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4 **Effect of ginger powder addition on quality, fatty acids profile, lipid oxidation and**
5 **antioxidant capacity of cooked pork burgers**

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21 **Abstract**

22 Ginger powder effects were evaluated in cooked pork burgers after a refrigerated storage as raw
23 meat products at 4 °C up to 7 days. Physical-chemical characteristics, fatty acids profile, lipid
24 oxidation and antioxidant capacity of burgers were tested in control samples (only meat, C) and in
25 two formulations containing 1% and 2% of ginger powder (G1 and G2). Ginger naturally brought
26 yellow pigments that increased b* index of G2 burgers. Colour variations between C and G2 were
27 also visible by human eyes as showed by ΔE indices. Ginger improved PUFA ω 3 and PUFA ω 6
28 percentages to the detriment of SFA with following decreases of atherogenicity and
29 thrombogenicity indices. Antioxidants present in ginger powder reduced lipid oxidation (TBARS) and
30 increased antioxidant capacity (FRAP, ABTS and DPPH) of burgers. Results highlighted that ginger

31 powder could express increasing the antioxidant capacity and reducing lipid oxidation of burgers
32 with the final income of healthier products than the control ones.

33

34 **Keywords**

35 Cooked burger; Meat quality; Natural antioxidant; Ginger; Antioxidant capacity.

36

37 **1. Introduction**

38 Ready to cook and ready to eat meat products are one of the more reliable protein foods sold in
39 markets. Particularly, burgers (or patties) represent one of the most consumed products due to their
40 easiness to be stored and the short time requested to be cooked. Due to grinding process burgers
41 are more susceptible to oxidation and microbial spoilage than unprocessed meat because of
42 disruption of muscle structure and the formation of a less stable food matrix that could occur more
43 easily into chemical and enzymatic oxidation [1, 2]. During the last decades several research studies
44 were conducted with the aims to enhance shelf life of burgers, as well as their nutritional value.
45 Production processes, packaging technologies and food additives were studied and employed with
46 the main aim to reduce oxidation [3–5].

47 In order to control food lipid oxidation and deterioration several synthetic additives with antioxidant
48 properties were widely used (i.e. butylated hydroxyanisole or BHA and butylated hydroxytoluene or
49 BHT). This trend changed few years ago after deeply evaluations of synthetic additives that
50 highlighted potential toxic effects and the following consumers behaviour to avoid food that contain
51 these types of additives [6, 7].

52 Natural antioxidants seem to be one of the most reliable responses to this issue, as most of them
53 are not related to side effects and are well accepted by consumers [8, 9].

54 Essential oils, extracts, powders and other plant products were largely studied, and several studies
55 had demonstrated their positive activity against lipid oxidation in meat products [4, 10]. Spices had
56 been used for centuries for their large number of properties. From a technological point of view,
57 they can modify the physical-chemical characteristics of the product or reduce the growth of
58 spoilage bacteria, from a sensory point of view they can affect the colour, taste, flavour and texture.
59 Among Zingiberaceae family ginger, *Zingiber officinale* Roscoe, is one of the most studied as food
60 additive due to its strong antioxidant profile [11–14] and it is a well-known spice used in different
61 types of dishes [15]. Commonly ginger is used both fresh or dried, anyhow, as powder it is more
62 stable and useful in food industries as ingredient in meat products. Ginger powder was largely

63 studied for its important content of antioxidant molecules such as phenols, terpenoids and
64 flavonoids [16, 17]. Furthermore, through the drying process antioxidant capacity of ginger could
65 be enhanced with the formation of shogaols from the dehydration of gingerols. Indeed, shogaols
66 exhibit higher biological properties than gingerols including anticancer and antioxidant activities
67 [18–20].

68 Several articles reported the effects of spices in raw meat burgers, anyhow, a small number of data
69 are available about how spices could contribute to the chemical and physical characteristics of
70 cooked products (i.e. ready to eat).

71 For the reasons reported above the main aims of this study were to quantify the effects of a well-
72 known spice, ginger, in a cooked meat product. Effects of two concentrations of ginger powder on
73 meat quality parameters (pH, colour and water holding capacity), fatty acid profile, lipid oxidation
74 and antioxidant capacity of burgers added with natural antioxidant before cooking were studied.
75 Furthermore, storage time up to 7 days was applied on raw burgers before cooking in order to assess
76 if ginger powder might enhance shelf life of burgers.

