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Effect of turmeric powder (*Curcuma longa* L.) and ascorbic acid on physical characteristics and oxidative status of fresh and stored rabbit burgers

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Abstract
The objective of this study was to evaluate the effect of *Curcuma longa* powder and ascorbic acid on some quality traits of rabbit burgers.
The burgers (burgers control with no additives; burgers with 3.5 g of turmeric powder/100 g meat; burgers with 0.1 g of ascorbic acid/100 g meat) were analyzed at Day 0 and 7 for pH, color, drip loss, cooking loss, fatty acid profile, TBARS, antioxidant capacity (ABTS, DPPH and FRAP) and microbial growth.
The addition of turmeric powder modified the meat color, produced an antioxidant capacity similar to ascorbic acid and determined a lower cooking loss than other formulations.
Turmeric powder might be considered as a useful natural antioxidant, increasing the quality and extending the shelf life of rabbit burgers.

Keywords
Rabbit burger; Meat quality; Natural antioxidant; Turmeric; Ascorbic acid; Antioxidant capacity

1. Introduction

Changes in eating habits have led people to consume processed products such as ready-to-cook and ready-to-eat meals, and simultaneously, the food industry has developed new formulations to improve the shelf life and food safety of these products. In an attempt to control the deterioration and lipid oxidation of food, synthetic additives with antioxidant properties are widely used. However, because synthetic antioxidants may have toxic effects and consumers are concerned with safety, the interest in products with natural antioxidants has increased (Dalle Zotte & Szendrő, 2011; Petracci & Cavani, 2013; Selani *et al.*, 2011).

Rabbit meat is characterized by excellent nutritive and dietetic properties associated with high protein content, high essential amino acid levels, low lipid content and high (60% of the total) unsaturated fatty acid (UFA) and polyunsaturated fatty acid (PUFA) contents (Dalle Zotte, 2002); therefore, it is a useful food in human diets. However, rabbit meat is susceptible to lipid oxidation and tends to produce an off-flavor more than other meat products, and consequently, the use of rabbit meat in processed products is very limited (Petracci & Cavani, 2013).

Different studies have evaluated the effect of dietary supplementation of natural antioxidants on rabbit performance and meat quality (Botsoglou, Florou-Paneri, Christaki, Giannenas, & Spais, 2004; Dal Bosco *et al.*, 2014; Dal Bosco *et al.,* 2012; Eid, 2008; Sgorlon, Stradaioli, Stefanon, Altimer, & Della Loggia, 2005; Zhang, Xiao, Samaraweera, Joo Lee, & Ahn, 2010), although none have evaluated the shelf-life or effect of natural antioxidants in processed food products derived from rabbit meat. Among the natural antioxidants, *Curcuma longa* L. (turmeric), an herbaceous perennial plant of the *Zingiberaceae* family, is a medicinal plant extensively used in Asian countries.

Turmeric powder is prepared by drying and grinding the plant's rhizomes and is commonly used as a spice for its flavor and color and as a preservative. Recently, *Curcuma longa* has been widely studied for its high antioxidant capacity and significant medical potential; it has been found to have anti-inflammatory, anti-infectious and anti-tumor properties (Jain, Shrivastava, Nayak, & Sumbhate, 2007). The curcuminoids are the major antioxidative compounds of turmeric, and the most widely studied is curcumin. Curcumin is a potent quencher of singlet oxygen species (Das & Das, 2002) and has the ability to inhibit lipid peroxidation and scavenge the superoxide anion and hydroxyl radicals (Ruby, Kuttan, Babu, Rajasekharan, & Kuttan, 1995; Motterlini, Foresti, Bassi, & Green, 2000). Additionally, curcumin (E 100) is a dicinnamoylmethane dye authorized as a food additive in the EU (EFSA, 2010) and is commonly used in the food industry as a yellow dye.

Several *in vitro* studies have analyzed the antioxidant effect of turmeric (Ruby *et al.*, 1995, Motterlini *et al.,* 2000); however, only a few studies have evaluated its effect on the shelf-life and antioxidant properties in meat (Daneshyar, 2012; Sharma, Pazhaniandi, Tanwar, Das, & Goswami, 2012).

The aim of this study was to evaluate the antioxidant effects of turmeric powder and ascorbic acid on the physical characteristics, FA profile, antioxidant status and microbial growth of fresh and stored rabbit burgers.

2. Materials and methods

Animals and sampling

In total, 36 hybrid rabbits weighing an average of 2.5 ± 0.10 kg, reared under intensive conditions, and fed a commercial pelleted feed were slaughtered in a farm abattoir. The farm was located near the Department of Veterinary Science of Pisa. The slaughter method was electrical stunning followed by cutting of the carotid arteries and jugular veins.

After chilling for 24 h at 4 ± 0.5 °C, the hindlegs were carefully dissected from the carcasses and deboned following standard procedures (Blasco and Ouayhoun, 1996).

Burger manufacture and experimental design

For the experiment, six batches of meat (B), consisting of ground meat from the hind legs of six rabbits, were generated, and the chemical composition was assessed. Three different types of meat formulations (F) were prepared from each batch: meat with no additives (control, C), meat with turmeric powder (3.5 g of turmeric powder per 100 g of meat, Tu) and meat with ascorbic acid (0.1 g of ascorbic acid per 100 g of meat, AA). The quantities of Tu and AA were chosen after preliminary evaluation of the antioxidant capacity of the two additives using ABTS, DPPH and FRAP methods to make them comparable.

