

1 **Assessment of the stability of fresh beef patties with the addition of**
2 **clove extract during frozen storage**

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18 **Abbreviated running title:** Inclusion of CE on the stability of fresh beef patties.

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23 **Abstract**

24 The study assessed the stability for fresh beef patties with the inclusion of clove extract
25 (CE) as a natural antioxidant in comparison to Butylated hydroxytoluene (BHT) and ascorbic acid
26 (AA) at frozen storage. Four different patties were made dependent on the added antioxidants:
27 control (added no antioxidants), added with 0.02% BHT, 0.05% AA, and 0.1% CE. Inclusion of
28 BHT, AA, and CE resulted in a significant reduction of TBARS and hue angle (h°) value and
29 increase of redness (CIE a^*) and chroma (C^*) values ($p < 0.05$). BHT, AA, and CE were observed
30 effectively to retard lipid oxidation and increase color stability. BHT and AA revealed significantly
31 ($p < 0.05$) higher thiol content than the control and CE. However, the reduction percentage for thiol
32 content in CE treated patties was lower than the control and AA-treated patties from first to last
33 time of storage. Moreover, inclusion of AA and CE led to significantly ($p < 0.05$) increased heme
34 iron content when compared to BHT and the control. In conclusion, CE can replace the application
35 of AA and BHT while improving lipid stability, heme iron content, and color stableness of fresh
36 beef patties throughout frozen storage.

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38 Keywords: Fresh beef patties, clove extract, oxidative stability, heme iron content, color value

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40 **1. Introduction:**

41 Meat is an important source of major nutrients and constituent of a daily diet. Change for
42 consumers' demands and increased market competitions have induced a requirement for
43 improving the quality of meat products as beef patty by developing their nutrient values and
44 advantageous-health qualities (Lopez-Lopez et al., 2011). Nevertheless, fresh meat and meat
45 products are highly responsive to quality deterioration because of the higher nutrient constitution
46 (Shah et al., 2014). Frozen storage has been allowed as the most efficient procedure for preserving
47 the attribute for meat products over an extended period of time. The meat quality can be
48 deteriorated because of the physico-chemical process and, consequently, various researches have
49 been presented that the oxidation of lipid is a prime cause for losing the qualities for several types
50 of meat plus processed meats during frozen storage (Ozer and Saricoban, 2010). In addition, auto-
51 oxidation is occurred broadly leading to a reduced functional quality of meats and meat-based
52 products throughout frozen storage (Mielnik et al., 2003).

53 One of the important factors for meat quality deterioration is the oxidative process which
54 affects lipid, protein, carbohydrate, vitamins, and pigments. Oxidative deterioration induces
55 mainly lipid oxidation and results in loss of nutritional quality and sensorial attributes and
56 reduction of shelf-life for meat-based products (Soriano et al., 2018). Lipid originated reactive
57 oxygen species and oxidizing myoglobin by-products lead to meat oxidation and dramatically
58 decrease quality properties such as color, flavor, and nutrient value of meat products (Seo et al.,
59 2019). Moreover, protein oxidation results in a change for amino acid structure, tenderness, and
60 water-retention capability for meats and processed meats causing a decrease in meat products
61 quality (Turgut et al., 2016).

62 The uses of antioxidants supplements have been proven to be an efficient plan for delaying
63 or preventing the oxidizing procedures. Therefore, ascorbic acid (AA) is usually employed as an
64 antioxidant supplement during processing of meats. AA is soluble in water, employed as the
65 additives based on the rule of 'proper amount' to hinder deterioration of meat product quality, and
66 is regarded for having no toxic impacts for the consumer (Carballo et al., 2018; Ozer and Saricoban,
67 2010). Moreover, Butylated hydroxytoluene (BHT) is a widely utilized artificial antioxidant which
68 is efficient to purify peroxy radical and restrain the origination of free radical. This is allowed to
69 incorporate into meat and meat product to slow or inhibit the oxidation and expand the storage

70 stability (Kumar et al., 2015). Nonetheless, consumer interests and requirements on naturally
71 originated antioxidant substances have been increasing owing to the antinutritional and
72 toxicological impacts for synthetically antioxidant substances like BHT, butylated hydroxyanisole
73 (BHA) or propyl gallate (Shah et al., 2014; Carballo et al., 2018).

