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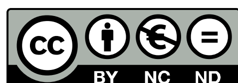

Mechanism of action of an antioxidant active packaging prepared with Citrus extract

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ABSTRACT

Active packaging consisting of polyethylene terephthalate (PET) trays coated with a Citrus extract, without and with plasma pre-treatment, can reduce lipid oxidation in cooked meat. The mechanism of action of the packaging was investigated by quantifying the extent of transfer of antioxidant components from the active packaging into cooked turkey meat. Kinetic studies revealed the affinity for water of phenolic compounds and carboxylic acids in the Citrus extract, suggesting their diffusion into the water phase of the meat facilitated their antioxidant effect. Analysis by high-performance liquid chromatography permitted the identification of carboxylic acids and flavanones as major components of the extract. Their quantification in meat after contact with the trays revealed a release of 100% of the total coated amount for citric acid, 30% for salicylic acid, 75% for naringin and 58% for neohesperidin, supporting the release of these components into cooked meat as a mechanism of action of the antioxidant active packaging.

26 *Keywords:* Citrus extract; PET tray; carboxylic acid; flavanones; HPLC.

27

28 **INTRODUCTION**

29 Interest in active packaging as an approach to improve the quality and increase the
30 shelf-life of food products has grown (De Kruijf, Van Beesty, Rijky, Sipiläinen-Malm,
31 Paseiro Losada, & De Meulenaer, 2002). Active packaging may be particularly useful in
32 processed food products, such as meat products, which can rapidly deteriorate due to
33 dehydration, discoloration, bacterial growth or degradation processes like lipid oxidation
34 (Seideman, Cross, Smith, & Durland, 1984; Ruban, 2009). Different solutions have been
35 proposed to minimize deteriorative processes in meat products, which include the use of drip
36 or taint adsorbents, oxygen scavengers and carbon dioxide emitters, often used in conjunction
37 with modified atmosphere packaging (Kerry, O'Grady, & Hogan, 2006). These solutions
38 prevent conditions that can cause colour changes, off flavour development and other
39 deteriorative processes in packaged meat. Other solutions include packaging with added
40 active compounds that come in contact with or are released into the food, where they can
41 carry out their protective action (Lee, 2005). Antioxidant active packaging falls into the latter
42 category.

43 Due to their negative perception among consumers, synthetic antioxidants are being
44 progressively replaced as ingredients in the formulation of antioxidant active packaging by
45 substances of natural origin, such as tocopherol or mixtures of plant and herbal extracts,
46 (Okabe, Watanabe, Shingu, Kushibiki, Hodate, Ishida et al., 2002; Georgantelis,
47 Ambrosiadis, Katikou, Blekas, & Georgakis, 2007). Packaging containing added antioxidants
48 can be prepared by the addition of the active substances to the packaging material before the
49 formation of the plastic film, with a subsequent release of the antioxidants through either
50 diffusion or film degradation (Pettersen, Mielnik, Eie, Skrede, & Nilsson, 2004; Van Aardt,

51 Duncan, Marcy, Long, O'Keefe, & Sims, 2007). The amount and rate of release of the
52 antioxidant compounds are fundamental to the extent and duration of the protective effect of
53 the packaging; studies have been undertaken on the production of controlled-release
54 packaging that optimizes the characteristics of the plastic polymer to regulate the release of
55 the active substances (Koontz, Moffitt, Marcy, O'Keefe, Duncan, & Long, 2010; Chen, Lee,
56 Zhu, & Yam, 2012;). Some types of active packaging have been prepared by adding the
57 antioxidant substances only to the layer of packaging in direct contact with the food,
58 facilitating the incorporation of the antioxidant in the packaging yet optimizing its release
59 into the food (Camo, Beltrán, & Roncalés, 2008; Soto-Cantú, Graciano-Verdugo, Peralta,
60 Islas-Rubio, González-Córdova, González-León et al., 2008). Alternative systems have been
61 developed by immobilizing the antioxidant compounds in the plastic polymer; these exert
62 their antioxidant activity by trapping free radicals responsible for the initiation and
63 propagation of the lipid peroxidation process (Nerín, Tovar, & Salafranca, 2008).
64 As cooked meat is highly susceptible to lipid oxidation (Gray, & Pearson, 1987), an
65 immediate interaction of the active compounds with the food could be advantageous to
66 protect the product. A coating of antioxidants on the surface of the packaging in contact with
67 the food can satisfy this requirement and permit an immediate protection against lipid
68 oxidation processes. An antioxidant active packaging developed in our laboratory by coating
69 polyethylene terephthalate (PET) trays with a Citrus extract has already been shown to reduce
70 lipid oxidation in cooked turkey meat (Contini, Katsikogianni, O'Neill, O'Sullivan, Dowling,
71 & Monahan, 2012). The natural antioxidant used is a mixture of carboxylic acids and
72 flavanones and has already been shown to exhibit antimicrobial activity in meat products
73 (Mexis, Chouliara, & Kontominas, 2012). The aim of the present study was to identify the
74 active components in the extract and investigate the mechanism of action of the active
75 packaging. Our hypothesis was that active components in the Citrus extract coated on PET

76 trays exert their antioxidant activity by migrating from the packaging surface into cooked
77 meat.
78
79 **2. MATERIALS AND METHODS**
80
81 2.1 Meat
82 Turkey breasts (~1.2 kg) were obtained from IGWT Poultry Service Ltd, County Monaghan,
83 Ireland. For the preparation of cooked meat, fresh turkey breast was wrapped in aluminum
84 foil, cooked for ~2 h to an internal temperature of 73 °C and immediately cooled at 4 °C in an
85 ice bath, as described in Contini et al. (2012).
86
87 2.2 Reagents
88 Citrus extract in powder form containing a mixture of flavanones and carboxylic acids
89 was obtained from Citrox Biosciences, Kimbolton, Cambridgeshire, England. A generic
90 composition of the Citrus extract, as per the manufacturer's specification, was naringin 3.6%,
91 neohesperidin 1.9%, rhoifolin 0.4%, poncirin 0.3%, naringenin 0.2%, hesperidin 0.2%, malic
92 acid 15%, ascorbic acid 15% and citric acid 15%. Chloroform (≥99%), gallic acid (≥98%),
93 methanol (≥99%), phosphoric acid (≥99%), sodium carbonate (≥99.5%), ascorbic acid
94 (≥99%), citric acid (≥99%), malic acid (≥99%), salicylic acid (≥99%), hesperidin (≥95%),
95 naringin (≥95%), neohesperidin (≥90%), poncirin (≥95%) and rhoifolin (≥99%) were
96 obtained from Sigma-Aldrich Ltd, Dublin, Ireland. Recycled polyethylene terephthalate
97 (PET) trays (100 × 150 × 25 mm) were supplied by Holfeld Plastic, Wicklow, Ireland and
98 low-density polyvinylchloride (PVC) catering film (thickness 7.0 µm; O_2 transmission 2000
99 cm^3 m^{-2} d^{-1} bar^{-1}) was supplied by Western Plastic Ltd, Galway, Ireland. Screw-cap plastic