Turmeric powder (commercial composition; protein 12.2%, fat 3.4%, ash 5.8%, and moisture 9.4%) and ascorbic acid were immediately added to the minced meat, and the batch was thoroughly mixed.

Six burgers (approximately 50 g each) per formulation from each batch were formed in Petri dishes (85 mm diameter) to obtain a total of 36 burgers per formulation (18 burgers per batch, for a total amount of 108 burgers).

95 The burgers were packaged in Styrofoam trays with polyethylene overwrap film and stored at 4 ± 1
96 °C for 0 and 7 days (Day 0, Day 7).
97 The samples (C, Tu, AA) were analyzed at Day 0 and 7 for pH, color, drip loss, cooking loss, fatty acid
98 (FA) profile, TBARS, antioxidant capacity (ABTS, DPPH and FRAP) and microbial growth.
99 For each formulation per batch two burgers were used for the determination of pH, color and drip
100 loss, two burgers were used for TBARS, antioxidant capacity, FA profile and microbial growth and
101 two burgers were cooked to quantify the cooking loss, at Day 0 and 7.
102
103 *Chemical composition and pH determination*
104 Moisture, ether extract and ash were determined according to the AOAC method (1995). Protein
105 content was calculated by difference.
106 pH was determined for each formulation using a pH meter (Hanna pH 211, Hanna Instruments,
107 Padova, Italy) equipped with a glass electrode (Hanna FC 200B, suitable for meat penetration) and
108 an automatic temperature compensator.
109
110 *Drip loss*
111 The drip loss was measured as the percentage of weight loss of burgers held under standardized
112 conditions (4 ± 0.5 °C for 24 hours and 7 days, Lundström, & Malmfors, 1985) and was expressed as
113 follows:
114 Drip loss = $[(W_b - W_a)/W_b] \times 100$,
115 where W_b and W_a are the weights of the burgers at Day 0 and Day 1 or Day 7, respectively, during
116 refrigerated storage.
117
118 *Cooking loss*
119 The burgers were weighed and then cooked in a preheated oven at 163°C to an internal temperature
120 of 71°C. The burgers were turned every 4 min to prevent excess surface crust formation. After
121 cooking, the burgers were held at room temperature for a few minutes, and the surfaces were dried
122 slightly with blotting paper before weighing. Cooking losses (%) were calculated as follows:
123 Cooking loss = $[(W_b - W_a)/W_b] \times 100$,
124 where W_b and W_a are the weights of the burgers before and after cooking, respectively (AMSA
125 1995).
126
127 *Color determination*
128 Meat color was expressed as L* (lightness), a* (redness), and b* (yellowness) according to the CIElab
129 system (CIE, 1976) and was measured in raw burgers using a Minolta CR300 chroma meter (Minolta,
130 Osaka, Japan).
131 The illuminant was D65, and an incidence angle of 0° was used. Each data point was the mean of
132 three replications measured on the surface of the burgers at randomly selected locations.
133 Prior to each session, the chroma meter was calibrated for the CIE color space system (CIE, 1976)
134 using a white tile (L* = 98.14, a* = -0.23 and b* = 1.89). The L* value indicates lightness (0 = darkness,
135 100 = lightness), the a* value indicates redness (+60 = red, -60 = green) and the b* value indicates
136 yellowness (+60 = yellow, -60 = blue). From these coordinates, hue (H*) and chroma (C*) were
137 calculated as follows:
138 Hue = $\tan^{-1} b^*/a^*$
139 Chroma = $(a^{*2} + b^{*2})^{½}$.
140 The numerical total color difference (ΔE) between burgers was calculated by:
141 $\Delta E_{\beta-\alpha} = [(L^*_\beta - L^*_\alpha)^2 + (a^*_\beta - a^*_\alpha)^2 + (b^*_\beta - b^*_\alpha)^2]^{0.5}$,
142 where L^*_α, a^*_α, b^*_α, and L^*_β, a^*_β, b^*_β are the values at Day 0 and 7, respectively, for each batch's

