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The Impact of Delactosed Whey Permeate Treatment on Shelf-life and Antioxidant Contents of Strawberries

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Title

**THE IMPACT OF DELACTOSED WHEY PERMEATE TREATMENT ON SHELF-
LIFE AND ANTIOXIDANT CONTENTS OF STRAWBERRIES**

Running title

Delactosed whey permeate treated strawberries

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26 **Summary**

27 The aim of this study was to investigate the effect of delactosed whey permeate (DWP)
28 treatment on antioxidant and physico-chemical properties of strawberries. Fresh strawberries
29 treated with 3 % DWP were analyzed for different quality, nutritional and microbiological
30 markers during 10 days of storage at 5 °C. The results showed that DWP treatment
31 significantly reduced incidences of decay (70 %) and numbers of total aerobic counts (~1.4
32 Log₁₀ CFU/g) and yeast and moulds (~1.8 Log₁₀ CFU/g). DWP treatment also inhibited the
33 loss of firmness (15 %) and maintained significantly ($p < 0.05$) higher levels of vitamin
34 C, total phenols and antioxidant activity of strawberries. Sensory scores confirmed that the
35 DWP treated strawberries retained a good appearance and overall quality. The aroma and
36 colour attributes were not reduced during storage. These results suggest that DWP treatment
37 has potential to extend the shelf-life and maintain the quality of strawberries during storage.

38

39 **Keywords:** Delactosed whey permeate, strawberry, antioxidant, quality, shelf-life.

40

41 **Introduction**

42 Strawberries (*Fragaria × ananassa* Duch.) are one of the most popular fruits worldwide due
43 to their high visual appeal and desirable flavour. The quality of strawberries for the market is
44 focused on physical qualities, such as size, colour, firmness, acidity, sweetness and aroma,
45 but there is an increasing interest in the health benefits of the fruit (Wang *et al.*, 2005).
46 Strawberries are very rich in nutrients such as amino acids, vitamins and anthocyanins
47 (Campaniello *et al.*, 2008). Fresh strawberries are also good source of ascorbic acid and
48 phenolic compounds. Ascorbic acid and anthocyanin have potent antioxidant properties and
49 phenolic content is positively related to total antioxidant activity of strawberries (Heo & Lee,
50 2005; Rekika *et al.*, 2005). However strawberries are highly perishable. The ripe fruits are
51 very susceptible to mechanical injury, water loss, microbiological decay and physiological
52 deterioration during storage. They can be easily contaminated with micro-organisms,
53 resulting in decreases in firmness, colour changes, and a shortened shelf-life (Hernandez-
54 Munoz *et al.*, 2008). Strawberry fruits have short ripening and senescent periods that make
55 marketing a challenge. Within the berry industry there is a huge demand to retain the quality
56 at the original level for a longer period. Therefore, post harvest treatment is necessary to
57 remove micro-organisms on the surfaces of the fruit and to extend shelf-life. The current
58 method of post harvest decay control for strawberries during storage and transport is the
59 application of synthetic fungicides. But problems related to development of pathogen
60 resistance to many currently used fungicides and potentially harmful effects on the
61 environment and human health have stimulated research to look for alternative measures
62 (Hernandez-Munoz *et al.*, 2008).

63 In the recent past, flavour and appearance were the most important attributes of fruits and
64 other fresh vegetables, but nowadays consumers are more concerned about food safety and
65 nutritional value. Several researchers have attempted to find the best compromise between

66 extended shelf-life and maintenance of nutritional value. However, none have yet gained
67 widespread acceptance by the industry. Refrigeration is widely used to reduce spoilage and
68 extend the shelf-life of fresh fruit and vegetables (Hernandez-Munoz *et al.*, 2006). Modified
69 atmospheres have been shown to be effective at inhibiting microbial growth, however, it
70 adversely affect the colour and flavour of strawberries (Pelayo *et al.*, 2003). Recently,
71 biologically active natural products have become an alternative for preservation of fresh
72 produce. Whey permeate is a by-product of the production of whey protein concentrate from
73 cheese whey. The main components of whey permeate are water, lactose, peptides and
74 minerals. Whey is used as a fermentation feedstock for the production of lactic acid, acetic
75 acid, propionic acid, ethanol, and single cell protein, etc (Nykänen *et al.*, 1998). However,
76 these applications still do not utilise all the whey produced and new uses for this by-product
77 are continually being sought.

78 Whey Permeate could be a promising natural bio-active alternative for the preservation of
79 fresh produce (Ahmed *et al.*, 2011[a](#),[b](#)). The application of whey into other products would
80 help the cheese industry to partially solve the problem of whey disposal. Whey and whey
81 ultra-filtration permeate have been proposed to be used as a natural antioxidant in foods
82 (Contreras *et al.*, 2011). Whey protein and peptides are widely used as bioactive and
83 nutritional ingredients in health and food products. Antimicrobial peptides have been
84 identified from whey (Kitts & Weiler, 2003; McCann *et al.*, 2006). These antimicrobial
85 peptides act against different gram-positive and gram-negative bacteria (*Escherichia*,
86 *Helicobacter*, *Listeria*, *Salmonella* and *Staphylococcus*), yeasts and filamentous fungi
87 (Rizzello *et al.*, 2005; Fitzgerald & Murray, 2006).

