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# 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 samples treated with the industry standard, chlorine (120 ppm). Samples treated with DP retained significantly higher antioxidant activity (FRAP) and total phenols (TP), when compared with those treated with PC and DC. DP showed significantly higher results than chlorine for DPPH, FRAP and TP. In DPPH assay, all whey permeate-treated samples showed similar antioxidant activity, while ascorbic acid and lycopene were unaffected by treatment. Among the three whey permeates, delactosed permeate showed the best results in maintaining the antioxidant properties of tomato, suggesting it could be used to enhance the antioxidant activity of fresh-cut tomato and retain the antioxidant components during storage.



## 1. 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 tomato 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 epidemiological studies and concluded that the intake of tomato products was consistently associated with a lower risk of a variety of cancers and, in particular, prostate cancer.

The fresh-cut industry claims their products are convenient and healthy alternatives to fulfil the dietary needs for fresh food and many fast food companies are diversifying their menu in order to offer a range of ready-to-eat salads to their clients. However, the many changes that happen in fruits and vegetables during harvesting, handling and processing can affect antioxidant status. Fresh-cut tissues are primarily exposed to oxidative stress, presumably causing membrane damage and altering the composition and content of antioxidant compounds, resulting in changes in the total antioxidant activity of the tissue. Decrease in the antioxidant activity after processing was reported for fresh-cut spinach (Gil, Aguayo, & Kader, 2002).

Many researchers have routinely used sodium hypochlorite for surface sanitation and sterilisation of fruits and vegetables. For tomato, a range of 0.105–1.05% sodium hypochlorite (1:50–1:5 dilutions of commercial bleach) is commonly used with washing or dipping for 1–3 min, to sterilise the surface of fruit before

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experimentation (Artes, Conesa, Hernandez, & Gil, 1999; Martin-Diana et al., 2006). Commercially, generally only 100–200 ppm chlorine is used for sanitation purposes. However, chlorine derivatives are not considered safe compounds, since they react with organic material, to form reactive by-products, such as chloramines and trihalomethanes, which are considered as potentially carcinogenic and may provoke detrimental effects in the organoleptic properties of food products (Hua & Reckhow, 2007). There is a need to investigate the efficiency of natural bio-active compounds and alternative techniques.

Alternatives or modified methods have been proposed, however, none have yet gained widespread acceptance by the industry. These include organic acids, essential oil treatments, irradiation, heat-shock, ozone treatment, etc. (Ponce, Roura, Del Valle, & Fritz, 2002; Singh, Singh, Bhunia, & Stroshine, 2002). Each has its advantages and disadvantages, where the disadvantages are dominating. The maintenance of the quality of fresh produce is still a major challenge for the food industry. In recent years, there is a renewed and growing interest in the use of natural products for the preservation of fresh-cut produce. Research and commercial applications have shown that natural components could replace traditional washing agents (Gil et al., 2002; Martin-Diana et al., 2006). The developments of chlorine-free fruit and vegetable products enriched with natural bio-products could contribute greatly to a new and growing market, where the consumers' health-related concerns are met. Although synthetic antioxidants are widely used in the food industry, consumers' concerns over their safety and toxicity have forced the food industry to find natural antioxidant sources (Mukhopadhyay, 2007, chap. 3).

Whey permeate is a by-product of the production of whey protein concentrates from cheese whey. The main ingredients of whey permeate are water, lactose, peptides and minerals. The high chemical oxygen demand (COD) (50 kg $O_2$/ton permeate) of whey makes its disposal a significant pollution problem. The cost-effective disposal of whey is a major problem for cheese manufacturers, despite the variety of techniques available. Whey is used as a fermentation feedstock for the production of lactic acid, acetic acid, propionic acid, ethanol, and single cell protein, etc. (Nykänen, Lapvetelainen, Hietnen, & Kallio, 1998). However, these applications still do not utilise all the whey produced and new uses for this by-product are continually being sought. Whey and whey ultrafiltration permeate have been proposed to be used as a natural antioxidant in foods (Del Mar Contreras, Hernández-Ledesma, Amigo, Martín-Álvarez, & Recio, 2010). Their application into other products would help the cheese industry to partially solve the problem of whey disposal.