100 tubes (50 ml) were supplied by Sarstedt Ltd, Wexford, Ireland. Membrane filters (0.2 μm)
101 were supplied by Pall Life Sciences, Cork, Ireland.
102
103 2.3 Preparation of the PET trays coated with Citrus extract
104 PET trays were coated with Citrus extract (PET-CIT) by spraying a methanolic
105 solution of the extract onto the polymer surface through a Teflon nebulizer mounted on a
106 computer numerical control system cncGraf (Boenigh Electronics, Bonn, Germany),
107 following the procedure described in Contini et al. (2012). A further set of trays was prepared
108 by a different procedure that involved a pre-treatment of the PET surface, consisting of
109 plasma activation of the tray surface with an atmospheric pressure plasma jet system
110 (PlasmaTreat GmbH, Steinhagen, Germany). Compressed air was used as reagent gas and the
111 plasma procedure carried out using the conditions described in Contini (2013). After the pre-
112 treatment, Citrus extract was nebulized onto tray surface (PET-PA-CIT) as above.
113
114 2.4 Measurement of total phenolic components of Citrus extract in meat
115 Our hypothesis was that if the antioxidant effect of the active packaging required
116 migration of antioxidants from the tray surface into meat then it should be possible to detect
117 Citrus extract components in the meat stored on Citrus coated surfaces. Since phenolic
118 components with known antioxidant effects (Nijveldt, van Nood, van Hoorn, Boelens, van
119 Norren, & van Leeuwen, 2001) are constituents of Citrus extract, an important first step was
120 to establish if it would be possible to detect these phenolics in meat at a level equivalent to
121 that obtained if all the phenolics on the tray surface migrated into the meat. To do this, Citrus
122 extract dissolved in methanol (13.5 mg ml^{-1}) was added to both raw and cooked turkey
123 muscle at a level of 1.35 mg g^{-1}. This level of addition was calculated from the density of the
124 coating applied to the PET tray area in contact with a 30 × 30 × 5 mm slice (3 g) of turkey

125 meat (Contini et al., 2012). Citrus extract was also added to distilled water for comparison
126 (1.35 mg g^{-1}).
127 A second experiment was carried out to quantify the release of phenolic compounds
128 into cooked turkey meat in contact with PET, PET-PA, PET-CIT and PET-PA-CIT trays. The
129 meat was cut into 5 mm thick slices using a meat slicer (Medoc, Logroños, Spain) and
130 subsequently cut into 3 g square (30 × 30 mm) pieces which were placed on the trays and
131 stored at 4 °C for 2 days. Meat pieces were removed immediately (day 0) and after 1 and 2
132 days of storage for measurement of total phenol (TP) content.
133 The extraction of phenol components from meat was performed following the
134 procedures described by Jang, Liu, Shin, Lee, Lee, Lee et al. (2008). Meat samples were
135 homogenized in 15 ml of distilled water for 1 min at 8000 rpm using an Ultraturrax T25
136 (IKA-Labortechnik) and subsequently 9 ml of chloroform were added. The mixture was
137 shaken vigorously and centrifuged for 10 min at 3000 rpm for phase separation. The
138 quantification of TP content in the upper aqueous phase was performed by the Folin-
139 Ciocalteu (FC) procedure described by Harbourne, Marete, Jacquier, & O'Riordan (2009).
140 This involved adding 0.2 ml of aqueous meat extract to 0.5 ml of FC reagent, 1.5 ml of 20%
141 sodium carbonate and 7.8 ml of distilled water. The solution was mixed and left for 2 h for
142 colour development. The absorbance was measured using a Shimadzu UV-1240
143 spectrophotometer (Kyoto, Japan) at a wavelength of 760 nm. Quantification was done based
144 on a standard curve generated with gallic acid (0.01 - 0.5 mg ml^{-1}) and TP content was
145 expressed as mg gallic acid equivalent (GAE) g^{-1} of meat.
146
147 2.5 Kinetics of release of total phenolic components from Citrus extract-coated trays into
148 water

149 Square pieces of the PET trays (30 × 30 mm), corresponding to the surface in contact
150 with the meat slices, were cut from uncoated (PET and PET-PA) and coated (PET-CIT and
151 PET-PA-CIT) trays. Each piece was then placed in a weighing boat with 2.1 ml of water
152 which corresponded to the moisture content of 3g of cooked turkey meat (McCance, &
153 Widdowson, 2002). The samples were then stored at 4 °C for 2 days, to mimic the conditions
154 of meat storage. The weighing boats were covered with PVC catering film to prevent
155 evaporation of the water during storage. The TP content in water was determined
156 immediately (day 0), after 10, 20, and 40 seconds, after 1, 2, 5, 10, 20 and 40 minutes, after 1,
157 2, 3, 4 and 6 hours and after 1 and 2 days, using the FC procedure described in section 2.4.
158
159 2.6 Identification and quantification of the Citrus extract components
160 The identification of Citrus extract components was performed by high-performance
161 liquid chromatography (HPLC) analysis of a solution of the extract and comparing the
162 retention times of the peaks obtained with those of pure standards of the components declared
163 by the provider of the Citrus extract. The Citrus extract (in powder form) was dissolved in
164 methanol (5 mg ml^{-1}), filtered through a 0.2 µm membrane filter and analysed by HPLC,
165 following the method described by Harbourne et al. (2009) with modifications to the mobile
166 phase. The analysis was carried out using an Agilent 1200 HPLC system (Agilent
167 Technologies, Palo Alto, CA, USA) equipped with an Agilent Synergi Hydro-RP 80A
168 analytical column (250 mm × 4.60 mm, 4 µm particle size) and a C18 guard column
169 (Phenomenex, Chesire, UK). The mobile phase was (A) 0.1% phosphoric acid in water and
170 (B) methanol. The separation was carried out at 37 °C at a flow rate of 0.8 ml min^{-1} with the
171 following gradient: 0-2 min, 90% A; 2-16 min, 90% A to 10% A; 16-22 min, 10% A to 90%
172 A. The detector used was a diode array (DAD) at a wavelength of 210 nm. For the

173 quantification of the main Citrus extract compounds identified, the instrument was calibrated
174 with 6 point calibration curves of their pure standards.

