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Lubna Ahmed
Technological University Dublin, lubna.ahmed@tudublin.ie

Ana Belen Martin-Diana
Technological University Dublin, anabelen.martindiana@tudublin.ie

Daniel Rico
Technological University Dublin, daniel.rico@tudublin.ie

Catherine Barry-Ryan
Technological University Dublin, Catherine.Barryryan@tudublin.ie

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Full Title

Optimization of Application of Delactosed Whey Permeate Treatment to Extend the Shelf-life of Fresh-cut Tomato using Response Surface Methodology

Names(s) Authors(s)

Lubna Ahmed[a]*, Daniel Rico[b], Ana B. Martin-Diana[c] and Catherine Barry-Ryan[a]

Author Affiliation(s)

[a]School of Food Science and Environmental Health, Dublin Institute of Technology (DIT), Cathal Brugha Street, Dublin 1, Ireland.

[b]Food Technology Department, Public University Nevarra, Pamplona, Spain.

[c]Agricultural Technological Institute of Castilla and Leon. Government of Castilla and Leon, Finca Zamadueñas, Valladolid, Spain.

***Corresponding Author: Lubna Ahmed**, School of Food Science and Environmental Health, Dublin Institute of Technology (DIT), Cathal Brugha Street, Dublin 1, Ireland. Phone: 35314024442, Fax: +35314024495, e-mail: lahmed@dit.ie

ABSTRACT

Optimization of delactosed whey permeate (DWP) treatment for fresh-cut tomato was accomplished by evaluating different quality, nutritional and microbial markers. Response surface methodology was applied to obtain polynomial model equations. DWP concentration (0 - 5 %) and storage (0 - 10 days) were used as independent factors in order to optimize the process. The analyses showed that increases in DWP concentration extended the quality of the fresh-cut tomato significantly ($p<0.05$) by maintaining texture, antioxidant activity (FRAP) and controlling the spoilage during the storage. However, concentrations >3 % were scored unacceptable by the sensory panel due to perceived off-odours. DWP treatment also improved retention of ascorbic acid and lycopene over storage. The total aerobic counts and yeast and moulds were reduced by ~1.5 log cfu/ g and ~1.0 log cfu/ g respectively after 10 days of storage treated with 3 % DWP. Predicted models were highly significant ($p<0.05$) for all the markers studied in fresh-cut tomato with high regression coefficients (R^2) ranging from 0.79 to 0.99. The study recommends the use of DWP at a concentration of 3 % to extend the shelf-life of fresh-cut tomato by preserving its quality and antioxidant properties during storage.

KEY WORDS: Whey permeate; Fresh-cut; Tomato; Preservation; RSM.

INTRODUCTION

Whey permeate is a by-product generated in the production of whey protein concentrate from cheese whey. The main ingredients of whey permeate are water, lactose, peptides and minerals. Whey and whey ultra-filtrated permeate have been proposed for use as a natural antioxidant in foods (*1*). Whey protein and peptides are widely used as bioactive and nutritional ingredients in health and food products. Lactoferrin, α-lactalbumin and β-lactoglobulin are proteins with antimicrobial properties. Casein macropeptide (CMP), α_1- and α_2- caseins are further examples of whey antimicrobial peptides (*2*). Whey peptides exhibit a growing number of biological effects including anti-hypertensive, anti-cancer, hypocholesterolemic, opiodergic, and anti-microbial activities (*3*). Whey is used as a fermentation feedstock for the production of lactic acid, acetic acid, propionic acid, ethanol, and single cell protein, etc. (*4*). However, these applications still do not utilize all the whey produced and new uses for this by-product are needed. Their application into other products would help the cheese industry to partially solve the problem of whey disposal.

Continued growth in 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 (*6*). The marketing of fresh-cut vegetables is limited by their short shelf-life due to quick decline in post-processing quality. 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 (*7*). Therefore the use of a novel alternative with a low-cost and as effective as chlorine is desired by industry. In recent years interest is growing in the use of natural

67 products for the preservation of fresh-cut produce. Research and commercial applications
68 have shown that natural components could replace traditional washing agents (*8*). The
69 development of chlorine-free fruit and vegetable products enriched with natural bio-
70 products could contribute greatly to a new and growing market, where the consumers'
71 concerns about their health are met.
72 Tomato is one of the most widely used and versatile vegetable crops. It is consumed fresh
73 and also used to manufacture a wide range of processed products. The consumption of
74 tomatoes is currently considered as an indicator of good dietary habit and healthy life style.
75 This fruit has undoubtedly assumed the status of a food with functional properties,
76 considering the overwhelming epidemiological evidence for its capacity to reduce the risk
77 of chronic diseases such as cardiovascular disease and cancer (*9*). This protective function
78 is attributed to antioxidant compounds like lycopene and other carotenoids (pro-vitamin A,
79 beta-carotene), ascorbic acid, vitamin E and flavonoids (*10*).
80 Response surface methodology (RSM) is a statistical technique which allows the user to
81 identify optimal conditions for a selected response while minimizing the number of
82 experiments required. When many factors and interactions affect desired response, RSM is
83 an effective tool for optimizing the process. Central composite design (CCD) is the most
84 popular form of RSM as it has been utilized by a number of researchers to optimize various
85 food processing methods such as, steamer jet-injection, milling, extraction, fermentation,
86 etc. (*11, 12*). In the present study, RSM was used to model the effect of DWP concentration
87 and storage time on fresh-cut tomato. The aim of this paper is to optimize the use of DWP
88 to extend the shelf-life of fresh-cut tomato with optimum quality, nutritional and microbial
89 properties for the industry.
90 **MATERIALS AND METHODS**

Sampling

Irish vine ripened tomatoes (*Lycopersicon esculentum* L. Mill.) cv. Moneymaker were purchased from a local supermarket (Dunnes Stores). According to the grower, the tomato plants were grown commercially in a greenhouse with a 14 h light period from February until November. The aerial environment of the greenhouse and crop irrigation and nutrition were precisely controlled. The temperature of the greenhouse was 16-21 °C which is optimum for lycopene synthesis in tomato fruits. The tomatoes were then brought to the food processing lab and stored at 4 °C before processing.

Preparation of treatment solution

Delactosed whey permeate (liquid) were kindly supplied by Glanbia Ltd. Ingredients, Ireland. Delactosed whey permeate (DWP) was obtained after removal of lactose crystals from whey permeate. The total solid, proteins, moisture content and pH of DWP solution were 32.9 %, 0.16 %, 72 % and 5.0 respectively. DWP liquid was diluted to different concentrations (0 - 5 %) with distilled water.