74 Clove (*Syzigium aromaticum L.*), is belonged to the Myrtaceae family, is a dried-up flower
75 bud and is largely utilized in the food products as it possesses a specific aroma and effective health
76 attributes. Clove contains phenolic constituents like tannins, sesquiterpenes, and triterpenoids
77 which can show antioxidant activities (Ramadan et al., 2013; Zhang et al., 2017). Clove extract
78 (CE) acquired from the entire clove bud has been widely examined to show high antioxidant
79 actions in meat products (Shi et al., 2014). In that context, clove extracts as natural antioxidant
80 have been employed for meats and meat-based products to increase lipid and protein stability
81 against oxidization, enhance color stabilization and sensorial properties, and lengthen the shelf-
82 life at the storage time (Zhang et al., 2017; Shi et al., 2014).

83 The uses of natural antioxidant have been expanded for improving the oxidizing stability
84 for meats and meat-based products over the past years (Armenteros et al., 2016). Zhang et al. (2016)
85 has recorded that CE as a natural antioxidant has been used potentially in meat products for
86 improving the oxidizing stability. However, it has been utilized BHT and AA as antioxidants in
87 the meat industries (Carballo et al., 2018; Cunha et al., 2018). Considering this, fresh beef patties
88 were formulated with BHT, AA, and CE, and it was examined the comparison for the impacts of
89 abovementioned antioxidants on oxidizing stability, color stableness, and heme iron content for
90 fresh beef patties. Nonetheless, no comparative analysis has been conducted for the antioxidative
91 effect of CE with AA and BHT on the stability of fresh beef patties, especially at frozen storage.

92 The purpose for the existing study was to determine the antioxidative effect of CE
93 compared to AA and BHT on the stability of fresh beef patties. Cooking loss, pH, lipid oxidization,
94 protein oxidization, heme iron level, and color values of fresh beef patties were evaluated during
95 6 months of frozen storage. Fresh beef patties with added no antioxidants were employed as the
96 control.

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99 **2. Materials and methods**

100 ***2.1. Formulation of clove extract***

101 Clove was purchased from the domestic market. The clove powder extract was got utilizing
102 the method of reflux extraction. After grinding of clove, the powder was mixed into the distilled
103 water (w/v, 1:5 ratios) and carried for extraction at 85°C for 7 h. Likewise, the extraction for
104 residue was conducted using distilled water (1:5 ratios) for 14 h at 85°C. The extracted two
105 solutions were filtrated with filter paper of Whatman No. 1. The eventual CE was collected after
106 condensing the solution through a void rotating evaporator at 85°C. The CE was preserved at -
107 60°C to continue analysis.

108

109 ***2.2. Chemicals***

110 Butylated hydroxytoluene (BHT), ascorbic acid (AA), 2-thiobarbituric acid (TBA), perchloric
111 acid (PCA), Sodium dodecyl sulfate (SDS), tris(hydroxymethyl)amino methane (TRIS) buffer,
112 and 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB) were obtained from Sigma Aldrich (St. Louis,
113 MO, USA). The whole chemicals utilized for the current research was of the highest purities of
114 analyzed properties.

115

116 ***2.3. Formulation of fresh beef patties***

117 Fresh beef loin and beef back fat were acquired from the domestic market and ground
118 independently utilizing a meat grinder (GG-22, German Knife, CA, USA) consisting of a plate set
119 in 8-mm diameter holes. The study included 4 treatments, and three batches for each treatment
120 were conducted 3 times for each storage month (mon). The preparation of all fresh beef patties
121 was performed employing the same formulations. The beef loin, back fat, and all fresh constituents
122 were combined absolutely in the correct proportion employing a patty mixer (5K5SS, KitchenAid,
123 Michigan, USA). The primary formulations contained 90.8% beef loin, 8.0% back fat, and 1.2%
124 sodium chloride. The fresh beef patties (4) were prepared as control (without BHT, AA, and CE),
125 added with 0.02% BHT (BHT), 0.05% AA (AA), and 0.1% CE (CE). The patties (45 g) were
126 structured through a hand held patties maker. After packaging in the polyethylene packets, the
127 fresh patties were kept at frozen (-21°C) storage for 0, 2, 4, and 6 mon to conduct the whole
128 experiments in the laboratory of Meat Processing.

129 **2.4. pH and cooking loss**

130 The pH value of beef patties was assessed employing an electronic pH meter (MP230,
131 Mettler Toledo, Greifensee, Switzerland). The distilled water (30 mL) mixed patties (3 g) were
132 homogenized utilizing an electronic homogenizer (T25 Ultra-Turrax, IKA, Germany) for 25 s. For
133 evaluating the pH of the patties, the pH meter was calibrated through standard buffers of pH 4.01,
134 7.00, and 9.21 at 21 °C.