143 formulation or the values at the same time (Day 0 or 7) of two different formulations within the
144 same batch. A variation in color (ΔE) equal to 2.3 units corresponds to a just-noticeable difference
145 (JND) for the human eye; higher variation is considered discernable (Sharma, 2003).
146
147 *Microbial assay*
148 For microbial assay 10 g of samples were used. The samples were analyzed for enumeration of total
149 aerobic plate counts (ISO 4833:2003) and the presence of beta glucuronidase-positive *Escherichia*
150 *coli* (ISO 16649-2:2001), *Enterobacteriaceae* (ISO 21528-2:2004) and coagulase-positive and -
151 negative staphylococci (ISO 6888-1:1999). All microbial counts were expressed as log CFU g^{-1}.
152
153 *Fatty acid composition*
154 The FA profile of the meat was determined using a gas chromatograph (Fisons mega 2, equipped
155 with a flame ionization detector; Fisons Instruments S.p.A., Rodano, Milano, Italy) after lipid
156 extraction (Folch, Lees, & Stanley, 1957) and consecutive hot derivatization with a methanolic
157 solution of sulfuric acid (3%). Separation of the resulting fatty acid methyl esters (FAMEs) was
158 performed on an Agilent (J&W) capillary column (30 m × 0.25 mm ID) coated with a DB-Wax
159 stationary phase (film thickness of 0.25 mm). The individual FAMEs were identified by referencing
160 the retention times of authentic FAME standards. The FA composition of the samples was expressed
161 as a percentage of the total FAs and calculated using Chrom-Card software.
162
163 *TBARS - Thiobarbituric acid reactive substances*
164 Thiobarbituric acid-reactive substances were measured for determination of malondialdehyde
165 (MDA) levels according to the method described by Ke, Ackman, Linke, & Nash (1977) and modified
166 by Dal Bosco *et al.* (2009). A 5 g sample was taken from raw burgers and homogenized for 45 sec at
167 9000 rpm (Polytron PT 3000, Kinematica AG, Eschbach, Deutschland) with 10 mL of 7.5%
168 trichloroacetic acid (TCA) and 0.1% diethylenetriaminepentaacetic acid (DTPA) in distilled water
169 (final concentration).
170 The homogenized sample was centrifuged (10000 rpm for 10 min) (4235A CWS, ALC International,
171 Milan, Italy) and filtered through Whatman number 1 filter paper, and 5 mL of the filtrate was mixed
172 with 2.5 mL of 2-thiobarbituric acid (TBA) solution (0.288% in distilled water) in capped test tubes.
173 The tubes were vortexed and placed in a water bath at 95 °C for 45 min, then cooled under tap
174 water. The absorbance was determined at 532 nm (V-530 Jasco International, Milan, Italy) against
175 a blank containing TCA/DTPA solution instead of a sample extract. A calibration curve was plotted
176 with TEP (1,1,3,3-tetraethoxypropane; 0-15 µM, final concentrations) to obtain the MDA
177 concentration, and the results were expressed as mg of MDA per kilogram of fresh meat. All
178 determinations were performed in triplicate.
179
180 *Antioxidant extraction*
181 Samples of fresh burgers (5 g) were homogenized in 10 mL of ethanol at 9000 rpm for 45 sec in a
182 tube wrapped in aluminum foil. Solid matter was separated by centrifugation at 10000 rpm for 10
183 min and filtered through Whatman number 4 filter paper. The filtrate was used to measure 2,2-
184 azinobis-(3 ethylbenzothiazoline-6-sulfonic acid) (ABTS) reducing activity, 1,1-diphenyl-2-
185 pircydrazyl (DPPH) radical scavenging activity, and ferric reducing ability (FRAP). All extractions were
186 performed in triplicate.
187
188 *$ABTS^{\bullet+}$ - radical cation decolorization assay*
189 The Trolox equivalent antioxidant capacity assay was used for the determination of meat
190 antioxidant activity, according to the method described by Re *et al.* (1999). The ABTS radical cation,

191 $ABTS^{•+}$, was produced by reacting 14 mM ABTS with an equal volume of 4.9 mM potassium
192 persulfate (final concentration 7 mM ABTS and 2.45 mM potassium persulfate in distilled water).
193 The mixture was incubated in the dark at room temperature, 12-16 h prior to use. The $ABTS^{•+}$
194 solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm. The diluted $ABTS^{•+}$
195 solution (3 mL) was reacted with 30 µL of the meat extracts, and the absorbance was read 6 min
196 after the initial mixing. The control absorbance was determined using the diluted $ABTS^{•+}$ solution
197 reacted with ethanol rather than with meat extract; ethanol alone was used as a blank. The
198 reduction of the $ABTS^{•+}$ radical was calculated as:
199 $Inhibition_{sample}(\%) = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$,
200 where $Abs_{control}$ is the absorbance of $ABTS^{•+}$ with ethanol rather than meat extract and Abs_{sample} is
201 the absorbance of the $ABTS^{•+}$ radical solution of the sample. Trolox (100-2000 µM, final
202 concentrations) was used for calibration, and the results were expressed as mmol of Trolox
203 equivalent per kilogram of fresh meat.
204
205 *$DPPH^{•}$ - radical scavenging activity*
206 The $DPPH^{•}$ radical scavenging activity was estimated using the method of Blois (1958) modified by
207 Jung *et al.* (2010). The control absorbance was detected using ethanol rather than meat extract in
208 the solution; ethanol alone was used as a blank. The absorbance of the solution was measured at
209 517 nm. The inhibition percentage of $DPPH^{•}$ radicals was calculated the same as for the $ABTS^{•+}$
210 radicals. Trolox (0-100 µM, final concentrations) was used for calibration and the results were
211 expressed as mmol of Trolox equivalent per kilogram of fresh meat.
212
213 *FRAP - ferric reducing ability assay*
214 The ferric antioxidant capacity of the samples was estimated according to the method described by
215 Benzie, & Strain (1996), modified for meat samples by Descalzo *et al.* (2007). Samples of the meat
216 extract (83 µl) were added to 2.5 mL of FRAP buffer containing 10 mM TPTZ (2,4,6-tris(2-pyridyl)-s-
217 triazine) in 40 mM HCl and 20 mM $FeCl_3$ added to 300 mM acetate buffer (pH 3.6), prepared daily
218 (1:1:10). The mixture was allowed to stand for 4 min at room temperature before the absorbance
219 was measured at 593 nm using a spectrophotometer. FRAP0 was estimated to measure endogenous
220 Fe^{II} that could react with TPTZ (Descalzo *et al.*, 2007) and determined using a TPTZ/HCl solution
221 without the addition of $FeCl_3$ to the mixture. FRAP values, derived from triplicate analyses, were
222 calculated according to the 0-100 µM calibration curve for $FeSO_4 \cdot 7H_2O$ (final concentrations) and
223 the results were expressed as mmol of Fe^{II} equivalent per kilogram of fresh meat.
224
225 *Statistical analysis*
226 ANOVA was performed using the SAS (2002) program and included the batches (B), the formulation
227 (F), the storage time (ST) and their interactions (F × ST). The batches did not show significant
228 differences and the *P* values were not reported in the tables. The statistical significance of
229 differences was assessed using Tukey's test (SAS, 2002).
230

