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2012

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Recommended Citation

Ahmed, L., Martin-Diana, A.B., Rico, D., and Barry-Ryan, C., 2012. Extending the shelf –life of tomato using by-product from cheese industry. The Journal of Food Processing and Preservation, 36, 141–151. DOI: 10.1111/j.1745-4549.2011.00562.x

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Full Title

EXTENDING THE SHELF-LIFE OF FRESH-CUT TOMATO USING BY-PRODUCT FROM CHEESE INDUSTRY

Running Title

Whey permeate on Quality of Fresh-cut tomato

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ABSTRACT

The effects of three whey permeates were investigated as potential natural washing treatment for fresh-cut tomato and compared with chlorine (120 ppm). Whey permeate treated samples resulted in equivalent or better than chlorine for all these attributes. Whey permeates were equally effective as chlorine to control the micro-organisms of fresh-cut tomato during storage. The microbial counts at day 10 were significantly reduced (~1.0 log CFU/g) in all the treated samples compared to the control (water treated) samples. Moreover whey permeate treated fresh-cut tomatoes showed lower water activity (2 %) and POD activities (21%) than chlorinated samples after 10 days of storage. Whey permeate also inhibited the loss of firmness of treated tomato slices. Sensory scores for aroma were significantly higher in whey permeate treated samples than chlorine treated samples. Among the three types of whey permeate, delactosed permeate (DP) showed the best results in maintaining the quality of fresh-cut tomato.

PRACTICAL APPLICATIONS

The market sales of ready-to-eat fresh vegetables have grown rapidly in recent decades as a result of changes in consumer attitudes. The marketing of fresh-cut vegetables is limited by their short shelf-life due to the quick decline in post-processing quality. Many attempts have been made to increase the shelf life of fresh-cut fruit and vegetables and many attempts have been made to increase the use of whey permeate, a valuable by-product of cheese processing industry. This paper takes an interesting approach by attempting to use whey permeate as a preserving agent of fresh-cut tomato quality during storage.

KEY WORDS: Whey permeate; Fresh-cut; Tomato; Shelf-life; Quality; Preservation.

1. INTRODUCTION

Continued growth in the ready-to-eat vegetable industry has been largely driven by increasing demand for convenient, fresh and healthy foods. Increasing the quality retention and shelf-life of these products during storage is an important demand of the industry and consumers (Rico *et al.* 2007). Chlorinated water (50–200 ppm) is widely used to wash fruits and vegetables as well as fresh-cut produce in order to preserve their quality. However, the possible formation of carcinogenic chlorinated compounds in water (chloramines and trihalomethanes) has called into question the use of chlorine for this purpose (Alegria *et al.* 2010). As soon as a suitable alternative to chlorine is available to industry, chlorine will be banned throughout the world. In several European countries including Germany, Netherlands, Switzerland and Belgium the use of chlorine in fresh-cut fruits and vegetables is already prohibited. Future regulatory restrictions on the use of chlorine for washing of 'ready-to-eat' vegetables are likely and will require the development of safe alternatives (Ölmez and Kretzschmar 2009). Therefore anything that is as cheap as chlorine and performs close to or equal to chlorine is desired by industry. In recent years interest is growing 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 (Rojas-Graü *et al.* 2009). The development of chlorine-free fruit and vegetable products enriched with natural bio-products could contribute greatly to a new and growing market, where the consumer's concerns about their health are met.

Whey permeate is a by-product of the production of whey protein concentrates from cheese whey. The main composition of whey permeate are water, lactose, low molecular 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 (Panesar *et al.* 2007). However, these

applications still do not utilize all the whey produced and new uses for this by-product are needed. Whey and whey ultrafiltrated permeate have been proposed to be used as a natural antioxidant in foods (Contreras *et al.* 2011). Whey proteins and peptides contain all the essential amino acids (Yalcin 2006). Lactoferrin, α-lactalbumin and β-lactoglobulin are a source of antimicrobial proteins. Casein macro peptide (CMP), α_{1-} and α_{2-} caseins are further examples of whey antimicrobial peptides (Rizzello *et al.* 2005). Martin-Diana *et al.* (2006) used acidic whey permeate for washing fresh-cut lettuce and carrots during storage. Whey protein has been found to reduce the enzymatic browning of 'Golden Delicious' apples (Perez-Gago *et al.* 2006). Coronadoa *et al.* (2002) successfully used rosemary extract and whey powder for the oxidative stability of wiener sausages during 10 months frozen storage.

