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Full Title

Effect of Delactosed Whey Permeate Treatment on Physico-chemical, Sensorial, Nutritional and Microbial Properties of Whole Tomatoes during Postharvest Storage

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ABSTRACT:

The objective of this study was to investigate the efficacy of delactosed whey permeate (DWP) treatment on the physico-chemical, microbial and antioxidant compounds of tomatoes stored at 15 °C for 21 days compared with traditional chlorine treatment. Fresh tomatoes were treated with 3 ml/100ml DWP or 120 mg/L chlorine solutions and packed in perforated polypropylene bags. The results showed that DWP treatment significantly reduced the number of total aerobic counts (~1.62 log cfu/g) and yeast and moulds (~1.66 log cfu/g) of tomatoes compared to chlorine during storage. Moreover, the tomatoes treated by DWP remained firmer (22 %) than the control fruits and maintained significantly ($p<0.05$) higher levels of vitamin C (15 %), total phenols (10 %) and antioxidant activity (26 %) at the end of storage. Sensory scores confirmed that the DWP treated tomato fruits retained a good appearance and overall quality compared to the chlorine treated samples. The aroma and texture attributes were maintained better in DWP treated tomatoes than chlorine treated tomatoes during storage. Therefore, DWP treatment could be used as a potential washing agent for fresh tomatoes to extend the shelf-life and maintain the nutritional quality during storage.

Key words: Delactosed **w**hey permeate; Tomato; Quality; Antioxidants; Shelf-life.

1. Introduction

The increasing growth in the consumption of fresh fruits and vegetables over the last century has driven commercial demand for improving the storage/transit conditions to manage postharvest disease proliferation and also maintain the quality (i.e. flavour, colour, nutritional aspects, firmness, 'shelf-life' and processing attributes) of fresh produces (Tzortzakis, Borland, Singleton, & Barnes, 2007). Flavour and appearance were the most important attributes of fresh fruits and vegetables, but now consumers are more concerned about food safety and nutritional value. Currently chemical treatments (mainly, 100–200 mg/L chlorine) are used to sanitise fresh produce. However, there are growing health and environmental concerns over current practices, mainly due to the risk of generating potentially harmful (carcinogenic) by-products and residues (Hua, & Reckhow, 2007). There are also growing practical concerns over the increasingly poor control achieved over a spectrum of spoilage organisms. As a consequence, there is considerable interest in alternative, safe, but effective, sanitising agents for use in the fresh produce industry (Tzortzakis et al., 2007). Several researchers have attempted to find the best compromise between extended shelf-life and maintenance of nutritional value. However, none have yet gained widespread acceptance by the industry. Nowadays, there is a renewed growing interest in the use of natural products for the preservation of fresh fruits and vegetables. Research and commercial applications have shown that natural components could replace traditional washing agents (Gil, Conesa, & Artes, 2002; Martin-Diana, Rico, Frias, Mulcahy, Henehan, & Barry- Ryan, 2006). Whey permeate is a by-product of the production of whey protein concentrate from cheese whey. The main components of whey permeate are water, lactose, peptides and minerals. 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. It could be a promising natural bio-active alternative for the preservation of fresh produce (Ahmed, Martin-Diana, Rico, & Barry-Ryan, 2011a, & b). Its application into other products would help the cheese industry to partially solve the problem of whey disposal. Contreras, Hernández-Ledesma, Amigo, Martín-Álvarez, & Recio (2011) proposed to use whey and whey ultrafiltration permeate as a natural antioxidant in foods. Whey proteins and peptides are commonly used in the food industry due to their wide range of chemical, physical and functional properties. The most important functional properties of whey proteins and peptides are viscosity, water holding capacity, emulsification, antimicrobial, immunostimulatory and anti-inflammatory activity (Almecija, Ibanez, Guadix, & Guadix, 2007). Whey antimicrobial peptides were reported to act against different gram-positive and gram-negative bacteria (*Escherichia, Helicobacter, Listeria, Salmonella* and *Staphylococcus*), yeasts and filamentous fungi (Rizzello, Losito, Gobbetti, Carbonara, Bari, & Zamboni, 2005; Fitzgerald, & Murray, 2006).

