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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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25 **ABSTRACT:**

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

41 **Key words:** Delactosed whey permeate; Tomato; Quality; Antioxidants; Shelf-life.

42

43 1. Introduction

44 The increasing growth in the consumption of fresh fruits and vegetables over the last century
45 has driven commercial demand for improving the storage/transit conditions to manage
46 postharvest disease proliferation and also maintain the quality (i.e. flavour, colour, nutritional
47 aspects, firmness, 'shelf-life' and processing attributes) of fresh produces (Tzortzakis,
48 Borland, Singleton, & Barnes, 2007). Flavour and appearance were the most important
49 attributes of fresh fruits and vegetables, but now consumers are more concerned about food
50 safety and nutritional value. Currently chemical treatments (mainly, 100–200 mg/L chlorine)
51 are used to sanitise fresh produce. However, there are growing health and environmental
52 concerns over current practices, mainly due to the risk of generating potentially harmful
53 (carcinogenic) by-products and residues (Hua, & Reckhow, 2007). There are also growing
54 practical concerns over the increasingly poor control achieved over a spectrum of spoilage
55 organisms. As a consequence, there is considerable interest in alternative, safe, but effective,
56 sanitising agents for use in the fresh produce industry (Tzortzakis et al., 2007). Several
57 researchers have attempted to find the best compromise between extended shelf-life and
58 maintenance of nutritional value. However, none have yet gained widespread acceptance by
59 the industry. Nowadays, there is a renewed growing interest in the use of natural products for
60 the preservation of fresh fruits and vegetables. Research and commercial applications have
61 shown that natural components could replace traditional washing agents (Gil, Conesa, &
62 Artes, 2002; Martin-Diana, Rico, Frias, Mulcahy, Henehan, & Barry- Ryan, 2006). Whey
63 permeate is a by-product of the production of whey protein concentrate from cheese whey.
64 The main components of whey permeate are water, lactose, peptides and minerals. Whey is
65 used as a fermentation feedstock for the production of lactic acid, acetic acid, propionic acid,
66 ethanol, and single cell protein, etc. (NyKänen, Lapvetelainen, Hietnen, & Kallio, 1998).
67 However, these applications still do not utilise all the whey produced and new uses for this

68 by-product are continually being sought. It could be a promising natural bio-active alternative
69 for the preservation of fresh produce (Ahmed, Martin-Diana, Rico, & Barry-Ryan, 2011a, &
70 b). Its application into other products would help the cheese industry to partially solve the
71 problem of whey disposal. Contreras, Hernández-Ledesma, Amigo, Martín-Álvarez, & Recio
72 (2011) proposed to use whey and whey ultrafiltration permeate as a natural antioxidant in
73 foods. Whey proteins and peptides are commonly used in the food industry due to their wide
74 range of chemical, physical and functional properties. The most important functional
75 properties of whey proteins and peptides are viscosity, water holding capacity, emulsification,
76 antimicrobial, immunostimulatory and anti-inflammatory activity (Almecija, Ibanez, Guadix,
77 & Guadix, 2007). Whey antimicrobial peptides were reported to act against different gram-
78 positive and gram-negative bacteria (*Escherichia*, *Helicobacter*, *Listeria*, *Salmonella* and
79 *Staphylococcus*), yeasts and filamentous fungi (Rizzello, Losito, Gobbetti, Carbonara, Bari,
80 & Zamboni, 2005; Fitzgerald, & Murray, 2006).

