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The Impact of Delactosed Whey Permeat 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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Summary

The aim of this study was to investigate the effect of delactosed whey permeate (DWP) treatment on antioxidant and physico-chemical properties of strawberries. Fresh strawberries treated with 3 % DWP were analyzed for different quality, nutritional and microbiological markers during 10 days of storage at 5 °C. The results showed that DWP treatment significantly reduced incidences of decay (70 %) and numbers of total aerobic counts (~1.4 Log_{10} CFU/g) and yeast and moulds (~1.8 Log_{10} CFU/g). DWP treatment also inhibited the loss of firmness (15 %) and maintained significantly (~~$p<0.05$~~$p\leq0.05$) higher levels of vitamin C, total phenols and antioxidant activity of strawberries. Sensory scores confirmed that the DWP treated strawberries retained a good appearance and overall quality. The aroma and colour attributes were not reduced during storage. These results suggest that DWP treatment has potential to extend the shelf-life and maintain the quality of strawberries during storage.

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

Introduction

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

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

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

Whey Permeate could be a promising natural bio-active alternative for the preservation of fresh produce (Ahmed *et al*., 2011a, b). The application of whey into other products would help the cheese industry to partially solve the problem of whey disposal. Whey and whey ultra-filtration permeate have been proposed to be used as a natural antioxidant in foods (Contreras *et al*., 2011). Whey protein and peptides are widely used as bioactive and nutritional ingredients in health and food products. Antimicrobial peptides have been identified from whey (Kitts & Weiler, 2003; McCann *et al.,* 2006). 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 & Murray, 2006).

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

Materials and methods

Sampling

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

Preparation of treatment solution

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

Processing and experimental setup

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

temperature. Processed strawberries were then pooled, mixed and ~ 200 grams placed in a polypropylene tray (180 mm length × 130 mm width × 25 mm depth) from Sharp Interpack Ltd., UK containing one layer of absorbent paper on the bottom (Fresh-R-Pax absorbent pads, Maxwell Chase Technologies, Atlanta). The principal ingredient in fresh-R-Pax absorbent pads is food grade sodium carboxymethyl cellulose (CMC), a common ingredient in ice-cream, sauces, low-fat foods, etc. The trays were then packaged in bags (200×320 mm^2) of 35 µm oriented polypropylene film (OPP) with permeability at 23 °C and 90 % RH of 3.3×10^{-12} mol/s/m^2/Pa for O_2 (Amcor Flexibles, UK). The packages were then heat-sealed under atmospheric conditions and stored at 5 °C for 10 days. ~~(Ahmed *et al.*, 2011)~~. ~~Three independent trials were carried out. Each experiment was conducted with 72 packages of strawberry and tested on day 1, 4, 7 and 10 (2 treatments × 3 replications × 3 batches × 4 days).~~

Markers analysis of strawberries

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

Quality markers

Headspace gas composition

Changes in O_2 and CO_2 concentration of the headspace of strawberry packages were monitored during the 10 days of storage. A Gaspace analyser (Systech Instruments, UK) was used to monitor O_2 and CO_2 levels. Gas extractions were performed with a hypodermic

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

Firmness

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

Colour

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

Sensory analysis

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

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

Nutritional markers

Ascorbic acid

The ascorbic acid content in strawberries was analysed by HPLC with a slight modification of the method described by Lee and Castle (2001). A strawberry sample (2.5 g) was weighed and 25 ml of 6 % metaphosphoric acid (pH 3.0) was added to it. The sample was then homogenised for 1 min at 24,000 rpm using an Ultra-Turrax T-25 Tissue homogeniser. Then the sample was shaken with a Gyratory Shaker G-2 (USA) for 2 hrs at 150 rpm and centrifuged for 15 min at 3,000 rpm at 4 °C (Sanio MSE Mistral 3000ii, UK). Following centrifugation, 10 ml of the supernatant was filtered through PTFE syringe filters (pore size 0.45 µm, Phenomenex, UK) and stored at -20 ºC in foil covered plastic test tubes for further analysis by HPLC. The analysis of ascorbic acid content was performed with Waters 600 Satellite HPLC, with a reversed phase analytical 5 µm particle diameter, polymeric C_{18}

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 Multituve 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 equivalents (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

strawberry. The FRAP reagent was prepared by mixing 38 mM sodium acetate (anhydrous) in distilled water (pH 3.6), 20 mM $FeCl_3.6H_2O$ in distilled water and 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl in proportions of 10:1:1. This reagent was freshly prepared before each experiment. In a 1.5 ml eppendorf tube 100 µl of appropriately diluted methanolic extract and 900 µl FRAP Reagent were added and vortex. After that they were kept for 40 min in the heating blocks at 37 °C, covered with tin foil. The absorbance of the supernatant was read at 593 nm in 1 ml plastic cuvettes. Each sample of the three batches was measured in triplicate. Results were expressed as mg Trolox 100 g^{-1} FW.