Tomato is one of the most widely used and versatile vegetable crops. It is consumed fresh and also used to manufacture a wide range of processed products. The consumption of tomatoes is currently considered as a nutritional indicator of good dietary habit and healthy life style. This fruit has undoubtedly assumed the status of a food with functional properties, considering the overwhelming epidemiological evidence for its capacity to reduce the risk of chronic diseases such as cardiovascular disease and cancer (Sgherri *et al.* 2008). This protective action is typically attributed to antioxidant components like lycopene and other carotenoids (pro-vitamin A, beta-carotene), ascorbic acid, vitamin E and flavonoids (Odriozola-Serrano *et al.* 2008).

Therefore the aim of this study was to examine the effect of whey permeate treatment on the quality retention of fresh-cut tomatoes during storage as an alternative to chlorine.

2. MATERIALS AND METHODS

2.1. Sampling and Treatment Design

92 Irish vine ripened tomatoes (*Lycopersicon esculentum* L. Mill.) cv. Moneymaker were purchased
93 from a local supermarket (Dunnes Stores). According to the grower, the tomato plants were
94 grown in a greenhouse with a 14 h light period from February until November. The atmosphere
95 of the greenhouse as well as crop irrigation and nutrition were precisely controlled. The
96 temperature of the greenhouse was 16–21 °C which is optimum for lycopene synthesis in tomato
97 fruit. The tomatoes were transported to the food processing lab and stored at 4 °C before
98 processing. An isolated and cleaned minimal-processing room was used for tomato processing.
99 The experiments were carried out between March and November, 2009. Three independent trials
100 were carried out. Each experiment was conducted with 180 fresh-cut tomato packages {4 test
101 days (1, 4, 7 and 10) × 5 treatments × 3 replications × 3 individual batches}.

102 **2.2. Preparation of Treatment Solution**

103 Three different types of whey permeate (liquid) were kindly supplied by Glanbia Ltd.
104 Ingredients, Ireland. The permeate concentrate (PC) was pre-concentrated by evaporation before
105 the lactose crystallisation process. Delactosed permeate (DP) was obtained after removal of
106 lactose crystals. The delactosed permeate (DP) was then concentrated further by evaporation to
107 give delactosed concentrate (DC). The main components of the three whey permeates were given
108 in Table 1.

109 Five washing treatments were conducted in parallel, using the same batch of product. The
110 samples were sanitised with water, chlorinated water (120 ppm) or whey permeates (PC, DP and
111 DC) at 3 % concentration (Ahmed *et al.* 2011). Chlorinated water was prepared by adding
112 sodium hypochlorite solution (≥ 5 % active chlorine, Aldrich Chemical Co., Dublin, Ireland) to
113 distilled water to obtain a final solution containing ~120 ppm free chlorine (pH 8.0) (Delaquis *et*
114 *al.* 2004). All treatments were prepared using distilled water.

115 **2.3. Processing and Experimental Setup**

116 Whole tomatoes were rinsed briefly (1 min) in water prior to washing to remove soil
117 contamination. Washing treatments were performed by immersion of the tomatoes in each
118 treatment solution for 1 min (with agitation). Each treatment was carried out in different baskets
119 (200 g tomatoes/ L). After washing, the tomatoes were dried for 5 min using a salad spinner.
120 Then they were sliced in 6 mm thick transversal slices with a commercial slicing machine
121 (Maxwell chase MCT-25, Baltimore Innovations, UK). Processed tomatoes were then pooled,
122 mixed and ~100 grams placed in a polypropylene tray (180 mm length × 130 mm width × 25 mm
123 depth) from Sharp Interpack Ltd., UK containing one layer of absorbent paper on the bottom
124 (Fresh-R-Pax absorbent pads, Maxwell Chase Technologies, Atlanta). The principal ingredient in
125 fresh-R-Pax absorbent pads is food grade sodium carboxymethyl cellulose (CMC), a common
126 ingredient in ice-cream, sauces, low-fat foods, etc. The trays were then packaged in bags (200 ×
127 320 mm) of 35 µm oriented polypropylene film (OPP) with permeability at 23 °C and 90 % RH
128 of 3.3×10^{-12} mol/s/m^2/Pa for O_2 and 3.1×10^{-9} mol/s/m^2/Pa for CO_2 (Amcor Flexibles Europe-
129 Brighouse, United Kingdom). The packages were then heat-sealed under atmospheric conditions
130 and stored at 4 °C for 10 days (Ahmed *et al.* 2011).