231 **3. Results**

232 The mean contents of moisture, protein, ether extract and ash of the meat batches used for the
233 preparation of experimental burgers are shown in Table 1. No significant differences among the six
234 meat batches were observed.
235 Table 2 presents the effects of formulation (F), storage time (ST) and the F × ST interaction on the
236 physical characteristics of burgers. Considering the main effects, the formulation affected all
237 parameters except for L* and drip loss, which were affected only by storage time (P<0.001). At Day

238 0, the burgers showed higher values of L* and lower values of drip loss than on Day 7 (data not
239 shown). A significant F × ST interaction was observed for pH, a*, b*, H*, C* (P<0.001) and cooking
240 loss (P<0.01). The pH values increased with storage time. At Day 0, the pH did not differ among
241 formulations, but at Day 7, the AA burgers presented higher pH values than the other formulations.
242 The meat presented discoloration with storage time. At Day 0, the burgers with turmeric powder
243 had higher values of a*, b*, H* and C* than the control and ascorbic acid burgers. At Day 7, the Tu
244 burgers presented values of a* similar to those of the AA burgers and higher values of b*, H* and
245 C* than the other formulations. The cooking loss was affected by storage time and formulation. At
246 Day 0, the Tu burgers presented a lower cooking loss than the burgers of the other formulations
247 (P<0.01); however, at Day 7, no difference in cooking loss was observed.
248 The total color differences (ΔE) are reported in Table 3. At Days 0 the ΔE values of Tu burgers
249 compared to AA and C burgers were higher than Day 7. No significant modification in color
250 difference between C and AA at the two times were observed. The ΔEs calculated between Tu and
251 the other formulations showed high values (ΔE above 40) for the yellow color due to turmeric
252 powder. The difference between C and AA at Day 0 was slightly over the JND threshold and
253 remained almost low during time. The ΔE calculated for each formulation, as a function of storage
254 time, showed that the AA burgers had a lower variation in color than C and Tu burgers.
255 The microbial analysis (Table 4) showed that microflora developed with storage time. At Day 0, the
256 AA burgers showed a significantly lower total aerobic plate count and coagulase-positive and -
257 negative staphylococci count, but at Day 7, they showed a higher total aerobic plate count and
258 coagulase-positive and -negative staphylococci count (P<0.001 and P<0.05, respectively) than the
259 other formulations. At Day 0, *Enterobacteriaceae* and beta glucuronidase-positive *E. coli* were below
260 the detection limit (<1 log CFU g^{-1}); however, after seven days of storage, the microbial counts
261 increased on an order of log 3 for *Enterobacteriaceae* in all formulations and for beta glucuronidase-
262 positive *E. coli* in the AA and Tu burgers.
263 The interaction FxST of FA profile (%) of the burgers was not significant and the data were discussed
264 considering only the effects of the main factors (Table 5).
265 The fatty acids of the burgers were composed mainly of linoleic acid (C18:2n-6) followed by palmitic
266 (C16:0) and oleic (C18:1) acids at contents of approximately 30%, 28% and 21%, respectively.
267 Considering the main factors, the formulations significantly affected the fatty acid composition. The
268 burgers with turmeric powder showed significantly higher values of C18:3n-3 (P<0.05), C20:2n-6
269 (P<0.001) and C20:3n-3 (P<0.01) than the C and AA burgers and also showed a significant reduction
270 of C14:0 (P<0.05) in comparison with the AA treatment. Tu and AA burgers presented significantly
271 higher proportions of arachidonic acid C20:4n-6 (P<0.05), EPA C20:5n-3 (P<0.05) and DHA C22:6n-3
272 (P<0.01) than the C burgers. Specifically, the Tu burgers were characterized by higher amounts of
273 total n-3 FA (P<0.001) and PUFAs (P<0.01) than the other formulations.
274 Considering the effect of Storage Time, the significant reduction of total n-3 (P<0.01) at Day 7
275 resulted from a reduction in the quantity of C20:3n-3 (P<0.01). The significantly lower quantity of
276 PUFAs at Day 7 was associated with the significant reduction of total n-3 (P<0.01), C20:2n-6 and
277 C20:4n-6 (P<0.05) content.
278 The lipid oxidation and antioxidant capacity of the burgers is reported in Table 6. Considering the
279 main effects, the storage time significantly affected TBARS, and all burgers presented higher
280 peroxidation at Day 7 (P<0.01). A significant F × ST interaction was observed for the radical
281 scavenging capacity assays. At Days 0 and 7, the Tu burgers showed the highest values of ABTS
282 (P<0.01). At Day 0, higher values of DPPH (P<0.05) and FRAP (P<0.001) were observed in the AA
283 burgers; however, at Day 7, these values were higher in the Tu burgers.
284