88 Therefore this study was carried out to investigate the efficacy of delactosed whey permeate
89 for extending the shelf-life by maintaining the quality and enhancing the antioxidant
90 components of strawberries during storage.

91 **Materials and methods**

92 Sampling

93 Irish Strawberries (*Fragaria × ananassa* Duch.) variety ‘Elsanta’ were purchased from a
94 local grower. ‘Elsanta’ is the most common variety growing in Ireland. It is a high-yielding,
95 long-lasting variety with excellent flavour. The strawberries were brought to the food
96 processing lab and stored at 5 °C before processing. The experiments were carried out
97 between April and September, 2010. [The strawberries were bought in three different batches.](#)
98 [And to ensure the consistency of the quality among batches, the same varieties of](#)
99 [strawberries were bought from the same grower. Also care has been taken to choose](#)
100 [homogeneous \(colour, size, free of mechanical damage and fungal decay\) samples every](#)
101 [time, which is the standard practice.](#)

102 Preparation of treatment solution

103 Delactosed whey permeate (liquid) was kindly supplied by Glanbia Ltd. Ingredients, Ireland.
104 Delactosed whey permeate (DWP) was obtained after removal of lactose crystals from cheese
105 whey permeate. In this experiment DWP was used at 3 % (v/v) concentration (Ahmed *et al.*,
106 2011c). The solution was prepared using distilled water stored at room temperature. The pH
107 of DWP solution was 5.0.

108 Processing and experimental setup

109 Whole Strawberries were rinsed briefly in tap water prior to washing in order to avoid soil
110 contamination. ~~Washing-DWP~~ treatment was performed by double treatment of 3 % DWP
111 solution. Firstly, the strawberries were immersed in DWP solution (200 g strawberries/ L) for
112 1 min (with agitation). Secondly, DWP solution was sprayed over the strawberries ([Ahmed *et*](#)
113 [al., 2011d](#)). For control treatment strawberries were washed with distilled water in same way
114 as DWP treatment. After the washing, the strawberries were dried for 15 min at ~~R~~[Room](#)

115 | temperature. Processed strawberries were then pooled, mixed and ~ 200 grams placed in a
116 | polypropylene tray (180 mm length × 130 mm width × 25 mm depth) from Sharp Interpack
117 | Ltd., UK containing one layer of absorbent paper on the bottom (Fresh-R-Pax absorbent pads,
118 | Maxwell Chase Technologies, Atlanta). The principal ingredient in fresh-R-Pax absorbent
119 | pads is food grade sodium carboxymethyl cellulose (CMC), a common ingredient in ice-
120 | cream, sauces, low-fat foods, etc. The trays were then packaged in bags (200×320 mm²) of 35
121 | μm oriented polypropylene film (OPP) with permeability at 23 °C and 90 % RH of 3.3×10^{-12}
122 | mol/s/m²/Pa for O₂ (Amcor Flexibles, UK). The packages were then heat-sealed under
123 | atmospheric conditions and stored at 5 °C for 10 days. ~~(Ahmed *et al.*, 2011). Three~~
124 | ~~independent trials were carried out. Each experiment was conducted with 72 packages of~~
125 | ~~strawberry and tested on day 1, 4, 7 and 10 (2 treatments × 3 replications × 3 batches × 4~~
126 | ~~days).~~

127 | Markers analysis of strawberries

128 | Different quality (headspace gas composition, firmness, colour changes and sensory
129 | analysis), nutritional (ascorbic acid, total phenols, antioxidant activity as measured by FRAP)
130 | and microbial (decay incidence, total aerobic bacteria and yeast and moulds) markers were
131 | monitored throughout the 10 days of storage of strawberry packages stored at 5 °C. Each
132 | marker was analyzed in three batches of strawberries with a total of 72 packages and tested
133 | on day 1, 4, 7 and 10 (2 treatments × 3 replications × 3 batches × 4 days).

134 | Quality markers

135 | Headspace gas composition

136 | Changes in O₂ and CO₂ concentration of the headspace of strawberry packages were
137 | monitored during the 10 days of storage. A Gaspacer analyser (Systech Instruments, UK) was
138 | used to monitor O₂ and CO₂ levels. Gas extractions were performed with a hypodermic

139 needle, inserted through an adhesive septum previously fixed to the bags, at a flow rate of
140 150 ml/min for 10 sec. Three bags per treatment were monitored for each experiment and all
141 bags for other analyses were checked before analysis (Martin-Diana *et al.*, 2006).

142 Firmness

143 Four strawberries of each pack were measured. The force necessary to cause a deformation of
144 3 mm with a speed of 0.02 mm/s was recorded using an Instron texture analyser (Instron
145 4302 Universal Testing Machine, Canton, MA, USA), with a 3.5 mm diameter flat faced
146 cylindrical probe. Data were analysed with the Instron series IX software for Windows.