Tomato is a versatile vegetable that is consumed fresh as well as in the form of processed products. Tomatoes are good sources of carotenoids (mainly, lycopene), ascorbic acid, vitamin E, folate and flavonoids. Moreover, tomatoes are rich in essential amino acids, and particularly high amounts of minerals (Fe, Mn, Zn, and Cu) and monounsaturated fatty acids (especially, oleic acid). Several epidemiological studies have reported that regular consumption of tomatoes reduces the incidence of degenerative diseases, including heart disease and cancer (Lavelli, Peri, & Rizzolo, 2000). The demand for fresh tomatoes has led to prolong the storage of tomato fruit, allowing long-distance shipping. Tomatoes for fresh consumption are commonly harvested at the early red-ripe stages, sanitized with chlorine and shipped to retailers under controlled conditions (temperature, atmosphere, relative humidity). All along the distribution chain, fresh tomatoes are maintained below ambient temperature, often under reduced levels of oxygen. The biochemical changes that occur under these conditions negatively affect flavour and other nutritional markers (Maul et al., 2000). For fresh tomatoes, texture, flavour and colour are the most important quality attributes, which directly relate to their marketing value (Liu, Zabaras, Bennett, Aguas, & Woonton, 2009). Tomato research has mainly focused on the selection of new varieties to increase firmness of the fruits and storage regimes to increase their shelf life.

Therefore this study was carried out to investigate the efficacy of whey permeate as an alternative to chlorine for extending the shelf-life by maintaining the quality and enhancing the antioxidant components of tomatoes during postharvest storage.

2. Materials and Methods

2.1. Sampling

Irish vine ripened tomatoes (*Lycopersicon esculentum* L. Mill.) cv. Moneymaker were purchased from a local supermarket (Dunnes Stores, Dublin, Ireland). 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. The experiments were carried out between July to December, 2010.

2.2. Preparation of treatment solution

Delactosed whey permeate (liquid) was kindly supplied by Glanbia Ltd. Ingredients, Kilkenny, Ireland. Delactosed whey permeate (DWP) was obtained after removal of lactose crystals from cheese whey permeate. In this experiment DWP was used at 3 ml/100ml concentration (Ahmed, Rico, Martin-Diana, & Barry-Ryan, 2011c). The solution was prepared using distilled water stored at room temperature. The pH of DWP solution was 5.0.

2.3. Processing and experimental set up

Whole tomatoes were rinsed briefly in water prior to washing in order to avoid soil contamination. Washing treatment was performed by double treatment of 3 ml/100ml DWP solution. Firstly, the tomatoes were immersed in DWP solution (200 g/L) for 1 min (with agitation). Secondly, DWP solution was sprayed over the tomatoes. Then the tomatoes were dried for 15 min at RT. Chlorinated water (120 mg/L) was used as a control treatment. Processed tomatoes were then pooled and ~ 200 grams placed in a polypropylene tray (180 mm length × 130 mm width × 25 mm depth) from Sharp Interpack Ltd., Canterbury, UK. The trays were then packaged in bags (200 × 320 mm^2) of 35 μm oriented polypropylene film (OPP) with permeability of 3.3×10^{-12} mol/s/m^2/Pa for O_2 at 23 °C and 90 % RH (Amcor Flexibles Europe-Brighouse, Gloucester, United Kingdom). The packages were then heat-sealed under atmospheric conditions and stored at 15 °C for 21 days. Three independent trials were carried out. Each experiment was conducted with 72 packages of tomatoes and tested on day 0, 7, 14 and 21 (2 treatments × 3 replications × 3 batches × 4 days).

2.4. Markers analysis of tomatoes

Different physico-chemical (headspace gas composition, firmness and colour), sensorial, nutritional (ascorbic acid, lycopene, total phenols, antioxidant activity as measured by DPPH) markers and microbial enumeration (total aerobic bacteria and yeast and moulds) were monitored throughout the 21 days of storage of tomato packages stored at 15 °C.