135 The cooking loss (%) for fresh beef patties was measured by the calculation of weight
136 difference between uncooked and cooked patties as followed:

137 $\text{Cooking loss (\%)} = [(\text{weight of uncooked beef patties} - \text{weight of cooked beef patties}) / \text{weight of}$
138 $\text{uncooked beef patties}] \times 100.$

139

140 **2.5. Lipid oxidation**

141 Thiobarbituric acid reactive substances (TBARS) of beef patties were assessed to measure
142 the lipid oxidation employing the minor modified method adopting from Cherian et al. (2007).
143 Fresh beef patties (3 g) were homogenized through 3.86% perchloric acid (27 mL) and stored for
144 1 h at 5 °C. The homogenate was centrifuged (1736R, Labogene Co., Seoul, Korea) at 2100 g for
145 10 min. After filtrating the supernatant, filtrate (2 mL) was admixed into 20 mM TBA (2 mL) and
146 allowed to stay at room-temperature for 14 h. The absorbance was evaluated at 531 nm
147 spectrophotometrically (Cary 60 UV-Vis, Agilent Technologies Inc., CA, USA). The TBARS
148 values were stated as mg malondialdehyde (MDA)/kg patties.

149

150 **2.6. Protein oxidation**

151 Thiol content for different patties was determined for measuring the protein oxidization
152 using the method described by Vossen and De Smet (2015) with some modifications. The patties
153 (2 g) were homogenized through 27 mL of 5% SDS in 0.10 M Tris buffer and transferred to the
154 water-bath at 80 °C for 30 min. The coolness formed homogenate was centrifuged at 6000 g for 20
155 min. The filtrated supernatant (0.5 mL) was added to 2 mL of 0.1 M TRIS buffer (pH 8.0) and 0.5
156 mL of 10 mM DTNB (5,5'-Dithiobis (2-nitrobenzoic acid) in 0.1M TRIS buffer to evaluate the
157 thiol content. Filtrated supernatant (0.5 mL) was incorporated into 0.1 M TRIS buffer (2.5 mL) to
158 evaluate the protein content. Moreover, the reagent blank was formed by mixing 5% SDS in TRIS

159 buffer (0.5 mL), 10 mM DTNB (0.5 mL), and 0.1 M TRIS buffer (2.0 mL). All solutions were
160 allowed for reacting in the dark place at 5°C for 30 min. Absorbance for thiol content was then
161 read at 412 nm. The calculation for thiol content was done employing the Lambert-Beer equation
162 of $\epsilon_{412} = 14000 \text{ M}^{-1} \text{ cm}^{-1}$, and the result was indicated in nmol of thiol/mg of protein. Protein
163 content was determined at 280 nm utilizing a BSA standard curve.

164

165 **2.7. Heme iron measurement**

166 Heme iron content of beef patties was measured employing the method explained by Ozer
167 and Saricoban (2010) with minor modification. Beef patties (1 g) were added to 5 mL of acidified
168 acetone solution (acetone: distilled water: HCl = 90:8:2) in polypropylene tube. The tube was
169 closed with a cap and permitted for standing in darkness condition at room-temperature for 1 h.
170 The tube content was filtrated using Whatman GFA as glass filter paper, and the absorbance was
171 evaluated at 640 nm.

172 The calculation for heme-iron content was performed by calculating the whole pigment as
173 hematin employing the following formulas:

174 Whole pigment (mg/kg) = absorbance \times 680.

175 Heme-iron (mg/kg) = whole pigment (mg/kg) \times 8.82/100.

176

177 **2.8. Color value**

178 Color values like lightness (CIE L^*), redness (CIE a^*), and yellowness (CIE b^*) for several
179 patties were determined utilizing a colorimeter (Konica Minolta CR-400, Tokyo, Japan). The
180 standard white plate ($Y = 81.2$; $x = 0.3191$; $y = 0.3263$) was employed for calibrating the
181 colorimeter, and each patty was measured twice. The measurement for chroma (C^*) value and hue
182 angle (h°) value was carried out utilizing two equations of $\{(a^* + b^*)^{1/2}\}$ and $\{\tan^{-1}(b^*/a^*)\}$,
183 respectively.

184

185 **2.9. Statistical analysis**

186 The experiments contained a sum of 48 observations (four treatments \times three batches \times
187 four storage periods) to conduct statistical analysis. All data were exhibited as mean values of 3
188 replications with the standard error of means. The data were examined utilizing a statistical

189 software of Statistical Analysis System (SAS) containing 9.3 version. One-way analysis of
190 variance (ANOVA) accompanied by Duncan's multiple range tests ($p < 0.05$) was utilized to assess
191 significant differences among different categories for fresh beef patties and to assess the effect of
192 storage period.