Processing

Whole tomatoes were rinsed briefly in water prior to washing in order to avoid soil contamination. Washing treatment was performed by double treatment of DWP treatment solution (0 – 5 %). First the tomatoes were immersed in DWP solution (200 g tomatoes/L) for 1 min (with agitation). The tomatoes were sliced 6 mm in thickness with a commercial slicing machine (Maxwell chase MCT-25, Baltimore Innovations, UK). Secondly the DWP treatment solution (0 – 5 %) were sprayed over the sliced tomato. The tomatoes were then air-dried for 30 mins in RT. Processed tomatoes were then pooled, mixed and ~100 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 packed in bags (200×320 mm) 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 and 3.1×10^{-9} mol/s/m^2/Pa for CO_2 (Amcor Flexibles Europe-Brighouse, United Kingdom). The packages were then heat-sealed under atmospheric conditions and stored at 4 °C for 10 days (*6*).

Experimental design

RSM was used in this work to study the effects of two independent variables [DWP concentration (0 - 5 %) and storage time (0 - 10 days)] on different quality, nutritional and microbial markers (dependent variables) on fresh-cut tomato using the Design Expert Version 7.1.3 software (Stat-Ease, Inc., Minneapolis, MN). The experimental design was based on a central composite design (CCD). The data obtained from the CCD design was fitted with a second order polynomial equation. The equation was as follows:

$$Y = \beta_0 + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_i \sum_{j=i+1} \beta_{ij} X_i X_j \quad \text{..... (1)}$$

where Y is the predicted response; β_0 is a constant; β_i is the linear coefficient; β_{ii} is the quadratic coefficient, β_{ij} is the interaction coefficient; and Xi and Xj are independent variables. The adequacy of the model was determined by evaluating the lack of fit, coefficient of regression (R^2) and the Fisher test value (F-value) obtained from the analysis of variance (ANOVA). Statistical significance of the model and model variables was determined at the 5 % probability level ($p<0.05$). The software uses the quadratic model equation (1) to build response surfaces. The complete design consisted of 11 experimental points including three replications of the central point. The actual values of the factors for the experimental designs are given in Table 1.

Markers analysis of fresh-cut tomato

Different quality (headspace gas composition, dry matter, pH, texture, color changes and sensory analysis), nutritional (ascorbic acid, lycopene, total phenols, antioxidant activity as measured by FRAP) and microbial (total aerobic bacteria and yeast and moulds) markers were monitored throughout the 10 days of storage of fresh-cut tomato stored at 4 °C.

Quality 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 fresh-cut tomatoes. A Gaspace analyzer (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 (*5*).

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.

Texture

Four measurements were made on each slice, two in the outer pericarp and two in the radial pericarp, applying the force in the axial direction. The force necessary to cause a deformation of 3mm with a speed of 0.02 mm/s was recorded using a an Instron texture analyzer (Instron 4302 Universal Testing Machine, Canton MA, USA), with a 3.5 mm

diameter flat faced cylindrical probe. Only the central slice in the stack was used in the analyses. The firmness measurement was performed immediately after removing the slice from the storage chamber (at storage temperature). Data were analyzed with the Instron series IX software for Windows.

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 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*) color and the b* parameter measures the degree of yellow (+b*) or blue (-b*) color. 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 fresh-cut tomato. 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 fresh-cut tomato. Panelists were required to score changes in fresh appearance, texture, color, aroma and general acceptability. Before starting of sensory experiments, panelists were familiarized with the product and scoring methods. This consisted of demonstration exercises involving examination of fresh-cut tomatoes at different levels of deterioration and agreeing appropriate scores. After becoming familiar with the test facilities and scoring regime, they were invited to score samples. This procedure was repeated several times until a level of consistency in scoring was obtained.

The same packages were scored during the entire trial for sensory analysis (10 days). 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 randomized order to minimize possible sequence influence.

Three DWP concentration (1, 3 and 5 %) and a control (chlorine 120 ppm) treated fresh-cut tomatoes were evaluated by the sensory panel by the sensory panel at regular intervals during storage (1, 4, 7 and 10). Fresh appearance, color, texture, aroma and general acceptability 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. (*13*). 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 odor-free environment. The results of the sensory analysis were reported as means of three separate trials. Data were analyzed using Compusense® Five software (Release 4.4, Ontario, Canada).

Nutritional markers

Ascorbic acid

The ascorbic acid content in fresh-cut tomatoes was analyzed by HPLC with a slight modification of the method described by Lee and Castle (*14*). A tomato sample (2.5 g) was weighed and 25 ml of 6 % meta-phosphoric acid (pH 3.0) was added to it. The sample was homogenized for 1 min at 24,000 rpm using an Ultra-Turrax T-25 Tissue homogenizer. Then the sample was shaken with a Gyrotory Shaker G-2 (USA) for 2 hrs at 150 rpm and centrifuged for 15 min at 785 ×g at 4 °C) (Sanio MSE Mistral 3000ii, UK). Following centrifugation, 10 ml of the supernatant was filtered through PTFE syringe filters (pore size

210 0.45 µm, Phenomenex, UK) and stored at - 20 ºC in foil covered plastic test tubes for
211 further analysis by HPLC.
212 The analysis of ascorbic acid content was performed with Waters 600 Satellite HPLC, with
213 a reverse phase analytical polymeric C_{18} column (150 × 4.6 mm, 5 µm) (Waters, Ireland)
214 with a UV-tunable absorbance detector (Waters 486) at 245 nm. Ten µl of the sample was
215 injected. An isocratic mobile phase of 25 mM monobasic potassium phosphate (pH 3.0)
216 with a flow rate of 1.0 ml/min was used. Five concentrations of ascorbic acid standard in 6
217 % meta-phosphoric acid in the range 10 - 50 µg/ml were injected.
218 **Lycopene**
219 Ten grams of tomato samples were weighed and transferred into a 100 mL beaker (wrapped
220 with aluminum foil). A 50-ml volume of hexane-acetone-ethanol solution (2:1:1 v/v/v)
221 containing 2.5 % BHT was added to solubilize the lycopene (*15*). Following this the
222 samples were homogenized with an Ultra-Turrax T-25 tissue homogenizer for 1 min at
223 20,500 rpm. The samples were then shaken with a Gyrotory Shaker G-2 (USA) for 2 hrs at
224 150 rpm followed by 10 ml of distilled water was added and stirred for additional 10 min.
225 The polar and non-polar layers were separated, and the upper hexane layer was collected
226 and filtered through a 0.45 µm PVDF membrane filter. It was transferred to a new 15 ml
227 aluminum wrapped test tubes and kept at - 80 °C for analysis.
228 The analysis of lycopene was performed with Waters 600 Satellite HPLC, with a reverse
229 phase analytical polymeric C_{18} column (150 × 4.6mm, 5 µm) (Waters, Ireland) with a UV
230 tunable absorbance detector (Waters 486) for spectrometric peak. The lycopene peaks were
231 identified at 475 nm. An isocratic mobile phase of methyl t-butyl ether/methanol/ethyl
232 acetate (40:50:10, v/v) with a flow rate of 1 ml/min was used. The column temperature and
233 mobile phase was maintained at 25 ºC. Analyses were performed under dim light to prevent

