart diseases (Lavelli, Peri & Rizzolo, 2000). These positive effects are believed to be attributable to the antioxidants, particularly carotenoids, flavonoids, lycopene and β-carotene (Odriozola-Serrano, Soliva-Fortuny & Martin-Belloso, 2008). Furthermore, recommendations to increase daily intake of fruits and vegetables rich in nutrients such as carotenoids and vitamins C and E to lower the markers of risk of cancer and cardiovascular diseases (CVD) have been made (Sgherri, Kadlecova, Pardossi, Navari-Izzo, & Izzo, 2008). Giovannucci (2002) reviewed a number of

68 epidemiological studies and concluded that the intake of tomato products was consistently
69 associated with a lower risk of a variety of cancers and in particular prostate cancer. The
70 fresh-cut industry claims their products are convenient and healthy alternatives to fulfil the
71 dietary needs for fresh food and many fast food companies are diversifying their menu in
72 order to offer a range of ready-to-eat salads to their clients. However, the many changes that
73 happen in fruits and vegetables during harvesting, handling and processing can affect
74 antioxidant status. Fresh cut tissues are primarily exposed to oxidative stress, presumably
75 causing membrane damage and altering the composition and content of antioxidant
76 compounds, resulting in changes in the total antioxidant activity of the tissue. Decrease in
77 the antioxidant activity after processing was reported for fresh-cut spinach (Gil, Aguayo &
78 Kader, 2002). Many researchers have routinely used sodium hypochlorite for surface
79 sanitation and sterilisation of fruits and vegetables. For tomato, a range of 0.105–1.05%
80 sodium hypochlorite (1:50– 1:5 dilutions of commercial bleach) is commonly used with
81 washing or dipping for 1 to 3 min to sterilise the surface of fruit before experimentation
82 (Artes, Conesa, Hernandez & Gil, 1999; Martin-Diana, Rico, Frias, Mulcahy, Henehan &
83 Barry-Ryan, 2006). Commercially, generally only 100–200 ppm chlorine is used for
84 sanitation purposes. However, the chlorine derivatives are not considered safe compounds
85 since they react with organic material to form reactive by-products such as chloramines and
86 trihalometanes, which are considered as potential carcinogenic and may provoke
87 detrimental effect in the organoleptic properties of food products (Hua & Reckhow, 2007).
88 There is a need to investigate the efficiency of natural bio-active compounds and alternative
89 techniques.
90 Alternatives or modified methods have been proposed, however, none have yet gained
91 widespread acceptance by the industry. These include organic acids, essential oil

92 treatments, irradiation, heat-shock, ozone treatment, etc. (Ponce, Roura, del Valle & Fritz,
93 2002; Singh, Singh, Bhunia & Stroshine, 2002). Each has its advantages and disadvantages,
94 where the disadvantages are dominating. The maintenance of the quality of fresh produce is
95 still a major challenge for the food industry. In recent years, there is a renewed growing
96 interest in the use of natural products for the preservation of fresh-cut produce. Research
97 and commercial applications have shown that natural components could replace traditional
98 washing agents (Gil et al., 2002; Martin-Diana et al., 2006). The developments of chlorine-
99 free fruit and vegetable products enriched with natural bio-products could contribute greatly
100 to a new and growing market, where the consumers' health-related concerns are met.
101 Natural antioxidants are of main interest nowadays. Synthetic antioxidants are widely used
102 in the food industry but consumers concerns over their safety and toxicity have forced the
103 food industry to find natural sources of antioxidant (Mukhopadhyay, 2007).
104 Whey permeate is a by-product of the production of whey protein concentrates from cheese
105 whey. The main ingredients of whey permeate are water, lactose, peptides and minerals.
106 The high chemical oxygen demand (COD) (50 kg O_2/ton permeate) of whey makes its
107 disposal a significant pollution problem. The cost-effective disposal of whey is a major
108 problem for cheese manufacturers, despite the variety of techniques available. Whey is used
109 as a fermentation feedstock for the production of lactic acid, acetic acid, propionic acid,
110 ethanol, and single cell protein, etc. (NyKänen, Lapvetelainen, Hietnen & Kallio, 1998).
111 However, these applications still do not utilise all the whey produced and new uses for this
112 by-product are continually being sought. Whey and whey ultrafiltration permeate have been
113 proposed to be used as a natural antioxidant in foods (del Mar Contreras, Hernández-
114 Ledesma, Amigo, Martín-Álvarez, & Recio, 2010). Their application into other products
115 would help the cheese industry to partially solve the problem of whey disposal. Whey

116 protein is widely used as a bioactive and nutritional ingredient in health and food products
117 (Marshall, 2004). β-Lactoglobulin (β-Lg) is a small, soluble globular protein with a variety
118 of useful nutritional and functional-food characteristics that have made it an ingredient of
119 choice in the formulation of modern foods and beverages. β-Lg exhibits a growing number
120 of biological effects including anti-hypertensive, anti-cancer, hypocholesterolemic,
121 opiodergic, and anti-microbial activities (Yalcin, 2006). α-Lactalbumin (α-La) is another
122 major whey protein which is one of the few proteins that remains intact upon pasteurisation,
123 and is a calcium binding protein that enhances calcium absorption. It is also a rich source of
124 the amino acids - lysine, leucine, threonine, tryptophan and cysteine (Permyakov &
125 Berliner, 2000). Whey could be a promising natural bio-active alternative to chlorine.
126 Martin-Diana et al., 2006 successfully used whey permeate for decontamination of fresh-cut
127 lettuce and carrots during storage.
128 Therefore this study was carried out to investigate the efficacy of whey permeate for
129 maintaining and also enhancing the antioxidant components and antioxidant activity during
130 storage of fresh-cut tomatoes.