131 **2.4. Markers Analysis**

132 Different physico-chemical (headspace gas composition, pH, moisture content, water activity),
133 instrumental analysis (color, texture, sensory), enzymatic activity (browning related enzyme –
134 peroxidase and texture related enzyme – pectin methyl esterase), and microbiological
135 (mesophillic, psychotropic, lactic acid bacteria, yeast and moulds) markers were monitored
136 throughout the storage (10 days) of fresh-cut packaged tomatoes stored at 4 °C.

137 **2.4.1. Physico-chemical Markers**

Headspace Gas Composition

Changes in O_2 and CO_2 concentration of the headspaces of the fresh-cut tomatoes packages were monitored during the shelf life of the product. A Gaspace analyser (Systech Instruments, UK) was used to monitor levels of O_2 and CO_2. 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 and all bags for other analyses were checked before analysis.

pH

Ten gram of tomato tissue was blended for 2 min. Then the pH was measured at room temperature using an Orion research pH-meter, UK.

Moisture Content

Moisture content was determined by AOAC method (1990) (Method 925.098). The tomato samples were dried at 105 °C overnight.

Water Activity

The water activity of the treated samples was measured with a fast water activity meter (GBX scientific FA-st/1, Cedex, France). One gram of tomato was placed in a small plastic cup onto the base of the air tight test chamber. The measuring head enclosed the sample and formed an airtight seal with the base. At least 15 samples were measured per treatment.

2.4.2 Instrumental Analysis

Color

For color analysis each piece of tomato in the storage pack was analyzed individually to minimize the variability of the product. Color was quantified using a Color Quest XE colorimeter (HunterLab, Northants, UK). A tomato slice was placed directly on the colorimeter

161 sensor (3.5 cm of diameter) and measured; 20 – 30 measurements were taken per treatment and
162 day. Before measuring the instrument was calibrated using a white tile (L* = 93.97, a* = 0.88
163 and b* = 1.21) and a black tile (L* = 56.23, a* = 21.85, b* = 8.31) standards. Hunter color
164 readings were recorded. The L* parameter (lightness index scale) range from 0 (black) to 100
165 (white). The a* parameter measures the degree of red (+a*) or green (−a*) color and the b*
166 parameter measures the degree of yellow (+b*) or blue (−b*) color. The CIE L* a* b*
167 parameters were converted to Hue (arctan b*/a*) and Chroma $(a^{*2}+b^{*2})^{1/2}$.
168 The translucency assessment was conducted visually on the outer pericarp of the tomato slices.
169 The evaluation was done under uniform light conditions.
170 *Texture*
171 Four measurements were made on each slice, two in the outer pericarp and two in the radial
172 pericarp, applying the force in the axial direction. Care was taken to standardize the
173 measurements in the radial pericarp at half radius and in the outer pericarp between two
174 junctions of outer and radial pericarp. The force necessary to cause a deformation of 3mm with a
175 speed of 0.02 mm/s was recorded using an Instron Texture Analyzer (Instron 4302 Universal
176 Testing Machine, Canton MA, USA), with a 3.5 mm diameter flat faced cylindrical probe. Only
177 the central slice in the stack was used in the analyses. The firmness measurement was performed
178 immediately after removing the slice from the storage chamber (at storage temperature). Data
179 were analysed with the Instron series IX software for Windows.
180 *Sensory Analysis*
181 Sensory analysis was performed for tomato samples over 10 days of storage time by a panel with
182 an age range of 25 – 40 years. Fresh appearance, colour, texture, aroma and general acceptability
183 of samples were scored on a hedonic scale of 1 to 9, where a score of one indicated a product of

very poor quality, etc (Ferreira *et al.* 2008). The evaluation was carried out in the sensory evaluation laboratory. Products were coded using random numbers to avoid bias. 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 sensory analysis was monitored with Compusense Five software (Release 4.4, Ontario, Canada).