77

78 **2. Material and methods**

79 2.1. Experiment design and burgers formulations

80 Burgers were formulated as reported by Mancini et al. [21]. Nine experimental units, each one
81 consisting in Longissimus lumborum muscle from one pig, were randomly assigned to three
82 formulations (3 experimental units per formulation). Each muscle was singularly minced for a total
83 of nine meat batches. Three formulations (F) were manufactured: control burgers (C, only meat),
84 burger added with 1% of ginger powder (G1, 10 g of ginger per kg of meat) and burgers added with
85 2% of ginger powder (G2, 20 g of ginger powder per kg of meat). Ginger powder was purchased
86 from wholesaler Drogheria e Alimentari S.p.A. (Scarperia e San Piero, Florence, Italy; rhizomes of
87 ginger from India, batch number L65069N, proximate composition, antioxidant capacity, colour and
88 fatty acids profile of ginger powder are reported in Table 1) as a ready to use food ingredient. From
89 each experimental unit 10 burgers were prepared (85 mm diameter, 100 g) for a total of 30 burgers
90 per F and an overall number of 90 burgers. Proximate analysis of meat batches reported in Mancini
91 et al. [21] (moisture: $71.56\% \pm 0.68$; ether extract: $2.23\% \pm 0.45$).

92 Samples were stored at 4 °C for 1, 4 and 7 days (storage time - ST, D1, D4, D7) in single Styrofoam
93 trays overwrapped with polyethylene film. At each ST three burgers for each experimental unit were
94 cooked in a preheated oven at 163 °C to an internal temperature of 71 °C (burgers were turned

95 every 4 min to prevent excess surface crust formation as reported by American Meat Science
96 Association, AMSA [22]). Internal temperature of burgers was monitored by opening the oven every
97 4 minutes, when burgers were turned, a variation of 1 °C of their internal temperature was tolerated
98 (measured by a portable thermocouple thermometer; HI 92704C, Hanna Instruments, Padova,
99 Italy). After cooking, the surfaces of the burgers were dried slightly with blotting paper and the
100 burgers were held at room temperature (set at 25 °C) until they cooled down. Cooked burgers were
101 analysed for the determination of pH, colour, fatty acids profile, lipid oxidation (TBARS) and
102 antioxidant capacity (ABTS, DPPH and FRAP).

103

104 2.2. pH and colour determination

105 The pH was determined with a puncture electrode (Double Pore Slim, Hamilton, Switzerland)
106 equipped on a pH meter with automatic temperature compensator (pH 2700, Eutech Instruments,
107 The Netherlands). pH meter was calibrated before each session with buffer solutions at pH 4.01 and
108 7.01 (HI7004L and HI7007L Hanna instruments, Italy).

109 Colour indices [23] as lightness (L^*), redness (a^*) and yellowness (b^*) were recorded with a Chroma
110 meter Minolta CR300 (Minolta, Japan) with an aperture size of 8 mm, illuminant D65 and incidence
111 angle of 0°. Before each session, the colorimeter was calibrated with a white tile ($L^* = 98.14$, $a^* =$
112 -0.23 and $b^* = 1.89$).

113 Chroma (C^*) and hue angle (H^*) were calculated from a^* and b^* indices as reported by Commission
114 Internationale de l'Éclairage (CIE) [23]. Moreover, numerical total colour difference (ΔE) was
115 calculated as proposed by Sharma and Bala [24] between different F at the same ST (effect of the
116 formulation) or between different ST of the same F (effect of the storage time). Moreover, colour
117 changes after cooking were calculated between raw and cooked samples for all the F and ST (colour
118 of raw samples were previously published by Mancini et al. [21]).

119

120 2.3. Fatty acids profile

121 Fatty acids were extracted and processed via transesterification with methanol [25]. FAMES (fatty
122 acid methyl esters) were analysed by gas chromatography and separated with an Agilent capillary
123 column (30 m × 0.25 mm I.D., CPS Analitica, Italy) coated with a DB-Wax stationary phase (film
124 thickness of 0.25 µm). The operating conditions of the column injection were as follows: the
125 temperatures of the injector and detector were 270 and 280 °C, respectively and the detector gas
126 flows were H₂ 50 mL/min and air 100 mL/min. The oven temperature was programmed to give good

127 peak separation; the initial temperature was set at 130 °C and then increased at a rate of 4.0 °C/min
128 until reaching a temperature of 180 °C, which was held for 5 min; the temperature was subsequently
129 increased at a rate of 5.0 °C/min until it reached 230 °C, which was held for 5 min. Helium was used
130 as a carrier gas at a constant flow rate of 1.5 mL/min. Individual fatty acid methyl esters were
131 identified with reference to the retention time of FAME mixture (Sigma-Aldrich, Germany) and
132 calculated with the internal standard method (nonadecanoic acid, C19:0).