81 Tomato is a versatile vegetable that is consumed fresh as well as in the form of processed
82 products. Tomatoes are good sources of carotenoids (mainly, lycopene), ascorbic acid,
83 vitamin E, folate and flavonoids. Moreover, tomatoes are rich in essential amino acids, and
84 particularly high amounts of minerals (Fe, Mn, Zn, and Cu) and monounsaturated fatty acids
85 (especially, oleic acid). Several epidemiological studies have reported that regular
86 consumption of tomatoes reduces the incidence of degenerative diseases, including heart
87 disease and cancer (Lavelli, Peri, & Rizzolo, 2000). The demand for fresh tomatoes has led to
88 prolong the storage of tomato fruit, allowing long-distance shipping. Tomatoes for fresh
89 consumption are commonly harvested at the early red-ripe stages, sanitized with chlorine and
90 shipped to retailers under controlled conditions (temperature, atmosphere, relative humidity).
91 All along the distribution chain, fresh tomatoes are maintained below ambient temperature,
92 often under reduced levels of oxygen. The biochemical changes that occur under these

93 conditions negatively affect flavour and other nutritional markers (Maul et al., 2000). For
94 fresh tomatoes, texture, flavour and colour are the most important quality attributes, which
95 directly relate to their marketing value (Liu, Zabaras, Bennett, Aguas, & Woonton, 2009).
96 Tomato research has mainly focused on the selection of new varieties to increase firmness of
97 the fruits and storage regimes to increase their shelf life.
98 Therefore this study was carried out to investigate the efficacy of whey permeate as an
99 alternative to chlorine for extending the shelf-life by maintaining the quality and enhancing
100 the antioxidant components of tomatoes during postharvest storage.

101 **2. Materials and Methods**

102 *2.1. Sampling*

103 Irish vine ripened tomatoes (*Lycopersicon esculentum* L. Mill.) cv. Moneymaker were
104 purchased from a local supermarket (Dunnes Stores, Dublin, Ireland). According to the
105 grower, the tomato plants were grown commercially in a greenhouse with a 14 h light period
106 from February until November. The aerial environment of the greenhouse and crop irrigation
107 and nutrition were precisely controlled. The temperature of the greenhouse was 16 - 21 °C
108 which is optimum for lycopene synthesis in tomato fruits. The tomatoes were then brought to
109 the food processing lab. The experiments were carried out between July to December, 2010.

110 *2.2. Preparation of treatment solution*

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

116 *2.3. Processing and experimental set up*

117 Whole tomatoes were rinsed briefly in water prior to washing in order to avoid soil
118 contamination. Washing treatment was performed by double treatment of 3 ml/100ml DWP
119 solution. Firstly, the tomatoes were immersed in DWP solution (200 g/L) for 1 min (with
120 agitation). Secondly, DWP solution was sprayed over the tomatoes. Then the tomatoes were
121 dried for 15 min at RT. Chlorinated water (120 mg/L) was used as a control treatment.
122 Processed tomatoes were then pooled and ~ 200 grams placed in a polypropylene tray (180
123 mm length × 130 mm width × 25 mm depth) from Sharp Interpack Ltd., Canterbury, UK. The
124 trays were then packaged in bags (200 × 320 mm²) of 35 µm oriented polypropylene film
125 (OPP) with permeability of 3.3×10^{-12} mol/s/m²/Pa for O₂ at 23 °C and 90 % RH (Amcor
126 Flexibles Europe-Brighthouse, Gloucester, United Kingdom). The packages were then heat-
127 sealed under atmospheric conditions and stored at 15 °C for 21 days. Three independent trials
128 were carried out. Each experiment was conducted with 72 packages of tomatoes and tested on
129 day 0, 7, 14 and 21 (2 treatments × 3 replications × 3 batches × 4 days).

130 2.4. *Markers analysis of tomatoes*

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

135 2.4.1. *Physico-chemical markers*

136 2.4.1.1. *Headspace gas composition*

137 Changes in O₂ and CO₂ concentration of the headspace of tomato packages were monitored
138 during the 21 days of storage. A Gaspacer analyser (Systech Instruments, Thame, UK) was
139 used to monitor O₂ and CO₂ levels. Gas extractions were performed with a hypodermic
140 needle, inserted through an adhesive septum previously fixed to the bags, at a flow rate of

141 150 ml/min for 10 sec. Three bags per treatment were monitored for each experiment
142 (Ahmed, Martin-Diana, Rico, & Barry-Ryan, 2012a, & b).