285 **4. Discussion**
286
287 As expected, our results confirm other findings reported in the literature indicating increases in
288 meat pH with storage time. The increase in pH may be attributable to both the hydrolysis of
289 proteins, which determines the alkalinization of meat resulting from an increase in ammoniacal
290 nitrogen levels and to the degradation of proteins and amino acids by gram-negative bacteria
291 (Cabanes, Ouhayoun, & Gilbert, 1996; Choe *et al.,* 2011; Dalle Zotte, 2002; Kilic, Simsek, Claus, &
292 Atilgan, 2014; Verma, & Sahoo, 2000).
293 In our study, no significant differences in pH values were observed among formulations at Day 0,
294 indicating that the pH of the rabbit meat was not affected by the substances added. The same result
295 was obtained in studies on the effects of natural antioxidants in pork patties and burgers (Carpenter,
296 O'Grady, O'Callaghan, O'Brien, & Kerry, 2007; Garrido, Auqui, Martí, & Linares, 2011). However,
297 after 7 days, the burgers with AA showed the highest degree of alkalinization, most likely because
298 of the hydrolysis of proteins and the degradation of amino acids by bacteria, with the subsequent
299 production of ammonia, amines and other basic substances (Rodriguez-Calleja, Garcia-Lopez,
300 Santos, & Otero, 2005; Nychas, Drosinos, & Board, 1998).
301 Our results indicated that ascorbic acid did not limit microbial growth; at Day 7, the AA burgers
302 showed the highest values of log CFU g^{-1}. A similar trend was observed in the Tu burgers. The
303 microbial growth in AA and Tu burgers could also be related to the slightly higher water holding
304 capacity (drip loss) at Day 7. The high water availability could have promoted the growth of bacteria,
305 and the close-to-neutral pH may have stimulated the growth of Staphylococci and *E. coli*, which have
306 an optimal pH of 6.5-7.0 (Valero *et al.,* 2009) and 5.5-8.0 (Buchanan, & Klawitter, 1992), respectively.
307 Moreover, ascorbic acid (at pH below 7.0) undergoes auto-oxidation with the formation of
308 dehydroascorbic acid and hydrogen peroxide in the presence of air or oxygen and metal ions.
309 Therefore, the lower values of log CFU g^{-1} in the AA burgers at Day 0 may also be associated with a
310 bactericidal effect from the formation of hydrogen peroxide. However, the increase in pH over time
311 could have decreased the effect of the ascorbic acid and turmeric on bacteria and promoted the
312 growth of Staphylococci and *E. coli*. These results are in agreement with those of other studies on
313 the antioxidant and antimicrobial activities of natural extracts and ascorbate; neither the natural
314 antioxidants nor the ascorbic acid inhibited the bacterial growth in beef patties (Banon, Diaz,
315 Rodriguez, Garrido, & Price, 2007; Sanchez-Escalante, Djenane, Torrescano, Beltran, & Roncales,
316 2001; Shivas *et al.*, 1984).
317 The differences in the a* and b* meat color indexes among the formulations may be ascribed to the
318 impact of curcumin, which increases yellowness; the powder itself showed a* and b* indexes of 18
319 and 69, respectively.
320 The lower L* values at Day 7 may be explained by the negative correlation between pH and
321 lightness; a high pH corresponds to less bright meat (Dal Bosco, Castellini, & Mugnai, 2002; Warriss,
322 2000). The trend observed for lightness and pH is similar to that reported in rabbit meat by Dal
323 Bosco et al. (2014) and in pork by Choe *et al.*, 2011. In these studies on the effect of natural
324 antioxidants and storage time on meat quality, a decrease of L* and an increase of pH during short
325 storage times were observed.
326 At Day 0, in all burgers, the a* values were higher than those previously reported for the *biceps*
327 *femoris* muscle of rabbits (Dalle Zotte *et al.,* 2009; Paci, Preziuso, D'Agata, Russo, & Dalle Zotte,
328 2013). The higher redness index could be attributed both to the histological composition of meat
329 used to prepare the burgers and to the manufacturing process for the ground meat. The burgers
330 were derived from the meat of the hind legs, which are partially constituted by muscles rich in red
331 fibers and characterized by a high oxidative activity (Ouhayoun, & Dalle Zotte, 1993). The grinding
332 process is known to incorporate oxygen and produce a bright red color linked to the formation of