133 Results were expressed as percentage of singular fatty acids on total FAME using the peak areas.
134 Atherogenicity (AI), thrombogenicity (TI), hypocholesterolemic (h), hypercholesterolemic (H) and
135 peroxidisability (PI) indices were calculated as reported below following the formulas proposed by
136 Ulbricht and Southgate [26] and Santos-Silva et al. [27]:

137 $AI: (C12:0 + C14:0 * 4 + C16:0) / (MUFA + PUFA\omega3 + PUFA\omega6)$

138 $TI: (C14:0 + C16:0 + C18:0) / (MUFA * 0.5 + PUFA\omega6 * 0.5 + PUFA\omega3 * 3 + PUFA\omega3 / PUFA\omega6)$

139 $h/H: (C18:1 + C18:2\omega6 + C18:3\omega3 + C18:3\omega6 + C20:4\omega6 + C20:5\omega3 + C22:6\omega3) / (C14:0 + C16:0)$

140 $PI: \sum monoenoic * 0.025 + \sum dienoic * 1 + \sum trienoic * 2 + \sum tetraenoic * 4 + \sum pentaenoic * 6 + \sum hexaenoic * 8$

142

143 2.4. Lipid oxidation (TBARS - Thiobarbituric acid reactive substances)

144 Samples (5 g) were extracted with trichloroacetic acid (TCA, 7.5%) and
145 diethylenetriaminepentaacetic acid (DTPA, 0.1%) as proposed by Dal Bosco et al. [28]. The extracted
146 samples were mixed with 2-thiobarbituric acid (TBA, 0.288%) and placed in water bath at 95 °C for
147 45 min. Samples absorbance were determined at 532 nm (V-530 Jasco International, Italy) against a
148 blank containing TCA/DTPA. Results were expressed as mg malondialdehyde equivalent (MDA-eq)
149 per kg of meat using a calibration curve of TEP (1,1,3,3-tetraethoxypropane, 0-15 µM).

150

151 2.5. Antioxidant capacity

152 Samples (5 g) were extracted with ethanol as reported by Mancini et al. [29] and extracts were used
153 to quantify the antioxidant capacity to reduce ABTS - 2,2'-azinobis(3-ethylbenzothiazoline-6-
154 sulphonic acid as reported by Re et al. [30], DPPH - 2,2-diphenyl-1-picrylhydrazyl as reported by Blois
155 [31] and TPTZ-FeCl₃ - complex 2,4,6-tris(2-pyridyl)-5-triazine with Fe(III) chloride (FRAP method) as
156 described by Descalzo et al. [32].

157 Antioxidant capacity was expressed as mmol equivalent of Trolox per kg of sample for both ABTS
158 and DPPH methods and as mmol equivalent of Fe(II) per kg of sample for FRAP method.

159

160 2.6. Statistical analysis

161 Two-ways ANOVA was used to analyse the effect of the formulation (F) and the storage time (ST) on
162 physical-chemical characteristics (pH, L*, a*, b*, C* and H*), fatty acids profile (single, sums and
163 calculated indices), lipid oxidation (TBARS) and antioxidant capacity (FRAP, ABTS and DPPH) of
164 burgers. The following linear model was used: $Y_{ijz} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + e_{ijz}$, where Y_{ijz} is the
165 dependent variable of the zth observation; μ is the overall mean; α_i is the effect of the F (i = C, G1,
166 G2); β_j is the effect of the ST (j = D1, D4, D7); $\alpha\beta_{ij}$ is the effect of the interaction between F and ST
167 (F × ST), and e_{ijz} is the random error. When the interaction F × ST was not significant (P<0.05) the
168 results were reported as functions of the main effects F and ST. When the effect showed a significant
169 variation (P<0.05) means were compared using Tukey's test.

170 A principal component analysis was conducted to determinate the relationship between pH, colour,
171 fatty acids profile, lipid oxidation (TBARS) and antioxidant capacity (ABTS, DPPH and FRAP); all the
172 data were mean centred and scaled to a unit standard deviation before analysis.

173 The R free software was used for the statistical analysis [33].

174

175 3. Results and discussion

176 Neither the F nor the ST affected the pH of the cooked burgers which presented an overall mean of
177 6.25 (Table 2). Considering the colour parameters, the addition of 2% of ginger powder to the meat
178 increased the yellowness of the burgers and consequently the levels of C* and H* (as b* is part of
179 the both C* and H* calculation formulas) (P<0.001, P<0.01 and P=0.001, respectively for b*, C* and
180 H*). No statistical difference was displayed between G1 and G2 in chroma.

181 Ginger as a spice is used in several different dishes and, as long with its pungent flavour, gives to the
182 product a yellow colour. This peculiarity modified the colour of G2 burgers with higher value of b*
183 (consequently higher values of C* and H*) than G1 and control burgers. These modifications were
184 also detected in other meat products with added plant extracts with a strong intrinsic colour-
185 pigments [34–36]. Changes in colour between control and burgers added with 2% of ginger powder
186 was also confirmed by the ΔE values between the F as function of ST. In regard to storage time L*,
187 b* and C* decreased between D4 and D7 (respectively P<0.01, P<0.05 and P<0.05).