193
194

195 **3. Results and discussion**

196 **3.1. pH and cooking loss**

197 The measurement of pH and cooking loss was conducted for 6 mon of frozen storage and
198 is displayed in Table 1. Non-significant change for pH value was noticed amongst all fresh beef
199 patties throughout frozen storage times ($p > 0.05$). Nonetheless, the pH value in all beef patty
200 samples was significantly increased ($p < 0.05$) from the first to last time of frozen storage. Ozer and
201 Saricoban (2010) recorded that pH value for meat products increased significantly during frozen
202 storage. This increasing for pH value is due to the production of ammonia arising from amino acids
203 deterioration as protein denaturation in meat products. The results are in accordance with Mokhtar
204 and Youssef (2014), who noticed that the beef burgers treated with BHT/BHA and CE showed no
205 significant changes for pH values compared with the control during storage.

206 The cooking loss considers the loss of moisture and fat after cooking of meat products. The
207 insignificant difference ($p > 0.05$) for cooking loss was seen among all patty samples at all storage
208 periods. Nevertheless, the cooking loss in all patties showed no significant changes among all
209 storage times ($p > 0.05$). It is reported that all antioxidants and storages times had no negative effects
210 in cooking loss. No cooking loss in beef patties formulated with BHT, AA, and CE can be related
211 to no fat and moisture loss. Non-significant change for cooking loss in chicken nugget was seen
212 after formulation with antioxidants of sage, rosemary, and tea catechin (O'Sullivan et al., 2004).
213 Basanta et al. (2018) also reported that patties sample with added plum pulp showed non-
214 significant change for cooking loss in comparison to the control sample.

215

216 **3.2. Lipid oxidation**

217 Lipid oxidization for fresh beef patties was assessed by measuring the TBARS value at
218 frozen storage, and the findings are exhibited in Fig. 1. Incorporated antioxidants and storage

219 months presented substantial ($p < 0.05$) effect on TBARS values. Maximal TBARS was shown in
220 control beef patties throughout the storage months; nonetheless, antioxidants formulated patties
221 exhibited obvious reduction for TBARS values. TBARS value for all kinds of beef patties was
222 significantly ($p < 0.05$) increased from mon 0 to mon 6 of storage. The antioxidants like BHT, AA,
223 and CE formulated fresh beef patties revealed significantly lower TBARS values during whole
224 frozen storage periods when compared with the control ($p < 0.05$). The findings noted that added
225 antioxidants presented a positive effect on the oxidative stability for beef patties. The increase for
226 TBARS value for the control could be due to the origination of increased MDA that has been
227 considered as secondary products for lipid oxidization (Zhang et al., 2016). However, non-
228 significant change for TBARS value was observed amongst BHT, AA, and CE contained beef
229 patties for all storage periods ($p > 0.05$). Ozer and Saricoban (2010) reported that chicken patties
230 with added AA had significantly reduced TBARS value compared to the control patties. The
231 substantial decrease for TBARS values was found in CE treated pork patties (Kong et al., 2010),
232 chicken meat sample (Zhang et al., 2016), buffalo patties (Tajik et al., 2014), beef burgers
233 (Mokhtar and Youssef, 2014), and pork sausages (Zhang et al., 2017). The current study is in
234 agreement with such results and indicates that the natural antioxidant like CE can have been
235 employed for enhancing the shelf-life for any meat product. This antioxidant effectiveness of CE
236 has been performed owing to the phenolic constituents and the capacity for the hydrogen molecule
237 donation to deactivate free radical (Baghshahi et al., 2014).

238

239 **3.3. Protein oxidation**

240 Protein oxidation of fresh beef patties was measured by the evaluation of thiol contents,
241 and the findings are presented in Table 2. The significant decline for thiol content in whole beef
242 patty samples were observed from mon 0 and 2 to mon 4 and 6 of frozen storage ($p < 0.05$), indicated
243 the proteins oxidization. A similar result has been found by Feng et al. (2016), who revealed that
244 proteins oxidization caused diminished thiol content for pork sausage at storage time. From first
245 to last time of storage, the reducing rate for thiol content in CE treated beef patties were lower
246 compared to AA supplemented beef patties and the control. The decline percentages for thiol
247 content were shown by ascending: control > AA > CE > BHT (12.31% > 11.20% > 9.51% > 8.72%,
248 respectively). At the end of the storage, thiol content reduction of 12.31% was seen for the control