234 sample degradation by photo-oxidation. Three concentrations of lycopene standard in the
235 range 0.01 - 0.03 mg/ml were injected.

236 **Total phenols**

237 For extraction, 1.25 g of tomato sample was weighed and 25 ml of methanol was added.
238 Following this the sample was homogenized in a 50 ml tube with an Ultra-Turrax T-25
239 tissue homogenizer for 1 min at 24,000 rpm. The samples were then thoroughly mixed with
240 a vortex mixer (V400 Multitude Vortexer, Alpha laboratories) for 2 hrs at 150 rpm. Then it
241 was centrifuged for 15 min at 785 ×g using a Sanyo MSE Mistral 3000i, UK. Following
242 centrifugation, 10 ml samples of the supernatant were filtered through PTFE syringe filters
243 (pore size 0.45µm, Phenomenex, UK). Finally the extracts were stored at – 20 °C in foil
244 covered plastic test tubes for further analysis.

245 Total phenol content of tomatoes was determined using the Folin-Ciocalteu method (*16*). In
246 a 1.5 ml Eppendorf tube, 100 µl of appropriately diluted methanolic extract, 100 µl of
247 MeOH and 100 µl of FC reagent were added and vortexed. After exactly 1 min, 700 µl of
248 sodium carbonate (20 %) was added, and the mixture was vortexed and allowed to stand at
249 room temperature in the dark for 20 min. Then the tubes were centrifuged at 14,737 ×g for
250 3 min. The absorbance of the supernatant was read at 735 nm in 1 ml plastic cuvettes.
251 Methanol was used in substitution of sample, undergoing the same procedure, for the blank
252 (MeOH + FCR + Na_2CO3). Each sample of the three batches was measured in triplicate.
253 Results were expressed as mg/L gallic acid equivalents (GAE).

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

255 The FRAP assay was carried out as described by Stratil et al. (*17*) with a slight
256 modification. Extraction was done same way as total phenol.

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 vortexed. 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.

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 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).

Validation of the model

The predictive performance of the developed models describing the combined effect DWP concentration (X_1) and storage time (X_2) on independent variables (quality, nutritional and microbiological markers) of fresh-cut tomato were validated in a separate set of selected conditions. The criterion used to characterize the fitting efficiency of the data to the model was the multiple correlation coefficients (R^2) and their average mean deviation (E , Eq. 2).

$$E(\%) = \frac{1}{n_e}\sum_{i=1}^{n}\left|\frac{V_E - V_P}{V_E}\right| \times 100 \quad \text{..... (2)}$$

where, n_e is the number of experimental data, V_E is the experimental value and V_P is the predicted value.

Statistical analysis

RSM was used to fit the experimental data to the quadratic polynomial equation to obtain coefficients of the equations. The model and statistical analyses and contour plots were analyzed using Design Expert, version 7.1.3 software (Stat-Ease, Inc., Minneapolis, MN). For comparison of DWP at optimum concentration with fresh-cut tomato in sensory analysis trials ANOVA (Multifactor and one-way) was performed to examine differences between treatment, storage time and interaction of both factors with each one of the variables studied. Means were compared by significant difference (LSD) test, at a significance level ($p<0.05$) using the Design Expert software.

RESULTS AND DISCUSSION

Quality markers

Headspace gas composition

Eqs. (2 and 3) described the models obtained for O_2 and CO_2 headspace composition. The models explained 99.33 % of variation of oxygen and 99.16 % of carbon dioxide due to the treatment effect of different concentrations (0 - 5) % of delactosed whey permeate and storage time (0 - 10 days). Significant linear effects ($p<0.05$) of storage were observed for oxygen. In case of carbon dioxide gas significant linear and quadratic effects ($p<0.05$) of storage were observed. DWP concentration did not affect significantly ($p>0.05$) the O_2 and CO_2 levels. The oxygen gas decreased and the carbon dioxide gas increased throughout storage, as expected. Oxygen decreased from atmospheric concentration (21 % - packaging conditions) to values around 14 % (Figure 1A) and carbon dioxide levels reached from 1 to 7 % at the end of the storage (Figure 1B).

303 $Y_{Oxygen} = 20.89017 - 0.76330\ X_2;\ R^2 = 99.33\ \%$ ……..…….. *(3)*

304 $Y_{Carbon\ dioxide} = 1.17682 + 0.29126\ X_2 + 0.030102\ X_2^2;\ R^2 = 99.16\ \%$ ……..…….. *(4)*

305 **pH**

306 The pH was significantly ($p<0.05$) affected by DWP concentration and storage time. The
307 polynomial model (Eq. 4) explained 84.17 % of pH data variation with these two factors. A
308 significant ($p<0.05$) linear effect of DWP concentration and storage were observed (Figure
309 1C). A general increase of pH was observed over storage, which could be due to an increase
310 in the bacterial growth (*18*). Similar results were found by Roura et al. (*19*), which
311 attributed the gradual increases in the pH values of spinach leaves and Swiss chard to the
312 microbial growth. DWP concentration had significantly ($p<0.05$) negative linear effect on
313 pH. Higher inhibition of bacterial growth with increased DWP concentrations could have
314 slowed down the increase of pH over storage.

315 $Y_{pH} = 4.52955 - 0.17759\ X_1 + 0.10786\ X_2;\ R^2 = 84.17\ \%$ ……..... *(5)*

316 **Texture**

317 The model (Eq. 5) explained 86.24 % of tomato texture variation. A significant ($p<0.05$)
318 decrease in texture was observed during storage (Figure 1D). DWP concentration affected
319 significantly ($p<0.05$) tomato firmness measurement.