175 For confirmatory purposes, liquid chromatography-mass spectrometry (LC-MS)
176 analysis was performed using a Waters Acquity HPLC system (Milford, MA, USA), coupled
177 with a triple-quadrupole mass-spectrometer Xevo TQ Waters-Micromass (Manchester, UK).
178 The analysis was carried out using the same column and mobile phase described for the
179 HPLC-DAD analysis. The mass spectrometry analysis was performed in negative
180 electrospray ionization (ESI^-) in scan acquisition mode with a desolvation temperature of 400
181 ºC and the desolvation gas flow of 800 l h^{-1}.

182

183 2.7 Quantification of Citrus extract components in cooked meat stored on Citrus extract-
184 coated trays

185 Turkey meat pieces (see section 2.4) were placed on PET (control) and PET-CIT trays
186 and removed immediately (day 0) or after 1 or 2 days of storage in a refrigerator at 4 °C.
187 After the exposure time, the Citrus extract components released into the meat were extracted
188 using the method of Folch, Lees, & Sloane-Stanley (1957) with modifications. The meat
189 pieces were transferred to 50 ml tubes and homogenized for 3 min at 8000 rpm with 19 ml of
190 chloroform, using an Ultraturrax T25. The mixture was shaken 3 times to dissolve the fat
191 contained in the tissue, 4 ml of distilled water were then added and the mixture shaken and
192 centrifuged for 10 min at 3000 rpm. An aliquot (2 ml) of the aqueous upper phase was
193 filtered through a 0.2 µm membrane filter and 10 µl were injected onto the HPLC column.
194 The recovery of the extraction was calculated as 61% for naringin, 64% for neohesperidin,
195 100% for citric acid and 53% for salicylic acid. Organic acids and flavanones were
196 determined as described above (see section 2.6).

197

198 2.8 Quantification of Citrus extract components remaining on coated trays after contact with
199 meat

200 To confirm the extent of release of Citrus extract components into the meat, the
201 components remaining on the trays after exposure to cooked turkey meat slices for up to 2
202 days at 4 °C (see section 2.4) were quantified. Pieces (30 x 30 mm) of PET-CIT trays,
203 previously in contact with the slices of meat, were cut and placed for 2 hours in weighing
204 boats containing 4 ml of water which corresponded to the final volume of the extract from
205 meat (see section 2.7). Pieces of PET-CIT trays of the same size which had not been in
206 contact with the meat were also immersed in 4 ml of water for 2 hours to act as a control. An
207 aliquot of the water was then collected from each weighing boat, filtered through a 0.2 µm
208 membrane filter and injected onto the HPLC column. The quantification of the components
209 was performed following the procedure described in section 2.4. The amount of the Citrus
210 extract components released into the meat was calculated as the difference between the
211 release into water from PET-CIT trays after contact with the meat and the release from PET-
212 CIT trays that had not been in contact with the meat (control).
213
214 2.9 Statistical analysis

215 Each experiment was carried out in triplicate, values were expressed as mean ±
216 standard deviation of the three repetitions. A one-way analysis of variance (ANOVA) and
217 Bonferroni's pair wise comparison test were used to determine significant differences
218 between the treatments, using SPSS (version 18) statistical software (IBM Inc. Chicago, IL,
219 USA).
220

221 **3. RESULTS AND DISCUSSION**

222

223 3.1 Direct addition of Citrus extract to raw and cooked meat

224 In the first experiment, the raw minced meat without the addition of Citrus extract
225 (control) had approximately 1.35±0.07 mg GAE g^{-1} of meat at day 0 and it did not change
226 significantly at day 1 and day 2 (Figure 1a). The response to the FC reagent in the control
227 meat is most likely due to of its reaction with substances naturally present in the meat. In fact,
228 meat is a complex matrix that contains different classes of substances which can react with
229 the FC reagent. This is supported by previous studies which showed that sugars, aromatic
230 amines, organic acids and Fe (II) can interfere in the reaction (Szydłowska-Czerniak,
231 Tułodziecka, & Szłyk, 2012). Contributions to the FC reaction have also been attributed to
232 amino acids, proteins and inorganic substances (Prior, Wu, & Schaich, 2005). Furthermore,
233 the reaction of the phenolic group of tyrosine with the FC reagent is also well known and the
234 basis of an assay for the quantification of soluble proteins (Lowry, Rosebrough, Lewis Farr,
235 & Randall, 1951); thus the presence of phenolic amino acids, in particular, would have
236 contributed to the relative high FC response of the control. The response in the meat with
237 added Citrus extract showed higher values compared to the control meat on each of the days,
238 however, the difference was significant only at day 2 ($p<0.05$). The difference between the
239 values found in the control meat and meat with added Citrus extract was lower than the value
240 obtained in distilled water to which the same amount of Citrus extract had been added. If all
241 the phenolics in the added Citrus extract were readily extractable and detectable, then one
242 would have expected the differences between the level of TP in the meat with added Citrus
243 extract and that in the control meat to be approximately equal to the level of TP in the water
244 control. As it was, the difference between Citrus extract added meat and control meat was
245 only approximately 40-50% of the value in water, this lower than expected level might be due
246 to the effect of the components in meat decreasing the efficiency of FC reaction or giving rise
247 to an incomplete recovery of the phenolic components of Citrus extract in the procedure for

248 their extraction from the meat. The lower variability of data obtained for the addition of
249 Citrus extract in water compared to that of the data for control meat and meat with Citrus
250 added extract could be a further indication of the impact of interfering components in the
251 meat reducing the accuracy of the assay.

