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Lubna Ahmed

Dublin Institute of Technology, lubna.ahmed@dit.ie

Ana Belen Martin-Diana

Dublin Institute of Technology, anabelen.martindiana@dit.ie

Daniel Rico

Dublin Institute of Technology, daniel.rico@dit.ie

Catherine Barry-Ryan

Dublin Institute of Technology, Catherine.Barryryan@dit.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)

^aSchool of Food Science and Environmental Health, Dublin Institute of Technology (DIT), Cathal Brugha Street, Dublin 1, Ireland.

^bFood Technology Department, Public University Navarra, Pamplona, Spain.

^cAgricultural 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

24 **ABSTRACT**

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

40

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

42

43 INTRODUCTION

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

57 Continued growth in ready-to-eat vegetable industry has been largely driven by increasing
58 demand for convenient, fresh and healthy foods. Increasing the quality retention and shelf-
59 life of these products during storage is an important demand of the industry and consumers
60 (6). The marketing of fresh-cut vegetables is limited by their short shelf-life due to quick
61 decline in post-processing quality. Chlorinated water (50–200 ppm) is widely used to wash
62 fruits and vegetables as well as fresh-cut produce in order to preserve their quality.
63 However, the possible formation of carcinogenic chlorinated compounds in water
64 (chloramines and trihalomethanes) has called into question the use of chlorine for this
65 purpose (7). Therefore the use of a novel alternative with a low-cost and as effective as
66 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**

91 **Sampling**

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

99 **Preparation of treatment solution**

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

105 **Processing**

106 Whole tomatoes were rinsed briefly in water prior to washing in order to avoid soil
107 contamination. Washing treatment was performed by double treatment of DWP treatment
108 solution (0 – 5 %). First the tomatoes were immersed in DWP solution (200 g tomatoes/L)
109 for 1 min (with agitation). The tomatoes were sliced 6 mm in thickness with a commercial
110 slicing machine (Maxwell chase MCT-25, Baltimore Innovations, UK). Secondly the DWP
111 treatment solution (0 – 5 %) were sprayed over the sliced tomato. The tomatoes were then
112 air-dried for 30 mins in RT. Processed tomatoes were then pooled, mixed and ~100 grams
113 placed in a polypropylene tray (180 mm length×130 mm width×25 mm depth) from Sharp
114 Interpack Ltd., UK containing one layer of absorbent paper on the bottom (Fresh-R-Pax

115 absorbent pads, Maxwell Chase Technologies, Atlanta). The principal ingredient in fresh-R-
116 Pax absorbent pads is food grade sodium carboxymethyl cellulose (CMC), a common
117 ingredient in ice-cream, sauces, low-fat foods, etc. The trays were then packed in bags
118 (200×320 mm) of 35 µm oriented polypropylene film (OPP) with permeability at 23 °C and
119 90 % RH of 3.3×10^{-12} mol/s/m²/Pa for O₂ and 3.1×10^{-9} mol/s/m²/Pa for CO₂ (Amcor
120 Flexibles Europe-Brighthouse, United Kingdom). The packages were then heat-sealed under
121 atmospheric conditions and stored at 4 °C for 10 days (6).

122 **Experimental design**

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

$$129 \quad Y = \beta_0 + \sum_{i=1}^2 \beta_i X_i + \sum_{i=1}^2 \beta_{ii} X_i^2 + \sum_i \sum_{j=i+1} \beta_{ij} X_i X_j \dots\dots\dots (1)$$

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

139 **Markers analysis of fresh-cut tomato**

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

144 **Quality markers**

145 **Headspace gas composition**

146 Changes in O₂ and CO₂ concentration of the headspaces of the fresh-cut tomatoes packages
147 were monitored during the shelf-life of fresh-cut tomatoes. A Gaspacer analyzer (Systech
148 Instruments, UK) was used to monitor O₂ and CO₂ levels. Gas extractions were performed
149 with a hypodermic needle, inserted through an adhesive septum previously fixed to the
150 bags, at a flow rate of 150 ml/min for 10 sec. Three bags per treatment were monitored for
151 each experiment and all bags for other analyses were checked before analysis (5).