320 $Y_{Texture} = 7.13840 + 0.39508\ X_1 - 0.53984\ X_2;\ R^2 = 86.24\ \%$ …..……..…… *(6)*

321 The presence of calcium in the whey permeates may have contributed to maintain the
322 firmness of tomato during storage (*20*). Calcium has positive effects of on the firmness of
323 fresh-cut fruits. Different calcium salts have been used for firmness improvement of fresh
324 fruits and vegetables. Calcium carbonate and calcium citrate are the main calcium salts
325 added to foods in order to enhance the nutritional value. Calcium chloride has been widely

used as preservative and firming agent in the fruit and vegetable industry for whole and fresh-cut commodities (*21*).

Color

The variations in color parameters (luminosity, a*, b*, Hue and Chroma) due to DWP concentration and storage time are shown in Table 2. The polymeric model explained 79.20 % of the variability of the luminosity due to the effect of concentration and storage time. Fresh-cut tomatoes showed significant decrease in luminosity during storage ($p<0.05$). This was in agreement with the findings of Lana et al. (*22*). The decrease in luminosity during the storage in fresh-cut tomato is attributed to the pigment break down, mainly carotenoids (*15*). There were no differences in L* values between DWP treatment concentrations.

A significant increase of a* was observed with increasing DWP concentrations. The model explained 87.59 % of the variability of a* due to the effect of DWP concentration and storage time. The parameter a* increased significantly ($p<0.05$) during storage. The a* value is an important parameter for red color development and the degree of ripening in tomato. Lana et al. (*22*) also showed increasing a* values of tomatoes during storage.

The b* values were analyzed through storage time in fresh-cut tomato enriched with different concentrations of DWP. The model explained 90.66 % of the changes of the b* value during storage. The parameter b* was not affected by DWP treatment concentrations. 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 (*23*).

Changes in Hue and Chroma were explained by 95.21 % and 85.32 % respectively by the model. The Hue and Chroma values were affected by the storage time. Hue has a negative correlation with the maturity of the tomato. As the tomatoes mature during storage, Hue

350 decreases. The concentration of DWP used did not induce significant ($p>0.05$) changes in
351 Hue and Chroma values.

352 **Sensory analysis**

353 All the attributes, fresh appearance, texture, aroma and general acceptability, except color
354 decreased significantly ($p<0.05$) during storage which is associated with a loss of quality
355 (Figure 2). However, the values at the end of the storage (10 days) were still above the
356 acceptability threshold of 5 for all the attributes scored. The non-hypoxic oxygen and
357 carbon-dioxide concentration in the packages might have helped to maintain acceptable
358 levels of color and aroma (*24*). Color increased during storage. The higher values for the
359 color parameter at the later stage of storage could be explained by the ripening of the fresh-
360 cut tomatoes during storage. Sensory scores of color was supported by the increased a*
361 value recorded by the colorimeter during storage of fresh-cut tomatoes. The treatments
362 affected significantly the sensory parameters of the samples. A significant ($p<0.05$)
363 reduction in aroma and general acceptability in samples treated with more than 3 % of
364 DWP concentrations was observed. The panelists considered best aroma of fresh-cut
365 tomatoes enriched with 3 % DWP. Samples treated with 3 % had significantly higher scores
366 for general acceptability and fresh appearance than samples treated with chlorine (control).
367 Other parameters evaluated by the sensory panel, such as, color had no significant
368 differences between treatments.

369 **Nutritional markers**

370 **Ascorbic acid**

371 The polynomial model explained 86.56 % of the variability of ascorbic acid due to storage
372 time and concentration of DWP (Eq. 6). The model predicted data showed in contour plots,
373 Figure 3A, where a significant ($p<0.05$) linear effect of the storage time was observed.

Ascorbic acid content is an indicator of quality in fresh-cut vegetables and considered one of the best sources of vitamin C by consumers. The initial (storage time 0) value of ascorbic acid was 19 mg/ 100 g FW. This is within the range of 6.96 to 21.23 mg/100 g FW for tomatoes as reported by Toor and Savage (*25*). The recovery of the method was 94.2 %. The LOD, LOQ and precision were <0.20 mg/100 g, <0.65 mg/100 g and 1.4 % respectively. Ascorbic acid content significantly (linearly) reduced during storage time. The highest ascorbic acid levels were found in 5 % DWP treated samples with no significant difference (p>0.05) using concentrations over 3 %.

$Y_{Ascorbic\ Acid} = 19.36484 + 0.12600\ X_1 - 0.45242\ X_2;\ R^2 = 86.56\ \%$ .... *(7)*

Lycopene

Lycopene content was evaluated throughout storage time at different DWP concentrations. The model for lycopene content with the two independent variables, storage and concentration of DWP is described in Eq. 7. A significant (p<0.05) linear effect of the storage time and quadratic effect of DWP concentration were observed (Figure 3B).

$Y_{Lycopene} = 3.83442 + 0.86401\ X_1 + 0.25972\ X_2 - 0.13375\ X_1^2;\ R^2 = 90.70\ \%$ .... *(8)*

Storage time was the most important factor affecting the samples. The lycopene content increased significantly (p<0.05) during storage. The increase in the lycopene concentration might be due to the biosynthesis of lycopene induced by ripening. DWP concentration also affected the lycopene content of the samples. The highest lycopene levels were found in 3 % DWP treated samples.

Total phenols

396 Model described in Eq. 8 explained 95.27 % of the total phenols. A significant ($p<0.05$)
397 linear effect of the storage time and quadratic effect of DWP concentrations on the total
398 phenol content was observed.

399 $Y_{Total\ Phenol} = 20.23503 + 1.19723\ X_1 - 0.32900\ X_2 - 0.17667\ X_1^2;\ R^2 = 95.27\ \%$ ..... (9)

400 Total phenol content (Figure 3C) of the samples significantly ($p<0.05$) decreased over
401 storage. The initial value of total phenols in samples was 20.3 mg GAE/100 g FW. This
402 result is in agreement with other studies (*25*). At the end of the storage the levels of total
403 phenols reached 17.8 mg GAE/100 g FW. Phenolics are the major antioxidant compounds
404 in plant extracts. Toor and Savage (*25*) reported that phenolic compounds might contribute
405 60 to 70% antioxidant activity of tomato extracts. The optimum DWP concentration was 3
406 % for total phenol retention of fresh-cut tomato.