252 The cooked meat without Citrus extract (control) showed a level of 0.36±0.05 mg
253 GAE g^{-1} of meat at day 0, which remained stable up to day 1 and day 2 (Figure 1b). The meat
254 with added Citrus extract showed significantly higher TP values during each sampling time
255 ($p<0.05$). Again, the difference between the values obtained in control meat and the meat
256 added with Citrus extract was lower than the value obtained in spiked water. The values
257 found in the unspiked meat (control) showed that the cooking process resulted in a decrease
258 of 74% in TP values compared to the raw meat. Previous studies have shown that cooking
259 processes can cause a decrease of phenolic compounds in vegetables (Yen, & Hung, 2000)
260 and in total antioxidant capacity of foodstuffs (Serpen, Gökmen, & Fogliano, 2012).
261 However, the decrease in TP values in cooked meat observed in this study is more likely due
262 to the well-established denaturation of soluble proteins that occurs during cooking (Tornberg,
263 2005) leading to a lower response to the FC reagent in cooked meat. The results of the
264 experiments showed higher TP values for the meat with Citrus extract added both in raw and
265 particularly in cooked meat. Therefore if substantial migration of phenolic compounds from
266 the tray surface into meat occurs it should be possible to detect an increase in phenolic
267 content of the meat.

268 The experiment carried out to quantify the release of phenolic compounds into cooked
269 meat in contact with the trays stored at 4 ºC for 2 days (see section 2.4), showed TP values at
270 day 2 of 0.345±0.058 mg GAE g^{-1} for meat stored on PET trays, 0.379±0.077 mg GAE g^{-1} for
271 meat stored on PET-CIT trays, 0.349±0.075 mg GAE g^{-1} for meat stored on PET-PA trays
272 and 0.404±0.061 mg GAE g^{-1} for meat stored on PET-PA-CIT trays. Although the TP values

273 of the meat in contact with the PET-CIT and PET-PA-CIT trays were always numerically
274 higher than those of the meat from the PET and PET-PA trays, the differences were not
275 statistically significant. These results suggest that while a release of phenolic substances from
276 the trays could be detected by FC assay, the high degree of variability due to the presence of
277 interfering components in meat meant that the differences between meat placed on uncoated
278 and coated trays were not statistically significant.

279

280 3.2 Kinetics of release of total phenol from the trays into water

281 The release of TP from PET-CIT and PET-PA-CIT trays into water occurred rapidly
282 during the first 20 min of contact, with the release from PET-PA-CIT trays being
283 significantly higher ($p<0.01$) than that from PET-CIT trays (Figure 2). After 20 min, the
284 release from the trays remained steady but the release from PET-PA-CIT trays was
285 significantly lower ($p<0.01$) than that from PET-CIT trays from that point onwards. A
286 previous study in our laboratory showed that a similar plasma pre-treatment resulted in a
287 greater coating of Citrus extract on the surface of trays, with 0.46 mg cm^{-2} and 0.78 mg cm^{-2}
288 on PET-CIT and PET-PA-CIT trays, respectively (Contini, 2013). The lower release from
289 PET-PA-CIT trays after 20 min, despite the higher initial coating density, suggests a higher
290 adhesion of the phenolic components of the Citrus extract to the plasma activated PET
291 surface. On the other hand, the faster initial release may indicate that some phenolic
292 molecules at the outer extremity of the thicker coating layer adhered less well to the surface
293 of the plasma activated PET.

294

295 3.3 Identification and quantification of Citrus extract components

296 The analysis of the Citrus extract by HPLC confirmed the presence of citric acid,
297 naringin, neohesperidin and traces of hesperidin and rhoifolin. Poncirin, ascorbic and malic

298 acid, if present, were not detected and no additional peaks were detected in the organic acid
299 or flavanones elution range. However, the presence of an unknown component with high
300 intensity required analysis by LC-MS for identification. On the basis of its deprotonated
301 molecular mass (m/z 137), the unknown component was identified as salicylic acid; a
302 common constituent of plants extracts (Harbourne et al., 2009). The quantification of the
303 components by HPLC analysis revealed the presence of 25 mg of citric acid, 36 mg of
304 salicylic acid, 1.5 mg of naringin and 1.4 mg of neohesperidin per 100 mg of Citrus extract.
305
306 3.4 Release of Citrus extract components from PET-CIT trays into meat
307 An attempt was made to confirm the presence and quantify by HPLC the levels of
308 specific constituents of the Citrus extract in meat which had been placed on the PET-CIT
309 trays for up to 2 days at 4 °C. The results of the analysis showed that the citric acid was
310 already detectable in the meat at day 0 and its content further increased during the following
311 days, to reach a maximum value of 370±12.5 μg g^{-1} of meat at day 2, with significantly
312 higher values ($p<0.01$) at day 1 and 2 compared to day 0 (Figure 3a). Salicylic acid showed
313 an immediate release (day 0), with no significant further increase during the study; the
314 highest value of 144±15.7 μg g^{-1} of meat was obtained at day 1. Uptake of flavanones was
315 lower than that of the organic acids as expected considering their lower level of occurrence,
316 with highest values of 15.1±5.4 μg g^{-1} meat for naringin and 11.1±1.5 μg g^{-1} meat for
317 neohesperidin after 2 days of storage (Figure 3b). The results did not show any significant
318 difference between the different storage times for flavanone uptake.
319 In the final experiment, the amount of Citrus extract constituents taken up by a 30 ×
320 30 × 5 mm (3g) slice of cooked turkey meat was calculated from the differences in the
321 amounts of coating constituents extractable into water from a control PET-CIT tray and a
322 PET-CIT tray which had been in contact with the slices of meat for 2 days at 4 ºC (section

323 2.8). The results showed a broadly similar release between citric and salicylic acid after 2
324 days (Figure 4a). The highest values of release, expressed on the basis of 3g meat, were
325 453±67.0 µg g^{-1} meat for citric acid, 408±29.4 µg g^{-1} meat for salicylic acid. The flavanones
326 showed similar trends, with a release of 21.2±3.7 µg g^{-1} meat for naringin and 18.1±3.3 µg g^{-1}
327 meat for neohesperidin (Figure 4b). The values calculated for citric acid, salicylic acid,
328 naringin and neohesperidin, measured directly in the cooked meat (Figure 3) represented
329 82%, 35%, 71% and 61%, respectively, of these values calculated indirectly through the
330 release into water (Figure 4). The amount of the substances released into meat, calculated as
331 the difference between the release in water from a tray after contact with meat and a tray that
332 has not been in contact with meat, could have been overestimated due to the presence of a
333 deposit remaining on the surface of the tray after its contact with the meat, which may have
334 hampered the release of the residual Citrus extract constituents. This hypothesis could explain
335 the apparent inconsistency of the results of the two experiments.