Whey protein is widely used as a bio-active and nutritional ingredient in health and food products (Marshall, 2004). β-Lactoglobulin (β-Lg) is a small, soluble globular protein with a variety of useful nutritional and functional-food characteristics that have made it an ingredient of choice in the formulation of modern foods and beverages. β-Lg exhibits a number of biological effects including anti-hypertensive, anti-cancer, hypocholesterolaemic, opioidergic, and antimicrobial activities (Yalcin, 2006). α-Lactalbumin (α-La) is another major whey protein which is one of the few proteins that remains intact upon pasteurisation, and is a calcium-binding protein that enhances calcium absorption. It is also a rich source of the amino acids lysine, leucine, threonine, tryptophan and cysteine (Permyakov & Berliner, 2000). Whey could be a promising natural bio-active alternative to chlorine. Martin-Diana et al. (2006) successfully used whey permeate for decontamination of fresh-cut lettuce and carrots during storage.

Therefore this study was carried out to investigate the efficacy of whey permeate for maintaining and also enhancing the antioxidant components and antioxidant activity during storage of fresh-cut tomatoes.

## 2. Materials and methods

### 2.1. Sampling and treatment design

Irish vine-ripened tomatoes (*Lycopersicon esculentum* L. Mill.) cv. Moneymaker was purchased from a local supermarket (Dunnes Stores). According to the grower, the tomato plants were grown commercially in a greenhouse with a 14-h light period from February until November. The aerial environment of the greenhouse and crop irrigation and nutrition were precisely controlled. The temperature of the greenhouse was 16–21 °C, which is optimum for lycopene synthesis in tomato fruits. The tomatoes were then brought to the 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 (four 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 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, Bourne End, UK). Processed tomatoes were then pooled, mixed and ~100 g placed in a polypropylene tray (180 mm length × 130 mm width × 25 mm depth) from Sharp Interpack Ltd. (Canterbury, UK) containing one layer of absorbent paper on the bottom (Fresh-R-Pax absorbent pads, Maxwell Chase Technologies, Atlanta, GA). 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 $3.3 \times 10^{-12}$ mol/s/m$^2$/Pa for $O_2$ and $3.1 \times 10^{-9}$ mol/s/m$^2$/Pa for $CO_2$ (Amcor Flexibles Europe, Gloucester, UK). 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 New Brunswick G-2 gyratory shaker (Edison, NJ) for 2 h at 150 rpm and centrifuged for 15 min at 3000 rpm at 4 °C (Sanyo MSE Mistral 3000ii; Sanyo E&E, Loughborough, UK). Following centrifugation, 10 ml of the supernatant was filtered through PTFE syringe filters (pore size 0.45 μm, Phenomenex, Torrance, CA) and stored at −20 °C in foil-covered plastic test tubes for further analysis by HPLC.

The analysis of ascorbic acid content was performed with a Waters 600 Satellite HPLC, with a reverse-phase analytical 5-μm particle diameter, polymeric $C_{18}$ column (150 × 4.6 mm, 5 μm) (Waters, Dublin, Ireland) with a UV-tuneable absorbance detector (Waters 486). Ten microlitres of the sample were injected on a reverse-phase $C_{18}$ HPLC column. An isocratic mobile phase of 25 mM monobasic potassium phosphate (pH 3.0) with a flow rate of 1.0 ml/min was used. The sample was detected at 230 nm on a Shimadzu SPD-10AV, UV–visible detector (Shimadzu, Kyoto, Japan). Five concentrations of ascorbic acid standard in 6% metaphosphoric acid over the range 10–50 μg/ml were injected and peak area and height were determined.

#### 2.4.2. Lycopene

Ten grams of tomato samples were weighed and transferred into a 100-ml beaker (wrapped with aluminium foil). A 50-ml volume of hexane:acetone:ethanol solution (2:1:1 v/v/v) containing 2.5% BHT was added to solubilise the lycopene (Shi & Le Maguer, 2000). Following this the samples were homogenised with an Ultra-Turrax T-25 tissue homogeniser for 1 min at 20,500 rpm. The samples were then shaken with a G-2 gyrotory shaker for 2 h at 150 rpm followed by the addition of 10 ml of distilled water and stirring for an additional 10 min. The upper hexane layer was collected and filtered through a 0.45-μm PVDF membrane filter. It was transferred to a new 15-ml aluminium-wrapped test tube and kept at −80 °C for analysis.