143 2.4.1.2. *Firmness*

144 Firmness measurement was performed immediately after removing the tomato packages from
145 the storage chamber. The force necessary to cause a deformation of 3 mm with a speed of
146 0.02 mm/s was recorded using an Instron texture analyser (Instron 4302 Universal Testing
147 Machine, Canton MA, USA), with a 3.5 mm diameter flat faced cylindrical probe. The
148 firmness Data were analysed with the Instron series IX software for Windows.

149 2.4.1.3. *Colour*

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

157 2.4.2. *Sensorial markers*

158 Analytical descriptive tests were used to discriminate between the sensory quality attributes
159 of tomatoes. DWP (3 ml/100ml concentration) and a control (chlorine - 120 mg/L) treated
160 tomatoes were evaluated by a panel of 12 trained judges (aged 20 - 35 years, eight females
161 and four males) all members of the School of Food Science and Environmental Health, DIT
162 at regular intervals during 21 days of storage. Samples were presented in randomised order
163 during analysis to minimise possible sequence influence. Appearance, colour, texture, aroma
164 and general acceptability of samples were scored on a scale of 1 to 9, where a score of one

165 indicated a product of very poor quality, etc. (Ferreira, Pinho, Amaral, & Martins, 2008;
166 Ahmed, Martin-Diana, Rico, & Barry-Ryan, 2012c). The sensory trial was carried out in the
167 sensory evaluation laboratory. Products were placed in plastic cups with lid, on a white
168 surface and judges were isolated from each-other in a booth in an odour-free environment.
169 The results of the sensory analysis were reported as means of three separate trials. Data were
170 analysed using Compusense® software (Release 4.4, Ontario, Canada).

171 2.4.3. *Nutritional markers*

172 2.4.3.1. *Ascorbic acid*

173 The ascorbic acid content in tomatoes was analysed by HPLC using the method described by
174 Lee, & Castle (2001) with a slight modification. 25 ml of 6 g/100ml metaphosphoric acid (pH
175 3.0) was added to 2.5 g of tomato sample. The sample was then homogenised for 1 min at
176 24,000 rpm using an Ultra-Turrax T-25 Tissue homogeniser (Fisher Scientific UK Ltd.,
177 Loughborough, UK). Then the sample was shaken with a Gyrotory Shaker G-2 (Edison, NJ,
178 USA) for 2 hrs at 150 rpm and centrifuged for 15 min at 3,000 ×g at 4 °C (Sanio MSE
179 Mistral 3000ii, Sanyo E&E, Loughborough, UK). Following centrifugation, 10 ml of the
180 supernatant was filtered through PVDF syringe filters (pore size 0.45 µm, Phenomenex,
181 Macclesfield Cheshire, UK) and stored at –20 °C in foil covered plastic test tubes for further
182 analysis by HPLC. The analysis of ascorbic acid content was performed with Waters 600
183 Satellite HPLC, with a reversed phase analytical 5 µm particle diameter, polymeric C₁₈
184 column (150 × 4.6 mm, 5 µm) (Waters, Dublin, Ireland) with a UV-tuneable absorbance
185 detector (Waters 486) at 230 nm. Ten µl of the tomato sample was injected. An isocratic
186 mobile phase of 25 mmol/L monobasic potassium phosphate (pH 3.0) with a flow rate of 1.0
187 ml/min was used. Five concentrations of ascorbic acid standard in 6 g/100ml metaphosphoric
188 acid in the range 10 - 50 µg/ml were injected and peak area and height were determined.
189 Each sample of the three batches was measured in triplicate.