336 The total amount of Citrus extract on the PET trays has been previously calculated
337 gravimetrically as 0.46 mg cm^{-2} (Contini, 2013). Using this value and the percentage of each
338 constituent of the Citrus extract calculated from HPLC analysis in the present study, in the
339 case of a complete release of the components, their amount in meat was estimated as 345 µg
340 g^{-1} of meat for citric acid, 497 µg g^{-1} of meat for salicylic acid, 20 µg g^{-1} of meat for naringin
341 and 19 µg g^{-1} of meat for neohesperidin. A comparison between the amount measured
342 directly in the meat and the values estimated on the basis of the Citrus extract coating density
343 indicated an almost complete release for citric acid, while the releases of naringin,
344 neohesperidin and salicylic acid were 75%, 58% and 30% respectively of their estimated
345 levels in the coating. The results indicate that the antioxidant effect of the packaging may
346 well be based on the release of the active substances from the trays into the food. The high
347 polarity of citric acid and flavanones, due to their carboxyl and glycoside groups,

348 respectively, may explain their rapid transfer into the water phase of cooked meat. Moreover,
349 the results of the experiment regarding the kinetics of TP release into water (see section 3.2)
350 also indicated the propensity of phenolic components of Citrus extract to migrate rapidly into
351 the aqueous phase. The lower release of salicylic acid could be explained by the lower
352 polarity of this molecule (Shalmashi, & Eliassi, 2008).

353 While the released substances can exert their antioxidant activity on the surface of the
354 meat, the antioxidant activity of the packaging could be also due to their diffusion deep into
355 the tissue of the meat. Regarding the antioxidant activity of the individual components, citric
356 acid has been described as an inhibitor of lipid oxidation in fatty food (Hras, Hadolin, Knez,
357 & Bauman, 2000) and studies on the antioxidant mechanism of citric acid have revealed that
358 its carboxyl or hydroxyl groups can exert a binding effect on metals thus inhibiting their
359 catalysis of oxidative reactions (Vareltzis, Hultin, & Autio, 2008). Flavanones have
360 antioxidant properties and their mechanism of action as free radical scavengers is well
361 documented (Cao, Sofic, & Prior, 1997). Salicylic acid has also been shown to have an
362 antioxidant activity in biological systems mainly by the stimulation of enzymes, such as
363 superoxide dismutase, catalase and peroxidase, as part of the antioxidative defense
364 mechanism (Tareen, Abbasi, & Hafiz, 2012). Previous work in our laboratory has established
365 that the Citrus extract coated PET trays can substantially reduce lipid oxidation in cooked
366 turkey meat over 4 days of storage period and its antioxidant effect is evident from the
367 earliest stages of the storage (Contini et al., 2012). The rapid release of the antioxidant
368 species from the coating into the meat, observed in the present work is consistent with and
369 indeed seems a pre-requisite for the immediacy of the antioxidant effect observed in the
370 earlier studies. However a further confirmation of the mechanism of the antioxidant effect of
371 the packaging came from the experiment on the kinetic of release of Citrus extract phenolics
372 into water, which showed a lower release from PET-PA-CIT trays compared to PET-CIT

373 trays (Figure 2). In fact, previous experiments showed that plasma pre-treatment enhanced
374 the antioxidant effect of the active packaging (Contini, 2013). These results suggested that an
375 antioxidant effect may also exerted by the Citrus extract components which remained on the
376 tray surface, where they could reduce lipid oxidation on the surface of the meat in contact
377 with the packaging, and that the extent of this effect depended on the amount of the coated
378 substances (0.46 mg cm^{-2} and 0.78 mg cm^{-2} on PET-CIT and PET-PA-CIT trays,
379 respectively).
380

381 **CONCLUSION**

382 The major components in the commercial Citrus extract, Citrox, quantified by HPLC,
383 are citric acid, salicylic acid, naringin and neohesperidin. The mechanism of action of
384 antioxidant active packaging containing Citrus extract appears to involve release to the
385 antioxidant molecules from the packaging into the food from its earliest exposure to the
386 packaging. At the same time, the lower release into water of Citrus extract components from
387 plasma pre-treated trays combined with their higher antioxidant effect suggests a contribution
388 to antioxidant activity from the substances remaining on the PET surface.
389
390

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393 Science Foundation Ireland grant 08/SRC/I1411.
394

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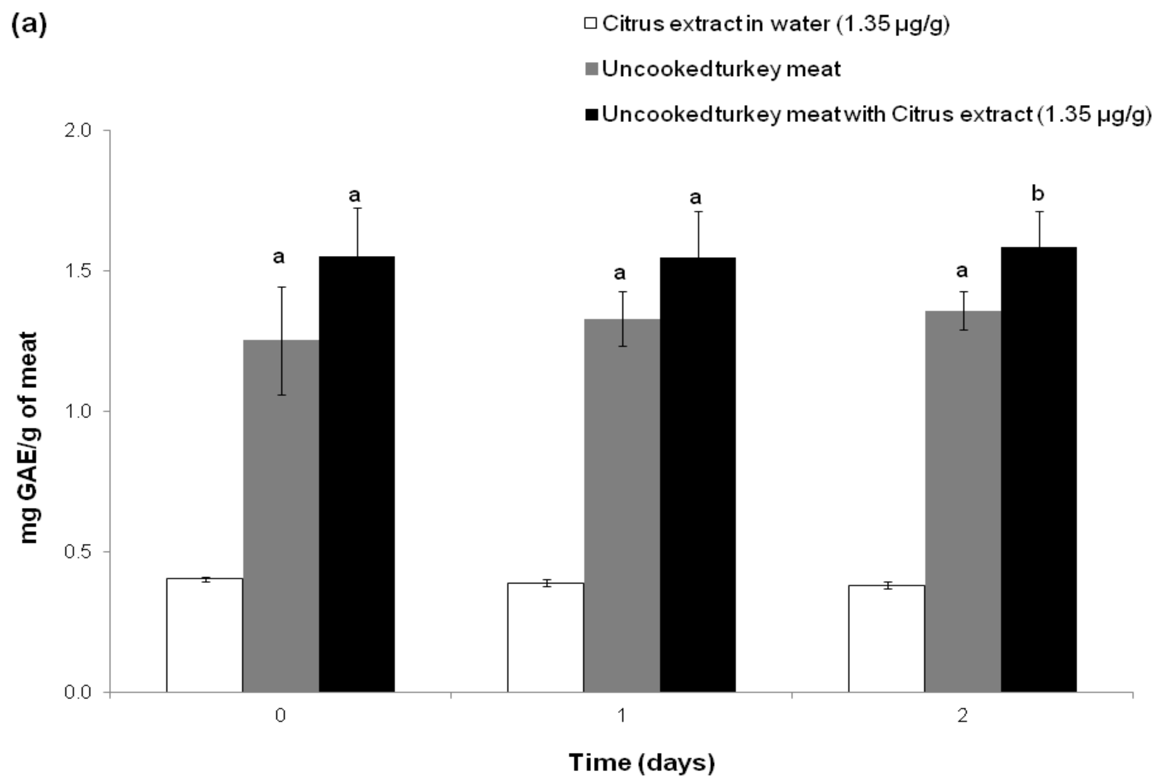
493