131 **2. Materials and Methods**

132 *2.1. Sampling and treatment design*

133 Irish vine ripened tomatoes (*Lycopersicon esculentum* L. Mill.) cv. Moneymaker were
134 purchased from a local supermarket (Dunnes Stores). According to the grower, the tomato
135 plants were grown commercially in a greenhouse with a 14 h light period from February
136 until November. The aerial environment of the greenhouse and crop irrigation and nutrition
137 were precisely controlled. The temperature of the greenhouse was 16-21°C which is
138 optimum for lycopene synthesis in tomato fruits. The tomatoes were then brought to the
139 food processing lab and stored at 4°C before processing. The experiments were carried out

between March and November. Three independent trials were carried out. Each experiment was conducted with 180 fresh-cut tomato packages (4 measurement days (day 1, day 3, day 7 and day 10) × 5 treatments × 3 replications × 3 Batches).

2.2. Preparation of treatment solution

Three different types of whey permeate (liquid) were kindly supplied by Glanbia Ltd. Ingredients, Ireland. The permeate concentrate (PC) was pre-concentrated by evaporation before the lactose crystallisation process. Delactosed permeate (DP) was obtained after removal of lactose crystals. The delactosed permeate (DP) was then concentrated further by evaporation to give delactosed concentrate (DC).

Five washing treatments were conducted in parallel, using the same batch of product. The samples were washed with water, chlorinated water (120 ppm) and whey permeates (PC, DP and DC) at 3% (v/v) concentration (Martin-Diana et al., 2006). The pH for permeate concentrate solution was 5.59, for delactosed permeate solution 5.08 and for delactosed concentrate solution 4.82. Chlorinated water was prepared by diluting sodium hypochlorite (13% free chlorine, Aldrich Chemical Co., Dublin, Ireland) with distilled water to obtain a ~120 ppm free chlorine solution (pH 8.0). For all treatments the solutions were prepared using distilled water stored at room temperature.

2.3. Processing and experimental set up

Whole tomatoes were rinsed briefly in water prior to washing in order to avoid soil contamination. Washing treatments were performed by immersion of the tomatoes in each treatment solution for 1 min (with agitation). Each treatment was carried out in different baskets (200 g tomatoes/L). After washing, the tomatoes were dried for 5 min using a salad spinner. The tomatoes were then sliced 6 mm in thickness with a commercial slicing

machine (Maxwell chase MCT-25, Baltimore Innovations, UK). Processed tomatoes were then pooled, mixed and ~100 grams placed in a polypropylene tray (180 mm length×130 mm width×25 mm depth) from Sharp Interpack Ltd., UK containing one layer of absorbent paper on the bottom (Fresh-R-Pax absorbent pads, Maxwell Chase Technologies, Atlanta). The principal ingredient in fresh-R-Pax absorbent pads is food grade sodium carboxymethyl cellulose (CMC), a common ingredient in ice-cream, sauces, low-fat foods, etc. The trays were then packaged in bags (200×320 mm) of 35 µm oriented polypropylene film (OPP) with permeability at 23°C and 90% RH of 3.3×10^{-12} mol/s/m^2/Pa for O_2 and 3.1×10^{-9} mol/s/m^2/Pa for CO_2 (Amcor Flexibles Europe-Brighouse, United Kingdom). The packages were then heat-sealed under atmospheric conditions and stored at 4°C for 10 days (Gil et al., 2002; Martin-Diana et al., 2006).

2.4. Nutritional markers of Fresh-cut Tomato

Different nutritional markers such as ascorbic acid, lycopene, total phenol, minerals and trace elements and antioxidant activity (DPPH and FRAP) were monitored throughout the 10 days of storage of fresh-cut tomato packages stored at 4°C.