249 patties; however, thiol content reduction for BHT, AA, and CE treated beef patties (8.72%, 11.20%,
250 and 9.51%, respectively) was lower than the control patties. After mon 4 and 6 of storage, BHT
251 and AA contained beef patties presented significantly higher thiol content by comparing with CE
252 contained patties and the control patties ($p < 0.05$), nevertheless, no significant change ($p > 0.05$) for
253 thiol content was found between BHT and AA contained beef patties. The CE formulated fresh
254 beef patties presented significantly lower thiol content than all other patties for all storage months
255 ($p < 0.05$). The outcomes are similar to the research analyzed by Zhang et al. (2017), who observed
256 that the significant reduction for thiol content was seen for CE formulated pork sausages compared
257 with the control, and this occurrence might be done because of the balancing of the antioxidants
258 and pro-oxidants effect for phenolic components in CE. Jongberg et al. (2011) stated that white
259 grapes extracts led to a reduction in thiol contents for beef patties, and it might be occurred owing
260 to the presence of ortho-phenolic substances in extracts. This thiol content reduction in beef patties
261 by the addition of CE was presented because of the ortho-phenolic component (eugenol), which
262 could react with thiol contents and produce thiol-quinone admixture; as a result, thiol content was
263 decreased (Zhang et al., 2017). Nonetheless, silver carp fillet formulated with CE was seen to
264 hinder the decline of thiol contents (Shi et al., 2014).

265

266 **3.4. Heme iron content**

267 Meats and meat-based products are important sources for heme iron connected to protein.
268 Heme iron content for fresh beef patties at frozen storage is presented in Fig. 2. The significant
269 decrease ($p < 0.05$) of heme-iron content was seen for all types of beef patties at frozen storage time
270 from mon 0 to mon 6. The heme iron content in CE treated patties was significantly increased
271 ($p < 0.05$) when compared to the control and BHT treated beef patties at all storage times, whereas
272 non-significant changes for heme iron contents were found between AA and CE treated beef patties
273 ($p > 0.05$). On mon 6, the beef patties formulated with AA showed significantly increased heme
274 iron content in comparison to BHT formulated patties and the control ($p < 0.05$). The results
275 specified that AA and CE prevented the free of iron from heme-iron. The increase in heme-iron
276 content for antioxidant contained patties could have been due to the increased level of soluble
277 heme pigments and the contribution for increased extractability of heme pigment (Ozer and
278 Saricoban, 2010). The reduction of heme-iron content occurred because of the releasing of iron

279 caused by disruption of heme and the increase of frozen storage times (Benjakul and Bauer, 2001;
280 Ozer and Saricoban, 2010). Purchas et al. (2003) stated that the drips freed from meat throughout
281 storage comprised a substantial quantity of iron, especially soluble heme-iron. Moreover, Buzala
282 et al. (2016) reported that heme-iron is mostly located in meat protein, is involved in the
283 contribution of the bright red color in meat and meat products.

284

285 **3.5. Color evaluation**

286 Color is a precious quality mostly of meat products and makes influencing the consumer
287 for instant purchasing or refusing the meat products through observation (Soriano et al., 2018).
288 The color values of fresh beef patties were evaluated for all storage months and are shown in Table
289 3. From mon 0 to mon 6, CIE a^* and C^* values were significantly reduced for all kinds of beef
290 patties ($p < 0.05$), nevertheless, non-significant change ($p > 0.05$) in CIE L^* , CIE b^* , and h° values
291 was seen for all beef patties. The CIE L^* value for all patties presented non-significant variation
292 ($p > 0.05$) throughout the storage. The patties formulated with CE showed significantly increased
293 ($p < 0.05$) CIE b^* value compared to the control at the final storage, while the non-significant change
294 ($p > 0.05$) for CIE b^* value was observed in BHT and AA processed patties compared with the
295 control. This result is in accordance with Radha Krishnan et al. (2014), who reported that the CIE
296 b^* values of the spice extract incorporated chicken meat samples were substantially higher in
297 comparison with the control. After 4th and 6th mon for frozen storage, BHT, AA, and CE
298 supplemented patties showed significantly increased ($p < 0.05$) CIE a^* and C^* value by comparing
299 with the control patties. Moreover, a significant decline ($p < 0.05$) for h° value was seen for BHT,
300 AA, and CE incorporated beef patties as compared to the control on mon 4 and 6. The lowered h°
301 value has been linked to a lowered decline for red color (Yousuf and Srivastava, 2017), indicated
302 that BHT, AA, and CE processed beef patties revealed lowered color decline compared to the
303 control. The result indicated that added antioxidants (BHT, AA, and CE) showed preventative
304 effects on discoloring for beef patties during frozen storage moment. This might have been related
305 to a reduction for lipid oxidization by the addition of BHT, AA, and CE, since reduced lipid
306 oxidization can cause reduced discoloration. The previous studies showed that lipid oxidization
307 for meat products caused redness degradation (Hayes et al., 2011; Jung et al., 2012). The
308 significant increase for CIE a^* value was also observed in CE incorporated pork patties (Kong et

309 al., 2010), chicken meat sample (Zhang et al., 2016), and pork sausages (Zhang et al., 2017).
310 Moreover, Falowo et al. (2014) stated that a preventative impact for natural plant extract on
311 discolorization for meats and meat-based products was found because of the antioxidative action
312 of phenolic substances.