The analysis of lycopene was performed with a Waters 600 Satellite HPLC, with a reverse-phase analytical 5-μm particle diameter, polymeric $C_{18}$ column (150 × 4.6 mm, 5 μm) with a UV-tuneable absorbance detector (Waters 486). 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 25 °C. Analyses were performed under dim light to prevent sample degradation by photo-oxidation. Three concentrations of lycopene standard over 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 were added. The sample was then homogenised in a 50-ml tube with an Ultra-Turrax T-25 tissue homogeniser for 1 min at 24,000 rpm. The samples were then thoroughly mixed with a vortex mixer (V400 multi-tube vortex, Alpha Laboratories, Eastleigh, UK) for 2 h at 150 rpm. Then it was centrifuged for 15 min at 3000 rpm using a Sanyo MSE Mistral 3000i. Following centrifugation, 10-ml samples of the supernatant were filtered through 0.45-μm PTFE syringe filters. 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 darkness. 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 methanol.

*2.4.3.2. Ferric ion reducing antioxidant power assay (FRAP).* The FRAP assay was carried out as described by Stratil, Klejdus, and Kuban (2006), with a slight modification. For extraction, 1.25 g of tomato sample was weighed and 25 ml of methanol were added. 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 were then thoroughly mixed with a V400 vortex mixer for 2 h at 150 rpm, before being centrifuged for 15 min at 3000 rpm. Following centrifugation, 10-ml samples of the supernatant were filtered through 0.45-μm PTFE syringe filters. The extracts were stored at −20 °C in foil-covered plastic test tubes for further analysis.

The FRAP reagent was prepared by mixing 38 mM sodium acetate (anhydrous) in distilled water pH 3.6, 20 mM $FeCl_3.6H_2O$ in distilled water and 10 mM 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ) in 40 mM HCl in proportions of 10:1:1. This reagent was freshly prepared before each experiment. In a 1.5-ml Eppendorf tube 100 μl of appropriately diluted methanolic extract and 900 μl FRAP reagent were added and vortexed. They were then kept for 40 min in heating blocks at 37 °C, covered with tin foil. The absorbance of the supernatant was read at 593 nm in 1-ml plastic cuvettes. Each sample of the three batches was measured in triplicate. The blank was methanol.

#### 2.4.4. Total phenols

For extraction, 1.25 g of tomato sample was weighed and 25 ml of methanol were added. 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 were then thoroughly mixed with a vortex mixer for 2 h at 150 rpm, before being centrifuged for 15 min at 3,000 rpm. Following centrifugation, 10-ml samples of the supernatant were filtered through 0.45-μm PTFE syringe filters. 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-Raventos, 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%) were 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 methanol. 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 of tomatoes (Hernandez-Suarez, Rodriguez, & Romero, 2007). The samples were ashed at 550 °C for 5 h and transferred quantitatively into a 100-ml volumetric flask. Then they were digested with a few drops of HCl and brought to volume with deionised $H_2O$. 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, Instrumentation Laboratory Inc., Wilmington, MA). 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 three 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 Sections 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. One-way analysis of variance (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 6.96–21.23 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 (Fig. 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. A higher amount of $O_2$ in the bags' headspace might cause a greater decrease in vitamin C content. Therefore, the initial decrease in the ascorbic acid content might be explained by higher $O_2$ concentration inside the packages initially (19% at Day 1). Consistently, Gil et al. (2002) reported a non-significant decrease of vitamin C due to cutting, in fruits such as mango, strawberry and watermelon. High titratable acidity is responsible for the stability of ascorbic acid in fruits. Tomato is a highly acidic fruit; it showed a relatively stable ascorbic acid content during post-harvest storage. In addition, phenolic substances have been reported to have a protective effect on ascorbic acid. Therefore, the presence of phenolics and flavanoids in tomato cells might have contributed to the maintenance of the ascorbic acid content. It has been reported that ascorbic acid contributes by 28–38% to the antioxidant activity, while the remaining activity is mainly due to phenolics (Toor & Savage, 2005).