188 Total colour difference (ΔE) values are reported in Table 3 as colour distance between cooked
189 samples of a F at two different time (ST effect) or between samples of different F a specific day of
190 analysis (F effect). Interestingly all the ΔE s calculated as function of the ST within the same F were

191 lower than the threshold of 2.3 points (threshold limit for a remarkable difference detectable by the
192 human eyes, as proposed by Sharma and Bala [24]); only the C burgers were near to that level (2.29,
193 colour difference between D1 and D7) highlighting a trend to modify their colour over time (even if
194 after 7 days that was not remarkable for the human eyes). On the other hand, as expected, the ΔE
195 between control burgers and the ones containing 2% of ginger were always over the threshold. No
196 discernible colour differences were detected between C and G1 at all the tested storage times. All
197 the ΔE s calculated between the raw and cooked samples showed that all the cooked samples were
198 discernible from their respectively raw samples, as the differences in colour were mainly dependent
199 on cooking and not on ST or the F.

200 During the storage time colour may change as a consequence of several chemical modifications that
201 occur to meat as depigmentation through oxidation of myoglobin to metmyoglobin [2].
202 Metmyoglobin is brown in colour and leads to a decrease of lightness of white meat. Burgers
203 presented lowest values of L^* , b^* and C^* at D7 as consequence of modification of the meat and, for
204 the two formulations added with ginger, also a discolouration of ginger pigment may contribute to
205 a decrease of yellowness. The values of ΔE between G1 and G2 also corroborate this hypothesis as
206 ΔE at D1 was higher (and over the recognizable by human) than the differences calculated at D4 and
207 D7. Anyway, these changes in ginger pigments did not affect the total colour differences as no values
208 of ΔE were higher than the threshold of discernibility for the calculation within the same F as
209 function of the ST.

210 As expected cooking session modified the colour of the burgers, with all the ΔE values over the
211 threshold. Colour modifications occurred in cooking are largely studied and very important in pork
212 meat as colour is commonly used in house cooking to determine when the product is ready to be
213 consumed [37]. Thus, discernible colour differences between raw and cooked samples might be a
214 useful, practical and well accepted characteristic. King and Whyte [37] reported that pH of raw meat
215 could affect colour parameter of cooked meat as it has a significant role in the formation of
216 ferrihemochrome from myoglobin. As pH of raw samples was not statistical different [21] the
217 differences observed in colour indices of cooked samples are likely ascribable to the F. Furthermore,
218 as reported by Andrés-Bello et al. [38], there is a strictly correlation between colour of raw and
219 cooked samples.

220 Addition of ginger powder strongly influenced fatty acids profile as reported in Table 4, also in this
221 determination no statistical interaction between formulation and storage time was showed. The
222 main saturated fatty acids in burgers were C14:0, C16:0 and C18:0. All these FA were at higher

223 concentrations in C burgers than G1 and G2 ones; between ginger burgers G2 showed lower values
224 than G1. Total SFA did not show statistical significance between C and G1, anyhow, lower value was
225 showed by G2. All monounsaturated fatty acids were reduced by both concentrations of ginger,
226 without differences between G1 and G2.

227 Ginger powder increased the contents of both PUFA ω 3 and PUFA ω 6 with minor differences
228 between single fatty acids trends. Indeed, as reported by Gur et al. [39] and Zachariah [15] ginger is
229 rich in PUFAs, both of the ω 3 and ω 6. Total PUFAs and ratio ω 3/ ω 6 were affected by these
230 modifications and showed significant differences between all the F, with lowest values of C, mid
231 values of G1 and higher values of G2. Variations in fatty acids profile affected all the calculated
232 indices with enhanced healthy characteristics in G1 and G2 burgers.

233 Storage time affected several fatty acids mainly in a progressive way, without strong differences
234 between grade of saturation (Table 4). Two main trends were showed as in one case FAs were
235 reduced day by day (C16:0, C18:0, C18:3 ω 3, total SFA and total PUFA ω 3; also, C18:2 ω 6 and total
236 PUFA ω 6 partially), and in the other case FAs were drastically decreased at D4 (C16:1, C22:5 ω 3 and
237 total PUFA). Only C14:0 reported to maintain its percentage between D1 and D4, with a following
238 decrease at D7. As consequences of these reductions the ratio ω 3/ ω 6 was reduced day by day and
239 PI index dropped at D4.

240 Lipid oxidation (TBARS) and antioxidant capacity (FRAP, ABTS and DPPH) were reported in Table 5.
241 No interaction F x ST was found, never the less for all the analyses both F and ST showed statistically
242 significant differences.