2.4.1. Physico-chemical markers

2.4.1.1. Headspace gas composition

Changes in O_2 and CO_2 concentration of the headspace of tomato packages were monitored during the 21 days of storage. A Gaspace analyser (Systech Instruments, Thame, UK) was used to monitor O_2 and CO_2 levels. Gas extractions were performed with a hypodermic needle, inserted through an adhesive septum previously fixed to the bags, at a flow rate of

150 ml/min for 10 sec. Three bags per treatment were monitored for each experiment (Ahmed, Martin-Diana, Rico, & Barry-Ryan, 2012a, & b).

2.4.1.2. Firmness

Firmness measurement was performed immediately after removing the tomato packages from the storage chamber. The force necessary to cause a deformation of 3 mm with a speed of 0.02 mm/s was recorded using an Instron texture analyser (Instron 4302 Universal Testing Machine, Canton MA, USA), with a 3.5 mm diameter flat faced cylindrical probe. The firmness Data were analysed with the Instron series IX software for Windows.

2.4.1.3. Colour

For colour analysis each tomato in the storage pack was analysed individually using a Colour Quest XE colorimeter (HunterLab, Northants, UK). A tomato was placed directly on the colorimeter sensor (3.5 cm of diameter) and measured. 20 – 30 measurements were taken per treatment and day. The L* parameter (lightness index scale) range from 0 (black) to 100 (white). The a* parameter measures the degree of red (+a*) or green (-a*) colour and the b* parameter measures the degree of yellow (+b*) or blue (-b*) colour. The CIE L* a* b* parameters were converted to Hue (arctan b*/a*) and Chroma $(a^{*2}+b^{*2})^{1/2}$.

2.4.2. Sensorial markers

Analytical descriptive tests were used to discriminate between the sensory quality attributes of tomatoes. DWP (3 ml/100ml concentration) and a control (chlorine - 120 mg/L) treated tomatoes were evaluated by a panel of 12 trained judges (aged 20 - 35 years, eight females and four males) all members of the School of Food Science and Environmental Health, DIT at regular intervals during 21 days of storage. Samples were presented in randomised order during analysis to minimise possible sequence influence. Appearance, colour, texture, aroma and general acceptability of samples were scored on a scale of 1 to 9, where a score of one indicated a product of very poor quality, etc. (Ferreira, Pinho, Amaral, & Martins, 2008; Ahmed, Martin-Diana, Rico, & Barry-Ryan, 2012c). The sensory trial was carried out in the sensory evaluation laboratory. Products were placed in plastic cups with lid, on a white surface and judges were isolated from each-other in a booth in an odour-free environment. The results of the sensory analysis were reported as means of three separate trials. Data were analysed using Compusense® software (Release 4.4, Ontario, Canada).

2.4.3. Nutritional markers

2.4.3.1. Ascorbic acid

The ascorbic acid content in tomatoes was analysed by HPLC using the method described by Lee, & Castle (2001) with a slight modification. 25 ml of 6 g/100ml metaphosphoric acid (pH 3.0) was added to 2.5 g of tomato sample. The sample was then homogenised for 1 min at 24,000 rpm using an Ultra-Turrax T-25 Tissue homogeniser (Fisher Scientific UK Ltd., Loughborough, UK). Then the sample was shaken with a Gyratory Shaker G-2 (Edison, NJ, USA) for 2 hrs at 150 rpm and centrifuged for 15 min at 3,000 ×g at 4 °C (Sanio MSE Mistral 3000ii, Sanyo E&E, Loughborough, UK). Following centrifugation, 10 ml of the supernatant was filtered through PVDF syringe filters (pore size 0.45 µm, Phenomenex, Macclesfield Cheshire, UK) and stored at –20 ºC in foil covered plastic test tubes for further analysis by HPLC. The analysis of ascorbic acid content was performed with Waters 600 Satellite HPLC, with a reversed 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) at 230 nm. Ten µl of the tomato sample was injected. An isocratic mobile phase of 25 mmol/L monobasic potassium phosphate (pH 3.0) with a flow rate of 1.0 ml/min was used. Five concentrations of ascorbic acid standard in 6 g/100ml metaphosphoric acid in the range 10 - 50 µg/ml were injected and peak area and height were determined. Each sample of the three batches was measured in triplicate.