313

314

315 **4. Conclusion**

316 The incorporated BHT, AA, and CE in fresh beef patties prompted a significant decline of
317 TBARS and h° values and increase of CIE a^* and C^* values at frozen storage for 6 mon as
318 compared with the control ($p < 0.05$). Inclusion of AA and CE led to significantly increased ($p < 0.05$)
319 heme iron content in beef patties when compared to BHT treated patties and the control. Moreover,
320 BHT and AA added patties and the control showed significantly increased ($p < 0.05$) thiol content
321 compared to CE treated patties. Nevertheless, the percentage in a decrease for thiol content of CE
322 treated patties was lower than the control and AA-treated patties from first to last time of storage.
323 It is definitely seen that BHT, AA, and CE showed the antioxidant effect on fresh beef patties. The
324 antioxidant impacts for three antioxidants were more pronounced for lipid oxidizing than protein
325 oxidizing. In Sum, the inclusion for CE could have been employed as a safe and substitution of
326 artificial antioxidants in beef patties preparation to efficiently prevent lipid oxidation and increase
327 heme iron content and color stability. Therefore, the results can be concluded that CE can replace
328 the application of AA and BHT when the formulation of fresh beef patties at frozen storage.

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330

331 **Acknowledgment**

332 This research was supported by the Korea Institute of Planning and Evaluation for
333 Technology in Food Agriculture, Forestry and Fisheries, Ministry of Agriculture, Food and Rural
334 Affairs (Project No.316064-02- 2-HD030).

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426

427 **Figure legends**

428

429 **Fig. 1:** Effect of different antioxidants on TBARS (mg MDA/kg of sample) value of fresh beef
430 patties during frozen storage. Error bars present standard deviations. Bar charts with different
431 letters present significant differences among the treatments (^{a-b}) at each storage month (p<0.05) or
432 storage months (^{A-C}) in each treatment (p<0.05). Con: control; BHT: added 0.02% BHT; AA:
433 added 0.05% ascorbic acid; CE: added 0.1% clove extract.

434

435 **Fig. 2:** Effect of different antioxidants on heme iron content (mg heme iron/kg of sample) of fresh
436 beef patties during frozen storage. Error bars present standard deviations. Bar charts with different
437 letters present significant differences among the treatments (^{a-c}) at each storage month (p<0.05) or
438 storage months (^{A-C}) in each treatment (p<0.05). Con: control; BHT: added 0.02% BHT; AA:
439 added 0.05% ascorbic acid; CE: added 0.1% clove extract.

440

441 **Table 1**442 **Effects of different antioxidants on pH and cooking loss of fresh beef patties at frozen storage**

	Storage month	Con	BHT	AA	CE	SEM
pH	0	5.56 ^B	5.53 ^B	5.50 ^B	5.54 ^B	0.03
	2	5.64 ^{AB}	5.64 ^{AB}	5.60 ^{AB}	5.63 ^{AB}	0.04
	4	5.63 ^{AB}	5.62 ^{AB}	5.59 ^{AB}	5.60 ^{AB}	0.03
	6	5.76 ^A	5.75 ^A	5.71 ^A	5.74 ^A	0.09
	SEM	0.04	0.05	0.05	0.05	
Cooking loss (%)	0	19.05	19.82	20.63	21.05	1.35
	2	20.01	22.05	23.38	18.50	1.66
	4	23.65	24.05	24.51	22.72	2.16
	6	19.75	19.81	20.68	18.09	1.43
	SEM	1.64	1.75	1.71	1.51	

443 ^{a-b}Mean values in the same row with different letters presented significant differences ($p < 0.05$).444 ^{A-B}Mean values in the same column with different letters presented significant differences ($p < 0.05$).