152 **pH**

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

155 **Moisture Content**

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

158 **Texture**

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

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

167 **Color**

168 For color analysis each piece of tomato in the storage pack was analyzed individually to
169 minimize the variability of the product. Color was quantified using a Color Quest XE
170 colorimeter (HunterLab, Northants, UK). A tomato slice was placed directly on the
171 colorimeter sensor (3.5 cm of diameter) and measured. 20 – 30 measurements were taken
172 per treatment and day. The L* parameter (lightness index scale) range from 0 (black) to 100
173 (white). The a* parameter measures the degree of red (+a*) or green (-a*) color and the b*
174 parameter measures the degree of yellow (+b*) or blue (-b*) color. The CIE L* a* b*
175 parameters were converted to Hue ($\arctan b^*/a^*$) and Chroma $(a^{*2}+b^{*2})^{1/2}$.

176 **Sensory analysis**

177 Analytical descriptive tests were used to discriminate between the sensory quality attributes
178 of fresh-cut tomato. A panel of 12 judges aged 20 - 35 years (eight females and four males,
179 all members of the School of Food Science and Environmental Health, DIT) was trained in
180 discriminate evaluation of fresh-cut tomato. Panelists were required to score changes in
181 fresh appearance, texture, color, aroma and general acceptability. Before starting of sensory
182 experiments, panelists were familiarized with the product and scoring methods. This
183 consisted of demonstration exercises involving examination of fresh-cut tomatoes at
184 different levels of deterioration and agreeing appropriate scores. After becoming familiar
185 with the test facilities and scoring regime, they were invited to score samples. This
186 procedure was repeated several times until a level of consistency in scoring was obtained.

187 The same packages were scored during the entire trial for sensory analysis (10 days).
188 During this training, the samples were presented to the panel to evaluate and measure the
189 reproducibility of the judges' answer and their capability in discriminating among samples.
190 During the analyses, samples were presented in randomized order to minimize possible
191 sequence influence.

192 Three DWP concentration (1, 3 and 5 %) and a control (chlorine 120 ppm) treated fresh-cut
193 tomatoes were evaluated by the sensory panel by the sensory panel at regular intervals
194 during storage (1, 4, 7 and 10). Fresh appearance, color, texture, aroma and general
195 acceptability of samples were scored on a hedonic scale of 1 to 9, where a score of one
196 indicated a product of very poor quality, etc. (13). The evaluation was carried out in the
197 sensory evaluation laboratory. Products were placed in plastic cups with lid, on a white
198 surface and judges were isolated from each-other in a booth in an odor-free environment.
199 The results of the sensory analysis were reported as means of three separate trials. Data
200 were analyzed using Compusense® Five software (Release 4.4, Ontario, Canada).

201 **Nutritional markers**

202 **Ascorbic acid**

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

210 0.45 μm , Phenomenex, UK) and stored at - 20 $^{\circ}\text{C}$ 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 \times 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 $\mu\text{g/ml}$ 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 $^{\circ}\text{C}$ 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 \times 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 $^{\circ}\text{C}$. 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 + Na₂CO₃). 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.

257 The FRAP reagent was prepared by mixing 38 mM sodium acetate (anhydrous) in distilled
258 water pH 3.6, 20 mM FeCl₃.6H₂O in distilled water and 10 mM 2,4,6-tri(2-pyridyl)-s-
259 triazine (TPTZ) in 40 mM HCl in proportions of 10:1:1. This reagent was freshly prepared
260 before each experiment. In a 1.5 ml Eppendorf tube 100 µl of appropriately diluted
261 methanolic extract and 900 µl FRAP Reagent were added and vortexed. After that they
262 were kept for 40 min in the heating blocks at 37 °C, covered with tin foil. The absorbance
263 of the supernatant was read at 593 nm in 1 ml plastic cuvettes. Each sample of the three
264 batches was measured in triplicate.

265 **Microbiological markers**

266 Microbiology analyses were carried out on the samples before and after the treatment at
267 regular intervals through the storage period. 25 g of tomatoes were blended in 225 ml of
268 peptone saline with a Stomacher circulator homogenizer. Enumeration and differentiation
269 of total aerobic counts were quantified at 30 °C in plate count agar (PCA) over 72 hrs. Yeast
270 and moulds were quantified at 25 °C in potato dextrose agar (PDA) over 72 hrs. The results
271 were expressed as log₁₀ colony forming units per gram (CFU/g).