243 TBARS values of C burgers were higher than the burgers of F G1 and G2 ($P < 0.01$), as well as the
244 antioxidant capacity of the control burgers, expressed as ABTS and DPPH, was lower than the
245 burgers added with ginger (both $P < 0.001$). No further differences were showed between the
246 addition of 1% or 2% of ginger powder. FRAP evaluation reported an increase in antioxidant capacity
247 in combination with the presence of the spice ($C < G1 < G2$, $P < 0.001$).

248 Regarding the ST, lipid oxidation and antioxidant capacity respectively increased and decreased in a
249 gradual way with no differences between D1-D4 and D4-D7 but with a statistical difference only
250 between D1 and D7.

251 Meat and more likely minced meat could be subject to a rapid deterioration of quality due to
252 enzymatic and microbial degradation [40, 41]. Moreover, lipid oxidation could occur as consequence
253 of several different factors, both endogenous and exogenous. Antioxidant molecules have the
254 peculiarity to stop the chain reaction of oxidation that typically occurs in organic matrix due to free

255 radicals formation. Ginger, as several other plants, contain a higher number of antioxidant
256 compound such as gingerol, paradol, shogaols, zingerone, zerumbone, terpenoids as well flavonoids
257 and phenols [16, 17]. The addition of ginger powder to the pork meat lead to a lower lipid oxidation
258 and to an increased antioxidant capacity due to the chemical characteristics of ginger. These
259 modifications were also evaluated in raw burgers [21] as well as in other cooked meat products
260 supplemented with plant additives [42–45]. Furthermore, previous studies have shown that ginger
261 powder addition to pork [21] or beef [46] burgers did not modified the appearance, the aroma and
262 the flavour intensities and seemed to contribute to maintain sensory characteristics during storage
263 time. However, further researches on sensory/consumers liking are suggested.

264 Principal components analysis (PCA) of the three first PC reached a cumulative percentage of 63.39%
265 of variability (Table 6). Eigenvectors reported in Table 6 and Figure 1 showed that the main factor
266 of variability (PC1) could be ascribed to the addition of ginger, without differentiation between G1
267 and G2. Indeed, negative values of PC1 represent data linked to the presence of ginger powder, such
268 as b* index (yellowness), antioxidant capacity (FRAP, ABTS and DPPH) and presence of PUFA ω 3
269 (C18:3 ω 3, C20:5 ω 3, C22:5 ω 3 and C22:6 ω 3) and PUFA ω 6 (C18:2 ω 6 and C20:4 ω 6). Positives
270 eigenvectors of MUFA (C14:0, C16:0 and C20:0), as well as TBARS could be more likely associated to
271 the absence of ginger powder and then to the C burger, as revealed also by Figure 1.

272 A lack of differentiation related to storage time was highlighted as nor PC2 or PC3 described its
273 contribution.

274

275 **4. Conclusions**

276 Ginger powder increases both nutritional and functional properties of pork burgers. Antioxidant
277 compounds, naturally contained in ginger, increased the capacity of the burgers to resist to lipid
278 oxidation during 7 day of refrigerated storage. Moreover, adding 2% ginger powder as ingredient in
279 the formulation of the burgers induced a reduction of the total SFA with increased healthy values
280 of these ready to cook products.

281

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286 **Disclosure statement**

287 No potential conflict of interest was reported by the authors.

288

289 **Reference**

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411 their application to ground beef patties. Food Chem 69:135–141 . doi: 10.1016/S0308-
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413

414 Table 1. Proximate composition, antioxidant capacity, colour and fatty acids profile of ginger
 415 powder.

| Proximate composition (%) | | Fatty acids profile (%) | |
|-----------------------------|--------|-------------------------|-------|
| Moisture | 6.47 | C16:0 | 20.49 |
| Fat | 6.51 | C18:0 | 10.08 |
| Protein | 13.80 | SFA | 37.52 |
| Ash | 8.02 | C18:1 | 15.85 |
| | | MUFA | 21.23 |
| | | C18:3 ω 3 | 2.90 |
| <u>Antioxidant capacity</u> | | C22:5 ω 3 | 2.02 |
| ABTS | 118.34 | PUFA ω 3 | 7.90 |
| DPPH | 10.99 | C18:2 ω 6 | 27.35 |
| FRAP | 75.51 | C20:2 ω 6 | 2.03 |
| | | C22:2 ω 6 | 2.00 |
| <u>Colour</u> | | PUFA ω 6 | 33.35 |
| L* | 73.04 | PUFA | 41.25 |
| a* | 5.08 | | |
| b* | 30.50 | | |

ABTS and DPPH in mmol of Trolox equivalent per kilogram of ginger powder; FRAP in mmol of Fe^{II} equivalent per kilogram of ginger powder.

Also C14:0, C15:0, C17:0, C20:0, C22:0, C24:0, C14:1, C16:1, C17:1, C22:1, C20:5 ω 3, C22:6 ω 3, C18:3 ω 6 and C20:4 ω 6 were detected in lower amounts. All the mentioned fatty acids have been utilized for calculating sum of lipid fractions.