445 Con: control; BHT: added 0.02% BHT; AA: added 0.05% ascorbic acid; CE: added 0.1% clove extract.

446 SEM: standard error of mean.

447

448 **Table 2**449 **Effects of different antioxidants on thiol content of fresh beef patties at frozen storage**

	Storage month	Con	BHT	AA	CE	SEM
Thiol content	0	93.60 ^{Ab}	95.98 ^{Aab}	98.67 ^{Aa}	76.02 ^{Ac}	1.41
	2	91.06 ^{Aa}	93.30 ^{ABa}	95.43 ^{Aa}	74.33 ^{Ab}	1.18
	4	86.93 ^{Bb}	90.51 ^{BCa}	90.15 ^{Ba}	71.11 ^{Bc}	0.69
	6	82.08 ^{Cb}	87.61 ^{Ca}	87.62 ^{Ba}	68.79 ^{Bc}	0.46
	SEM	0.79	0.76	1.33	0.86	

450 Values presented as nmol thiol/mg of protein in fresh beef patties

451 ^{a-c}Mean values in the same row with different letters presented significant differences ($p < 0.05$).452 ^{A-C}Mean values in the same column with different letters presented significant differences ($p < 0.05$).

453 Con: control; BHT: added 0.02% BHT; AA: added 0.05% ascorbic acid; CE: added 0.1% clove extract.

454 SEM: standard error of mean.

455

456 **Table 3**

457 **Effects of different antioxidants on color values of fresh beef patties at frozen storage**

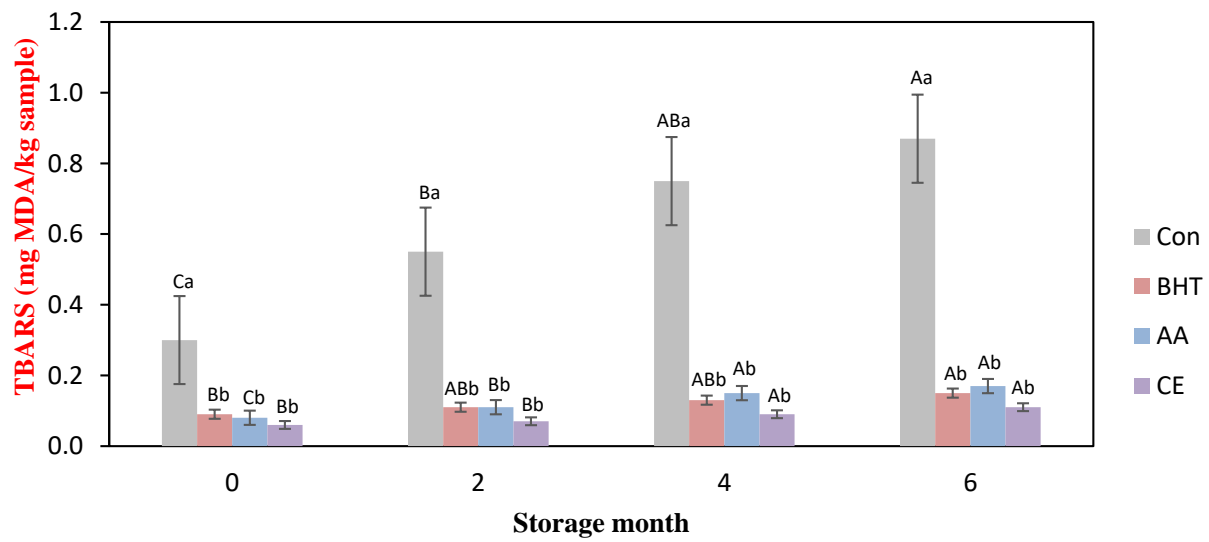
	Storage month	Con	BHT	AA	CE	SEM
Lightness (CIE L*)	0	41.07 ^{Aa}	41.25 ^{Aa}	41.51 ^{Aa}	40.63 ^{Aa}	1.98
	2	39.70 ^{Aa}	40.38 ^{Aa}	39.78 ^{Aa}	40.28 ^{Aa}	2.15
	4	39.27 ^{Aa}	41.07 ^{Aa}	40.54 ^{Aa}	40.50 ^{Aa}	1.66
	6	39.07 ^{Aa}	40.65 ^{Aa}	40.13 ^{Aa}	38.98 ^{Aa}	1.14
	SEM	1.64	1.44	1.80	2.06	
Redness (CIE a*)	0	22.13 ^{Aa}	23.84 ^{Aa}	23.13 ^{Aa}	20.98 ^{Aa}	0.73
	2	21.98 ^{Aa}	23.13 ^{ABa}	20.40 ^{ABa}	22.26 ^{Aa}	1.17
	4	12.56 ^{Bb}	19.78 ^{BCa}	18.47 ^{Ba}	18.44 ^{Ba}	1.01
	6	11.42 ^{Bb}	17.90 ^{Ca}	18.56 ^{Ba}	17.65 ^{Ba}	0.78
	SEM	1.07	1.04	0.84	0.74	
Yellowness (CIE b*)	0	15.37 ^{Aa}	16.22 ^{Aa}	16.19 ^{Aa}	15.82 ^{Aa}	0.75
	2	19.22 ^{Aa}	19.49 ^{Aa}	19.61 ^{Aa}	20.76 ^{Aa}	5.43
	4	11.66 ^{Aa}	13.39 ^{Aa}	12.69 ^{Aa}	13.34 ^{Aa}	0.77
	6	11.50 ^{Ab}	12.43 ^{Aab}	12.65 ^{Aab}	12.98 ^{Aa}	0.38
	SEM	1.90	1.67	1.88	1.88	
Chroma (C*)	0	26.94 ^{Aa}	28.85 ^{Aa}	28.24 ^{Aa}	26.29 ^{Aa}	0.97
	2	23.58 ^{Aa}	24.95 ^{Ba}	22.19 ^{Ba}	24.54 ^{ABa}	1.73
	4	17.19 ^{Bb}	23.90 ^{Ba}	22.45 ^{Ba}	22.79 ^{ABa}	1.03
	6	16.21 ^{Bb}	21.82 ^{Ba}	22.22 ^{Ba}	21.39 ^{Ba}	0.75
	SEM	1.27	1.06	0.98	1.18	
Hue angle (h°)	0	34.83 ^{ABa}	34.18 ^{Aa}	34.91 ^{Aa}	36.92 ^{Aa}	0.86
	2	25.92 ^{Ba}	26.80 ^{Aa}	27.15 ^{Aa}	28.27 ^{Aa}	7.46
	4	43.09 ^{Aa}	34.14 ^{Ab}	34.67 ^{Ab}	35.82 ^{Ab}	1.86
	6	45.36 ^{Aa}	34.76 ^{Ab}	34.56 ^{Ab}	36.35 ^{Ab}	1.51
	SEM	2.90	2.29	3.27	3.23	