147 Colour

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

155 Sensory analysis

156 Analytical descriptive tests were used to discriminate between the sensory quality attributes
157 of strawberries. A panel of 12 judges aged 20 - 35 years (eight females and four males, all
158 members of the School of Food Science and Environmental Health, DIT) was trained in
159 discriminate evaluation of strawberry. Before starting the sensory experiments, panellists
160 were familiarised with the product and scoring methods. This consisted of demonstration
161 exercises involving examination of strawberries at different levels of deterioration and
162 agreeing appropriate scores. After becoming familiar with the test facilities and scoring

163 regime, they were invited to score strawberry samples. This procedure was repeated several
164 times until a level of consistency in scoring was obtained. During this training, the samples
165 were presented to the panel to evaluate and measure the reproducibility of the judges' answer
166 and their capability in discriminating among samples. During the analyses, samples were
167 presented in randomised order to minimise possible sequence influence. DWP concentration
168 (3 %) and a control (water) treated strawberries were evaluated by the sensory panel during
169 storage. Colour, texture, aroma and general acceptability of samples were scored on a scale of
170 1 to 9, where a score of one indicated a product of very poor quality, etc (Ferreira *et al.*,
171 2008). The evaluation was carried out in the sensory evaluation laboratory. Products were
172 placed in plastic cups with lid, on a white surface and judges were isolated from each-other in
173 a booth in an odour-free environment. The results of the sensory analysis were reported as
174 means of three separate trials. Data were analysed using Compusense® software (Release
175 4.4, Ontario, Canada).

176 Nutritional markers

177 Ascorbic acid

178 The ascorbic acid content in strawberries was analysed by HPLC with a slight modification
179 of the method described by Lee and Castle (2001). A strawberry sample (2.5 g) was weighed
180 and 25 ml of 6 % metaphosphoric acid (pH 3.0) was added to it. The sample was then
181 homogenised for 1 min at 24,000 rpm using an Ultra-Turrax T-25 Tissue homogeniser. Then
182 the sample was shaken with a Gyrotory Shaker G-2 (USA) for 2 hrs at 150 rpm and
183 centrifuged for 15 min at 3,000 rpm at 4 °C (Sanio MSE Mistral 3000ii, UK). Following
184 centrifugation, 10 ml of the supernatant was filtered through PTFE syringe filters (pore size
185 0.45 µm, Phenomenex, UK) and stored at -20 °C in foil covered plastic test tubes for further
186 analysis by HPLC. The analysis of ascorbic acid content was performed with Waters 600
187 Satellite HPLC, with a reversed phase analytical 5 µm particle diameter, polymeric C₁₈

188 column (150 × 4.6 mm, 5 μm) (Waters, Ireland) with a UV-tuneable absorbance detector
189 (Waters 486) at 230 nm. Ten μl of the strawberry sample was injected. An isocratic mobile
190 phase of 25 mM monobasic potassium phosphate (pH 3.0) with a flow rate of 1.0 ml/min was
191 used. Five concentrations of ascorbic acid standard in 6 % metaphosphoric acid in the range
192 10 - 50 μg ml⁻¹ were injected and peak area and height were determined.

193 Total phenols

194 For extraction, 25 ml of methanol was added to 2.5 g of strawberry samples and homogenised
195 in a 50 ml tube with an Ultra-Turrax T-25 tissue homogeniser for 1 min at 24,000 rpm. The
196 samples were then thoroughly mixed with a vortex mixer (V400 Multitube Vortexer, Alpha
197 laboratories) for 2 hrs at 150 rpm. Then they were centrifuged for 15 min at 3,000 rpm using
198 a Sanyo MSE Mistral 3000i, UK. Following centrifugation, 10 ml samples of the supernatant
199 were filtered through PTFE syringe filters (pore size 0.45μm, Phenomenex, UK). Finally the
200 extracts were stored at -20 °C in foil covered plastic test tubes for further analysis. Total
201 phenol content of strawberries was determined using the Folin-Ciocalteu method (Singleton
202 *et al.*, 1999). In a 1.5 ml eppendorf tube, 100 μl of appropriately diluted methanolic extract,
203 100 μl of MeOH and 100 μl of FC reagent were added and vortexed. After exactly 1 min, 700
204 μl of sodium carbonate (20 %) was added, and the mixture was vortexed and allowed to stand
205 at room temperature in the dark for 20 min. Then the tubes were centrifuged at 13,000 rpm
206 for 3 min. The absorbance of the supernatant was read at 735 nm in 1 ml plastic cuvettes.
207 Each sample of the three batches was measured in triplicate. Results were expressed as mg l⁻¹
208 gallic acid equivalent (GAE).