#### 3.1.2. Lycopene

Lycopene content of fresh-cut tomato was analysed during 10 days of storage after treatments with different types of whey

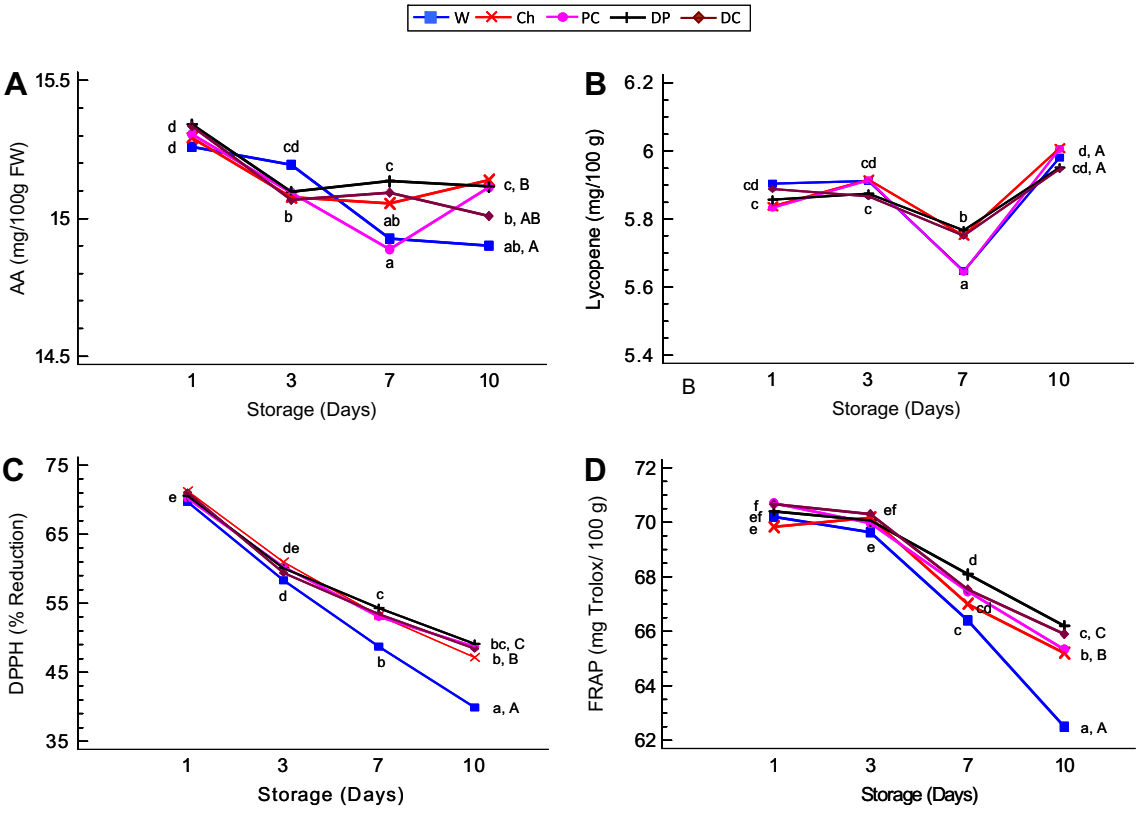


**Fig. 1.** Ascorbic acid (A), lycopene (B), DPPH (C) and FRAP (D) in fresh-cut tomatoes treated with chlorine (Ch), water (W) and whey permeate concentrate (PC), whey delactosed permeate (DP) and whey delactosed concentrate (DC) during the 10 days of storage at 4 °C. Points designated on any curve by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

permeate. The average amount of lycopene in the samples was 5.86 mg/100 g FW. The treatments did not show any significant effect on the lycopene concentration of the samples, as they followed the same pattern during storage. However, storage time had significant effect ($p < 0.05$) on the samples. The lycopene content increased slightly at Day 3, though not significantly (Fig. 1B). At Day 7, lycopene concentration of the samples decreased by around 0.2 mg/100 g FW in all the treatments. This could be explained by slow degradation of lycopene during storage. The samples showed a moderate increase at Day 10. Fruits biosynthesise carotenoids during ripening, throughout storage time. On the other hand, Shi and Le Maguer (2000) observed that carotenoids are susceptible to oxidation in the presence of light, oxygen and low pH. 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 et al., 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 (Fig. 1C). All three whey permeates showed significantly ($p < 0.05$) higher DPPH reduction than chlorine and water treatment. The higher antioxidant activity of whey permeate-treated samples could be associated with the intrinsic antioxidant activity of whey permeates (41.6–53.8 mg Trolox/l, as measured by DPPH). Whey permeates might have also helped to maintain 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.