494 **Figure 1.** Effect of the direct addition of Citrus extract on the total phenol content of (a) raw
495 and (b) cooked turkey meat, stored for 2 days at 4 °C. White column, Citrus extract added
496 into water (control); grey column, turkey meat; black column, turkey meat with Citrus
497 extract. Bars indicate mean ± SD. [a,b] Within each storage time, bars with different letters are
498 significantly different due to treatment. Within each treatment there was no significant
499 difference due to storage time.
500
501 **Figure 2.** Release of the total phenols from the PET-CIT and PET-PA-CIT trays into water,
502 stored for 2 days at 4 °C. Points indicate mean ± SD. [a,b] Within each storage time, bars with
503 different letters are significantly different due to treatment. [r,s,t,u,v,w,x,y,z] Within each treatment,
504 bars with different letters are significantly different due to storage time.
505
506 **Figure 3.** Release of (a) organic acids and (b) flavanones from PET-CIT trays into meat
507 stored for up to 2 days at 4 °C. Bars indicate mean ± SD. [y,z] Within each treatment, bars with
508 different letters are significantly different due to the storage time.
509
510 **Figure 4.** Release of (a) organic acids and (b) flavanones from PET-CIT trays into meat
511 stored for 2 days at 4 °C, calculated from the release into water. Bars indicate mean ± SD.
512 Within each treatment there was no difference due to storage time.
513
514

515 **Figure 1.** Contini et al.



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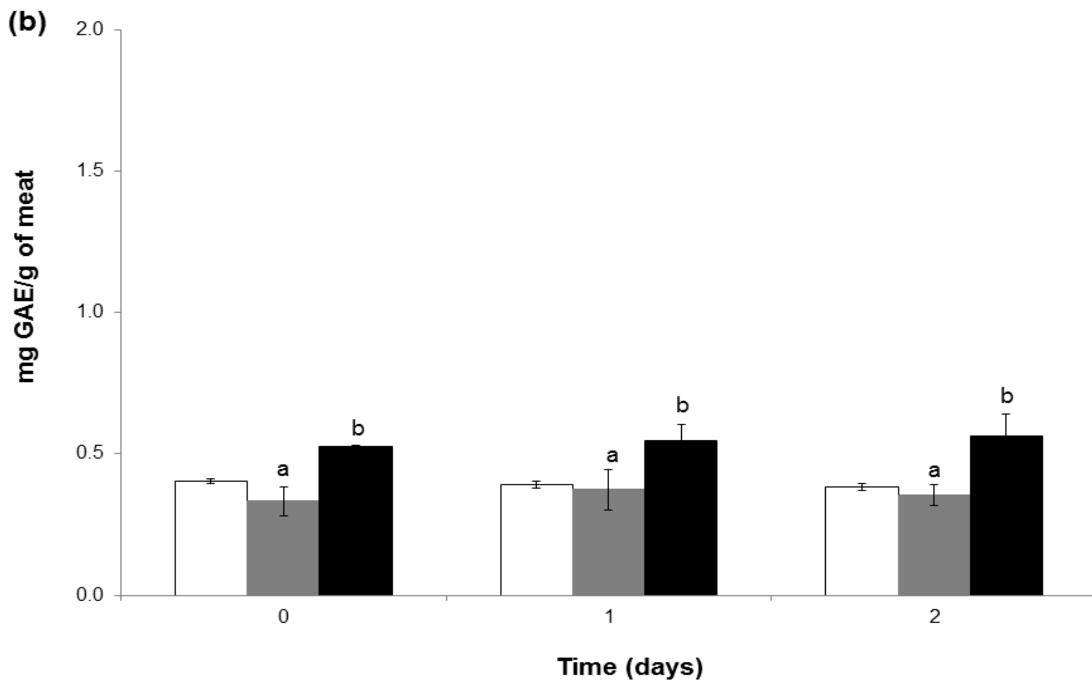

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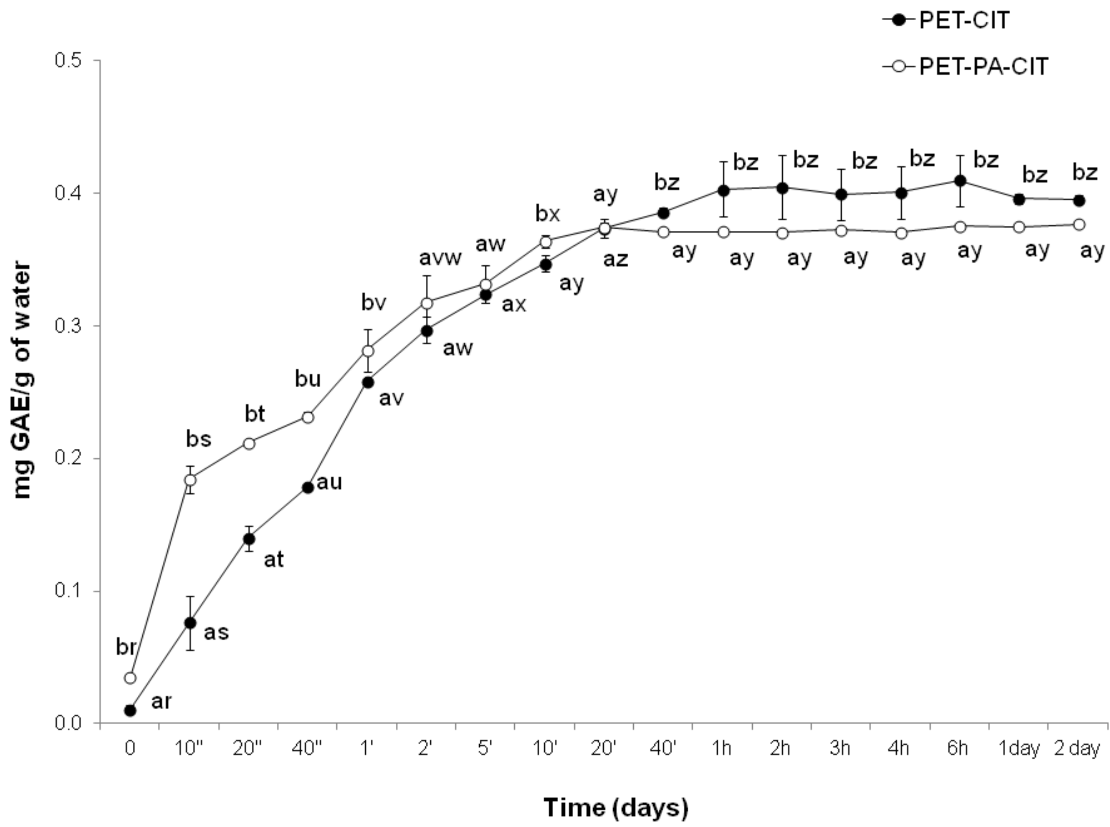