209 Antioxidant activity test - ferric ion reducing antioxidant power assay (FRAP)

210 The FRAP assay was carried out as described by Stratil *et al.* (2006) with a slight
211 modification. The extraction for the FRAP assay was done as per the phenol content of

212 strawberry. The FRAP reagent was prepared by mixing 38 mM sodium acetate (anhydrous)
213 in distilled water (pH 3.6), 20 mM FeCl₃.6H₂O in distilled water and 10 mM 2,4,6-tri(2-
214 pyridyl)-s-triazine (TPTZ) in 40 mM HCl in proportions of 10:1:1. This reagent was freshly
215 prepared before each experiment. In a 1.5 ml eppendorf tube 100 µl of appropriately diluted
216 methanolic extract and 900 µl FRAP Reagent were added and vortex. After that they were
217 kept for 40 min in the heating blocks at 37 °C, covered with tin foil. The absorbance of the
218 supernatant was read at 593 nm in 1 ml plastic cuvettes. Each sample of the three batches was
219 measured in triplicate. Results were expressed as mg Trolox 100 g⁻¹ FW.

220 Microbiological markers

221 Decay incidences (%)

222 10 fruits of each treated and control samples were inspected at day 1, 4, 7 and 10 of storage
223 and the fruits were considered infected when a visible lesion was observed. The visible
224 microbial attack on the fruit was characterised as brown spots and a softening of the injured
225 zone. The results (with LSD mean comparison test) were expressed as the percentage of
226 infected fruit (Tanada-Palmu & Grosso, 2005).

227 Micro-organisms

228 Microbiology analyses were carried out on the treated and control samples during 10 days of
229 storage. 25 g of strawberries were blended in 225 ml of peptone saline with a Stomacher
230 circulator homogeniser. Enumeration and differentiation of total aerobic counts were
231 quantified at 30 °C in plate count agar (PCA) over 72 hrs. Yeast and moulds were quantified
232 at 25 °C in potato dextrose agar (PDA) over 72 hrs. The results were expressed as Log₁₀
233 colony forming units per gram (CFU/g).

234

235 Statistical analysis

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

240 **Results and discussion**

241 Quality markers

242 Headspace gas composition

243 Headspace gas (O_2 and CO_2) composition within strawberry packages significantly
244 ($p < 0.05$) changed over storage. Oxygen decreased from atmospheric levels (21 % -
245 packaging conditions) to values around 20 % at day 1 and levels around 16 % by day 10
246 (Figure 1). An increase in carbon dioxide was observed, from 0 % to 2 % in 24 hours and to
247 values around 5 % at the end of storage. These results were in agreement with previous
248 studies (Campaniello *et al.*, 2008; Hernandez-Munoz *et al.*, 2008). The DWP treatment did
249 not show any significant ($p > 0.05$) difference to the control samples for headspace gas
250 composition as the pattern of change was the same for both samples over time. Low storage
251 temperatures and modified atmospheres with elevated CO_2 levels are common tools for
252 avoiding, at least partially, mould growth and senescence, and extending fruit shelf-life.
253 However, prolonged exposure of strawberries to high CO_2 concentrations can cause off-
254 flavour development (Hernandez-Munoz *et al.*, 2008).

255 Firmness

256 Firmness of strawberries decreased rapidly during storage (Table 1). DWP treatment
257 markedly inhibited fruit softening and maintained significantly ($p < 0.05$) higher levels
258 of firmness throughout the storage compared to control. The firmness in DWP-treated fruits
259 was 15 % higher than that in control fruits at day 10. This result correlated with the sensory

260 panel scores for texture. Texture is a critical quality attribute in the consumer acceptability of
261 fresh fruit and vegetables. Strawberry is a soft fruit that suffers a rapid loss of firmness during
262 storage which contributes greatly to its short post-harvest life and susceptibility to fungal
263 contamination (Hernandez-Munoz *et al.*, 2008). The change in the texture of strawberries
264 after storage is related to the gravitational collapse of the cell due to the absence of turgor
265 with the corresponding loss of liquid and the senescence causes the alteration/softening of the
266 tissue (Castello *et al.*, 2010). The presence of calcium in the whey permeate might have
267 contributed to maintenance of this firmness of strawberry during storage (Evans *et al.*, 2010).
268 This effect of Ca-calcium can be explained by the formation of cross links between the
269 carboxyl groups of polyuronide chains found in the middle lamella of cell wall. Ca-Calcium
270 also increases cell turgor pressure and stabilises the cell membrane (Shafiee *et al.*, 2010).

271 Colour

272 Colour is an important factor in the perception of strawberry fruit quality. In the present
273 study, strawberries showed significant decrease ($p < 0.05$) in luminosity during storage.
274 This was in agreement with the findings of (Nunes *et al.*, 2005). There were no significant
275 ($p > 0.05$) differences in L* values between DWP treated and control samples (Table
276 1). But the parameters a* and b* were significantly ($p < 0.05$) affected by the DWP
277 treatment, both decreasing significantly ($p < 0.05$) during storage (data not shown). Hue
278 and chroma also decreased during storage and the decrease was more prominent in control
279 fruits. The greatest colour changes during storage occurred in control samples. This can be
280 associated with an acceleration of senescence, which caused a loss of intracellular liquid and
281 tissue collapse. The control fruits darkened and surface browning developed at the end of
282 storage. Anthocyanin degradation and oxidation of soluble phenolic compounds, caused
283 possibly by increased polyphenol oxidase (PPO) activity as a result of water loss, contributed
284 to the development of strawberry surface browning during storage (Nunes et al., 2005). The