2.4.1. Ascorbic acid

The ascorbic acid content in fresh-cut tomatoes was analysed by HPLC with a slight modification of the method described by Lee and Castle (2001). A tomato sample (2.5 g) was weighed and 25 ml of 6% metaphosphoric acid (pH 3.0) was added to it. The sample was then homogenised for 1 min at 24,000 rpm using an Ultra-Turrax T-25 Tissue homogeniser. Then the sample was shaken with a Gyrotory Shaker G-2 (USA) for 2 hrs at 150 rpm and centrifuged for 15 min at 3,000 rpm at 4°C) (Sanio MSE Mistral 3000ii, UK). Following centrifugation, 10 ml of the supernatant was filtered through PTFE syringe filters

186 (pore size 0.45 µm, Phenomenex, UK) and stored at -20ºC in foil covered plastic test tubes
187 for further analysis by HPLC.
188 The analysis of ascorbic acid content was performed with Waters 600 Satellite HPLC, with
189 a reversed phase analytical 5 µm particle diameter, polymeric C_{18} column (150 × 4.6mm, 5
190 µm) (Waters, Ireland) with a UV-tuneable absorbance detector (Waters 486) for
191 spectrometric peak. Ten µl of the sample was injected on a reverse phase C_{18} HPLC
192 column. An isocratic mobile phase of 25 mM monobasic potassium phosphate (pH 3.0)
193 with a flow rate of 1.0 ml/min was used. The sample was detected at 230 nm on a Shimadzu
194 SPD-10AV, UV–visible detector. Five concentrations of ascorbic acid standard in 6%
195 metaphosphoric acid in the range 10-50 µg/ml were injected and peak area and height were
196 determined.
197 *2.4.2. Lycopene*
198 Ten grams of tomato samples were weighed and transferred into a 100 mL beaker (wrapped
199 with aluminium foil). A 50-mL volume of hexane-acetone-ethanol solution (2:1:1 v/v/v)
200 containing 2.5% BHT was added to solubilise the lycopene (Shi & Le Maguer, 2000).
201 Following this the samples were homogenised with an Ultra-Turrax T-25 tissue
202 homogeniser for 1 min at 20,500 rpm. The samples were then shaken with a Gyrotory
203 Shaker G-2 (USA) for 2 hrs at 150 rpm followed by 10 mL of distilled water was added and
204 stirred for additional 10 min. The polar and non-polar layers were separated, and the upper
205 hexane layer was collected and filtered through a 0.45 µm PVDF membrane filter. It was
206 transferred to a new 15ml aluminium wrapped test tubes and kept at −80°C for analysis.
207 The analysis of lycopene was performed with Waters 600 Satellite HPLC, with a reversed
208 phase analytical 5 µm particle diameter, polymeric C_{18} column (150×4.6mm, 5 µm)
209 (Waters, Ireland) with a UV tuneable absorbance detector (Waters 486) for spectrometric

peak. The lycopene peaks were identified at 475 nm. An isocratic mobile phase of methyl t-butyl ether/methanol/ethyl acetate (40:50:10, v/v) with a flow rate of 1 ml/min was used. The column temperature and mobile phase was maintained at 25ºC. Analyses were performed under dim light to prevent sample degradation by photo-oxidation. Three concentrations of lycopene standard in the range 0.01–0.03 mg/mL were injected and peak area and peak height were determined. Lycopene content in the samples were identified by comparing peak retention time. The contents of lycopene were expressed as milligrams per 100 g wet weight.

2.4.3. Antioxidant activity test

2.4.3.1. 2, 2-Diphenyl-l-picrylhydrazyl radical scavenging capacity assay (DPPH)

DPPH scavenging activity assay was performed as per the method described by Sanchez-Moreno (2002) with a slight modification. For extraction, 1.25 g of tomato sample was weighed and 25 ml of methanol was added to it. Following this the sample was homogenised in a 50 ml tube with an Ultra-Turrax T-25 tissue homogeniser for 1 min at 24,000 rpm. The samples are then thoroughly mixed with a vortex mixer (V400 Multitude Vortexer, Alpha laboratories) for 2 hrs at 150 rpm. Then it was centrifuged for 15 min at 3,000 rpm using a Sanyo MSE Mistral 3,000i, UK. Following centrifugation, 10 ml samples of the supernatant were filtered through PTFE syringe filters (pore size 0.45 μm, Phenomenex, UK). Finally the extracts were stored at -20°C in foil covered plastic test tubes for further analysis. In a 1.5-mL Eppendorf tube 500 μl of appropriately diluted methanolic extract and 500 μl DPPH Reagent were added and vortexed. After that they were kept for 30 min in dark. The absorbance of the supernatant was read at 515 nm in 1 ml plastic cuvettes. Each sample of the three batches was measured in triplicate. The blank was MeOH.