2.4.3. Enzymatic Activity

Browning-related Enzyme - Peroxidase (POD)

POD enzyme was assayed in homogenates that were prepared as follows: 10 g of tomato puree was placed in a 100 mL beaker in a 1:2 (w:v) ratio with 0.5 M phosphate buffer, pH 6.5, containing 50 g/L polyvinylpyrrolidone. Then homogenization was carried out twice with an Ultra-Turrax T-25 tissue homogenizer at 4 °C and 20,500 rpm, for 1 min each time with a break of 3 min between homogenizations to avoid excess heating of the sample. The homogenate was centrifuged at 12,720 g for 30 min at 4 °C. It was then filtered through Whatman no. 4 filter paper. The resulting crude extract was used without further purification. All the extracts were kept at 4 °C in the dark and used immediately (within 1 h). POD activity was assayed spectrophotometrically by a modified method based on (Martin-Diana *et al.* 2006). The reaction mixture contained 0.2 mL of extract and 2.7 mL of 0.05 M phosphate buffer, pH 6.5, containing 1.85 mL of hydrogen peroxide (1.5 %, v/v) as oxidant and 3.7 mL of p-phenylendiamine as hydrogen donor. The oxidation of p-phenylendiamine was monitored at 485 nm and 25 °C. A unit of enzyme activity was defined as an increase of 0.1 absorbance units per minute.

Texture-related Enzyme - Pectin Methyl Esterase (PME)

PME activity was measured using the method described by Yoo *et al.* (2009). Ten grams of tomato puree was diluted in an extraction solution (0.2 M sodium phosphate buffer, pH 7.5

containing 1 M sodium chloride and 10 mM dithiothreitol) and homogenized at 4 °C for 2 min at 5,500 rpm. The macerate was incubated at 4 °C for 30 min with agitation and centrifuged at 12,720 g for 30 min at 4 °C. It was then filtered through Whatman no. 4 filter paper. One milliliter of this extract was mixed with 40 mL of substrate solution (1 % pectin). The solution was adjusted to pH 7.0 with 1.0 M NaOH, and the pH of the solution was readjusted to pH 7.5 with 0.05 M NaOH. After the pH reached 7.5; 0.2 mL of 0.05 N NaOH was added. The time required to return to pH 7.5 was recorded. Activity was quantified as carboxyl groups formed by the hydrolysis of methyl esters of pectin and was measured tritrimetrically using a pH electrode to monitor the production of H^+ (Martin-Diana *et al.* 2005). The enzymatic activity can be described by the following equation:

$$PME = \frac{0.2[mL]NaOH * 0.05[Mol \cdot L^{-1}]NaOH \cdot X[mL] \cdot 10^3[mMol \cdot Mol^{-1}] \cdot 10^3[L \cdot mL^{-1}]}{Y[mL] \cdot Z[g] \cdot time[\min]} \quad (1)$$

Where, X = total volume (mL) extracted, Y = volume used (1 mL) in the assay, Z = sample used in the assay (10 g). Three macerates per treatment and day were prepared. Triplicates of the enzymatic activity were analyzed.

2.4.4. Microbiological Markers

Microbiology analyses were carried out on the samples before and after the treatment at regular intervals through the storage period. 25 g of tomatoes were blended in 225 mL of peptone saline with a Stomacher circulator homogenizer. Enumeration and differentiation of mesophilic bacteria were quantified at 30 ºC in plate count agar (PCA) over 72 hrs. Psychrotrophic bacteria were quantified on plate count agar (PCA) at 4 ºC over 7 days. Enumeration of lactic acid bacteria was carried out using deMan Rogosa Sharpe Agar (MRS) at 35 ºC over 48 hrs. Yeast and moulds were quantified at 25 ºC in potato dextrose agar (PDA) over 72 hrs. The results were expressed as log10 colony forming units per gram (CFU/g).

2.5. Statistical Analysis

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

3. RESULTS AND DISCUSSION

3.1. Physico-chemical markers

Headspace Gas Composition

Headspace gas (O_2 and CO_2) composition within fresh-cut tomato packages significantly ($p<0.05$) changed over storage. Oxygen decreased from atmospheric levels (21 % - packaging conditions) to values around 19 % at day 1 and levels around 14 % by day 10. A sharp increase in carbon dioxide was observed in 24 hours (day 1), from 0 to 3 %, reaching values around 7 % at the end of storage (day 10). This increase in CO_2 after cutting of fresh tomatoes has been previously described (Gil *et al.* 2002). Similar gas concentration levels at day 10, with good quality retention on fresh-cut tomatoes, were attained in previous work by Artés *et al.* (1999) using also passive MAP. The treatments did not show any significant ($p<0.05$) effect on headspace gas composition as the pattern of change was the same over time.