272 **Validation of the model**

273 The predictive performance of the developed models describing the combined effect DWP
274 concentration (X₁) and storage time (X₂) on independent variables (quality, nutritional and
275 microbiological markers) of fresh-cut tomato were validated in a separate set of selected
276 conditions. The criterion used to characterize the fitting efficiency of the data to the model
277 was the multiple correlation coefficients (R²) and their average mean deviation (E , Eq. 2).

$$278 \quad E(\%) = \frac{1}{n_e} \sum_{i=1}^n \left\| \frac{V_E - V_P}{V_E} \right\| \times 100 \dots\dots\dots (2)$$

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

281 **Statistical analysis**

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

290 **RESULTS AND DISCUSSION**

291 **Quality markers**

292 **Headspace gas composition**

293 Eqs. (2 and 3) described the models obtained for O_2 and CO_2 headspace composition. The
294 models explained 99.33 % of variation of oxygen and 99.16 % of carbon dioxide due to the
295 treatment effect of different concentrations (0 - 5) % of delactosed whey permeate and
296 storage time (0 - 10 days). Significant linear effects ($p < 0.05$) of storage were observed for
297 oxygen. In case of carbon dioxide gas significant linear and quadratic effects ($p < 0.05$) of
298 storage were observed. DWP concentration did not affect significantly ($p > 0.05$) the O_2 and
299 CO_2 levels. The oxygen gas decreased and the carbon dioxide gas increased throughout
300 storage, as expected. Oxygen decreased from atmospheric concentration (21 % - packaging
301 conditions) to values around 14 % (Figure 1A) and carbon dioxide levels reached from 1 to
302 7 % at the end of the storage (Figure 1B).

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

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

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

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

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

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

328 **Color**

329 The variations in color parameters (luminosity, a^* , b^* , Hue and Chroma) due to DWP
330 concentration and storage time are shown in Table 2. The polymeric model explained 79.20
331 % of the variability of the luminosity due to the effect of concentration and storage time.
332 Fresh-cut tomatoes showed significant decrease in luminosity during storage ($p < 0.05$). This
333 was in agreement with the findings of Lana et al. (22). The decrease in luminosity during
334 the storage in fresh-cut tomato is attributed to the pigment break down, mainly carotenoids
335 (15). There were no differences in L^* values between DWP treatment concentrations.

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

341 The b^* values were analyzed through storage time in fresh-cut tomato enriched with
342 different concentrations of DWP. The model explained 90.66 % of the changes of the b^*
343 value during storage. The parameter b^* was not affected by DWP treatment concentrations.
344 The decreasing trend of b^* values throughout the storage showed that the fresh-cut
345 tomatoes did not have any chilling injury stored at 4 °C as it is the optimum storage
346 temperature of fresh-cut fruits and vegetables (23).

347 Changes in Hue and Chroma were explained by 95.21 % and 85.32 % respectively by the
348 model. The Hue and Chroma values were affected by the storage time. Hue has a negative
349 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.

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

382 $Y_{Ascorbic\ Acid} = 19.36484 + 0.12600 X_1 - 0.45242 X_2; R^2 = 86.56 \% \dots\dots\dots (7)$

383 **Lycopene**

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

388 $Y_{Lycopene} = 3.83442 + 0.86401 X_1 + 0.25972 X_2 - 0.13375 X_1^2; R^2 = 90.70 \%$
 389 $\dots\dots\dots (8)$

390 Storage time was the most important factor affecting the samples. The lycopene content
 391 increased significantly ($p<0.05$) during storage. The increase in the lycopene concentration
 392 might be due to the biosynthesis of lycopene induced by ripening. DWP concentration also
 393 affected the lycopene content of the samples. The highest lycopene levels were found in 3
 394 % DWP treated samples.

395 **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.

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

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 % (R²) of the
410 variability of antioxidant activity as measured by FRAP due to storage time and DWP
411 treatment concentration.