234 *2.4.3.2. Ferric ion reducing antioxidant power assay (FRAP)*

235 The FRAP assay was carried out as described by Stratil, Klejdus and Kuban (2006) with a
236 slight modification. For extraction, 1.25 g of tomato sample was weighed and 25 ml of
237 methanol was added to it. Following this the sample was homogenised in a 50 ml tube with
238 an Ultra-Turrax T-25 tissue homogeniser for 1 min at 24,000 rpm. The samples are then
239 thoroughly mixed with a vortex mixer (V400 Multitude vortexer, Alpha laboratories) for 2
240 hrs at 150 rpm. Then it was centrifuged for 15 min at 3,000 rpm using a Sanyo MSE Mistral
241 3000i, UK. Following centrifugation, 10 ml samples of the supernatant were filtered
242 through PTFE syringe filters (pore size 0.45 μm, Phenomenex, UK). The extracts were
243 stored at -20°C in foil covered plastic test tubes for further analysis.
244 The FRAP reagent was prepared by mixing 38 mM sodium acetate (anhydrous) in distilled
245 water pH 3.6, 20 mM $FeCl_3.6H_2O$ in distilled water and 10 mM 2,4,6-tri(2-pyridyl)-s-
246 triazine (TPTZ) in 40 mM HCl in proportions of 10:1:1. This reagent was freshly prepared
247 before each experiment. In a 1.5 mL Eppendorf tube 100 μl of appropriately diluted
248 methanolic extract and 900 μl FRAP Reagent were added and vortex. After that they were
249 kept for 40 min in the heating blocks at 37°C, covered with tin foil. The absorbance of the
250 supernatant was read at 593 nm in 1 ml plastic cuvettes. Each sample of the three batches
251 was measured in triplicate. The blank was MeOH.

252 *2.4.4. Total phenols*

253 For extraction, 1.25 g of tomato sample was weighed and 25 ml of methanol was added.
254 Following this the sample was homogenised in a 50 ml tube with an Ultra-Turrax T-25
255 tissue homogeniser for 1 min at 24,000 rpm. The samples are then thoroughly mixed with a
256 vortex mixer (V400 Multituve Vortexer, Alpha laboratories) for 2 hrs at 150 rpm. Then it
257 was centrifuged for 15 min at 3,000 rpm using a Sanyo MSE Mistral 3000i, UK. Following

centrifugation, 10 ml samples of the supernatant were filtered through PTFE syringe filters (pore size 0.45μm, Phenomenex, UK). Finally the extracts were stored at -20°C in foil covered plastic test tubes for further analysis. Total polyphenol content of tomatoes was determined using the Folin-Ciocalteu method (Singleton, Orthofer & Lamuela-Ravento, 1999). In a 1.5 mL Eppendorf tube, 100 μl of appropriately diluted methanolic extract, 100 μl of MeOH and 100 μl of FC reagent were added and vortexed. After exactly 1 min, 700 μl of sodium carbonate (20%) was added, and the mixture was vortexed and allowed to stand at room temperature in the dark for 20 min. Then the tubes were centrifuged at 13,000 rpm for 3 min. The absorbance of the supernatant was read at 735 nm in 1 ml plastic cuvettes. The blank was MeOH. Each sample of the three batches was measured in triplicate. Results were expressed as mg/L gallic acid equivalents (GAE).

2.4.5. Minerals

Mineral analysis was performed on 3 g tomatoes (Hernandez-Suarez, Rodriguez & Romero, 2007). The samples were ashed at 550°C for 5 hrs and transferred quantitatively into 100 ml volumetric flask. Then they were digested with a few drops of HCl and brought to volume with deionized H_2O. The sample solution was analysed for Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), Iron (Fe), Copper (Cu), Zinc (Zn) and Manganese (Mn) using an Atomic Absorption Spectrophotometer (Model IL357, USA). Calibration was done according to the operator's manual. The standards were prepared from the standard solutions taking into account the linear range for each element. Both samples and standards contained 1 ml of lanthanum solution/100 ml.

2.5. Antioxidant Activity of Whey Permeates (DPPH and FRAP)

The 3 whey permeate (PC, DP and DC) solutions were prepared individually in distilled water to give a final concentration of 3% (v/v). The antioxidant activity was measured by DPPH and FRAP as mentioned in 2.4.3.1. and 2.4.3.2.

2.6. Statistical analysis

Data were analysed by multivariate analysis of variance (MANOVA) using Statgraphics software (centurium XV; Statistical Graphics Co., Rockville, USA) for different washing treatments. Analysis of variance one-way (ANOVA) was used to analyse each treatment over storage. In the case of significant differences LSD range test ($p < 0.05$) was used.

3. Results and Discussion

3.1. Nutritional markers of Fresh-cut Tomato

3.1.1. Ascorbic acid

The average concentration of ascorbic acid was found to be 15 mg/100 g FW in the current study, which is within the range of 6.96 to 21.23 mg/100 g FW reported by Toor and Savage (2005). No significant difference was observed between whey permeate and chlorine treatments in terms of vitamin C content. However, samples treated with water showed the lowest ascorbic acid content after 10 days of storage (Figure 1A). Vitamin C concentration showed no substantial variations throughout the storage time in all the treatments. This trend was in accordance with the values observed by other authors (Gil et al., 2002; Toor & Savage 2005). The maintenance of vitamin C concentration in fresh-cut tomatoes may be explained through the low presence of O_2 inside the trays (12% at day 10). Soliva-Fortuny, Oms-Oliu and Martin-Belloso (2002) reported that the magnitude of vitamin C degradation can be related to the O_2 concentrations inside the packages. The higher amount of O_2 in the bags headspace might cause the greater decrease in vitamin C

303 content. Therefore, the initial decrease in the ascorbic acid content might be explained by
304 higher O_2 concentration inside the packages initially (19% at day 1). Consistently, Gil et al.
305 (2002) reported a non-significant decrease of vitamin C due to cutting in fruits such as
306 mango, strawberry and watermelon. High titratable acidity is responsible for the stability of
307 ascorbic acid in fruits. Tomato is a highly acidic fruit; it showed a relatively stable ascorbic
308 acid content during post-harvest storage. In addition, phenolic substances have been
309 reported to have a protective effect on the ascorbic acid. Therefore, the presence of
310 phenolics and flavanoids in tomato cells might have contributed to the maintenance of the
311 ascorbic acid content. It has been reported that ascorbic acid contributes by 28–38% to the
312 antioxidant activity, while the remaining activity is mainly due to phenolics (Toor &
313 Savage, 2005).