416

417 Table 2. pH and colour parameters of burgers in relation to the formulation and the storage time.

| | Formulation (F) | | | Storage time (ST) | | | P-value | | RMSE |
|----|--------------------|---------------------|--------------------|---------------------|--------------------|--------------------|---------|-------|-------|
| | C | G1 | G2 | D1 | D4 | D7 | F | ST | |
| N | 9 | 9 | 9 | 9 | 9 | 9 | | | |
| pH | 6.20 | 6.31 | 6.23 | 6.26 | 6.29 | 6.19 | 0.885 | 0.905 | 0.475 |
| L* | 26.81 | 26.94 | 26.52 | 26.72 ^{xy} | 27.37 ^x | 26.17 ^y | 0.461 | 0.008 | 0.723 |
| a* | 12.92 | 12.85 | 11.99 | 13.25 | 12.08 | 12.43 | 0.155 | 0.087 | 1.076 |
| b* | 15.29 ^b | 16.39 ^b | 17.90 ^a | 16.60 ^{xy} | 17.17 ^x | 15.80 ^y | <0.001 | 0.020 | 0.929 |
| C* | 20.03 ^b | 20.84 ^{ab} | 21.59 ^a | 21.30 ^x | 21.04 ^x | 20.13 ^y | 0.005 | 0.029 | 0.877 |
| H* | 49.83 ^b | 51.94 ^b | 56.16 ^a | 51.03 | 54.83 | 51.79 | 0.001 | 0.054 | 2.975 |

C: cooked burgers of only meat; G1: cooked burgers of meat added with 1% (w/w) of ginger powder; G2: cooked burgers of meat added with 2% (w/w) of ginger powder.

^{a, b} In the same row indicate significant differences for F.

^{x, y} In the same row indicate significant differences for ST.

419 Table 3. Total colour difference (ΔE) of burgers as function of the storage time or the formulation
 420 and between raw and cooked samples with fixed time and formulation.

| | | ΔE Storage time (ST) | | |
|-----------------|-------|------------------------------|-------|--|
| Formulation (F) | D1–D4 | D4–D7 | D1–D7 | |
| C | 1.58 | 1.68 | 2.29 | |
| G1 | 1.77 | 1.69 | 0.49 | |
| G2 | 1.13 | 1.55 | 1.33 | |

| | | ΔE Formulation (F) | | |
|-------------------|------|----------------------------|-------|--|
| Storage time (ST) | C–G1 | C–G2 | G1–G2 | |
| D1 | 1.12 | 3.37* | 2.28 | |
| D4 | 1.07 | 2.51* | 1.45 | |
| D7 | 1.97 | 2.74* | 1.72 | |

Raw-Cooked

| | | Storage time (ST) | | |
|-----------------|-------|-------------------|-------|--|
| Formulation (F) | D1 | D4 | D7 | |
| C | 5.24* | 7.30* | 5.33* | |
| G1 | 4.64* | 5.93* | 4.20* | |
| G2 | 3.93* | 4.93* | 3.31* | |

C: cooked burgers of only meat; G1: cooked burgers of meat added with 1% (w/w) of ginger powder;
 G2: cooked burgers of meat added with 2% (w/w) of ginger powder.

*Value over the threshold (2.3 points) with a noticeable difference in colour between the samples.