2.4.3.2. 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 (20 ml: 10ml: 10ml) containing 2.5 g/100ml 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 (Fisher Scientific UK Ltd., Loughborough, UK) for 1 min at 20,500 rpm. The samples were then shaken with a Gyrotory Shaker G-2 (Edison, NJ, USA) for 2 hrs at 150 rpm followed by 10 ml of distilled water was added and stirred for additional 10 min. The polar and non-polar layers were separated, and 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 foil wrapped test tubes and kept at –80 °C for analysis. The analysis of lycopene was performed with Waters 600 Satellite HPLC, with a reversed phase analytical polymeric C_{18} column (150 × 4.6mm, 5 µm) (Waters, Dublin, 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 (400 ml: 500 ml: 100 ml) with a flow rate of 1 ml/min was used. The column temperature and mobile phase was maintained at 25 ºC. 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 lycopene content was expressed as ml/100 g wet weight. Each sample of the three batches was measured in triplicate.

2.4.3.3. Total phenols

For extraction, 25 ml of methanol was added to 1.25 g of tomato samples and homogenised in a 50 ml tube with an Ultra-Turrax T-25 tissue homogeniser (Fisher Scientific UK Ltd., Loughborough, UK) for 1 min at 24,000 rpm. The samples were then thoroughly mixed with a vortex mixer (V400 Multituve Vortexer, Alpha laboratories, Eastleigh, UK) for 2 hrs at 150 rpm. Then they were centrifuged for 15 min at 3,000 ×g using a Sanyo MSE Mistral 3000i (Sanyo E&E, Loughborough, UK). Following centrifugation, 10 ml samples of the supernatant were filtered through PVDF syringe filters (pore size 0.45μm, Phenomenex, Macclesfield Cheshire, UK). Finally the extracts were stored at - 20 °C in foil covered plastic test tubes for further analysis. Total phenol 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 g/100ml) 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 12,720 ×g for 3 min. The absorbance of the supernatant was read at 735 nm in 1 ml plastic cuvettes. Each sample of the three batches was measured in triplicate. Results were expressed as mg/L gallic acid equivalents (GAE).

2.4.3.4. Antioxidant Activity Test - 2, 2-Diphenyl-l-picrylhydrazyl radical scavenging capacity assay (DPPH)

The extraction for DPPH scavenging activity was done as per the total phenol content of tomatoes (section 2.4.3.3). The assay was performed using the method of Sanchez-Moreno (2002) with a slight modification. 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 measured at 515 nm in 1 ml plastic cuvettes. Each sample of the three batches was measured in triplicate.

2.4.4. Microbial enumerations

Microbiology analyses were carried out on the DWP treated and chlorine treated (control) samples at day 0, 7, 14 and 21 of storage. 25 g of tomatoes were blended in 225 ml of peptone saline with a Stomacher circulator homogeniser (Fisher Scientific UK Ltd., Loughborough, UK). Enumeration and differentiation of total aerobic counts were quantified at 30 ºC in plate count agar (PCA) over 72 hrs. Yeast and moulds were quantified at 25 ºC in potato dextrose agar (PDA) over 72 hrs. The results were expressed as log colony forming units per gram (log cfu/g).

2.5. 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. Physico-chemical markers

3.1.1. Headspace Gas composition

Headspace gas (O_2 and CO_2) composition within tomato packages significantly ($p<0.05$) changed over storage. Oxygen decreased from atmospheric levels (21 % - packaging conditions) to values around 18 % during the 1st week and to levels around 13 % by day 21 (Fig. 1A). An increase in carbon dioxide was observed, from 1 % to 4 % in 7 days and to values around 9 % at the end of storage (Fig. 1B). These results were in agreement with previous studies (Boukobza, Dunphy, & Taylor, 2001). The DWP treated tomatoes did not show any significant ($p>0.05$) difference to the chlorine treated samples (treated with chlorine) for headspace gas composition as the pattern of change was the same for both samples over time. With the development of modified atmosphere packaging, tomatoes can be kept for several weeks depending on the maturity when harvested. However, a lack of flavour has been associated with these storage procedures (Maul, Sargent, Sims, Baldwin, Balaban, & Huber, 2000). This is not surprising as both temperature and atmospheric conditions have a direct effect on the fruit metabolism, leading to changes in the formation of flavour compounds.