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

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**

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

$$422 \quad Y_{Total\ Aerobic\ Counts} = 6.39038 - 1.83525 X_1 + 0.11953 X_2 + 0.22859 X_1^2; R^2 = 96.91 \% \dots (11)$$

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

429 The antimicrobial application of whey has received considerable attention. Whey
430 antimicrobial properties have been reported widely in the literature but mainly based on the
431 in vitro trials (2, 26). Although the mechanism of antimicrobial activity of whey permeate is
432 still unknown, several have been proposed. The most likely factor is the acid pH of the
433 wash treatment which can have a direct effect on the initial microbial count reduction and
434 on subsequent growth during storage. Another factor can be the presence of lactic acid,
435 which can enter the cells in an un-dissociated form. And finally, the presence of
436 antibacterial peptides in the whey permeate might contribute to its antimicrobial capacity
437 (27). Antimicrobial peptides have been identified from whey protein hydrolysates. The
438 most studied are the lactoferrins. Additionally, a few antimicrobial peptides have been
439 identified from α_{S1} -casein and α_{S2} -casein (28). These antimicrobial peptides act against
440 different gram-positive and gram-negative bacteria (*Escherichia*, *Helicobacter*, *Listeria*,
441 *Salmonella* and *Staphylococcus*), yeasts and filamentous fungi (2, 26). The amphipathic
442 nature of these peptides presumably underlies their biological activities which enables them
443 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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- 575

576 **Figure captions**

577 Figure 1. Contour plots showing the effect of DWP concentration (0 – 5 %) and storage
578 time (0 – 10 days) on O₂ (A), CO₂ (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