285 [low pH of DWP might inhibit the PPO activity in treated strawberries therefore prevented](#)
286 [darkening \(Ahmed et al., 2011b\). Since the optimal pH for PPO activity is between 5 and 7,](#)
287 [acidification to low pH may inhibit, prevent, or minimize PPO activity \(Guerrero-Beltran et](#)
288 [al., 2005\).](#)

289 Sensory

290 All the attributes evaluated (such as, colour, aroma, texture and general acceptability)
291 decreased significantly ($p < 0.05$) during storage for both treatments which is associated
292 with a loss of quality (Figure 2). Significant differences ($p < 0.05$) were observed
293 between DWP treated and control samples for colour, aroma, texture and general
294 acceptability scores. DWP treated samples scored significantly higher ($p < 0.05$) than
295 the control samples. The panellists scored the aroma and colour of strawberries treated with
296 DWP was higher than the control samples. This was in agreement with most of the physico-
297 chemical markers of strawberries studied. The values at the end of the storage (10 days) were
298 above the acceptability threshold of 5 for all the attributes scored.

299 Nutritional markers

300 Ascorbic acid

301 The initial content of ascorbic acid in strawberries was found to be 85 mg/100 g FW ([fresh](#)
302 [weight](#)), which is within the reported range of 45 to 90 mg/100 g FW (Cao *et al.*, 2010;
303 Cordenunsi *et al.*, 2003). Significantly ($p < 0.05$) higher levels of ascorbic acid was
304 found in DWP treated samples compared to control samples at the end of storage. There was
305 a decrease in ascorbic acid content in strawberry fruit over storage (Figure 3A). Temperature
306 management after harvest is considered to be the most important factor in the maintenance of
307 ascorbic acid content in fruits and vegetables. It is commonly assumed that low temperature
308 has a protective effect on ascorbic acid content in fruits and vegetables, except for some

309 chilling-sensitive crops (Lee & Kader, 2000). In addition, phenolic substances have been
310 reported to have protective effects on the ascorbic acid (Ahmed *et al.*, 2011a).

311 Total phenols

312 Strawberries are good sources of natural antioxidants (Wang & Lin, 2000). In addition to the
313 usual nutrients, such as vitamins and minerals, strawberries are also rich in phenolic
314 compounds (Ayala-Zavala *et al.*, 2004). Phenols are the major antioxidant compounds in
315 plant extracts and might contribute 60 to 70 % antioxidant activity of extracts (Toor &
316 Savage, 2005). In the present study total phenol content of the DWP treated strawberries was
317 significantly ($p < 0.05$) higher than the control samples at the end of storage. The initial
318 concentration of total phenols in samples was 290 mg GAE/100 g FW (Figure 3B). This
319 value was in accordance with other studies (Allende *et al.*, 2007; Ayala-Zavala *et al.*, 2004).
320 Total phenol content of strawberries decreased over storage. Control samples decreased more
321 to a value of approx 30 mg GAE/100 g FW after 10 days of storage. Previous studies showed
322 that both temperature and storage time had a significant effect ($p < 0.05$) on total
323 phenolic compounds of strawberry fruits (Ayala-Zavala *et al.*, 2004).

324 Antioxidant activity test - ferric ion reducing antioxidant power assay (FRAP)

325 Ferric ion reducing antioxidant power (FRAP) is one of the most commonly used antioxidant
326 activity assay (Stratil *et al.*, 2006). DWP treated samples retained significantly
327 ($p < 0.05$) better antioxidant activity than control samples (Figure 3C). FRAP value of
328 strawberries decreased during storage in both samples. Strawberries have shown a
329 remarkably high scavenging activity toward chemically generated radicals, thus making them
330 effective in inhibiting oxidation of human low-density lipoproteins (Ayala-Zavala *et al.*,
331 2004). Wang and Lin (2000) reported that strawberries have high oxygen radical absorbance
332 activity against peroxy radicals (ROO^{\bullet}), superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide

333 (H₂O₂), hydroxyl radicals (OH^{*}), and singlet oxygen (¹O₂); and antioxidant activities were
334 different among varieties. The strawberries treated with DWP retained more nutrients during
335 storage than the control samples because of the protective effect from DWP and also because
336 the strawberries have been infused with the antioxidants of DWP during treatment. The
337 antioxidant activity (FRAP) of DWP solution was 179.86±1.2 mg Trolox/ L (Ahmed et al.,
338 2011a). There is a positive correlation between antioxidant activity and total phenols (Wang
339 & Lin, 2000). The total phenol value of DWP solution was 114.19±1.09 mg Gallic Acid/ L.