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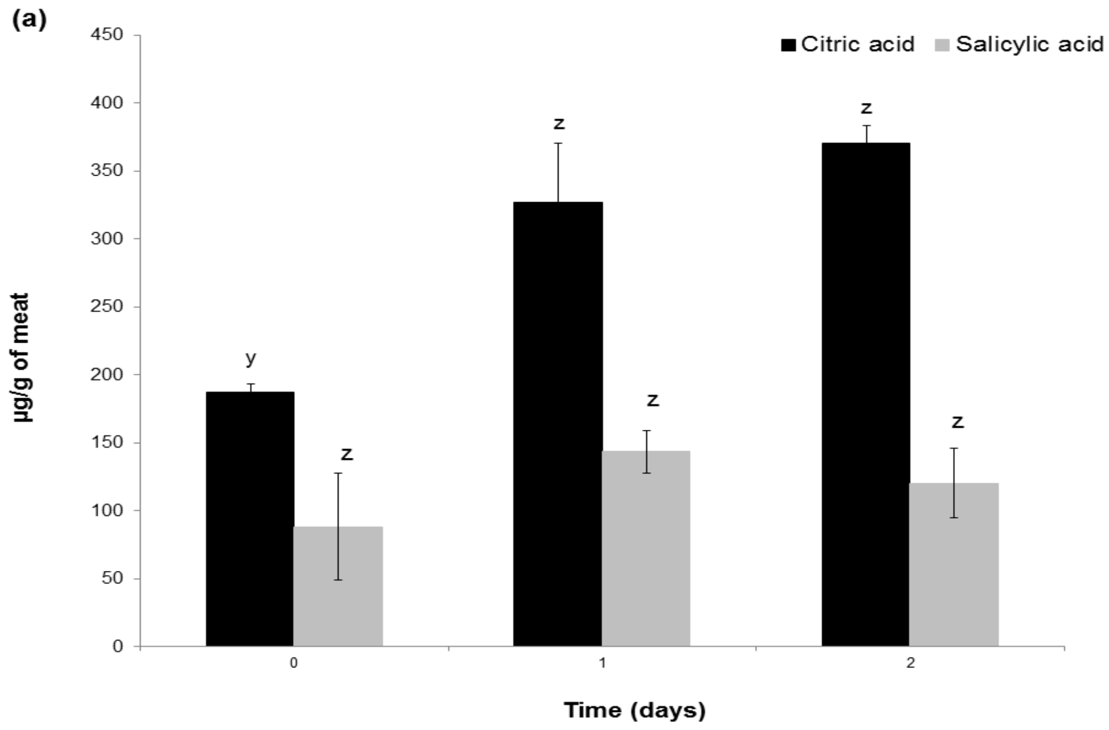

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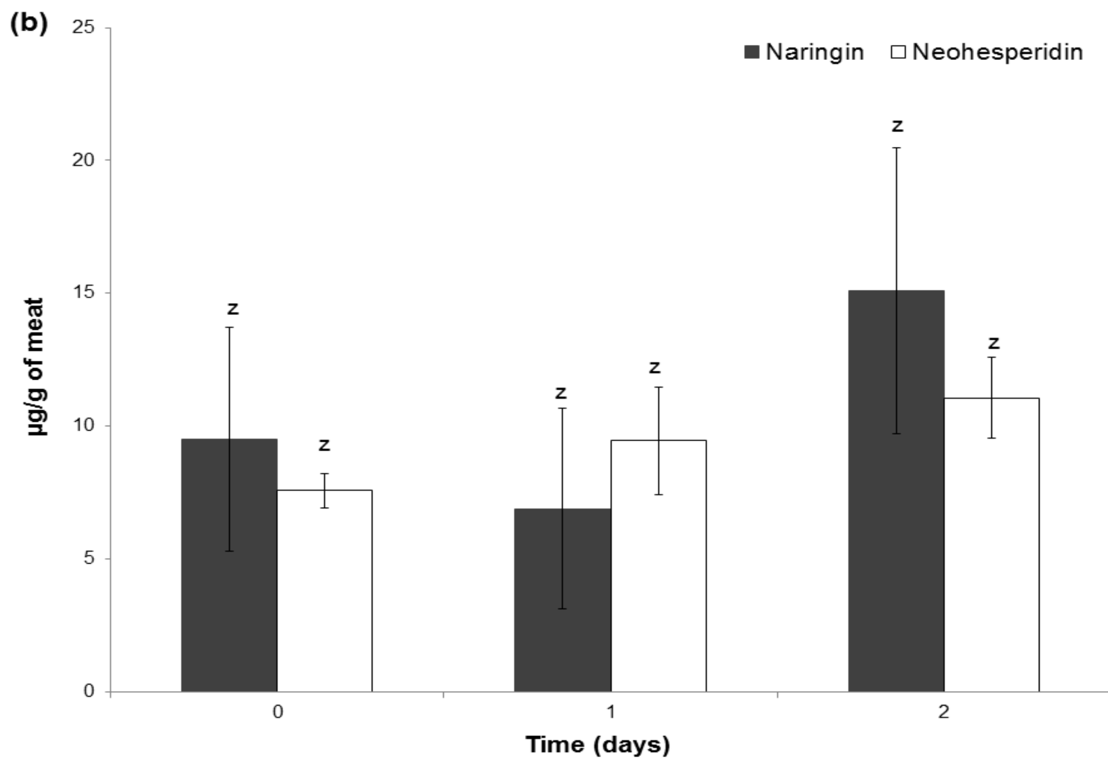