pH

The pH values of the samples treated with chlorine were significantly ($p<0.05$) higher than those of samples treated with whey permeate during all storage. The pH increased significantly ($p<0.05$) over storage in all the samples. This is in agreement with other authors (Artés *et al.* 1999). At day 10 the lowest pH appeared in samples washed with DP followed by DC and PC,

253 although the difference among the whey permeate treated samples was not significant (Fig. 1C).
254 The increase in pH during storage might be associated with bacterial growth (Rico *et al.* 2007).
255 *Moisture Content*
256 All the samples in the present study maintained moisture content values around 94 %. This was
257 in agreement with findings reported for tomatoes by Hernandez-Suarez *et al.* (2008). In fact,
258 tomato is one of the highest water containing vegetables. Samples treated with whey permeates
259 had significantly ($p<0.05$) higher water content values than those treated with chlorine and water
260 (Fig. 1D). The possible reason for the higher moisture content in the whey treated samples could
261 be that whey might form a thin layer on the sample surface which could prevent water loss
262 (Rojas-Graü *et al.* 2007). Samples treated with water showed the lowest water content at the end
263 of the storage.
264 *Water Activity*
265 Significantly ($p<0.05$) lower water activity was observed in samples treated with whey permeate
266 (no differences among the whey permeates) than those treated with chlorine or water (Fig. 2A).
267 A significant increase was observed in all samples during storage. Water activity is considered
268 an indirect indicator of growth of micro-organisms and most degradation reactions of a chemical,
269 enzymatic and physical nature (Rico *et al.* 2007). In agreement to this, results obtained for POD
270 activity and microbial counts showed a similar trend (section 3.3 and 3.4). The lower water
271 activity obtained by the permeate treatments positively affected the textural properties of fresh-
272 cut tomatoes during storage, according to data from sensorial and instrumental texture (section
273 3.2).
274 **3.2. Instrumental analysis**
275 *Color*

Fresh-cut tomatoes showed a significant decrease in luminosity during storage ($p<0.05$). This was in agreement the work of Lana *et al.* (2006) using video image analysis of fresh-cut tomato. Luminosity of the samples (L*) was not affected by treatment (Table 2), as well as a* and b* parameters. However, the parameter a* increased significantly ($p<0.05$) during storage. This is an indicator for red color development and the degree of ripening in tomato (Lana *et al.* 2006). Hue has a negative correlation with the maturity of the tomato. As tomato matures during storage, Hue decreases. The decreasing trend of b* values throughout the storage showed that the fresh-cut tomatoes did not have any chilling injury stored at 4 °C as it is the optimum storage temperature of fresh-cut fruits and vegetables (Silviera *et al.* 2010). The authors found that the temperature used during storage did not influence significantly ($p<0.05$) the development of translucency, indicating that the water soaking of the pericarp tissue is not a result of chilling injury. The intensity of translucency depends on the maturity of the tomatoes.

Texture

Texture (firmness) decreased significantly ($p<0.05$) during storage for all treatments (Fig. 2B). These instrumental results correlated with the sensory panel results (Fig. 3). All three whey permeates treatment maintained significantly ($p<0.05$) better texture than chlorine or water treatment. The presence of calcium in whey permeates might helped to maintain the firmness of tomato during storage (Evans *et al.* 2010). Different calcium salts have been used for firmness improvement of fresh fruits and vegetables. Calcium carbonate and calcium citrate are the main calcium salts added to foods in order to enhance the nutritional value. Calcium chloride has been widely used as preservative and firming agent in the fruits and vegetables industry for whole and fresh-cut commodities (Chardonnet *et al.* 2003). Differences between the whey permeate treatments were also observed. Samples washed with DP maintained significantly ($p<0.05$)