333 oxymyoglobin.
334 A F × ST interaction effect on the redness index was observed. The significant decrease of a* values
335 at Day 7 observed in the C and Tu burgers may be related to the oxidation of the C burgers and to
336 the natural pigments of curcumin in the Tu burgers. Several studies have found reductions in the
337 redness of ground meat with storage time and ascribed these effects to the metmyoglobin produced
338 by oxidation of myoglobin (Choe *et al.,* 2011).
339 The significant reduction of the yellowness index in Tu burgers during the storage period most likely
340 resulted from changes in the natural pigments of turmeric associated with the enzymatic oxidation
341 of phenolic compounds (Dogan, Ayyildiz, Dogan, Alan, & Diken, 2013).
342 The interaction effect indicated that the a*and b* indexes were more stable during storage in
343 burgers with ascorbic acid, confirming that ascorbic acid contributes to color stabilization by
344 delaying discoloration. This trend is similar to that reported for the effect of different doses of
345 ascorbic acid in ground pork, ground beef and beef steaks, where ascorbic acid was found to
346 efficiently retard the oxidation of meat pigments (Ahn, & Nam, 2004; Banon *et al.,* 2007; Mitsumoto,
347 O'Grady, Kerry, & Buckley, 2005; Mitsumoto, Cassens, Schaefer, & Scheller 1991; Sanchez-Escalante
348 *et al.,* 2001).
349 The changes in the color of the burgers observed during storage were confirmed by the hue and
350 chroma results. Among the color coordinates, the use of H* is recommended for monitoring meat
351 discoloration (Ortuno, Serrano, Jordan, & Banon 2014) because human evaluators are better able
352 to understand color (hue) and lightness (L*) (Ripoll, Joy, & Munoz, 2011). In this study, the increase
353 in H* values during storage suggested an increase in meat discoloration that was less noticeable in
354 the Tu and AA burgers than in the control burgers, which underwent intense discoloration during
355 storage. The decrease in C* values indicated a decrease of color saturation, mainly in the Tu burgers.
356 The high ΔE between Tu burgers and the other formulations at both storage times resulted from the
357 high b* index value of the turmeric powder. The unnoticeable color difference at Day 0 between
358 the C and AA treatments confirmed that ascorbic acid did not change the color of the meat. At Day
359 7, the difference between the C and AA treatments was over the threshold, possibly because of the
360 increase of metmyoglobin content in the C burgers. The increase of metmyoglobin content during
361 storage in the control burgers and the color protection provided by ascorbic acid were also
362 confirmed from the lowest ΔE in the AA burgers compared with the C burgers between 0 and 7 days.
363 In the Tu burgers, the ΔE values indicated color differences between Day 0 and 7, most likely
364 resulting from the decrease in the a* and b* indexes of the natural pigments of the turmeric powder.
365 The storage time affected the weight loss of the burgers. At Day 7, the drip loss was significantly
366 higher than that observed at Day 0. Different factors may affect the drip loss such as fat content,
367 pH, storage time and processing factors. The fat content plays a key role in water retention and
368 oxidative processes occurring in both lipid and protein fractions during storage, and it may alter the
369 water holding capacity in fresh meat and burgers in which the exudate losses are exacerbated by
370 grinding processes (Lonergan, Huff-Lonergan, Rowe, Kuhlers, & Jungst, 2001; Traore *et al.,* 2012;
371 Troy, & Kerry, 2010). Rabbit meat has a low fat content and the lipids are composed mainly of
372 polyunsaturated fatty acids. For these reasons, there is a need to preserve the integrity of cellular
373 membranes to reduce weight loss during storage (Lo Fiego *et al.*, 2004). Our results did not show
374 significant differences among formulations; however, the drip losses of burgers treated with
375 ascorbic acid and turmeric powder tended to be low, suggesting a protective effect against protein
376 and lipid oxidation.
377 The low cooking losses of the Tu burgers at Day 0 and 7 most likely resulted from the presence of
378 turmeric powder, which may have improved the water holding capacity.
379 As regard fatty acid profile, the data did not show a significant interaction F*ST probably due to the
380 number of samples, at Day 0 the burger showed slightly differences while at Day 7 the differences

381 between formulations tended to increase (data not shown).
382 The higher content of EPA (C20:5n-3), DHA (C22:6n-3) and arachidonic acids (C20:4n-6) observed in
383 the Tu and AA burgers compared with the control burgers might be attributed to the protective
384 effect of the antioxidants. Curcumin is a strong quencher of singlet oxygen species and the major
385 antioxidant of turmeric powder, and ascorbic acid is a scavenger of the peroxyl radical and can
386 reduce or prevent H_2O_2-induced lipid peroxidation (Daneshyar, 2012; Lean, & Mohamed, 1999;
387 Sharma *et al.,* 2012; Yen, Duh, & Tsai, 2002).
388 Moreover, the PUFA and total n-3 FA levels might have been the highest in the Tu burgers because
389 of the protective effect on C18:3n-3, C20:2 n-6 and C20:3 n-3 and because of the polyunsaturated
390 acid content of turmeric powder (Chaundhry, & Khan, 2012; Richmond, & Pombo-Villar, 1997).
391 The significant decrease of PUFA and total n-3 levels during storage was associated with a significant
392 reduction of C20:2n-6, C20:3 n-3 and C20:4 n-6 levels, even though the PUFA and total n-3 levels of
393 burgers with Tu tended to be slightly higher than those of the other formulations.
394 Tu burgers had higher antioxidant capacity than AA burgers over the storage period. The ABTS
395 values remained high, and the FRAP and DPPH values remained at high levels after 7 days (although
396 at Day 0 they were lower than those in the AA burgers), suggesting higher antioxidant capacity in
397 Tu burgers than in AA burgers. Fasseas, Mountzouris, Tarantilis, Polissiou, & Zervas (2007) and Jung
398 *et al.* (2010) similarly reported that the antioxidant capacity in meat products supplemented with
399 antioxidant plants and vegetal extracts (oregano, sage and gallic acid) remained constant over short
400 storage times. Nevertheless the antioxidant capacity observed during storage time the lipid
401 oxidation (TBARS) increased, independently by formulation. Neither ascorbic acid nor turmeric
402 prevented the oxidative processes in burgers. These findings are in agreement with those of Haak,
403 Raes, & De Smet (2009), who observed an increase in the lipid oxidation of pork patties with the
404 addition of natural antioxidants during storage time.
405