340 Microbiological markers

341 Decay incidences (%)

342 The decay incidence of strawberries treated with DWP were reduced significantly ($p < 0.05$)
343 compared to control fruit (Figure 4A). However, decay incidences of strawberry fruits
344 increased with storage time for both samples. At the end of 10 days of storage, 92 % of
345 control fruits showed visual signs of decay, while the DWP treated fruits showed decay
346 incidence of less than 22 %. This lower decay incidence result was well correlated with the
347 inhibited microbial populations in strawberries treated with DWP. Storage life of the
348 strawberry fruits was significantly ($p < 0.05$) increased by the use of DWP treatment.
349 The control fruits were not suitable to be exposed in the market more than 7 days of storage,
350 while fruits treated with DWP were still suitable to be exposed in the market after 10 days of
351 storage.

352 Micro-organisms

353 DWP treatment significantly ($p < 0.05$) affected the aerobic counts and yeast and
354 moulds of strawberry, resulting in a positive effect for the extension of the shelf-life. The
355 numbers of aerobic counts and yeast and moulds on strawberries were significantly
356 ($p < 0.05$) decreased by DWP treatment at day 0, when compared to the control fruit.

357 Strawberries stored at 5 °C had initial loads of total aerobic bacteria ~ 2.0 Log₁₀ CFU/g and
358 yeast and moulds ~ 2.2 Log₁₀ CFU/g. This result was in agreement with the finding of
359 Allende *et al.* (2007). Total aerobic counts and yeast and moulds on strawberries treated with
360 DWP were significantly ($p < 0.05$) lower than control fruits during the whole storage,
361 indicating that DWP treatment effectively reduced the microbial load and subsequently
362 improving the quality of the products. Strawberries treated with DWP showed ~ 1.4 Log₁₀
363 CFU/g (Figure 4B) and ~1.8 Log₁₀ CFU/g (Figure 4C) higher reduction in total aerobic
364 counts and yeast and moulds respectively, than the control samples after 10 days of storage.
365 However, the numbers of the micro-organisms increased during storage in both samples. This
366 increase was more obvious between days 7 and 10. The values of whey permeate treated
367 samples at the end of the storage were lower than the recommended 10⁸ CFU/g for consumer
368 consumption of fresh-cut vegetables (Alegria *et al.*, 2010). The presence of antimicrobial
369 peptides in the whey permeate might have contributed to its antimicrobial capacity (Clare &
370 Swaisgood, 2000). The amphipathic nature of these peptides presumably underlies their
371 biological activities which enables them to associate with lipid membranes and disrupt
372 normal membrane functions of bacteria (Saint-Sauveur *et al.*, 2008; Gauthier *et al.*, 2006).

373 **-Conclusion**

374 The post-harvest application of DWP significantly reduced the incidence of decay, microbial
375 population and maintained overall quality and antioxidant components of strawberries and
376 thereby extended the shelf-life of the fruits. The presence of antimicrobial peptides
377 (caseinmacropeptide or bacteriocins) in DWP might contribute to its antimicrobial capacity.
378 Although further investigations on pathogens are recommended, DWP treatment seems to be
379 a promising technique to extend the shelf-life of strawberries during cold storage.

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383 permeate, to Amcor Flexible Ltd. for providing OPP film and to Sharp Interpack for the
384 polypropylene trays.

385 **References**

386 Ahmed, L., Martin-Diana, A. B., Rico, D. & Barry-Ryan, C. (2011a). The antioxidant
387 properties of whey permeate treated fresh-cut tomatoes. *Food Chemistry*, **124**, 1451–
388 1457.

389 [Ahmed, L., Rico, D., Martin-Diana, A. B. & Barry-Ryan, C. \(2011b\). Extending the shelf-life
390 of fresh-cut tomato using by-product from cheese industry. *Journal of Food
391 Processing and Preservation*, DOI: 10.1111/j.1745-4549.2011.00562.x.](#)

392 [Ahmed, L., Martin-Diana, A. B., Rico, D. & Barry-Ryan, C. \(2011c\). Optimization of
393 Application of Delactosed Whey Permeate Treatment to Extend the Shelf-life of
394 Fresh-Cut Tomato using Response Surface Methodology. *Journal of Agricultural and
395 Food Chemistry*, **59**, 2377–2385.](#)

396 [Ahmed, L., Martin-Diana, A. B., Rico, D. & Barry-Ryan, C. \(2011d\). Quality and Nutritional
397 Status of Fresh-cut Tomato as Affected by Spraying of Delactosed Whey Permeate
398 Compared to Industrial Washing Treatment. *Food and Bioprocess Technology*, DOI:
399 \[10.1007/s11947-011-0623-6.\]\(#\)](#)

400 Alegria, C., Pinheiro, J., Gonçalves, E. M., Fernandes, I. & Moldão, M. (2010). Evaluation of
401 a pre-cut heat treatment as an alternative to chlorine in minimally processed shredded
402 carrot. *Innovative Food Science and Emerging Technologies*, **11**, 155–161.

403 Allende, A., Marin, A., Buendia, B., Tomas-Barberan, T. & Gil, M. I. (2007). Impact of
404 combined postharvest treatments (UV-C light, gaseous O₃, super atmospheric O₂ and

405 high CO₂) on health promoting compounds and shelf-life of strawberries. *Postharvest*
406 *Biology and Technology*, **46**, 201–211.