190 2.4.3.2. *Lycopene*

191 Ten grams of tomato samples were weighed and transferred into a 100 ml beaker (wrapped
192 with aluminium foil). A 50-ml volume of hexane-acetone-ethanol solution (20 ml: 10ml:
193 10ml) containing 2.5 g/100ml BHT was added to solubilise the lycopene (Shi, & Le Maguer,
194 2000). Following this the samples were homogenised with an Ultra-Turrax T-25 tissue
195 homogeniser (Fisher Scientific UK Ltd., Loughborough, UK) for 1 min at 20,500 rpm. The
196 samples were then shaken with a Gyrotory Shaker G-2 (Edison, NJ, USA) for 2 hrs at 150
197 rpm followed by 10 ml of distilled water was added and stirred for additional 10 min. The
198 polar and non-polar layers were separated, and the upper hexane layer was collected and
199 filtered through a 0.45 µm PVDF membrane filter. It was transferred to a new 15 ml
200 aluminium foil wrapped test tubes and kept at -80 °C for analysis. The analysis of lycopene
201 was performed with Waters 600 Satellite HPLC, with a reversed phase analytical polymeric
202 C₁₈ column (150 × 4.6mm, 5 µm) (Waters, Dublin, Ireland) with a UV tuneable absorbance
203 detector (Waters 486) for spectrometric peak. The lycopene peaks were identified at 475 nm.
204 An isocratic mobile phase of methyl t-butyl ether/methanol/ethyl acetate (400 ml: 500 ml:
205 100 ml) with a flow rate of 1 ml/min was used. The column temperature and mobile phase
206 was maintained at 25 °C. Analyses were performed under dim light to prevent sample
207 degradation by photo-oxidation. Three concentrations of lycopene standard in the range 0.01
208 - 0.03 mg/ml were injected and peak area and peak height were determined. Lycopene
209 content in the samples were identified by comparing peak retention time. The lycopene
210 content was expressed as ml/100 g wet weight. Each sample of the three batches was
211 measured in triplicate.

212 2.4.3.3. *Total phenols*

213 For extraction, 25 ml of methanol was added to 1.25 g of tomato samples and homogenised in
214 a 50 ml tube with an Ultra-Turrax T-25 tissue homogeniser (Fisher Scientific UK Ltd.,

215 Loughborough, UK) for 1 min at 24,000 rpm. The samples were then thoroughly mixed with
216 a vortex mixer (V400 Multitube Vortexer, Alpha laboratories, Eastleigh, UK) for 2 hrs at 150
217 rpm. Then they were centrifuged for 15 min at 3,000 ×g using a Sanyo MSE Mistral 3000i
218 (Sanyo E&E, Loughborough, UK). Following centrifugation, 10 ml samples of the
219 supernatant were filtered through PVDF syringe filters (pore size 0.45µm, Phenomenex,
220 Macclesfield Cheshire, UK). Finally the extracts were stored at - 20 °C in foil covered plastic
221 test tubes for further analysis. Total phenol content of tomatoes was determined using the
222 Folin-Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999). In a 1.5 ml
223 Eppendorf tube, 100 µl of appropriately diluted methanolic extract, 100 µl of MeOH and 100
224 µl of FC reagent were added and vortexed. After exactly 1 min, 700 µl of sodium carbonate
225 (20 g/100ml) was added, and the mixture was vortexed and allowed to stand at room
226 temperature in the dark for 20 min. Then the tubes were centrifuged at 12,720 ×g for 3 min.
227 The absorbance of the supernatant was read at 735 nm in 1 ml plastic cuvettes. Each sample
228 of the three batches was measured in triplicate. Results were expressed as mg/L gallic acid
229 equivalents (GAE).

230 *2.4.3.4. Antioxidant Activity Test - 2, 2-Diphenyl-1-picrylhydrazyl radical scavenging*
231 *capacity assay (DPPH)*

232 The extraction for DPPH scavenging activity was done as per the total phenol content of
233 tomatoes (section 2.4.3.3). The assay was performed using the method of Sanchez-Moreno
234 (2002) with a slight modification. In a 1.5 ml Eppendorf tube 500 µl of appropriately diluted
235 methanolic extract and 500 µl DPPH Reagent were added and vortexed. After that they were
236 kept for 30 min in dark. The absorbance of the supernatant was measured at 515 nm in 1 ml
237 plastic cuvettes. Each sample of the three batches was measured in triplicate.