299 higher texture throughout the storage than DC and PC. There was no significant difference
300 between PC and DC treatments.
301 *Sensory Analysis*
302 All the attributes evaluated (such as, texture, aroma, first impression, general acceptability)
303 except for color (probably due to ripening) decreased significantly ($p<0.05$) during storage which
304 is associated with a loss of quality (Fig. 3). However, the values at the end of the storage (10
305 days) were still above the acceptability threshold of 5 for all the attributes scored. The non-
306 hypoxic O_2 and CO_2 concentration in the packages might have helped to maintain acceptable
307 levels of colour and aroma (Aguayo *et al.* 2006). The treatments did not affect the visual changes
308 and color parameter of the samples (data not shown). Significant differences were observed
309 between treatments for aroma, texture and general acceptability scores. Lowest scores of texture
310 and general acceptability were observed in water treated samples throughout the storage. In
311 general, whey permeate treatments scored significantly higher ($p<0.05$) or equivalent to chlorine
312 treatments. Among the whey permeates the general acceptability of delactosed permeate (DP)
313 was the highest, this was in agreement with most of the physico-chemical markers of fresh-cut
314 tomatoes studied in the current research. The use of whey permeate for food preservation has
315 been examined by Nykänen *et al.* (1998). These authors analyzed the effect of nisin-whey
316 permeate washing solutions on total counts and sensory characteristics in rainbow trout. They
317 found that nisin-whey treatment caused no negative effect on sensory attributes.
318 **3.3. Enzymatic activity**
319 *Browning-related Enzyme- Peroxidase (POD)*
320 Fresh-cut tomato treated with whey permeate had lower ($p<0.05$) POD activity compared to
321 chlorine and water treated samples (Fig. 2C). This lower POD activity might be associated with

322 the potential antioxidant activity of the whey permeate (Ahmed *et al.* 2011). A significant
323 ($p<0.05$) increase in POD activity in all the treatments was observed during storage. The initial
324 increase at day 3 might be due to mechanical stress during minimal processing (Cantos *et al.*
325 2001). The depletion of antioxidants might attribute to the sharp increase of POD activity in
326 whey treated samples at the end of storage (day 7 to 10). Differences in POD activity were not
327 observed for different types of whey permeate. Perez-Gago *et al.* (2006) found that edible
328 composite coatings prepared from whey protein concentrate (WPC) and beeswax (BW) with
329 ascorbic acid or 0.5 % cystein (Cys) reduced the enzymatic browning of 'Golden Delicious'
330 apples.

331 *Texture-related Enzyme- Pectin Methyl Esterase (PME)*

332 Water and chlorine-treated samples showed significantly ($p<0.05$) lower PME activity than those
333 treated with whey permeate (Fig. 2D). Differences between treatments were observed as samples
334 washed with DP had the highest PME activity of all the treatments followed by DC and PC.
335 PME activity increased significantly ($p<0.05$) during storage for all treatments, although
336 showing fluctuations over storage, which might be due to a wounding response and/or to changes
337 in the solubility of the enzyme during storage (Cantos *et al.* 2001). Such behaviour has been
338 observed for certain browning related enzymes (Perez-Gago *et al.* 2006). Other authors have
339 attributed this variability to intrinsic factors and to pre- and postharvest factors, which can affect
340 enzyme activity, vitamin content, etc (Yoo *et al.* 2009).

341 **3.4. Microbiological Markers**

342 Fresh-cut tomatoes stored at 4 °C had initial loads of total mesophilic bacteria approximately ~ 4
343 log CFU/g (Fig. 4A). At day 10 samples treated with water reached the highest values (~ 8.5 log
344 CFU/g). The whey permeate treatments were equally effective as chlorine in controlling the

345 bacterial growth. In fresh-cut tomatoes, total mesophilic counts increased during storage for all
346 the washing treatments. Samples treated with whey permeates and chlorine showed better
347 reduction in the mesophillic counts (~ 1.2 log CFU/g) than samples treated with water, with
348 significant ($p<0.05$) differences at days 7 and 10 of storage. The values of whey permeate-treated
349 samples at the end of the storage were lower than the recommended 10^8 CFU/g for consumer
350 consumption of fresh-cut vegetables (Alegria *et al.* 2009).
351 Psychrotrophic counts on sliced tomatoes at day 10 were significantly reduced in all the treated
352 samples compared to the control (water treated) samples (Fig. 4B). The highest reduction (1.2
353 log CFU/g) at day 10 was observed in samples treated with whey permeates followed by samples
354 treated with chlorine (~ 0.8 log CFU/g). Psychrotrophic bacteria initial (day 1) load was
355 approximately 3.5 log CFU/g. The highest microbial load was observed in control samples
356 (water treated), which at day 10 reached ~ 7.0 log CFU/g. The three whey permeate and chlorine
357 treatments resulted in significantly lower psychrotrophic counts than water over storage
358 ($p<0.05$), and by the end of storage (day 10) whey-treated samples showed lower counts than
359 those treated with water and chlorine ($p<0.05$).
360 Fresh-cut tomatoes stored at 4 °C had initial loads of LAB approximately 4 log CFU/g. In all the
361 treatments LAB counts significantly ($p<0.05$) increased during storage. Chlorine, DP and DC
362 treatments showed a significant reduction (~ 1.0 log CFU/g) in LAB growth compared to water
363 and PC treatments (Fig. 4C). Chlorine treatment maintained the lowest LAB load, followed by
364 DC and DP. PC and water-treated samples showed the highest LAB counts. This might be
365 explained by the presence of lactose in PC which could facilitate the growth of LAB. High LAB
366 loads are associated with fermentation processes (off-odours). However, controlled growth of