Microbiological markers

Decay incidences (%)

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

Micro-organisms

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

Statistical analysis

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

Results and discussion

Quality markers

Headspace gas composition

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

Firmness

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

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

Colour

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

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

Sensory

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

Nutritional markers

Ascorbic acid

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

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

Total phenols

Strawberries are good sources of natural antioxidants (Wang & Lin, 2000). In addition to the usual nutrients, such as vitamins and minerals, strawberries are also rich in phenolic compounds (Ayala-Zavala *et al*., 2004). Phenols are the major antioxidant compounds in plant extracts and might contribute 60 to 70 % antioxidant activity of extracts (Toor & Savage, 2005). In the present study total phenol content of the DWP treated strawberries was significantly (~~$p<0.05$~~$p\leq0.05$) higher than the control samples at the end of storage. The initial concentration of total phenols in samples was 290 mg GAE/100 g FW (Figure 3B). This value was in accordance with other studies (Allende *et al*., 2007; Ayala-Zavala *et al*., 2004). Total phenol content of strawberries decreased over storage. Control samples decreased more to a value of approx 30 mg GAE/100 g FW after 10 days of storage. Previous studies showed that both temperature and storage time had a significant effect (~~$p<0.05$~~$p\leq0.05$) on total phenolic compounds of strawberry fruits (Ayala-Zavala *et al*., 2004).

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

Ferric ion reducing antioxidant power (FRAP) is one of the most commonly used antioxidant activity assay (Stratil *et al*., 2006). DWP treated samples retained significantly (~~$p<0.05$~~$p\leq0.05$) better antioxidant activity than control samples (Figure 3C). FRAP value of strawberries decreased during storage in both samples. Strawberries have shown a remarkably high scavenging activity toward chemically generated radicals, thus making them effective in inhibiting oxidation of human low-density lipoproteins (Ayala-Zavala *et al*., 2004). Wang and Lin (2000) reported that strawberries have high oxygen radical absorbance activity against peroxyl radicals ($ROO^{\bullet}$), superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide

(H_2O_2), hydroxyl radicals ($OH^{\bullet}$), and singlet oxygen ($^1O_2$); and antioxidant activities were different among varieties. The strawberries treated with DWP retained more nutrients during storage than the control samples because of the protective effect from DWP and also because the strawberries have been infused with the antioxidants of DWP during treatment. The antioxidant activity (FRAP) of DWP solution was 179.86±1.2 mg Trolox/ L (Ahmed et al., 2011a). There is a positive correlation between antioxidant activity and total phenols (Wang & Lin, 2000). The total phenol value of DWP solution was 114.19±1.09 mg Gallic Acid/ L.

Microbiological markers

Decay incidences (%)

The decay incidence of strawberries treated with DWP were reduced significantly ($p < 0.05$) compared to control fruit (Figure 4A). However, decay incidences of strawberry fruits increased with storage time for both samples. At the end of 10 days of storage, 92 % of control fruits showed visual signs of decay, while the DWP treated fruits showed decay incidence of less than 22 %. This lower decay incidence result was well correlated with the inhibited microbial populations in strawberries treated with DWP. Storage life of the strawberry fruits was significantly (~~$p<0.05$~~$p\leq0.05$) increased by the use of DWP treatment. The control fruits were not suitable to be exposed in the market more than 7 days of storage, while fruits treated with DWP were still suitable to be exposed in the market after 10 days of storage.

Micro-organisms

DWP treatment significantly (~~$p<0.05$~~$p\leq0.05$) affected the aerobic counts and yeast and moulds of strawberry, resulting in a positive effect for the extension of the shelf-life. The numbers of aerobic counts and yeast and moulds on strawberries were significantly (~~$p<0.05$~~$p\leq0.05$) decreased by DWP treatment at day 0, when compared to the control fruit.

Strawberries stored at 5 °C had initial loads of total aerobic bacteria ~ 2.0 Log_{10} CFU/g and yeast and moulds ~ 2.2 Log_{10} CFU/g. This result was in agreement with the finding of Allende *et al*. (2007). Total aerobic counts and yeast and moulds on strawberries treated with DWP were significantly (~~$p<0.05$~~$p\leq0.05$) lower than control fruits during the whole storage, indicating that DWP treatment effectively reduced the microbial load and subsequently improving the quality of the products. Strawberries treated with DWP showed ~ 1.4 Log_{10} CFU/g (Figure 4B) and ~1.8 Log_{10} CFU/g (Figure 4C) higher reduction in total aerobic counts and yeast and moulds respectively, than the control samples after 10 days of storage. However, the numbers of the micro-organisms increased during storage in both samples. This increase was more obvious between days 7 and 10. The values of whey permeate treated samples at the end of the storage were lower than the recommended 10^8 CFU/g for consumer consumption of fresh-cut vegetables (Alegria *et al*., 2010). The presence of antimicrobial peptides in the whey permeate might have contributed to its antimicrobial capacity (Clare & Swaisgood, 2000). The amphipathic nature of these peptides presumably underlies their biological activities which enables them to associate with lipid membranes and disrupt normal membrane functions of bacteria (Saint-Sauveur *et al*., 2008; Gauthier *et al*., 2006).

Conclusion

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

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FIGURE LEGENDS

Figure 1. Effect of DWP treatment on headspace gas composition in strawberries during 10 days of storage at 5 °C. Points designated on any curve by different letters are significantly different (~~p<0.05~~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.

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

Figure 3. Effect of DWP treatment on (A) ascorbic acid, (B) total phenols and (C) antioxidant activity - FRAP in strawberries during 10 days of storage at 5 °C. Points designated on any curve by different letters are significantly different (~~p<0.05~~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.

Figure 4. Effect of DWP treatment on (A) decay incidence, (B) total aerobic counts and (C) yeast and moulds in strawberries during 10 days of storage at 5 °C. Points designated on any curve by different letters are significantly different (~~p<0.05~~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.