238 *2.4.4. Microbial enumerations*

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

246 2.5. *Statistical analysis*

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

251 3. **Results and Discussion**

252 3.1. *Physico-chemical markers*

253 3.1.1. *Headspace Gas composition*

254 Headspace gas (O_2 and CO_2) composition within tomato packages significantly ($p < 0.05$)
255 changed over storage. Oxygen decreased from atmospheric levels (21 % - packaging
256 conditions) to values around 18 % during the 1st week and to levels around 13 % by day 21
257 (Fig. 1A). An increase in carbon dioxide was observed, from 1 % to 4 % in 7 days and to
258 values around 9 % at the end of storage (Fig. 1B). These results were in agreement with
259 previous studies (Boukobza, Dunphy, & Taylor, 2001). The DWP treated tomatoes did not
260 show any significant ($p > 0.05$) difference to the chlorine treated samples (treated with
261 chlorine) for headspace gas composition as the pattern of change was the same for both
262 samples over time. With the development of modified atmosphere packaging, tomatoes can

263 be kept for several weeks depending on the maturity when harvested. However, a lack of
264 flavour has been associated with these storage procedures (Maul, Sargent, Sims, Baldwin,
265 Balaban, & Huber, 2000). This is not surprising as both temperature and atmospheric
266 conditions have a direct effect on the fruit metabolism, leading to changes in the formation of
267 flavour compounds.

268 3.1.2. Firmness

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

283 3.1.3. Colour

284 Tomatoes showed a significant decrease ($p < 0.05$) in luminosity during storage which is in
285 agreement with the findings of Lana, Tijskens, & Van Kooten (2006). There were no
286 significant ($p > 0.05$) differences in L^* values between DWP treated and chlorine treated

287 samples (Table 1). The parameters a^* and b^* were also not affected by the DWP treatment.
288 However, the parameter a^* increased significantly ($p<0.05$) during storage. This is an
289 indicator for red colour development and the degree of ripening in tomato (Lana et al., 2006).
290 Hue and chroma also decreased significantly ($p<0.05$) during storage for both samples.

291 3.2. *Sensorial markers*

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

306 3.3. *Nutritional Markers*

307 3.3.1. *Ascorbic Acid*

308 Significantly ($p<0.05$) higher levels of ascorbic acid was found in DWP treated samples
309 compared to chlorine treated samples during storage (Fig. 3A). By day 21, there was 12.9
310 mg/100 g FW of vitamin C in the DWP treated tomato sample, which is about 1.2-fold higher

311 than in the control samples. The DWP treatment might have formed a thin layer on tomatoes
312 which prevented the loss of ascorbic acid during storage. Also the calcium content of DWP
313 could influence the retention of ascorbic acid of tomatoes. Storage organs such as fruits
314 compared to leaves are relatively poor sinks for calcium. Exogenous applications of calcium
315 markedly increase the calcium content in the flesh and affect some of the changes associated
316 with ripening and senescence (Ramezani, Rahemi, & Vazifeshenas, 2009). Vitamin C
317 retention can also be greatly favoured by the presence of O₂ and the coating on the surface of
318 fresh fruits and vegetables may reduce O₂ diffusion and consequently better preserve the
319 vitamin C content. Oms-Oliu, Soliva-Fortuny, & Martin-Belloso (2008a, & b) reported
320 similar results in pears treated with the polysaccharide-based edible coatings and found that
321 the treatments significantly reduced the loss of vitamin C from pears after more than 1 week.
322 However, there was a decrease in ascorbic acid content in tomatoes for both treatments over
323 storage. This trend was in accordance with the values observed by other authors (Gil et al.,
324 2002; Toor, & Savage, 2005). Ascorbic acid contributes by 28–38 % to the antioxidant
325 activity, while the remaining activity is mainly due to phenolics (Toor, & Savage, 2005).