these bacteria can have a positive antimicrobial effect on the vegetable due to their production of bacteriocins (Rico *et al.* 2007).

Fresh-cut tomatoes stored at 4 °C had initial loads of yeast and moulds approximately 4 log CFU/g. This result was in agreement with the finding of Prakash *et al.* (2002) in diced tomato. Yeast and moulds count increased significantly ($p<0.05$) with storage time (Fig. 4D). Water showed the highest yeast and moulds growth (~ 8.3 log CFU/g, at day 10). DP treated tomatoes showed significantly ($p<0.05$) lower counts than chlorine treated samples. DC and PC treatments showed similar effect on yeast and moulds counts to chlorine treatment. Whey permeate and chlorine treatments exhibited significant ($p<0.05$) reduction (~1.0 log CFU/g) in yeast and moulds growth than the control (water treated) samples.

The antimicrobial capacity of whey permeate can be explained by a variety of factors. The most likely factor is the acid pH of the wash treatment which can have a direct effect on the initial microbial count reduction and on subsequent growth during storage. Another factor can be the presence of lactic acid, which can enter the cells in an un-dissociated form. And finally, the presence of antibacterial peptides in the whey permeate might contribute to its antimicrobial capacity (Clare and Swaisgood 2000). Different antimicrobial peptides have been identified from whey protein hydrolysates. These antimicrobial peptides act against different gram-positive and gram-negative bacteria (*Escherichia, Helicobacter, Listeria, Salmonella* and *Staphylococcus*), yeasts and filamentous fungi (Rizzello *et al.* 2005; Fitzgerald and Murray 2006) and different mechanisms of action have been suggested for whey antimicrobial peptides (Saint-Sauveur *et al.* 2008; Gauthier *et al.* 2006).

4. CONCLUSION

In this study, delactosed permeate performed better or similar to the industrial standard chlorine in retaining the quality of fresh-cut tomatoes. Although (DP) treatment showed similar results to chlorine in retaining some of the quality markers, considering the potential harmful effect of chlorine DP could be considered as the best treatment for keeping fresh-cut tomato quality acceptable during 10 days of storage. The quality retention capacity of whey permeate can be explained by a variety of factors. The most likely factor is the acid pH of the wash treatment which can have a direct effect on the initial microbial count reduction and on subsequent growth during storage. Another factor could be the presence of antibacterial peptides (caseinmacropeptide or bacteriocins) in the whey permeate might contribute to its antimicrobial capacity. Although further investigations on pathogens are recommended, whey permeate seems to be a good quality retention method of fresh-cut tomato and to control the growth of bacteria associated with its quality deterioration.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the financial support of the DIT Strand I Research Project (2006–2010). Thanks to Glanbia (Ltd Ingredients, Ireland) for supplying the whey permeate, to Amcor Flexible Ltd. for providing the OPP film and to Sharp Interpack for the polypropylene trays.

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506 TABLE 1. MAIN COMPOSITION OF THE THREE WHEY PERMEATES

Whey Permeates	pH	Total Solid %	Protein %	Dry Matter %	Ash %
Permeate concentrate (PC)	5.48	34.61	1.06	3.06	3.25
Delactosed concentrate (DP)	5.01	32.90	2.83	8.60	8.14
Delactosed concentrate (DC)	5.75	53.25	3.77	7.08	10.96

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TABLE 2. CHANGES IN COLOR IN FRESH-CUT TOMATO STORED AT 4 °C, TREATED WITH 120 PPM CHLORINE (CH), WATER (W) AND WHEY PERMEATE CONCENTRATE (PC), DELACTOSED PERMEATE (DP) AND DELACTOSED CONCENTRATE (DC)[1].