406 **Conclusion**

407 This experiment showed that the addition of 3.5% of turmeric powder had significant positive
408 effects on the oxidative status and on some quality characteristics of rabbit burgers under
409 refrigerated storage. The addition of turmeric powder modified the meat color and produced an
410 antioxidant capacity similar to ascorbic acid: this last finding is important, mainly for rabbit meat
411 that is rich in polyunsaturated fatty acids, as EPA and DHA.
412 The results lead to think that turmeric powder might be considered as a useful natural antioxidant,
413 increasing the quality and extending the shelf life of rabbit burgers; moreover rabbit burgers with
414 turmeric powder might be considered a functional food for anti-infiammatory, anti-infectious and
415 antitumor properties derived from Curcumin. Further studies might be interesting to test different
416 doses of turmeric and to verify if the changes of color and flavor in rabbit burgers added with Tu are
417 negligible or well accepted by consumers of different countries.
418

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Table 1.
Chemical composition of the meat batches used for the preparation of experimental burgers (n=6: mean ± standard deviation).

Component (%)	Mean		SD
Moisture	74.79	±	0.476
Protein	20.79	±	0.744
Ether extract	2.75	±	0.781
Ash	1.67	±	0.475

593 Table 2.

594 Physical characteristics of the burgers.

595

Formulations		Storage time						*P*-value			RMSE[1]
		Day 0			Day 7			F	ST	F x ST	
		C	AA	Tu	C	AA	Tu				
Burgers	n	6	6	6	6	6	6				
pH		5.77[d]	5.78[d]	5.84[d]	6.08[c]	6.69[a]	6.28[b]	***	***	***	0.058
L*		55.51	54.29	54.15	50.82	50.21	51.32	ns	***	ns	1.093
*a**		8.94[b]	9.14[b]	13.17[a]	5.02[d]	7.99[bc]	7.30[c]	***	***	***	0.718
*b**		6.28[c]	6.23[c]	53.17[a]	7.35[c]	7.04[c]	48.52[b]	***	*	***	0.890
Hue (H*)		35.09[d]	34.40[d]	76.12[a]	56.15[b]	41.38[c]	81.50[a]	***	***	***	2.536
Chroma (C*)		10.94[cd]	11.08[c]	54.79[a]	8.94[d]	10.66[cd]	49.07[b]	***	***	***	0.956
Drip loss	%	2.50	2.00	1.90	10.40	7.60	7.20	ns	***	ns	2.295
Cooking loss	%	25.60[a]	26.00[a]	18.40[b]	13.31[c]	13.40[c]	11.60[c]	***	***	**	1.798

C: Control burgers, meat only; AA: burgers with ascorbic acid; Tu: burgers with turmeric powder; ns: not significant; *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; different letters in the same row indicate significant differences for F x ST interaction ($P<0.05$); [1]: root mean square error.

596

597 Table 3.
598 Numerical total color difference (ΔE) between formulations at Day 0 or 7 and between storage times within
599 the same formulation.
600

ΔE within Storage time				
Formulations	Day 0	Day 7	*P*-value	RMSE[1]
C-AA	2.34	3.62	ns	1.150
C-Tu	47.14[a]	41.26[b]	***	1.750
AA-Tu	47.13[a]	41.53[b]	***	1.367

ΔE within Formulations					
Storage time	C	AA	Tu	*P*-value	RMSE[1]
Day 0-Day 7	6.50[a]	4.48[b]	8.14[a]	***	1.223

C: Control burgers, meat only; AA: burgers with ascorbic acid; Tu: burgers with turmeric powder. ΔE calculated at time 0 or 7 between formulations (C-AA, C-Tu and AA-Tu) and within the same formulation between storage times (Day 0 – Day 7); ns: not significant; *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; different letters in the same row indicate significant differences ($P<0.05$); [1]: root mean square error.