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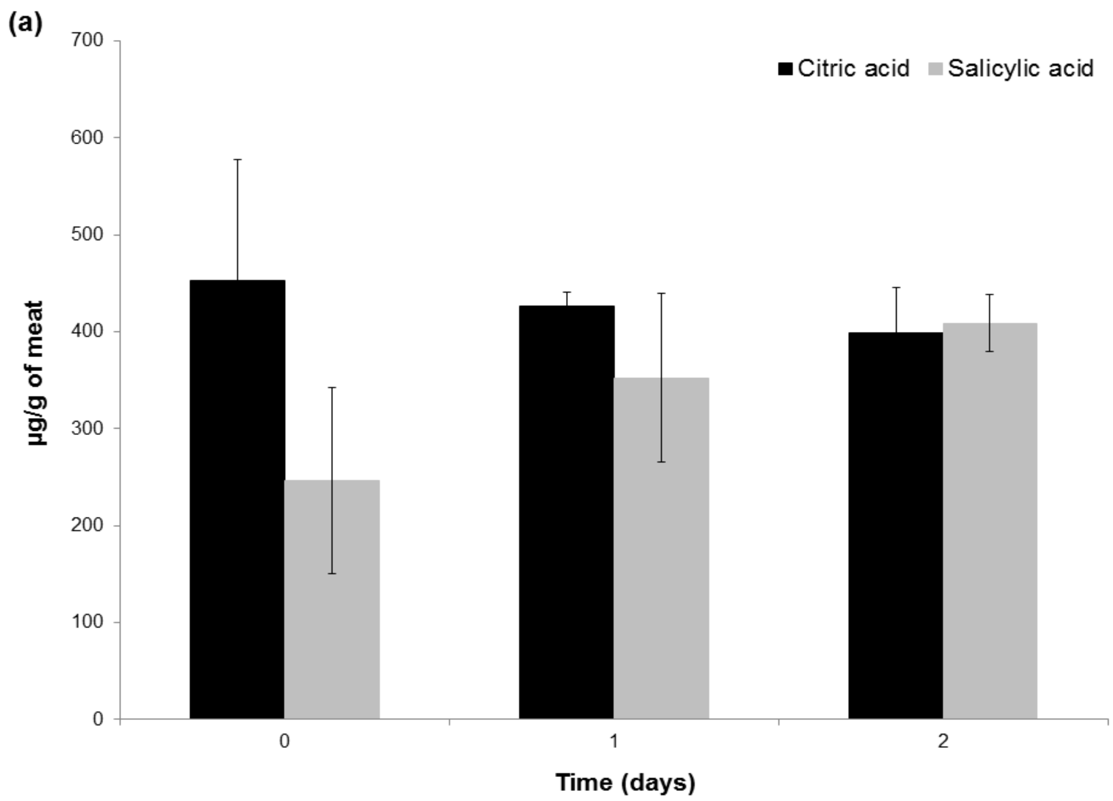

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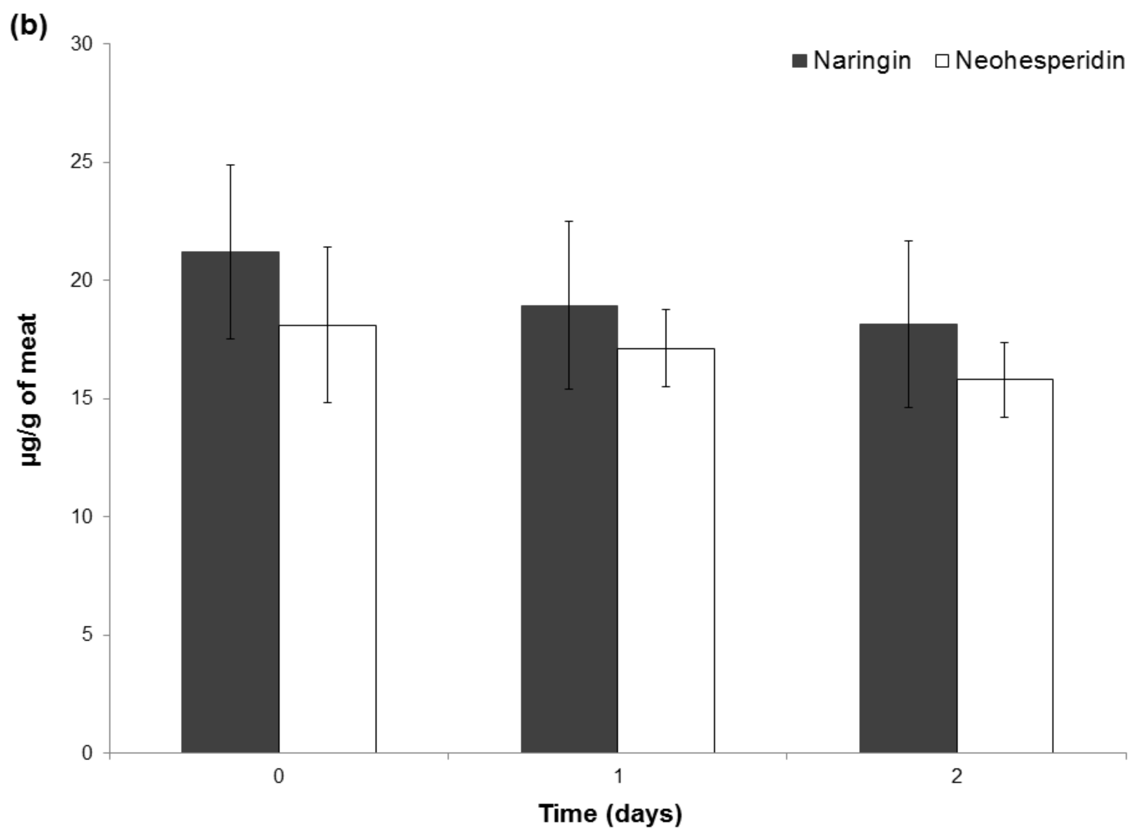

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