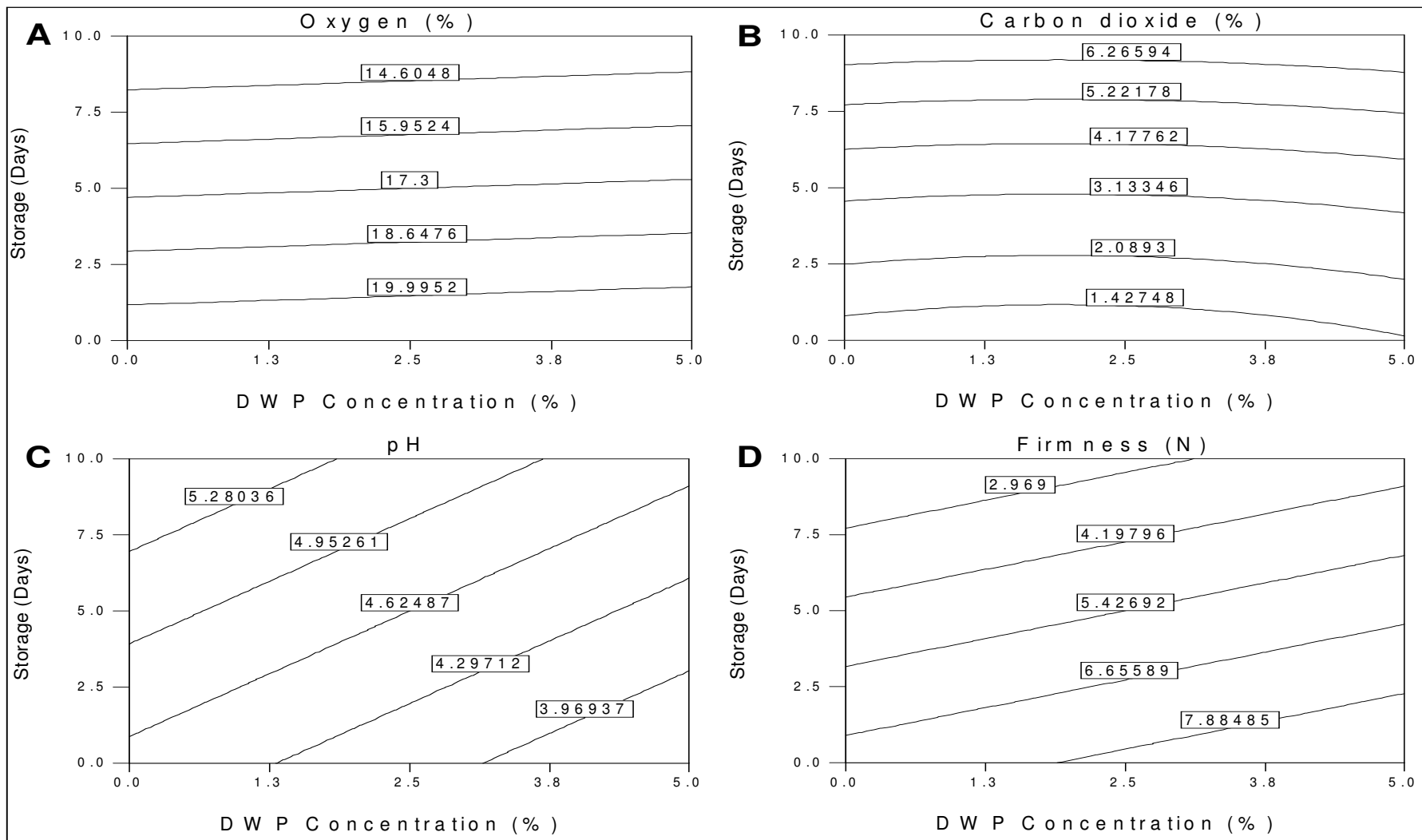


Figure 2

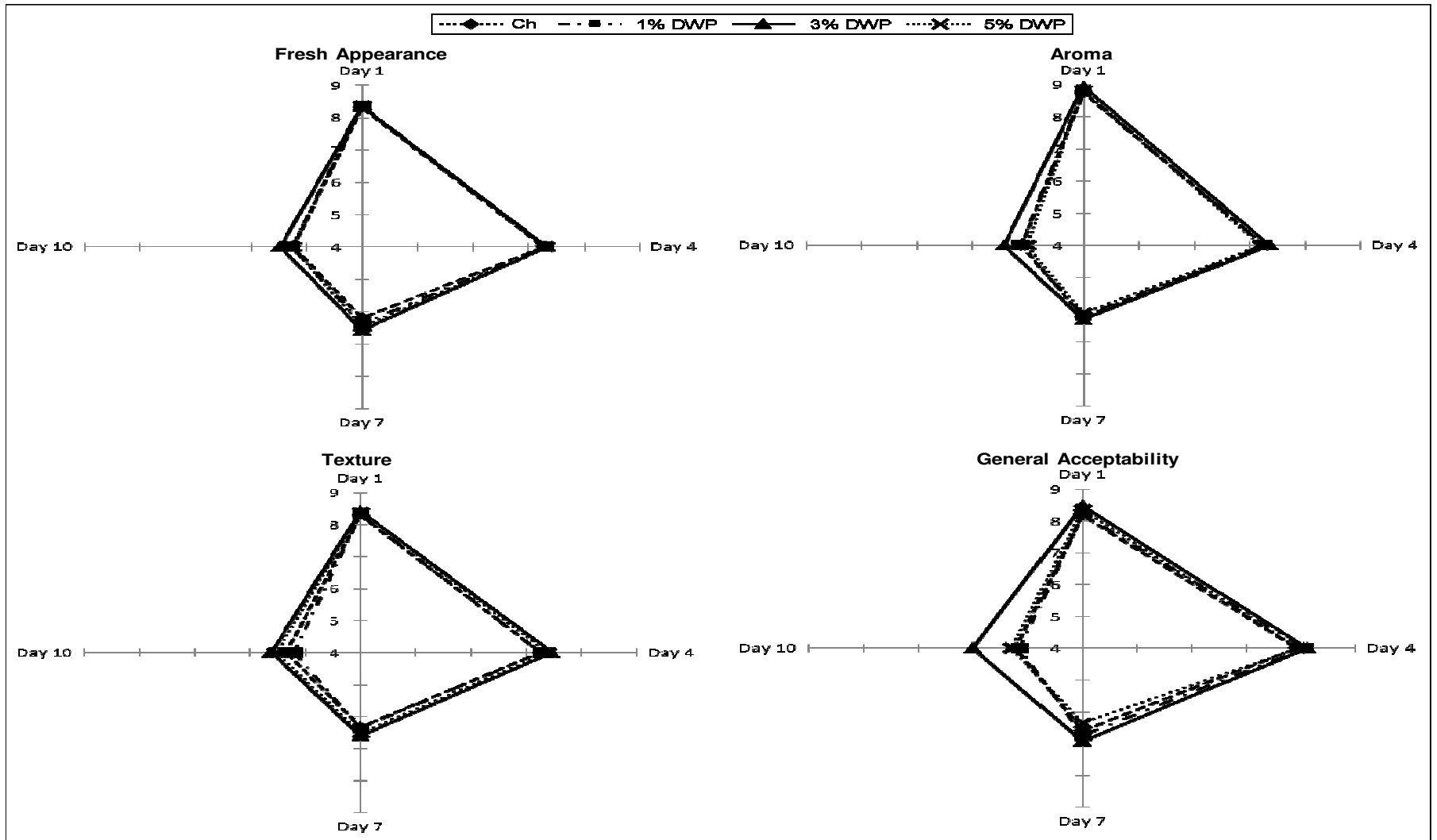