3.1.2. Firmness

The firmness of tomatoes decreased gradually during storage (Table 1). DWP treatment significantly ($p<0.05$) inhibited fruit softening and maintained higher levels of firmness throughout the storage compared to chlorine treatment. The firmness in DWP-treated fruits was around 22 % higher than that in chlorine treated fruits at the end of storage. This result is well correlated with the sensory panel scores for texture (Section 3.1.4). For fresh tomatoes, texture and colour are the most important quality attributes, which directly relate to their marketing value (Liu et al., 2009). The possible reason of DWP treated tomatoes for remaining firmer during storage than the chlorine treated fruits is the calcium content of DWP (Ahmed et al., 2011b). Calcium has been used for firmness improvement of fresh fruits and vegetables by many researchers (Evans, Zulewska, Newbold, Drake, & Barbano, 2010; Martin-Diana et al., 2006). This effect of Ca can be explained by the formation of cross links between the carboxyl groups of polyuronide chains found in the middle lamella of cell wall. Calcium also increases cell turgor pressure and stabilises the cell membrane (Shafiee, Taghavi, & Babalar, 2010).

3.1.3. Colour

Tomatoes showed a significant decrease ($p<0.05$) in luminosity during storage which is in agreement with the findings of Lana, Tijskens, & Van Kooten (2006). There were no significant ($p>0.05$) differences in L* values between DWP treated and chlorine treated samples (Table 1). The parameters a^* and b^* were also not affected by the DWP treatment. However, the parameter a^* increased significantly ($p<0.05$) during storage. This is an indicator for red colour development and the degree of ripening in tomato (Lana et al., 2006). Hue and chroma also decreased significantly ($p<0.05$) during storage for both samples.

3.2. Sensorial markers

Significant differences ($p<0.05$) were observed between DWP treated and chlorine treated samples for all the attributes evaluated (such as, appearance, aroma, texture and general acceptability) except for colour (Fig. 2). The treatments did not affect the colour of the samples. DWP treated samples scored significantly higher ($p<0.05$) than the chlorine treated samples. The panellists scored the DWP treated tomatoes higher than the chlorine treated samples for aroma and texture. There was no negative effect of DWP on the tomato samples. This was in agreement with most of the physico-chemical markers of tomatoes studied. The appearance, aroma, texture and general acceptability of tomato samples decreased significantly ($p<0.05$) during storage for both treatments which is associated with a loss of quality. For the control sample the aroma and general acceptability decreased slowly in the first 7 days of storage but thereafter declined rapidly until the end of storage. However, the texture decreased gradually. At the end of storage, the DWP treated tomatoes kept a good appearance and overall quality while in control fruit these parameters fell below the limit of marketability.

3.3. Nutritional Markers

3.3.1. Ascorbic Acid

Significantly ($p<0.05$) higher levels of ascorbic acid was found in DWP treated samples compared to chlorine treated samples during storage (Fig. 3A). By day 21, there was 12.9 mg/100 g FW of vitamin C in the DWP treated tomato sample, which is about 1.2-fold higher than in the control samples. The DWP treatment might have formed a thin layer on tomatoes which prevented the loss of ascorbic acid during storage. Also the calcium content of DWP could influence the retention of ascorbic acid of tomatoes. Storage organs such as fruits compared to leaves are relatively poor sinks for calcium. Exogenous applications of calcium markedly increase the calcium content in the flesh and affect some of the changes associated with ripening and senescence (Ramezanian, Rahemi, & Vazifehshenas, 2009). Vitamin C retention can also be greatly favoured by the presence of O_2 and the coating on the surface of fresh fruits and vegetables may reduce O_2 diffusion and consequently better preserve the vitamin C content. Oms-Oliu, Soliva-Fortuny, & Martin-Belloso (2008a, & b) reported similar results in pears treated with the polysaccharide-based edible coatings and found that the treatments significantly reduced the loss of vitamin C from pears after more than 1 week. However, there was a decrease in ascorbic acid content in tomatoes for both treatments over storage. This trend was in accordance with the values observed by other authors (Gil et al., 2002; Toor, & Savage, 2005). Ascorbic acid contributes by 28–38 % to the antioxidant activity, while the remaining activity is mainly due to phenolics (Toor, & Savage, 2005).