Color parameter	Treatments	Storage (days)			
		1	4	7	10
L*	W	44.18^{b}	43.70^{ab}	43.17^{ab}	42.57^{a}
	Ch	45.39^{b}	44.25^{b}	43.53^{ab}	43.29^{ab}
	PC	45.19^{b}	44.02^{ab}	43.50^{ab}	43.46^{ab}
	DP	44.90^{b}	43.70^{ab}	43.23^{ab}	43.19^{ab}
	DC	44.60^{b}	43.88^{ab}	43.78^{ab}	42.63^{a}
a*	W	13.72^{a}	13.81^{ab}	13.93^{b}	14.14^{b}
	Ch	13.45^{a}	13.85^{ab}	14.05^{b}	14.23^{b}
	PC	13.69^{a}	13.74^{a}	13.80^{ab}	14.19^{b}
	DP	13.51^{a}	13.67^{a}	13.83^{ab}	14.36^{b}
	DC	13.70^{a}	13.85^{ab}	14.09^{b}	14.23^{b}
b*	W	21.84^{ef}	20.06^{cd}	18.63^{b}	17.84^{a}
	Ch	22.25^{f}	20.07^{cd}	18.91^{bc}	18.14^{ab}
	PC	22.16^{ef}	20.04^{cd}	18.86^{bc}	17.93^{ab}
	DP	21.44^{e}	19.87^{cd}	19.37^{c}	18.39^{ab}
	DC	22.22^{f}	20.27^{d}	18.55^{ab}	18.45^{ab}
Hue	W	57.48^{d}	55.42^{c}	53.7^{bc}	51.60^{a}
	Ch	58.08^{d}	55.00^{bc}	54.54^{bc}	52.27^{ab}
	PC	58.19^{d}	55.43^{c}	54.01^{bc}	51.68^{a}
	DP	57.16^{d}	55.49^{c}	55.12^{bc}	52.01^{ab}
	DC	57.95^{d}	54.92^{bc}	53.51^{b}	52.36^{ab}
Chroma	W	25.92^{de}	24.37^{c}	23.16^{ab}	22.76^{a}
	Ch	26.21^{e}	24.52^{c}	23.26^{ab}	22.94^{a}
	PC	26.08^{de}	24.34^{c}	23.31^{ab}	22.87^{a}
	DP	25.51^{d}	24.14^{bc}	23.63^{b}	23.34^{ab}
	DC	26.20^{e}	24.78^{c}	23.07^{ab}	23.30^{ab}

[1]Values designated by the 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. 1. EFFECT OF TREATMENTS ON O_2 (A), CO_2 (B), PH (C) AND MOISTURE CONTENT (D) IN FRESH-CUT TOMATO PACKAGES OVER 10 DAYS STORAGE AT 4 °C. POINTS DESIGNATED ON ANY CURVE 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.

FIG. 2. EFFECT OF TREATMENTS ON WATER ACTIVITY (A), TEXTURE (B), POD (C) AND PME ACTIVITY (D) IN FRESH-CUT TOMATO PACKAGES, MONITORED OVER 10 DAYS STORAGE AT 4 °C. POINTS DESIGNATED ON ANY CURVE 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.

FIG. 3. SENSORY EVALUATION OF FRESH-CUT TOMATOES AFTER TREATMENT STORED AT 4 °C FOR 10 DAYS. VALUES DESIGNATED ON ANY CURVE BY THE 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. FRESH APPEARANCE (9 = EXCELLENT, 1 = POOR); AROMA (9 = FRESH, 1 = ROTTEN); COLOUR (9 = DARK, 1 = LIGHT); TEXTURE (9 = VERY CRISPY, 1 = SOFT); OVERALL ACCEPTABILITY (9 = EXCELLENT, 1 = POOR).

FIG. 4. EFFECT OF WASHING TREATMENTS ON MICROBIAL LOAD: MESOPHILIC (A), PSYCHROTROPHIC (B), LACTIC ACID BACTERIA (C) AND YEAST AND MOULDS (D) DURING 10 DAYS STORAGE OF FRESH-CUT TOMATO AT 4 °C. POINTS DESIGNATED ON ANY CURVE BY THE 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.