Figure 3

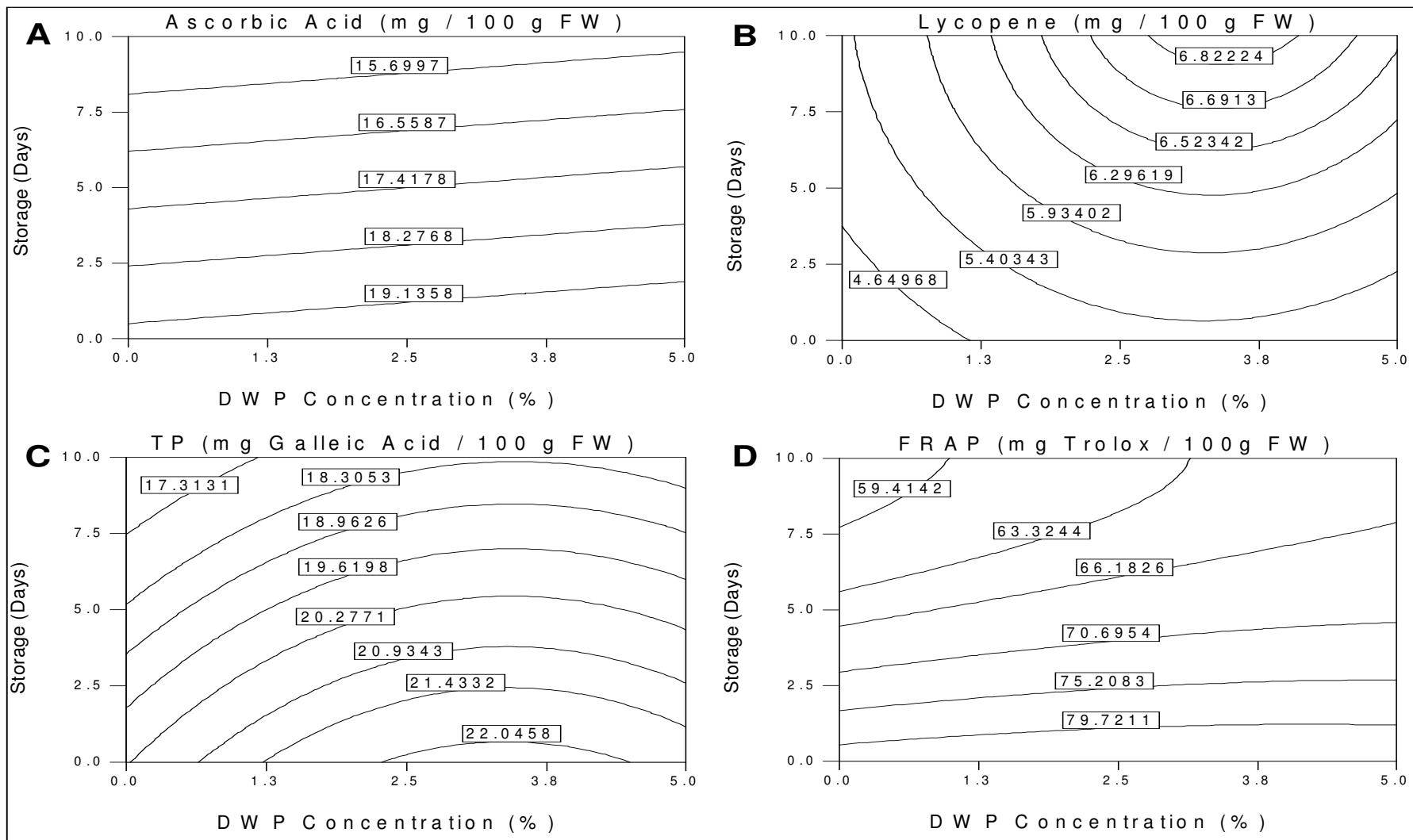


Figure 4