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

408 Ferric ion reducing antioxidant power (FRAP) is one of the most commonly used
409 antioxidant capacity assay (*17*). The polynomial model explained 96.88 % (R^2) of the
410 variability of antioxidant activity as measured by FRAP due to storage time and DWP
411 treatment concentration.

412 $Y_{FRAP} = 82.11696 + 1.14875\ X_1 - 4.43818\ X_2 + 0.19422\ X_2^2;\ R^2 = 96.88\ \%$ ..... (10)

413 Figure 3D shows the variation of FRAP at different DWP concentrations and over storage
414 time. Storage had significant ($p<0.05$) linear and quadratic effects on the FRAP values of
415 fresh-cut tomatoes. Antioxidant activity as measured by FRAP decreased significantly
416 during storage. DWP concentrations showed only linear effect with significant increase
417 with increasing concentrations.

418 **Microbiological markers**

419 **Total aerobic counts**

Figure 4A shows a significant linear increase of total aerobic counts over storage time. The model described in Eq. 10 explained 96.91 % of aerobic load variation.

$$Y_{Total\ Aerobic\ Counts} = 6.39038 - 1.83525\ X_1 + 0.11953\ X_2 + 0.22859\ X_1^2;\ R^2 = 96.91\ \%\ \ldots.\ (11)$$

The initial loads of total aerobic counts were approximately 6.25 log CFU/g in fresh-cut tomatoes stored at 4 °C. DWP concentration also significantly ($p<0.05$) affected the aerobic counts of fresh-cut tomato (linear and quadratic effects), resulting in a positive effect for the extension of the shelf-life. DWP concentration (3 %) reduced (linear and quadratic effects) aerobic counts by ~1.5 log cfu/ g after 10 days of storage. DWP treatment of 3 % had similar microbial load values to chlorine over storage (data not shown).

The antimicrobial application of whey has received considerable attention. Whey antimicrobial properties have been reported widely in the literature but mainly based on the in vitro trials (*2, 26*). Although the mechanism of antimicrobial activity of whey permeate is still unknown, several have been proposed. 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 (*27*). Antimicrobial peptides have been identified from whey protein hydrolysates. The most studied are the lactoferrins. Additionally, a few antimicrobial peptides have been identified from α_{S1}-casein and α_{S2}-casein (*28*). These antimicrobial peptides act against different gram-positive and gram-negative bacteria (*Escherichia, Helicobacter, Listeria, Salmonella* and *Staphylococcus*), yeasts and filamentous fungi (*2, 26*). 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. The

444 mechanism of action has been investigated for whey antimicrobial peptides by Saint-
445 Sauveur et al. (*29*). The killing mechanism found for most peptides investigated consists of
446 attacks on the outer and inner membranes, ultimately resulting in lysis of the bacteria. The
447 disruption of normal membrane permeability is at least partly responsible for the
448 antibacterial mechanism of lactoferricins.

449 **Yeast and moulds**

450 The model described in Eq. 11 explained 96.62 % of yeast and moulds load variation. A
451 significant ($p<0.05$) linear increase of yeast and moulds over storage was observed. A
452 significant ($p<0.05$) reduction (linear and quadratic effects) with increasing DWP treatment
453 concentration occurred (Figure 4B).

454 $Y_{Yeast\ and\ Moulds} = 5.80510 - 1.23220\ X_1 + 0.40297\ X_2 - 0.099643\ X_1 \times X_2 + 0.18917\ X_1^2;\ R^2 =$

455 *96.62 %* ..... *(12)*

456 Fresh-cut tomatoes stored at 4 °C had initial loads of yeast and moulds approximately 5.59
457 log CFU/g. This result was in agreement with the finding of Prakash et al. (*30*) for diced
458 tomato. Yeast and moulds load increased in all the samples over storage. DWP treatment
459 reduced (3 %) yeast and moulds counts by ~1.0 log cfu/ g after 10 days of storage. The
460 values of DWP treated samples at the end of the storage were lower than the recommended
461 10^8 CFU/g for consumer consumption of fresh-cut vegetables (*7*).

462 **Validation of the model**

463 Despite some variations, results obtained from the validated predicted model and actual
464 experimental values showed that the established models reliably predicted the markers
465 studied. The predicted values were in close agreement with experimental values (Table 3)
466 and were found to be not significantly different at $p>0.05$ using a paired t-test. In addition
467 variations between the predicted and experimental values obtained for all the markers

468 studied were within acceptable error range as depicted by average mean deviation (E%,
469 Table 3). Therefore, the predictive performance of the established model may be considered
470 acceptable.
471 Application of the response surface methodology indicated the suitability of 3 % DWP as a
472 natural preservative ingredient to extend the shelf-life of fresh-cut tomato. Variations in
473 DWP concentration in the range evaluated (0 to 5 %) were critical in some of the markers
474 studied, such as, texture, sensory, aerobic counts and yeast and moulds. Higher DWP
475 concentrations maintained the quality better than lower concentrations, i.e. maintaining
476 texture, total aerobic counts and yeast and moulds. However, perceived off-odors due to
477 DWP addition over 3 %, and so the reduction of sensory scores in general acceptability,
478 suggested that the use of 3 % of DWP in order to obtain a balance between quality and
479 nutritional values. Also the naturally present antioxidants, such as ascorbic acid and
480 lycopene were retained best within the range of 3 to 5 % of DWP treatment. Further
481 research with pathogens to assess the efficacy of DWP as a natural preservative for fresh-
482 cut tomato is recommended.

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488

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576 **Figure captions**

577 Figure 1. Contour plots showing the effect of DWP concentration (0 – 5 %) and storage
578 time (0 – 10 days) on O_2 (A), CO_2 (B), pH (C) and texture (D) in fresh-cut tomato packaged
579 and stored at 4 °C.

580 Figure 2. Sensory evaluation of fresh-cut tomatoes packaged and stored for 10 days at 4 °C
581 and washed with 3 different concentrations of DWP and 120 ppm chlorine.

582 Figure 3. Contour plots showing the effect of DWP concentration (0 – 5 %) and storage
583 time (0 – 10 days) on Ascorbic acid (A), lycopene (B), TP (C) and antioxidant activity -
584 FRAP (D) in fresh-cut tomato packaged and stored at 4 °C.