314 *3.1.2. Lycopene*

315 Lycopene content of fresh-cut tomato was analysed during 10 days of storage after
316 treatments with different types of whey permeate. The average amount of lycopene in the
317 samples was 5.86 mg/100 g FW. The treatments did not show any significant effect
318 ($p<0.05$) on the lycopene concentration of the samples as they followed the same pattern
319 during storage. However, storage time had significant effect ($p<0.05$) on the samples. The
320 lycopene content increased slightly at day 3, though not significantly (Figure 1B). At day 7,
321 lycopene concentration of the samples decreased by around 0.2 mg/100 g FW in all the
322 treatments. This could be explained by slow degradation of lycopene during storage. The
323 samples showed a moderate increase at day 10. Fruits biosynthesise carotenoids during
324 ripening throughout storage time. On the other hand, Shi and Le Maguer (2000) observed
325 that carotenoids are susceptible to oxidation in the presence of light, oxygen and low pH.
326 Consequently, the increase in the lycopene concentration at day 10 might be due to the

biosynthesis of lycopene induced by ripening and the low oxidation of this carotenoid as a result of low availability of O_2 in the package headspace (Odriozola-Serrano, Soliva-Fortuny & Martin-Belloso, 2008).

3.1.3. Antioxidant activity test

3.1.3.1. 2, 2-Diphenyl-1-picrylhydrazyl radical scavenging capacity assay (DPPH)

The antioxidant capacity as measured by DPPH radical scavenging activity differed significantly ($p<0.05$) between treatments (Figure 1C). All three whey permeates showed significantly ($p<0.05$) higher DPPH reduction than chlorine and water treatment. The higher antioxidant activity of whey permeates treated samples could be associated with the intrinsic antioxidant activity of whey permeates (41.60 – 53.82 mg Trolox/L, as measured by DPPH). Whey permeates might have also helped to retain the antioxidant activity of tomato slices. The water treated samples had the lowest DPPH reduction of all the treatments. These results could be related to the total phenolic content of the samples since the samples containing higher phenolic content exhibited stronger DPPH reduction and vice versa.

On the other hand, the antioxidant capacity of fresh-cut tomatoes depleted with storage time irrespective of the treatments. The antioxidant activity was reduced by around 11% from day 1 to day 3. This initial reduction could be associated with the stress caused by minimal processing. Significant differences on antioxidant capacity were observed by other researchers as a consequence of minimal processing. A decrease in the antioxidant capacity after processing was observed in fresh-cut spinach (Gil et al., 2002). Lana and Tijskens (2006) reported that fresh-cut tissues are primarily subjected to oxidative stress, causing membrane damage and altering the composition and content of antioxidant compounds, resulting in the reduction of the total antioxidant activity of the tissue.

3.1.3.2. Ferric ion reducing antioxidant power assay (FRAP)

Ferric ion reducing antioxidant power (FRAP) is one of the most commonly used antioxidant capacity assay (Kong & Xiong, 2006). FRAP value of fresh-cut tomatoes decreased significantly during storage in all treatments (Figure 1D). Similar results were observed in the other antioxidant capacity assay (DPPH). The decrease of FRAP value was slow until day 3. After day 3, the samples showed a sharp decrease in their antioxidant capacity (FRAP). The treatments showed a significant difference ($p<0.05$) in terms of their FRAP values during storage, DP exhibited the highest FRAP value after 10 days of storage followed by DC and PC. Chlorine treated samples showed a significantly lower FRAP value compared to whey permeates (DP and DC). In general whey permeates and chlorine treatments showed significantly ($p<0.05$) higher antioxidant capacity measured by FRAP than water treatment.

3.1.4. Total phenols

The average concentration of total phenols in samples at day 1 was 20.3 mg GAE/100 g FW. This value was in accordance with other studies (Toor & Savage, 2005, Gil et al., 2002). Martinez-Valverde, Periago, Provan and Chesson (2002) reported a concentration of 27.2 mg/100 g FW for phenolic compounds in tomatoes. The treatments differed significantly over storage time (Figure 2). Water treatment showed the lowest phenolic content of all the treatments. DP and PC maintained significantly ($p<0.05$) higher total phenols than the chlorine treatment, while DC showed similar results to chlorine. The total phenol content decreased significantly ($p<0.05$) during storage irrespective of treatments. Phenolic content was not significantly affected by minimal processing. The decrease was slow until day 3. After this all the treatments demonstrated a rapid decrease in the total phenolic content. Water treated samples decreased the most to a value of around 15 mg

375 GAE/100 g FW after 10 days of storage. Phenolics are the major antioxidant compounds in
376 plant extracts. Toor and Savage (2005) reported that phenolic compounds might contribute
377 60 to 70% antioxidant activity of tomato extracts.