407 Ayala-Zavala, J. F., Wang, S. Y., Wang, C. Y. & Gonzalez-Aguilar, G. A. (2004). Effect of
408 storage temperatures on antioxidant capacity and aroma compounds in strawberry
409 fruit. *LWT - Food Science and Technology*, **37**, 687–695.

410 Campaniello, D., Evillacqua, A. B., Sinigaglia, M. & Corbo, M. R. (2008). Chitosan:
411 Antimicrobial activity and potential applications for preserving minimally processed
412 strawberries. *Food Microbiology*, **25**, 992–1000.

413 Cao, S., Hu, Z., Pang, B., Wang, H., Xie, H. & Wu, F. (2010). Effect of ultrasound treatment
414 on fruit decay and quality maintenance in strawberry after harvest. *Food Control*, **21**,
415 529–532.

416 Castelló, M. L., Fito, P. J. & Chiralt, A. (2010). Changes in respiration rate and physical
417 properties of strawberries due to osmotic dehydration and storage. *Journal of Food*
418 *Engineering*, **97**, 64–71.

419 Clare, D. A. & Swaisgood, H. E. (2000). Bioactive milk peptides (6). *Journal of Dairy*
420 *Science*, **83**, 1187–1195.

421 Contreras, M. del M., Hernández-Ledesma, B., Amigo, L., Martín-Álvarez, P. J. & Recio, I.
422 (2011). Production of antioxidant hydrolyzates from a whey protein concentrate with
423 thermolysin: Optimization by response surface methodology. *LWT - Food Science*
424 *and Technology*, **44**, 9–15.

425 Cordenunsi, B. R., Nascimento, J. R. O. & Lajolo, F.M. (2003). Physico-chemical changes
426 related to quality of five strawberry fruit cultivars during cool-storage. *Food*
427 *Chemistry*, **83**, 167–173.

- 428 Evans, J., Zulewska, J., Newbold, M., Drake, M. A. & Barbano, D. M. (2010). Comparison
429 of composition and sensory properties of 80 % whey protein and milk serum protein
430 concentrates. *Journal of Dairy Science*, **93**, 1824–1843.
- 431 Ferreira, V. O., Pinho, O., Amaral, M. & Martins, I. (2008). Application of blended-learning
432 strategies on sensory analysis teaching. In M. Munoz, I. Jelinek, and F. Ferreira
433 (Eds.). *Proceedings of the IASK International Conference Teaching and Learning*, pp.
434 262–270, Aveiro, Portugal.
- 435 Fitzgerald, R. J. & Murray, B. A. (2006). Bioactive peptides and lactic fermentations.
436 *International Journal of Dairy Technology*, **59**, 118-125.
- 437 Gauthier, S. F., Pouliot, Y. & Saint-Sauveur, D. (2006). Immunomodulatory peptides obtained
438 by the enzymatic hydrolysis of whey proteins. *International Dairy Journal*, **16**,
439 1315–1323.
- 440 [Guerrero-Beltran, J. A., Swanson, B. G. & Barbosa-Canovas, G. V. \(2005\). Inhibition of](#)
441 [polyphenoloxidase in mango puree with 4-hexylresorcinol, cysteine and ascorbic](#)
442 [acid. *LWT - Food Science and Technology*, **38**, 625–630.](#)
- 443 Heo, H. J. & Lee, C. Y. (2005). Strawberry and its anthocyanins reduce oxidative stress
444 induced apoptosis in PC₁₂ cells. *Journal of Agricultural and Food Chemistry*, **53**,
445 1984–1989.
- 446 Hernandez-Munoz, P., Almenar, E., Ocio, M. J. & Gavara, R. (2006). Effect of calcium dips
447 and chitosan coatings on postharvest life of strawberries (*Fragaria × ananassa*).
448 *Postharvest Biology and Technology*, **39**, 247–253.
- 449 Hernandez-Munoz, P., Almenar, E., Valle, V. del, Velez, D. & Gavara, R. (2008). Effect of
450 chitosan coating combined with postharvest calcium treatment on strawberry
451 (*Fragaria × ananassa*) quality during refrigerated storage. *Food Chemistry*, **110**, 428–
452 435.

453 Kitts, D. D. & Weiler, K. (2003). Bioactive proteins and peptides from food sources.
454 Applications of bioprocesses used in isolation and recovery. *Current Pharmaceutical*
455 *Design*, **9**, 1309–1323.

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

459 Lee, K. S. & Kader, A. A. (2000). Pre-harvest and postharvest factors influencing vitamin C
460 content of horticultural crops. *Postharvest Biology and Technology*, **20**, 207–220.

461 Martin-Diana, A. B., Rico, D., Frias, J. M., Mulcahy, J., Henehan, G. T. M. & Barry-Ryan, C.
462 (2006). Whey permeate as a bio-preservative for shelf life maintenance of fresh-cut
463 vegetables. *Innovative Food Science and Emerging Technology*, **7**, 112-123.

464 McCann, K. B., Shiell, B. J., Michalski, W. P., Lee, A., Wan, J., Roginski, H. & Coventry,
465 M.J. (2006). Isolation and characterization of a novel antibacterial peptide from
466 bovine α_{s1} -casein. *International Dairy Journal*, **16**, 316-323.