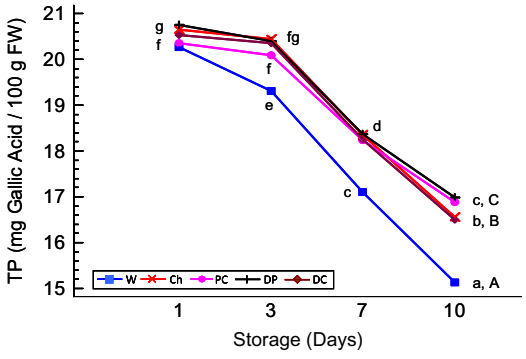


**Fig. 2.** Total phenols content after treatment with chlorine (Ch), water (W) and whey permeate concentrate (PC), whey delactosed permeate (DP) and whey delactosed concentrate (DC) at 4 °C in fresh-cut tomatoes over 10 days of storage. Points designated on any curve by different letters are significantly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

*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 assays (Kong & Xiong, 2006). FRAP value of fresh-cut tomatoes decreased significantly during storage in all treatments (Fig. 1D). Similar results were observed in the DPPH assay. The decrease of FRAP value was slow until Day 3, after which, the samples showed a sharp decrease in their antioxidant capacity. 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 (Fig. 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 GAE/100 g FW after 10 days of storage. Phenolics are the major antioxidant compounds in plant extracts. Toor and Savage (2005) reported that phenolic compounds might contribute 60–70% antioxidant activity of tomato extracts.

### 3.1.5. Correlation among DPPH, FRAP and total phenol

A significant correlation ($p < 0.05$) between radical-scavenging activities as measured using the FRAP and DPPH assays was observed ($r^2 = 0.8005$, $p < 0.05$). In addition, antioxidant capacities were strongly correlated with phenol content ($r^2 = 0.9173$, $p < 0.05$ for FRAP vs. phenols and $r^2 = 0.8227$, $p < 0.05$ for DPPH vs. phenols; Table 1). These results suggest that the DPPH and FRAP antioxidant activity could be predicted on the basis of total phenol assay. The results emphasised the importance of phenolic compounds in the antioxidant behaviour of fresh-cut tomato and indicated that the phenolic compounds contributed significantly to the total antioxidant activity. Total phenol assay though used for

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

| Correlation between | Linear equations of relationship | Correlation coefficient ($r^2$) |
|---|---|---|
| FRAP and DPPH | $Y$ (FRAP) = 0.2294$X$ (DPPH) + 54.971 | 0.8005 |
| FRAP and TP | $Y$ (FRAP) = 1.2672$X$ (TP) + 44.194 | 0.9173 |
| DPPH and TP | $Y$ (DPPH) = 4.6816$X$ (TP) − 31.05 | 0.8227 |

**Table 2**
Changes in mineral content (mg/100 g FW) in fresh-cut tomato treated with 120 ppm chlorine (Ch), water (W) and whey permeate concentrate (PC), whey delactosed permeate (DP) and whey delactosed concentrate (DC) during storage at 4 °C).[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 | $0.73^{a}$ | $0.74^{a}$ | $0.73^{a}$ | $0.73^{a}$ |

[1] Values designated by the different letters are significantly different ($p < 0.05$). Lower-case letters are used for comparisons during storage and upper-case letters for treatment comparisons. Three independent trials were carried out in triplicate.

determining total phenolic content, follows the same principle as electron-transfer-based antioxidant activity tests.

### 3.1.6. Minerals

Table 2 shows the results for the concentrations of the minerals and trace elements studied during storage for 10 days after treatments with whey permeate and chlorine. Ca, Mn and Zn were the most stable elements, with no significant variation due to storage time. Samples treated with PC showed the highest Zn content while the DC-treated samples had the lowest. Fresh-cut tomatoes did not show any significant difference in Ca, Mg, Cu, Fe and Mn content after the washing treatments. However, treatments did affect the Na, K and Zn content of the samples. Samples treated with whey permeate showed significantly ($p < 0.05$) higher values of Na and K, compared with water-treated samples throughout the 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 is that minerals could be leaching out of tomato juice during storage.