585 Figure 4. Contour plots showing the effect of DWP concentration (0 – 5 %) and storage
586 time (0 – 10 days) on total aerobic counts (A) and yeast and moulds (B) in fresh-cut tomato
587 packaged and stored at 4 °C.

Table 1. Response surface methodology design

Points	DWP Concentration (%)	Storage (Days)
1	0.550253	3
2	5.5	3
3	5.5	0.171573
4	5.5	3
5	10.4497	3
6	9	1
7	2	1
8	9	5
9	5.5	3
10	2	5
11	5.5	5.82843

Table 2**.** Analysis of variance of the regression coefficients of the fitted quadratic equation for color.

Coefficient	L*	a*	b*	Hue	Chroma
β_0 (intercept)	44.5288	13.48	21.8602	57.0723	25.0536
Linear					
β_1 (Concentration)	0.280479 ^{ns}	0.0410426 ^{s}	0.302278 ^{ns}	0.483391 ^{ns}	0.0628907 ^{ns}
β_2 (Storage)	-0.28366 ^{s}	-0.0030048 ^{s}	-0.580779 ^{s}	-0.250071 ^{s}	-0.17084 ^{s}
Quadratic					
β_{11} (Concentration)	-0.0539062 ^{ns}	0.00307281^{ns}	-0.0234375 ^{ns}	-0.0614579 ^{ns}	0.00119798 ^{ns}
β_{22} (Storage)	0.00566294 ^{ns}	0.00651372 ^{ns}	-0.0234375 ^{ns}	-0.0357834 ^{ns}	0.00464286 ^{ns}
Cross product					
β_{12}	0.0075 ^{ns}	0.00214286 ^{ns}	0.00821429 ^{ns}	-0.00392857 ^{ns}	0.00464286 ^{ns}
R^2	79.21	87.60	90.66	95.21	85.32
P-value	0.0061	0.0008	0.0001	< 0.0001	0.0005

^{s} = significant at $p<0.05$
^{ns} = non-significant

Table 3. Experimental and predicted values and average mean deviation (E %) for all the markers studied of fresh-cut tomatoes treated with 3 % DWP at day 10.

Markers	Experimental Value	Predicted Value	E%
O_2 (%)	13.2	13.53	0.83
CO_2 (%)	7.2	7.01	0.88
pH	4.82	4.98	1.11
Firmness (N)	2.9	2.93	0.34
L*	43.19	42.62	0.44
a*	14.36	14.25	0.26
b*	17.93	18.39	0.86
Hue	52.5	52.01	0.31
Chroma	23.08	23.34	0.38
Ascorbic acid (mg/ 100 g FW)	16.22	16.87	1.34
Lycopene (mg/ 100 g FW)	6.86	6.99	0.63
TP (mg Gallic acid/ 100 g FW)	18.2	18.09	0.20
FRAP (mg Trolox/ 100 g FW)	63.11	63.25	0.07
Total aerobic counts (log cfu/ g)	7.18	6.88	1.39
Yeast and moulds (log cfu/ g)	7.38	7.34	0.18