421

422 Table 4. Fatty acids profile (% of total FAME) and correlated indices of burgers.

| Fatty acids | Formulation (F) | | | Storage Time (ST) | | | P-value | | RMSE |
|---------------------------------|--------------------|--------------------|--------------------|--------------------|--------------------------------|--------------------|---------|--------|-------|
| | C | G1 | G2 | D1 | D4 | D7 | F | ST | |
| N | 9 | 9 | 9 | 9 | 9 | 9 | | | |
| C14:0 | 1.93 ^a | 1.72 ^b | 1.20 ^c | 1.66 ^x | 1.68 ^x | 1.51 ^y | <0.001 | <0.001 | 0.098 |
| C16:0 | 22.99 ^a | 22.91 ^b | 21.94 ^c | 22.87 ^x | 22.61 ^y | 22.35 ^z | <0.001 | <0.001 | 0.040 |
| C18:0 | 13.46 ^a | 13.29 ^b | 12.95 ^c | 13.73 ^x | 13.13 ^y | 12.85 ^z | <0.001 | <0.001 | 0.037 |
| <i>SFA</i> | 39.48 ^a | 38.93 ^a | 37.45 ^b | 39.64 ^x | 38.42 ^y | 37.80 ^z | <0.001 | <0.001 | 0.514 |
| C16:1 | 2.21 ^a | 2.10 ^b | 2.09 ^b | 2.18 ^x | 2.14 ^a ^y | 2.09 ^y | <0.001 | 0.0041 | 0.047 |
| C18:1 ω 9 | 38.67 ^a | 37.87 ^b | 37.94 ^b | 38.07 | 38.16 | 38.26 | <0.001 | 0.546 | 0.047 |
| C20:1 | 0.71 ^a | 0.65 ^b | 0.67 ^b | 0.69 | 0.68 | 0.66 | 0.019 | 0.205 | 0.036 |
| <i>MUFA</i> | 42.36 ^a | 41.49 ^b | 41.70 ^b | 41.87 | 41.85 | 41.81 | <0.001 | 0.911 | 0.269 |
| C18:3 ω 3 | 1.29 ^c | 1.41 ^b | 1.84 ^a | 1.68 ^x | 1.52 ^y | 1.34 ^z | <0.001 | <0.001 | 0.039 |
| C22:5 ω 3 | 0.17 ^b | 0.62 ^a | 0.54 ^a | 0.53 ^x | 0.41 ^y | 0.39 ^y | <0.001 | 0.003 | 0.077 |
| C22:6 ω 3 | 0.53 ^b | 0.51 ^b | 0.86 ^a | 0.67 | 0.62 | 0.61 | <0.001 | 0.187 | 0.078 |
| <i>PUFAω3</i> | 2.03 ^c | 2.60 ^b | 3.29 ^a | 2.93 ^x | 2.59 ^y | 2.39 ^z | <0.001 | <0.001 | 0.132 |
| C18:2 ω 6 | 14.99 ^c | 16.35 ^b | 16.69 ^a | 16.04 ^x | 16.02 ^{xy} | 15.98 ^y | <0.001 | 0.014 | 0.041 |
| C20:4 ω 6 | 0.98 ^c | 1.53 ^b | 1.72 ^a | 1.43 | 1.40 | 1.40 | <0.001 | 0.108 | 0.032 |
| <i>PUFAω6</i> | 16.68 ^c | 18.87 ^a | 18.95 ^a | 18.27 ^x | 18.17 ^{xy} | 18.06 ^y | <0.001 | 0.044 | 0.165 |
| <i>PUFA</i> | 18.72 ^c | 21.47 ^b | 22.24 ^a | 21.20 ^x | 20.76 ^y | 20.46 ^y | <0.001 | <0.001 | 0.266 |
| <i>AI</i> | 0.49 ^a | 0.47 ^a | 0.42 ^b | 0.47 | 0.45 | 0.46 | <0.001 | 0.461 | 0.025 |
| <i>TI</i> | 1.07 ^a | 0.99 ^b | 0.90 ^c | 0.99 | 0.98 | 0.99 | <0.001 | 0.893 | 0.141 |
| <i>h/H</i> | 2.31 ^c | 2.36 ^b | 2.57 ^a | 2.39 | 2.42 | 2.43 | <0.001 | 0.144 | 0.037 |
| <i>PI</i> | 29.14 ^c | 35.78 ^b | 39.64 ^a | 36.19 ^x | 34.50 ^y | 33.87 ^y | <0.001 | <0.001 | 0.957 |
| ω 3/ ω 6 | 0.12 ^c | 1.14 ^b | 0.17 ^a | 0.16 ^x | 0.14 ^y | 0.13 ^z | <0.001 | <0.001 | 0.006 |

C: cooked burgers of only meat; G1: cooked burgers of meat added with 1% (w/w) of ginger powder; G2: cooked burgers of meat added with 2% (w/w) of ginger powder.

C15:0, C17:0, C20:0, C22:0, C24:0, C14:1, C15:1, C17:1, C22:1 ω 9, C24:1, C18:3 ω 6, C20:5 ω 3, C20:2 ω 6, C22:2 ω 6 were detected but not summarized because found below 1% of total FAME. All the mentioned fatty acids have been utilized for calculating sum of lipid fraction.

AI: Atherogenicity index; TI: Thrombogenicity index; h/H: hypocholesterolemic index/Hypercholesterolemic index; PI: Peroxidisability index.

a, b, c In the same row indicate significant differences for F.

x, y, z In the same row indicate significant differences for ST.