458 ^{a-b}Mean values in the same row with different letters presented significant differences ($p < 0.05$).

459 ^{A-C}Mean values in the same column with different letters presented significant differences ($p < 0.05$).

460 Con: control; BHT: added 0.02% BHT; AA: added 0.05% ascorbic acid; CE: added 0.1% clove extract.

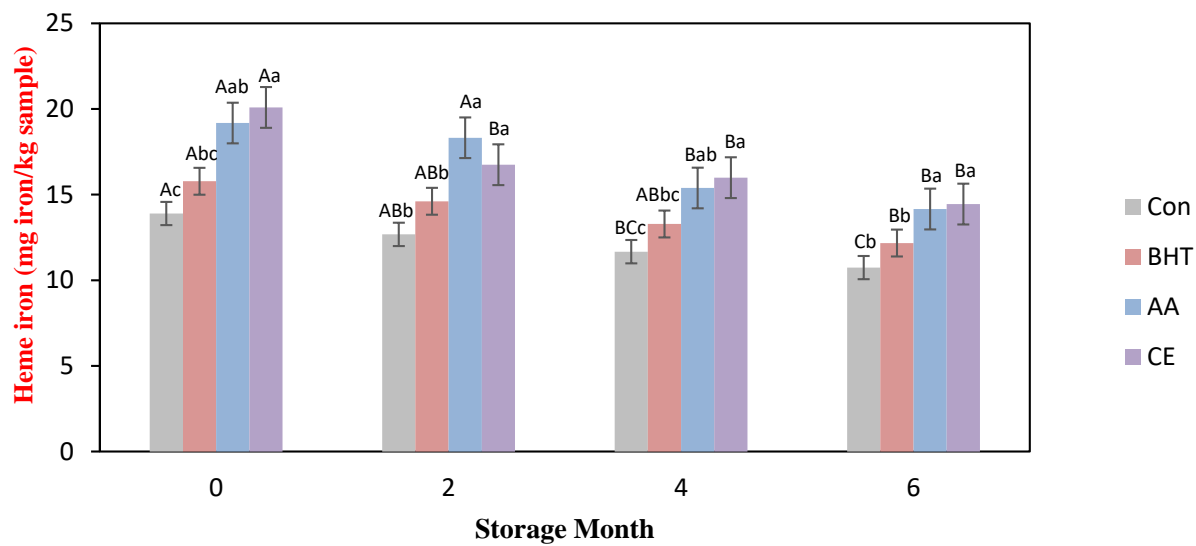
461 SEM: standard error of mean.



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