Figure 1

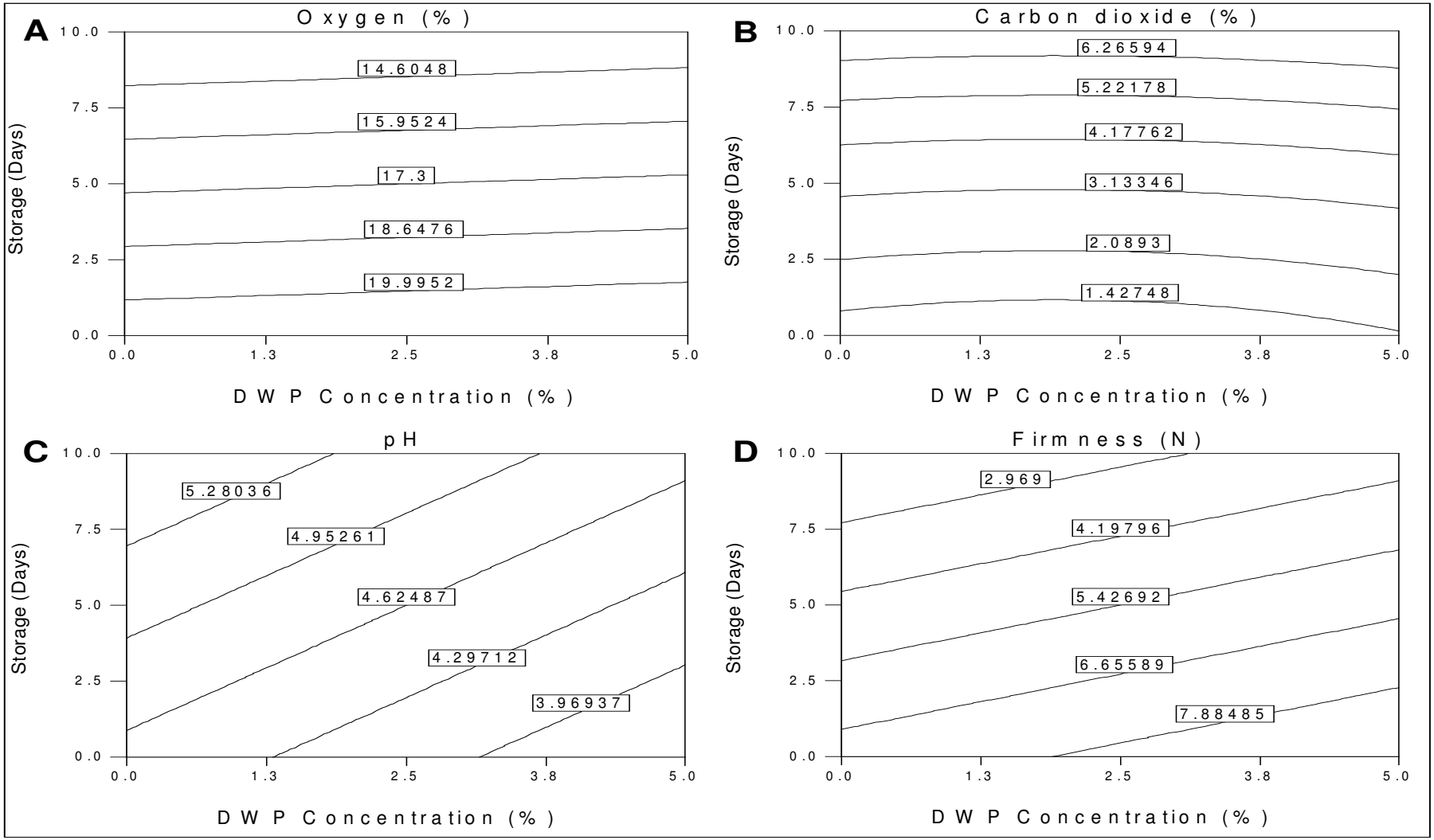

A
Oxygen (%)
Storage (Days)
10.0
7.5
5.0
2.5
0.0
14.6048
15.9524
17.3
18.6476
19.9952
0.0
1.3
2.5
3.8
5.0
DWP Concentration (%)
B
Carbon dioxide (%)
6.26594
5.22178
4.17762
3.13346
2.0893
1.42748
C
pH
5.28036
4.95261
4.62487
4.29712
3.96937
D
Firmness (N)
2.969
4.19796
5.42692
6.65589
7.88485

Figure 2

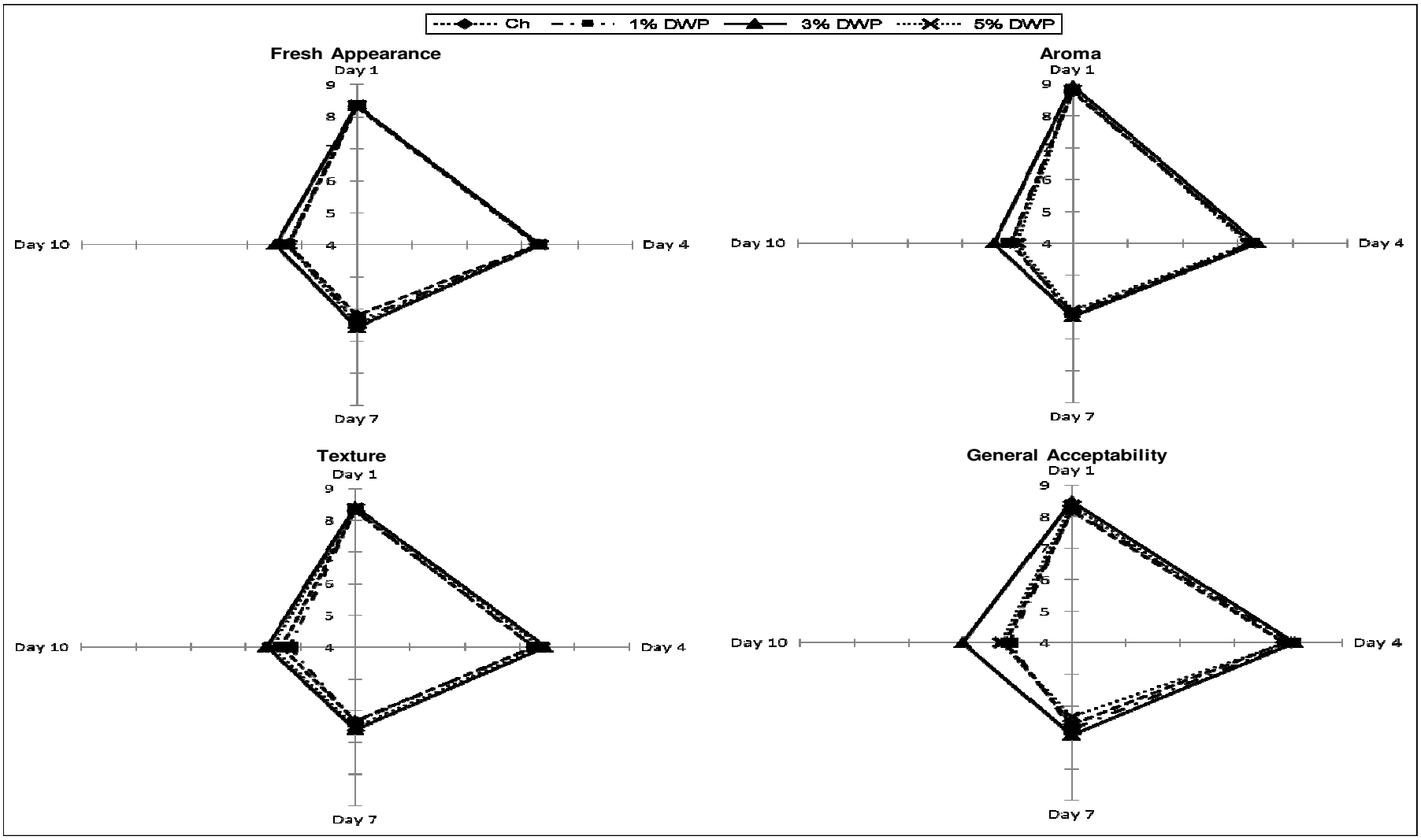

Ch
1% DWP
3% DWP
5% DWP
Fresh Appearance
Aroma
Texture
General Acceptability
Day 1
Day 4
Day 7
Day 10
9
8
6
5
4