378 *3.1.5. Correlation among DPPH, FRAP and Total phenol*

379 A significant correlation ($p<0.05$) between radical scavenging activities as measured using
380 the FRAP and DPPH assays was observed (Correlation Co-efficient, $R^2=0.8005$, $p<0.05$).
381 In addition, antioxidant capacities were strongly correlated with phenol content ($R^2=0.9173$,
382 $p<0.05$ for FRAP vs Phenols and $R^2=0.8227$, $p<0.05$ for DPPH vs Phenols) (Table 1).
383 These results suggest that the DPPH and FRAP antioxidant activity could be predicted on
384 the basis of total phenol assay. The results emphasised the importance of phenolic
385 compounds in the antioxidant behaviour of fresh-cut tomato and indicated that the phenolic
386 compounds contributed significantly to the total antioxidant activity. Total phenol assay
387 though used for determining total phenolic content follows the same principle as electron-
388 transfer based antioxidant activity tests.

389 *3.1.6. Minerals*

390 Table 2 shows the results for the concentrations of the minerals and trace elements studied
391 during storage for 10 days after treatments with whey permeate and chlorine. Ca, Mn and
392 Zn were the most stable elements, with no significant variation due to storage time.
393 Samples treated with PC showed the highest Zn content while the DC treated samples had
394 the lowest. Fresh-cut tomatoes did not show any significant ($p<0.05$) difference in Ca, Mg,
395 Cu, Fe and Mn content after the washing treatments. However, treatments did affect the Na,
396 K and Zn content of the samples. Samples treated with whey permeate showed significantly
397 ($p<0.05$) higher values of Na and K compared with water treated samples throughout the
398 storage. The intrinsic mineral content of the whey permeates might have contributed to

these higher values. Na, K, Mg and Fe showed a significant decreasing trend ($P<0.05$) during storage regardless of the treatments. Since minerals are not metabolised and therefore their contents should not change, variations of mineral content of fresh-cut tomatoes during storage have been attributed to redistribution of mineral elements in the fruit slices (Hernández Suarez et al., 2007). Another explanation of the minerals could be leaching out of tomato juice during storage.

3.2. Antioxidant activity test of whey permeates (DPPH and FRAP)

Radical quenching is a primary mechanism of antioxidants to inhibit oxidative processes. DPPH is a relatively stable organic radical, thus widely used as a substrate to evaluate the efficacy of antioxidants (Sanchez-Moreno, 2002). In our DPPH test, whey permeates reduced the DPPH radical to a yellow-coloured compound, apparently due to the DPPH radical accepting an electron or hydrogen to become a stable diamagnetic molecule. The reduced DPPH radical electron spin resonance (ESR), signal intensity in the presence of the whey permeate samples indicated that mixed peptides/amino acids were capable of quenching DPPH presumably by pairing the odd electron of the DPPH radicals (Table 3).

The antioxidant potential of the whey permeates were also estimated using the FRAP assay, which measures their ability to reduce a TPTZ–Fe(III) complex to a TPTZ–Fe(II) complex. The reducing power (reported as FRAP values) of the whey permeates are presented in Table 3. The reducing power assay is the most effective means to evaluate the ability of antioxidants to donate electrons. The strong reducing power of the whey may be due to the increased availability of hydrogen ions produced by peptide hydrolysis (Kong & Xiong, 2006). Colbert and Decker (1991) found that the efficacy of proteins or peptides depended on their molecular weights, and peptides of lower molecular weight have strongest

antioxidant activity. Whey permeate was found to contain peptides that were heat stable and had a molecular weight of 500–5,000 Daltons.

Previous studies have shown that whey contains a broad range of antioxidant activity in an iron-catalysed liposome oxidation system (Peña-Ramos & Xiong, 2003) or a copper-catalysed liposome emulsion (Colbert & Decker, 1991), depending on the proteases used. Whey hydrolysates applied to cooked meat pork patties could suppress lipid oxidation (Peña-Ramos & Xiong, 2003). Individually, both hydrolysed lactalbumin and lactoglobulin could act as antioxidants (Hernández-Ledesma, Dávalos, Bartolomé, & Amigo, 2005). Coronadoa, Troutb, Dunsheac and Shaha (2002) used rosemary extract and whey powder for the oxidative stability of wiener sausages during 10 months frozen storage and they found improved sensory scores as compared to wieners without additional antioxidant. They suggested that whey powder could have acted as a potential antioxidant.