424 Table 5. Lipid peroxidation (TBARS) and antioxidant capacity of burgers (FRAP, ABTS and DPPH).

| | Formulation (F) | | | Storage time (ST) | | | P-value | | RMSE |
|-------|-------------------|-------------------|-------------------|-------------------|--------------------|-------------------|---------|-------|-------|
| | C | G1 | G2 | D1 | D4 | D7 | F | ST | |
| N | 9 | 9 | 9 | 9 | 9 | 9 | | | |
| TBARS | 0.10 ^a | 0.07 ^b | 0.07 ^b | 0.07 ^y | 0.08 ^{xy} | 0.10 ^x | 0.007 | 0.030 | 0.020 |
| FRAP | 0.24 ^c | 1.50 ^b | 2.62 ^a | 1.63 ^x | 1.44 ^{xy} | 1.29 ^y | <0.001 | 0.029 | 0.244 |
| ABTS | 1.50 ^b | 2.53 ^a | 2.63 ^a | 2.49 ^x | 2.23 ^{xy} | 1.94 ^y | <0.001 | 0.018 | 0.369 |
| DPPH | 0.11 ^b | 0.14 ^a | 0.14 ^a | 0.14 ^x | 0.13 ^{xy} | 0.12 ^y | <0.001 | 0.031 | 0.013 |

C: cooked burgers of only meat; G1: cooked burgers of meat added with 1% (w/w) of ginger powder; G2: cooked burgers of meat added with 2% (w/w) of ginger powder.

TBARS expressed in mg of MDA-eq. 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.

^{a, b, c} In the same row indicate significant differences for F.

^{x, y} In the same row indicate significant differences for ST.

425

426 Table 6. Eigenvalues and eigenvectors of the first three principal components (PC) of principal
 427 components analysis conducted on the physical-chemical characteristics (pH, L*, a*, b*), single fatty
 428 acids, lipid oxidation (TBARS) and antioxidant capacity (FRAP, ABTS and DPPH) of burgers stored up
 429 to 7 days.

| | PC | | |
|------------------|---------|---------|---------|
| | PC 1 | PC 2 | PC 3 |
| Eigenvalues | 11.5377 | 5.4846 | 3.8962 |
| Eigenvectors | | | |
| L* | 0.0108 | 0.1002 | -0.1883 |
| a* | 0.0732 | 0.1182 | -0.2153 |
| b* | -0.2235 | -0.0076 | 0.0430 |
| pH | -0.0450 | 0.1156 | -0.1067 |
| TBARS | 0.1802 | 0.0744 | 0.1751 |
| FRAP | -0.2775 | -0.0194 | 0.0218 |
| ABTS | -0.2419 | 0.0259 | -0.2036 |
| DPPH | -0.2128 | -0.0146 | -0.1439 |
| C14:0 | 0.2343 | 0.0343 | -0.1904 |
| C15:0 | -0.2070 | 0.1375 | 0.2169 |
| C16:0 | 0.1868 | 0.0072 | -0.3147 |
| C17:0 | 0.0044 | 0.3410 | 0.1172 |
| C18:0 | 0.0486 | 0.0546 | -0.1207 |
| C20:0 | 0.1992 | 0.2668 | 0.0987 |
| C22:0 | -0.1614 | 0.2843 | -0.1130 |
| C24:0 | -0.1378 | 0.2653 | -0.1295 |
| C14:1 | -0.0683 | 0.2842 | 0.1084 |
| C15:1 | -0.2222 | 0.1369 | 0.0972 |
| C16:1 | 0.1329 | 0.1759 | -0.0657 |
| C17:1 | 0.1817 | 0.2884 | -0.0037 |
| C18:1 ω 9 | 0.1392 | -0.0409 | 0.2587 |
| C20:1 | 0.0722 | 0.3478 | -0.0800 |
| C22:1 ω 9 | -0.1253 | 0.0545 | -0.2846 |
| C24:1 | -0.2688 | 0.0452 | -0.0283 |

| | | | |
|------------------|---------|---------|---------|
| C18:3 ω 3 | -0.2531 | 0.0633 | 0.1390 |
| C20:5 ω 3 | -0.0507 | 0.1631 | -0.0364 |
| C22:5 ω 3 | -0.2295 | -0.1152 | -0.2062 |
| C22:6 ω 3 | -0.2156 | 0.0756 | 0.2474 |
| C18:2 ω 6 | -0.2074 | 0.0047 | -0.1699 |
| C18:3 ω 6 | -0.0554 | 0.3628 | -0.0279 |
| C20:2 ω 6 | 0.1032 | 0.2316 | -0.1567 |
| C20:4 ω 6 | -0.2575 | -0.0566 | -0.0973 |
| C22:2 ω 6 | 0.0599 | -0.1215 | -0.4394 |
| Cumulative % | 34.96 | 51.58 | 63.39 |

430

431 Figure 1. Biplot (Loading and Score plots) of the principal component analysis (PCA) performed on
432 the physical-chemical characteristics (pH, L*, a*, b*), single fatty acids, lipid oxidation (TBARS) and
433 antioxidant capacity (FRAP, ABTS and DPPH).

434

435 **Figure Captions**

436 **Fig 1**

437 C: cooked burgers of only meat; G1: cooked burgers of meat added with 1% (w/w) of ginger powder;
438 G2: cooked burgers of meat added with 2% (w/w) of ginger powder. Storage time as D1, D4 and D7,
439 respectively for day 1, 4 and 7.