326 3.3.2. *Lycopene*

327 The initial amount of lycopene in the samples was around 5.8 mg/100 g FW which is within
328 the reported range of 2 to 10 mg/100 g FW (Toor, & Savage, 2005). The DWP treatment did
329 not show any significant effect ($p < 0.05$) on the lycopene concentration of the samples as they
330 followed the same pattern for chlorine treated sample during storage (Fig. 3B). However,
331 storage time had a significant effect ($p < 0.05$) on the samples. The lycopene content increased
332 significantly over the storage. The increase in the lycopene concentration might be due to the
333 biosynthesis of lycopene induced by ripening and the low oxidation of this carotenoid as a
334 result of low availability of O₂ in the package headspace (Odriozola-Serrano, Soliva-Fortuny,
335 & Martin-Belloso, 2008). On the other hand Toor, & Savage (2005) found temperature plays

336 an important role in lycopene accumulation during tomato ripening, in association with the
337 internal membrane system. They have reported that the mean lycopene content of tomatoes
338 stored at 15 °C was 1.8-fold higher than that of refrigerated (4 °C) tomatoes.

339 3.3.3. *Total phenols*

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

358 3.3.4. *Antioxidant Activity Test - 2, 2-Diphenyl-1-picrylhydrazyl radical scavenging* 359 *capacity assay (DPPH)*

360 The antioxidant capacity as measured by DPPH radical scavenging activity differed
361 significantly ($p < 0.05$) between the DWP treated and the chlorine treated tomato samples (Fig.
362 3D). The DWP treated samples showed significantly ($p < 0.05$) higher DPPH reduction than
363 chlorine treated samples. The higher antioxidant activity of the whey permeate treated
364 samples could be associated with the intrinsic antioxidant activity of whey permeate (Ahmed
365 et al., 2011c). Whey permeate might have also helped to retain the antioxidant activity of
366 tomato fruits. This result correlated with the total phenols results, since the DWP treated
367 sample containing higher phenolic content exhibited stronger DPPH reduction. During
368 storage, the amount of ascorbic acid was higher in DWP treated tomatoes and the possible
369 synergistic interactions between ascorbic acid and phenols may have been responsible for the
370 observed higher antioxidant activity in this sample. Previous studies showed that soluble
371 antioxidant activity contributes around 92 % toward the total antioxidant activity of tomatoes,
372 while the lipophilic antioxidants, mainly lycopene and lipophilic phenolics, contributed only
373 about 8 % to the total antioxidant activity of tomatoes (Toor, & Savage, 2005).

374 3.4. *Microbial Enumerations*

375 DWP treatment significantly ($p < 0.05$) reduced the growth of total aerobic counts and yeast
376 and moulds of tomatoes, resulting in a positive effect on the extension of shelf-life. Tomatoes
377 stored at 15 °C had initial loads of total aerobic bacteria ~ 3.4 log cfu/g and yeast and moulds
378 ~ 3.8 log cfu/g. The DWP treatment maintained a low level of microbial count compared to
379 the chlorine treatment over the storage. On the day 7 the counts of DWP treated tomatoes
380 were lower than the chlorine treated tomatoes by 0.57 and 0.53 log cfu/g in total aerobic
381 bacteria and yeast and moulds, respectively. The difference in counts between the two
382 treatments was higher when storage time was prolonged. On day 14, the counts of DWP
383 treated samples were lower than the chlorine treated samples by 1.01 and 0.79 log cfu/g for
384 total aerobic bacteria and yeast and moulds, respectively and at the end of 21 days of storage

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

396 **4. Conclusion**

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

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

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529

530 **Figure Legends**

531 **Fig. 1.** Effect of chlorine () and DWP () treatment on headspace gas composition
532 (A) O₂ and (B) CO₂ in tomatoes during 21 days of storage at 15 °C. Points designated on any
533 curve by different letters are significantly different (p<0.05). Lowercase letters are used for
534 comparisons during storage and uppercase letters for treatment comparisons. Three
535 independent trials were carried out in triplicate.

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

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

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