4. Conclusion

The total phenol content and antioxidant activities measured by DPPH and FRAP were significantly ($p<0.05$) higher in DP treated tomato samples than the industrial standard, chlorine treated samples during storage. DP showed significantly better or similar results to the other whey permeates (PC and DC) in all the markers tested. Water treated samples retained the least antioxidants. These results suggested whey permeate could be a promising alternative to maintain and also to enhance the nutritional quality of fresh-cut tomatoes. However, further research is required to identify and characterise those low molecular weight antioxidants. Methods to isolate these compounds commercially are also needed in order to bring these antioxidants to the market.

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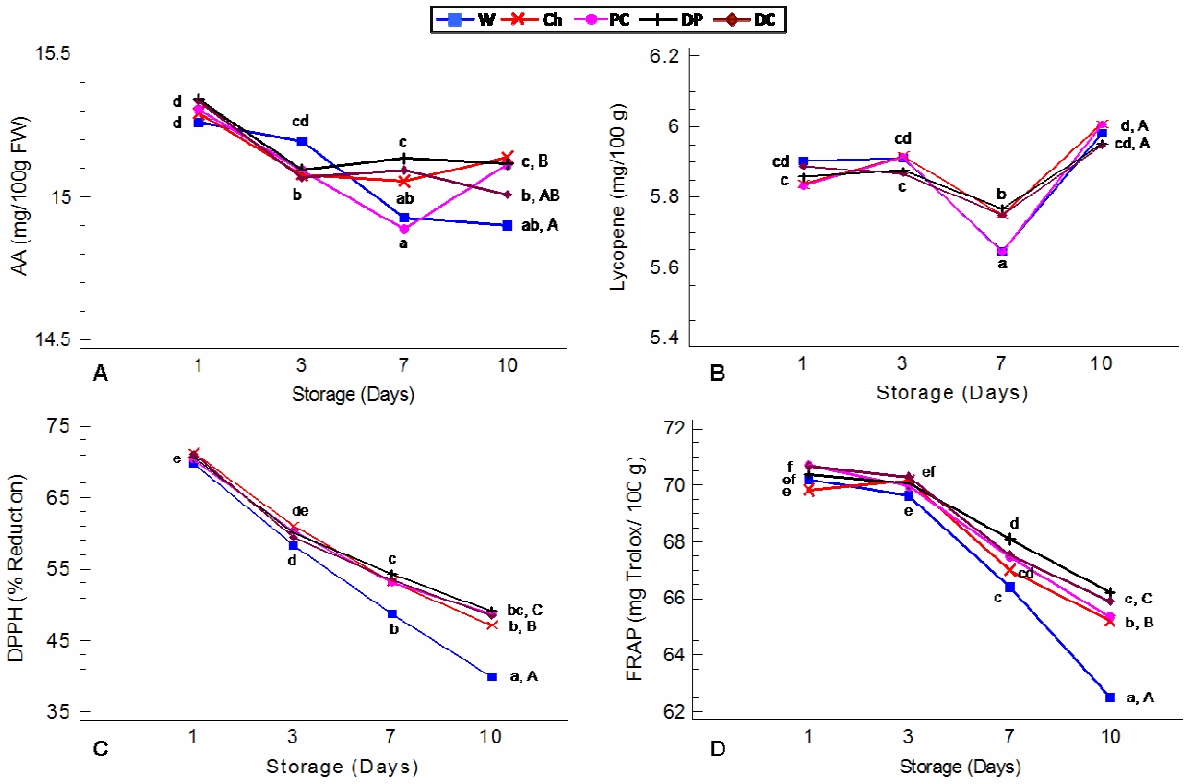

546

547 **Figure 1.** Ascorbic acid (**A**), lycopene (**B**), DPPH (**C**) and FRAP (**D**) in fresh-cut tomatoes
548 treated with chlorine (Ch), water (W) and whey permeate concentrate (PC), whey
549 delactosed permeate (DP) and whey delactosed concentrate (DC) during the 10 days of
550 storage at 4°C. Points designated on any curve by the different letters are significantly
551 different ($p<0.05$). Lowercase letters are used for comparisons during storage and uppercase
552 letters for treatment comparisons. Three independent trials were carried out in triplicate.

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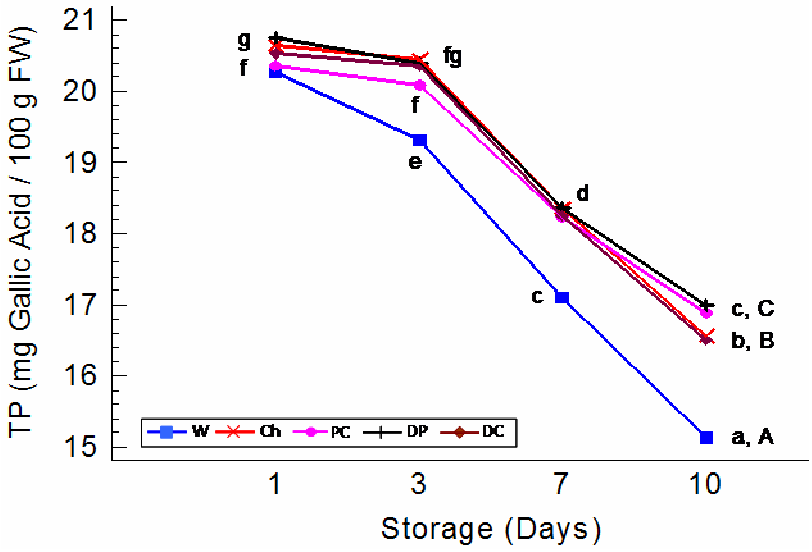