3.3.2. Lycopene

The initial amount of lycopene in the samples was around 5.8 mg/100 g FW which is within the reported range of 2 to 10 mg/100 g FW (Toor, & Savage, 2005). The DWP treatment did not show any significant effect ($p<0.05$) on the lycopene concentration of the samples as they followed the same pattern for chlorine treated sample during storage (Fig. 3B). However, storage time had a significant effect ($p<0.05$) on the samples. The lycopene content increased significantly over the storage. The increase in the lycopene concentration 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). On the other hand Toor, & Savage (2005) found temperature plays an important role in lycopene accumulationin during tomato ripening, in association with the internal membrane system. They have reported that the mean lycopene content of tomatoes stored at 15 ºC was 1.8-fold higher than that of refrigerated (4 ºC) tomatoes.

3.3.3. Total phenols

Impacts of storage treatments on the antioxidant properties of tomato fruit and tomato products are of concern because tomato fruit are recognised to be particularly rich in several antioxidants (Tzortzakis et al., 2007). In addition to the usual nutrients, such as vitamins and carotenoids, tomatoes also contain phenolic compounds (Ahmed et al., 2012a, & b). Phenols are the major antioxidant compounds in plant extracts and might contribute 60 to 70 % of the antioxidant activity of extracts (Toor, & Savage, 2005). In the present study the initial concentration of total phenols in tomato samples was 21 mg gallic acid/100 g FW (Fig. 3C). This value was in accordance with other studies (George, Kaur, Khurdiya, & Kapoor, 2004; Toor, & Savage, 2005). Total phenol content of the DWP treated tomatoes was significantly ($p<0.05$) higher than the chlorine treated samples at the end of storage. The calcium content of DWP might have helped to retain for higher phenolic content of the samples during storage. It has been reported that calcium has positive effects on the accumulation of carotenoids, vitamin C and phenolic acids in fruits and vegetables (Singh, Beloy, McInerney, & Day, 2012; Marin, Rubio, Martinez, & Gil, 2009). However, the total phenol content of tomatoes decreased over storage. The decrease is more obvious in chlorine treated samples (approx 8 mg GAE/100 g FW) after 21 days of storage. The decrease in total phenols observed toward the end of storage may be due to the breakdown of the cellular structure and the over-ripening of fruits.

3.3.4. Antioxidant Activity Test - 2, 2-Diphenyl-l-picrylhydrazyl radical scavenging capacity assay (DPPH)

The antioxidant capacity as measured by DPPH radical scavenging activity differed significantly ($p<0.05$) between the DWP treated and the chlorine treated tomato samples (Fig. 3D). The DWP treated samples showed significantly ($p<0.05$) higher DPPH reduction than chlorine treated samples. The higher antioxidant activity of the whey permeate treated samples could be associated with the intrinsic antioxidant activity of whey permeate (Ahmed et al., 2011c). Whey permeate might have also helped to retain the antioxidant activity of tomato fruits. This result correlated with the total phenols results, since the DWP treated sample containing higher phenolic content exhibited stronger DPPH reduction. During storage, the amount of ascorbic acid was higher in DWP treated tomatoes and the possible synergistic interactions between ascorbic acid and phenols may have been responsible for the observed higher antioxidant activity in this sample. Previous studies showed that soluble antioxidant activity contributes around 92 % toward the total antioxidant activity of tomatoes, while the lipophilic antioxidants, mainly lycopene and lipophilic phenolics, contributed only about 8 % to the total antioxidant activity of tomatoes (Toor, & Savage, 2005).