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

| Whey permeates (3%) | Significance of difference | DPPH mg Trolox | FRAP mg Trolox |
|---|---|---|---|
| PC | S | 41.6 ± 1.38 | 136 ± 1.6 |
| DP | NS | 62.3 ± 1.55 | 180 ± 1.2 |
| DC | NS | 53.8 ± 2.55 | 178 ± 0.6 |

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

### 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 was 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 500–5000 Da.

Previous studies have shown that whey contains a broad range of antioxidant activities in an iron-catalysed liposome oxidation system (Pen~a-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 (Pen~a-Ramos & Xiong, 2003). Individually, both hydrolysed lactalbumin and lactoglobulin could act as antioxidants (Hernández-Ledesma, Dávalos, Bartolomé, & Amigo, 2005). Coronado, Trout, Dunshea, and Shah (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 industry 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 that 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 in the whey permeates. Methods to isolate these compounds commercially are also needed in order to bring these antioxidants to the market.

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## References


Artes, F., Conesa, M. A., Hernandez, S., & Gil, M. (1999). Keeping quality of fresh-cut tomato. *Postharvest Biology and Technology, 17*, 153–162.

Colbert, L. B., & Decker, E. A. (1991). Antioxidant activity of an ultrafiltration permeate from acid whey. *Journal of Food Science, 56*, 1249–1250.

Coronado, S. A., Trout, G. R., Dunshea, F. R., & Shah, N. P. (2002). Antioxidant effects of rosemary extract and whey powder on the oxidative stability of wiener sausages during 10 months frozen storage. *Meat Science, 62*, 217–224.

del Mar Contreras, M., Hernández-Ledesma, B., Amigo, L., Martín-Álvarez, P. J., & Recio, I. (2010). Production of antioxidant hydrolyzates from a whey protein concentrate with thermolysin: Optimization by response surface methodology. *LWT – Food Science and Technology*. doi:10.1016/j.lwt.2010.06.017.

Gil, M. I., Aguayo, E., & Kader, A. A. (2002). Quality and nutrient retention in fresh-cut versus whole fruit during storage. *Journal Agricultural and Food Chemistry, 54*, 4284–4296.

Giovannucci, E. (2002). A review of epidemiologic studies of tomatoes, lycopene, and prostate cancer. *Experimental Biology and Medicine, 227*(10), 852–859.

Hernandez-Suarez, M., Rodriguez, R. E. M., & Romero, D. C. (2007). Mineral and trace element concentrations in cultivars of tomatoes. *Food Chemistry, 104*, 489–499.

Hernández-Ledesma, B., Dávalos, A., Bartolomé, B., & Amigo, L. (2005). Preparation of antioxidant enzymatic hydrolysates from α-lactalbumin and β-lactoglobulin. Identification of active peptides by HPLC–MS/MS. *Journal of Agricultural and Food Chemistry, 53*, 588–593.

Hua, G., & Reckhow, D. A. (2007). Comparison of disinfection by product formation from chlorine and alternative disinfectants. *Water Research, 41*, 1667–1678.

Kong, B. H., & Xiong, Y. L. (2006). Antioxidant activity of zein hydrolysates in a liposome system and the possible mode of action. *Journal of Agricultural and Food Chemistry, 54*, 6059–6068.

Lana, M. M., & Tijskens, L. M. M. (2006). Effect of cutting and maturity on antioxidant activity of fresh-cut tomatoes. *Food Chemistry, 97*, 203–211.

Lavelli, V., Peri, C., & Rizzolo, A. (2000). Antioxidant activity of tomato products as studied by model reactions using xanthine oxidase, myeloperoxidase, and copper-induced lipid peroxidation. *Journal of Agricultural and Food Chemistry, 48*, 1442–1448.

Lee, H. S., & Castle, W. S. (2001). Seasonal changes of carotenoid pigments and color in Hamlin, Eartygold, and Budd Blood orange juices. *Journal of Agricultural and Food Chemistry, 49*, 877–882.