467 Nunes, M. C. N., Brecht, J. F., Morais, A. M. M. B. & Sargent, S. A. (2005). Possible
468 influences of water loss and polyphenol oxidase activity on anthocyanin content and
469 discolouration in fresh ripe strawberry (cv. Oso Grande) during storage at 1 °C.
470 *Journal of Food Science*, **70**, 79–84.

471 Nykänen, A., Lapveteläinen, A., Hietnen, R. M. & Kallio, H. (1998). The effect of acetic
472 acid, nisin-whey permeates sodium chloride and related combinations on aerobic plate
473 count and the sensory characteristics of rainbow trout. *LWT - Food Science and*
474 *Technology*, **3**, 286–290.

475 Pelayo, C., Ebeler, S. E. & Kader, A. A. (2003). Postharvest life and flavour quality of three
476 strawberry cultivars kept at 5 °C in air or air + 20 kpa CO₂. *Postharvest Biology and*
477 *Technology*, **27**, 171–183.

478 Rekika, D., Khanizadeh, S., Deschenes, M., Levasseur, A., Charles, M. T., Tsao, R. & Yang,
479 R. (2005). Antioxidant capacity and phenolic content of selected strawberry
480 genotypes. *Hortscience*, **40**, 1777–1781.

481 Rizzello, C. G., Losito, I., Gobbetti, M., Carbonara, T., De Bari, M. D. & Zambonin, P. G.
482 (2005). Antibacterial activities of peptides from the water-soluble extracts of Italian
483 cheese varieties. *Journal of Dairy Science*, **88**, 2348-2360.

484 Saint-Sauveur, D., Gauthier, S. F., Boutin, Y. & Montoni, A. (2008). Immunomodulating
485 properties of a whey protein isolate, its enzymatic digest and peptide fractions.
486 *International Dairy Journal*, **18**, 260–270.

487 Shafiee, M., Taghavi, T. S. & Babalar, M. (2010). Addition of salicylic acid to nutrient
488 solution combined with postharvest treatments (hot water, salicylic acid, and calcium
489 dipping) improved postharvest fruit quality of strawberry. *Scientia Horticulturae*, **124**,
490 40–45.

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

494 Stratil, P., Klejdus, B. & Kuban, V. (2006). Determination of total content of phenolic
495 compounds and their antioxidant activity in vegetables – evaluation of
496 spectrophotometric methods. *Journal of Agricultural and Food Chemistry*, **54**, 607-
497 616.

498 Tanada-Palmu, P. S. & Grosso, C. R. F. (2005). Effect of edible wheat gluten-based films and
499 coatings on refrigerated strawberry (*Fragaria × ananassa*) quality. *Postharvest
500 Biology and Technology*, **36**, 199–208.

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

503 Wang, S.Y. & Lin, H. S. (2000). Antioxidant activity in fruits and leaves of blackberry,
504 raspberry, and strawberry varies with cultivar and developmental stage. *Journal of*
505 *Agricultural and Food Chemistry*, **48**, 140–146.

506 Wang, S. Y., Feng, R. T., Lu, Y. J., Bowman, L. & Ding, M. (2005). Inhibitory effect on
507 activator protein-1, nuclear factor-kappaB, and cell transformation by extracts of
508 strawberries (*Fragaria x ananassa* Duch.). *Journal of Agricultural and Food*
509 *Chemistry*, **53**, 4187–4193.

510

511 **FIGURE LEGENDS**

512 **Figure 1.** Effect of DWP treatment on headspace gas composition in strawberries during 10
513 days of storage at 5 °C. Points designated on any curve by different letters are significantly
514 different ($p < 0.05$). Lowercase letters are used for comparisons during storage and
515 uppercase letters for treatment comparisons. Three independent trials were carried out in
516 triplicate.

517 **Figure 2.** Sensory evaluation of strawberries after DWP treatment and stored at 5 °C for 10
518 days. Three independent trials were carried out in triplicate. Colour (9 = bright red, 1 =
519 darkened); Aroma (9 = strawberry like, 1 = fermented); Texture (9 = very crispy, 1 = soft);
520 General acceptability (9 = excellent, 1 = poor).

521 **Figure 3.** Effect of DWP treatment on (A) ascorbic acid, (B) total phenols and (C)
522 antioxidant activity - FRAP in strawberries during 10 days of storage at 5 °C. Points
523 designated on any curve by different letters are significantly different ($p < 0.05$).
524 Lowercase letters are used for comparisons during storage and uppercase letters for treatment
525 comparisons. Three independent trials were carried out in triplicate.

526 **Figure 4.** Effect of DWP treatment on (A) decay incidence, (B) total aerobic counts and (C)
527 yeast and moulds in strawberries during 10 days of storage at 5 °C. Points designated on any
528 curve by different letters are significantly different ($p < 0.05$). Lowercase letters are
529 used for comparisons during storage and uppercase letters for treatment comparisons. Three
530 independent trials were carried out in triplicate.