601

602 Table 4.
603 Log CFU g^{-1} of total aerobic count, beta glucuronidase-positive *Escherichia coli*, *Enterobacteriaceae*, and
604 coagulase-positive and -negative Staphylococci in raw burgers stored for 0 and 7 days.
605

	Storage time						*P*-value			
	Day 0			Day 7			F	ST	F x ST	RMSE[1]
Formulations	C	AA	Tu	C	AA	Tu				
Burgers n	6	6	6	6	6	6				
Total aerobic count	3.54^{d}	3.04^{e}	3.57^{d}	6.46^{c}	8.53^{a}	7.03^{b}	***	***	***	0.170
E. coli	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	2.97^{a}	2.98^{a}	***	***	***	0.015
Enterobacteriaceae	0.00	0.00	0.00	2.99	3.02	3.02	ns	***	ns	0.020
Staphylococci	2.74^{ab}	1.51^{b}	1.97^{ab}	2.04^{ab}	4.56^{a}	3.76^{ab}	ns	**	*	0.953

C: Control burgers, meat only; AA: burgers with ascorbic acid; Tu: burgers with turmeric powder; ns: not significant; *: *P*<0.05; **: *P*<0.01; ***: *P*<0.001; different letters in the same row indicate significant differences for F x ST interaction (*P*<0.05); [1]: root mean square error.

606

607 Table 5.
608 Effect of main factors (formulation and storage time) on burgers Fatty acid profile (%).
609

	Formulation			Storage time (days)		*P*-value		
	C	AA	Tu	0	7	F	ST	RMSE[1]
Burgers n.	12	12	12	18	18			
C 14:0	1.80[ab]	1.99[a]	1.51[b]	1.83	1.70	*	ns	0.314
C 16:0	27.67	28.25	28.17	27.85	28.21	ns	ns	0.689
C 16:1	2.45	2.62	2.27	2.36	2.53	ns	ns	0.460
C 17:0	0.66	0.63	0.63	0.61	0.67	ns	ns	0.074
C 18:0	8.04	7.52	7.43	7.57	7.75	ns	ns	0.610
C 18:1 n-9	21.10	20.76	20.76	20.65	21.10	ns	ns	0.726
C 18:2 n-6	30.26	30.15	29.67	30.22	29.83	ns	ns	0.880
C 18:3 n-3	2.14[b]	2.13[b]	2.38[a]	2.21	2.23	*	ns	0.204
C 20:0	0.67	0.65	0.47	0.53	0.67	ns	ns	0.095
C 20:2 n-6	0.29[b]	0.23[c]	0.51[a]	0.37[a]	0.31[b]	***	*	0.561
C 20:3 n-3	2.84[b]	3.05[b]	3.89[a]	3.59[a]	2.92[b]	**	**	0.060
C 20:4 n-6	0.29[b]	0.42[a]	0.41[a]	0.43[a]	0.32[b]	*	*	0.097
C 20:5 n-3	0.21[b]	0.30[a]	0.30[a]	0.30	0.24	*	ns	0.079
C 22:6 n-3	0.11[b]	0.16[a]	0.21[a]	0.17	0.14	**	ns	0.054
C 24:0	1.48	1.15	1.41	1.31	1.38	ns	ns	0.326
∑SFA	40.31	40.19	39.62	39.70	40.38	ns	ns	0.997
∑MUFA	23.55	23.38	23.02	23.01	23.63	ns	ns	0.805
∑n3	5.30[b]	5.64[b]	6.78[a]	6.27[a]	5.54[b]	***	**	0.655
∑n6	30.84	30.79	30.59	31.02	30.46	ns	ns	0.852
∑PUFA	36.14[b]	36.43[b]	37.36[a]	37.29[a]	36.00[b]	*	***	0.851

C: Control burgers, meat only; AA: burgers with ascorbic acid; Tu: burgers with turmeric powder; ns: not significant; *: P<0.05; **: P<0.01; ***: P<0.001; different letters in the same row indicate significant differences for F and ST (*P*<0.05); [1]: root mean square error.

610

611 Table 6.

612 Lipid peroxidation (TBARS) and antioxidant capacity (ABTS, DPPH and FRAP) of the burgers.

613

	Storage time						*P*-value			
	Day 0			Day 7			F	ST	F x ST	RMSE[1]
Formulation	C	AA	Tu	C	AA	Tu				
Burgers n.	6	6	6	6	6	6				
TBARS	0.07	0.08	0.08	0.13	0.18	0.18	ns	**	ns	0.024
ABTS	1.75^{d}	3.80^{b}	4.65^{a}	2.87^{c}	3.48^{b}	4.83^{a}	***	*	**	0.196
DPPH	0.44^{c}	1.22^{a}	1.20^{ab}	0.58^{c}	1.06^{b}	1.11^{ab}	***	ns	*	0.059
FRAP	0.17^{e}	5.73^{a}	3.89^{b}	0.49^{e}	1.86^{d}	3.37^{c}	***	***	***	0.200

TBARS expressed in mg of MDA per kilogram of fresh meat; ABTS and DPPH in mmol of Trolox equivalent per kilogram of fresh meat; FRAP in mmol of Fe^{II} equivalent per kilogram of fresh meat; C: Control burgers, meat only; AA: burgers with ascorbic acid; Tu: burgers with turmeric powder; ns: not significant; *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; different letters in the same row indicate significant differences for F x ST interaction ($P<0.05$); [1]: root mean square error.

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