Marshall, K. (2004). Therapeutical applications of whey protein. *Alternative Medicine Review, 9*, 136–156.

Martin-Diana, A. B., Rico, D., Frias, J. M., Mulcahy, J., Henehan, G. T. M., & Barry-Ryan, C. (2006). Whey permeate as a bio-preservative for shelf life maintenance of fresh-cut vegetables. *Innovative Food Science and Emerging Technology, 7*, 112–123.

Martinez-Valverde, I., Periago, M. J., Provan, G., & Chesson, A. (2002). Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). *Journal of the Science of Food and Agriculture, 82*, 323–330.

Meléndez-Martínez, A. J., Fraser, P. D., & Bramley, P. M. (2010). Accumulation of health promoting phytochemicals in wild relatives of tomato and their contribution to in vitro antioxidant activity. *Phytochemistry, 71*, 1104–1114.

Mukhopadhyay, A. K. (2007). *Antioxidants – Natural and synthetic*. Germany: Amani Int. Publishers.

Nykänen, A., Lapvetelainen, A., Hietnen, R. M., & Kallio, H. (1998). The effect of lactic acid, nisin whey permeate, sodium chloride and related combinations on aerobic plate count and the sensory characteristics of rainbow trout. *Lebensmittel-Wissenschaft und-Technologie, 31*, 286–290.

Odriozola-Serrano, I., Soliva-Fortuny, R., & Martin-Belloso, O. (2008). Effect of minimal processing on bioactive compounds and color attributes of fresh-cut tomatoes. *Lebensmittel-Wissenschaft und-Technologie, 41*, 217–226.

Pen~a-Ramos, E. A., & Xiong, Y. L. (2003). Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. *Meat Science, 64*, 259–263.

Permyakov, E. A., & Berliner, L. J. (2000). α-Lactalbumin: Structure and function. *FEBS Letter, 473*, 269–274.

Ponce, A. G., Roura, S. I., del Valle, C. E., & Fritz, R. (2002). Characterization of native microbial population of Swiss Chaed (*Beta vulgaris*, type cicla). *Lebensmittel-Wissenschaft und-Technologie, 37*, 199–204.

Sanchez-Moreno, C. (2002). Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Science and Technology International, 8*, 121–137.

Sgherri, C., Kadlecova, Z., Pardossi, A., Navari-Izzo, F., & Izzo, R. (2008). Irrigation with diluted sea water improves the nutritional value of cherry tomatoes. *Journal of Agricultural and Food Chemistry, 56*, 3391–3397.

Shi, J., & Le Maguer, M. (2000). Lycopene in tomatoes: Chemical and physical properties affected by food processing. *Critical Reviews in Food Science and Nutrition, 40*(1), 1–42.

Singh, N., Singh, R. K., Bhunia, A. K., & Stroshine, R. L. (2002). Efficacy of chlorine dioxide, ozone, and thyme essential oil or a sequential washing in killing *Escherichia coli* O157:H7 on lettuce and baby carrots. *Lebensmittel-Wissenschaft und-Technologie, 35*, 720–729.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. R. (1999). Analysis of total phenols and other oxidation substrates and oxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology, 299*, 152–178.

Soliva-Fortuny, R. C., Oms-Oliu, G., & Martin-Belloso, O. (2002). Effects of ripeness stages on the storage atmosphere, colour, and textural properties of minimally processed apple slices. *Journal of Food Science, 67*(5), 1958–1963.

Stratil, P., Klejdus, B., & Kuban, V. (2006). Determination of total content of phenolic compounds and their antioxidant activity in vegetables – Evaluation of spectrophotometric methods. *Journal of Agricultural Food Chemistry, 54*, 607–616.

Toor, R. K., & Savage, G. P. (2005). Antioxidant activities in different fractions of tomato. *Food Research International, 38*, 487–494.

WHO (2003). *Diet, nutrition and the prevention of chronic diseases* (p. 916). World Health Organization. ISSN: 0512-3054. [*Technical report series* (p. 149). Geneva: World Health Organization].

Yalcin, A. S. (2006). Emerging therapeutic potential of whey proteins and peptides. *Current Pharmaceutical Design, 12*(13), 1637–1643.