3.4. Microbial Enumerations

DWP treatment significantly ($p<0.05$) reduced the growth of total aerobic counts and yeast and moulds of tomatoes, resulting in a positive effect on the extension of shelf-life. Tomatoes stored at 15 °C had initial loads of total aerobic bacteria ~ 3.4 log cfu/g and yeast and moulds ~ 3.8 log cfu/g. The DWP treatment maintained a low level of microbial count compared to the chlorine treatment over the storage. On the day 7 the counts of DWP treated tomatoes were lower than the chlorine treated tomatoes by 0.57 and 0.53 log cfu/g in total aerobic bacteria and yeast and moulds, respectively. The difference in counts between the two treatments was higher when storage time was prolonged. On day 14, the counts of DWP treated samples were lower than the chlorine treated samples by 1.01 and 0.79 log cfu/g for total aerobic bacteria and yeast and moulds, respectively and at the end of 21 days of storage

DWP treated tomatoes had ~ 1.62 log cfu/g (Fig. 4A) and ~1.66 log cfu/g (Fig. 4B) lower counts of total aerobic bacteria and yeast and moulds respectively than the chlorine treated samples. However, the numbers of the micro-organisms increased during storage in both samples. This increase was more obvious during the later days of storage. The values of DWP treated samples at the end of the storage were within the considered limit (10^8 cfu/g) for consumer consumption of fresh fruits and vegetables (Alegria, Pinheiro, Gonçalves, Fernandes, & Moldão, 2010). The antimicrobial peptides present in DWP might contribute to its antimicrobial capacity (Clare, & Swaisgood, 2000). The amphipathic nature of these peptides presumably underlies their biological activities which enables them to associate with lipid membranes and disrupt normal membrane functions of bacteria (Saint-Sauveur, Gauthier, Boutin, & Montoni, 2008; Gauthier, Pouliot, & Saint-Sauveur, 2006).

4. Conclusion

The post-harvest application of DWP significantly reduced microbial population and maintained overall quality and antioxidant components of tomatoes. Sensory scores confirmed that DWP treatment had no negative effect on aroma of tomatoes. The presence of antimicrobial peptides (caseinmacropeptide or bacteriocins) in DWP might contribute to its antimicrobial capacity. DWP treatment seems to be a promising technique to extend the shelf-life of tomatoes during storage. Further research on antimicrobial and antioxidant properties of DWP and their effect on pathogens are recommended.

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Figure Legends

Fig. 1. Effect of chlorine ( ) and DWP ( ) treatment on headspace gas composition (A) O_2 and (B) CO_2 in tomatoes during 21 days of storage at 15 °C. Points designated on any curve by different letters are significantly different ($p<0.05$). Lowercase letters are used for comparisons during storage and uppercase letters for treatment comparisons. Three independent trials were carried out in triplicate.

Fig. 2. Sensory evaluation of tomatoes after chlorine ( ) and DWP ( ) treatment and stored at 15 °C for 21 days. Points designated by different letters are significantly different ($p<0.05$) for each attribute. Three independent trials were carried out in triplicate. Colour (9 = bright red, 1 = darkened); Aroma (9 = fresh, 1 = rotten); Texture (9 = very crispy, 1 = soft); General acceptability (9 = excellent, 1 = poor).

Fig. 3. Effect of chlorine ( ) and DWP ( ) treatment on (A) ascorbic acid, (B) lycopene, (C) total phenols and (D) antioxidant activity - DPPH in tomatoes during 21 days of storage at 15 °C. Points designated on any bar by different letters are significantly different ($p<0.05$). Lowercase letters are used for comparisons during storage and uppercase letters for treatment comparisons. Three independent trials were carried out in triplicate.

Fig. 4. Effect of chlorine ( ) and DWP ( ) treatment on (A) total aerobic counts and (B) yeast and moulds in tomatoes during 21 days of storage at 15 °C. Points designated on any curve by different letters are significantly different ($p<0.05$). Lowercase letters are used for comparisons during storage and uppercase letters for treatment comparisons. Three independent trials were carried out in triplicate.