554

555 **Figure 2.** Total phenols content after treatment with chlorine (Ch), water (W) and whey
556 permeate concentrate (PC), whey delactosed permeate (DP) and whey delactosed
557 concentrate (DC) at 4°C in fresh-cut tomatoes in 10 days of storage. Points designated on
558 any curve by the different letters are significantly different ($p<0.05$). Lower case letters are
559 used for comparisons during storage and upper case letters for treatment comparisons.
560 Three independent trials were carried out in triplicate.

561 **Table 1.** Correlations between the results obtained from total phenolic content (TP), 2, 2-
562 Diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH) and ferric reducing
563 antioxidant power (FRAP) assays in fresh-cut tomato packages throughout the 10 days of
564 storage at 4°C treated with water, chlorine and whey permeates.

Correlation between	**Linear equations of relationship**	**Correlation coefficient (R^2)**
FRAP and DPPH	Y (FRAP) = 0.2294X (DPPH) + 54.971	0.8005
FRAP and TP	Y (FRAP) = 1.2672X (TP) + 44.194	0.9173
DPPH and TP	Y (DPPH) = 4.6816X (TP) – 31.05	0.8227

565

566 **Table 2.** Changes in mineral content (mg/100 g FW) in fresh-cut tomato during storage at
567 4°C treated with 120 ppm chlorine (Ch), water (W) and whey permeate concentrate (PC),
568 whey delactosed permeate (DP) and whey delactosed concentrate (DC).[1]

Mineral	Treatment	Differences among treatments	Storage (Days)			
			1	3	7	10
Na	W	A	11.32^{f}	9.26^{d}	8.06^{ab}	7.70^{a}
	Ch	AB	10.70^{e}	9.44^{d}	8.50^{bc}	7.99^{ab}
	PC	B	11.19^{f}	9.17^{d}	8.66^{c}	8.25^{bc}
	DP	B	11.04^{ef}	9.26^{d}	8.78^{cd}	8.17^{b}
	DC	B	11.18^{f}	9.37^{d}	8.51^{bc}	8.24^{bc}
K	W	A	246^{ef}	187^{d}	153^{c}	932^{a}
	Ch	AB	253^{f}	193^{de}	173^{cd}	113^{ab}
	PC	B	253^{f}	219^{e}	186^{d}	133^{bc}
	DP	B	253^{f}	227^{ef}	160^{cd}	126^{b}
	DC	B	226^{ef}	219^{e}	166^{cd}	140^{bc}
Mg	W	A	126^{d}	121^{b}	121^{b}	118^{a}
	Ch	A	126^{d}	120^{ab}	121^{b}	119^{ab}
	PC	A	126^{d}	120^{ab}	121^{b}	120^{ab}
	DP	A	126^{d}	122^{c}	119^{ab}	121^{b}
	DC	A	127^{d}	121^{b}	118^{a}	120^{ab}
Fe	W	A	2.16^{d}	2.03^{c}	1.94^{b}	1.88^{a}
	Ch	A	2.16^{d}	2.03^{c}	1.95^{b}	1.90^{a}
	PC	A	2.16^{d}	2.04^{c}	1.94^{b}	1.90^{a}
	DP	A	2.17^{d}	2.05^{c}	1.94^{b}	1.88^{a}
	DC	A	2.16^{d}	2.04^{c}	1.94^{b}	1.90^{a}
Zn	W	AB	0.74^{a}	0.73^{a}	0.75^{a}	0.73^{a}
	Ch	AB	0.74^{a}	0.74^{a}	0.74^{a}	0.76^{a}
	PC	B	0.74^{a}	0.76^{a}	0.76^{a}	0.75^{a}
	DP	AB	0.74^{a}	0.75^{a}	0.76^{a}	0.74^{a}
	DC	A	0.73^{a}	0.74^{a}	0.73^{a}	0.73^{a}

569 [1]Values designated by the different letters are significantly different ($p<0.05$). Lowercase
570 letters are used for comparisons during storage and uppercase letters for treatment
571 comparisons. Three independent trials were carried out in triplicate.

572 **Table 3.** Antioxidant Activity (DPPH and FRAP) of whey permeate concentrate (PC),
573 whey delactosed permeate (DP) and whey delactosed concentrate (DC).[1]

Whey Permeates (3%)	**Significance of difference**	**DPPH mg Trolox/ L**	**FRAP mg Trolox/ L**
PC	S	41.60±1.38	135.68±1.6
DP	NS	62.29±1.55	179.86±1.2
DC	NS	53.82±2.55	178.46±0.6

574 [1]S and NS for each sample denote significant ($p<0.05$) and non-significant ($p>0.05$)
575 difference respectively.