Figure 3

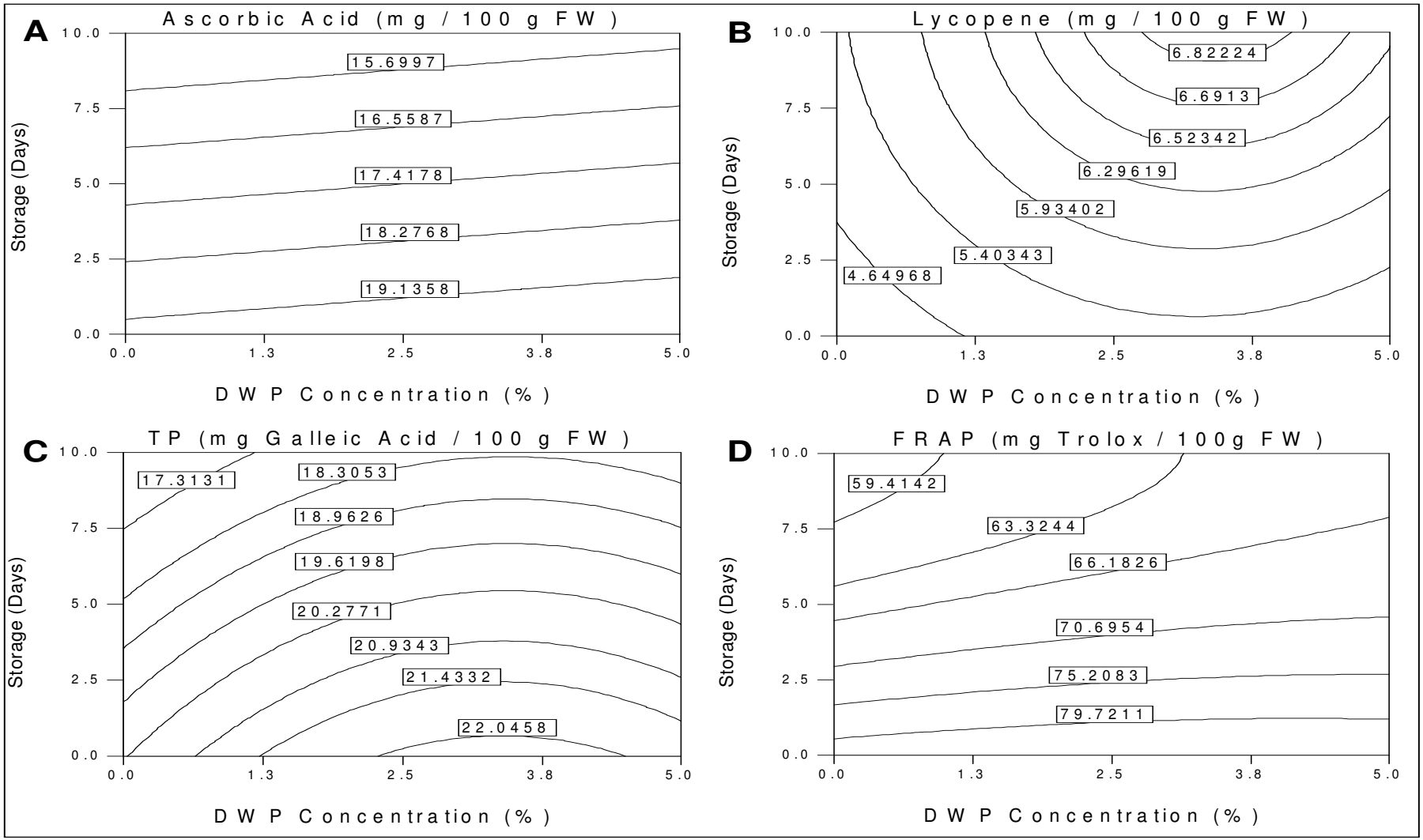
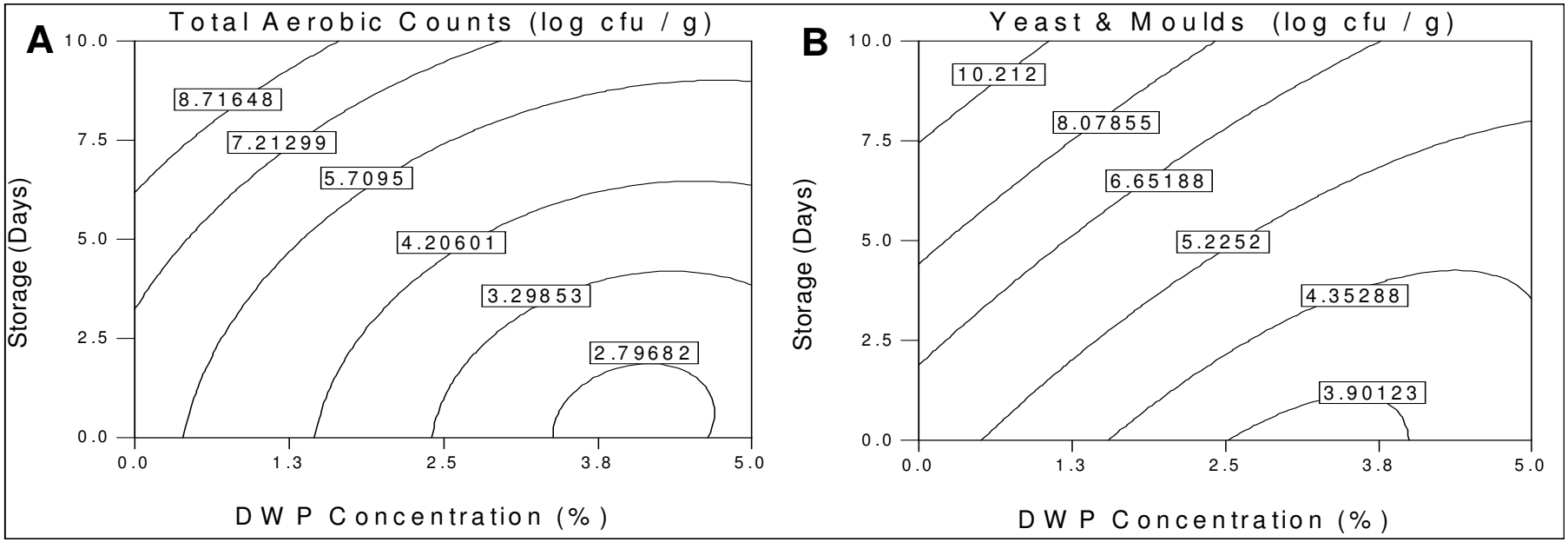

A
Ascorbic Acid (mg / 100 g FW)
15.6997
16.5587
17.4178
18.2768
19.1358
B
Lycopene (mg / 100 g FW)
6.82224
6.6913
6.52342
6.29619
5.93402
5.40343
4.64968
C
TP (mg Galleic Acid / 100 g FW)
17.3131
18.3053
18.9626
19.6198
20.2771
20.9343
21.4332
22.0458
D
FRAP (mg Trolox / 100g FW)
59.4142
63.3244
66.1826
70.6954
75.2083
79.7211
Storage (Days)
DWP Concentration (%)
0.0
1.3
2.5
3.8
5.0
2.5
5.0
7.5
10.0

Figure 4


A
Total Aerobic Counts (log cfu / g)
8.71648
7.21299
5.7095
4.20601
3.29853
2.79682
Storage (Days)
10.0
7.5
5.0
2.5
0.0
0.0
1.3
2.5
3.8
5.0
DWP Concentration (%)
B
Yeast & Moulds (log cfu / g)
10.212
8.07855
6.65188
5.2252
4.35288
3.90123
Storage (Days)
DWP Concentration (%)