http://dx.doi.org/10.1016/j.meatsci.2017.03.004
Accepted Version

Improving pork burgers quality using *Zingiber officinale* Roscoe powder (ginger)

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Abstract

Pork burgers were evaluated for physical-chemical characteristics, fatty acids profile, lipid oxidation, antioxidant capacity, microbiological growth and sensory evaluation during storage time of seven days at 4°C as function of three formulations as only meat (control, B) and meat added with ginger powder at the percentage of 1 and 2% (BG1 and BG2).

BG1 and BG2 were less redness than control ones with incremented yellow hue. These modifications in color parameters did not modify sensory characteristics of burgers. PUFA were incremented (both PUFAω3 and PUFAω6) by the addition of ginger. Furthermore, BG1 and BG2 burgers showed to be less sensitive to lipid oxidation and to possess an increase in antioxidant capacity. Microbial growth evaluation of total aerobic count and *Pseudomonas* spp. showed that ginger powder delayed in time the bacterial contamination. Results highlighted that the presence of ginger led to an enhanced shelf life and health characteristics of burgers (increasing peroxidisability, ratio hypocholesterolemic/hypercholesterolemic and ratio ω3/ω6; reducing atherogenicity and thrombogenicity).

Keywords
Burger; Meat quality; Natural antioxidant; Ginger; Antioxidant capacity.

1. Introduction

Ready-to-cook products represent an important percentage of food production for their high usage and acceptance by high number of consumers. Burgers are one of the most consumed meat product for their practicality to be cooked and for their ease of consumption.

As well known grinding process, as a result of disruption on muscle structure, leads to a less stable food matrix that could occur more easily to chemical and enzymatic oxidation processes and in an increased microbial growth (Emswiler, Pierson, & Kotula, 1976; Mancini & Hunt, 2005). Several factors as production processes, packaging and food additives were studied during the last decades in order to reduce oxidations and enhance shelf life of meat products (Hygreeva & Pandey, 2016; Jiang & Xiong, 2016; Overholt et al., 2016; Shahidi & Ambigaipalan, 2015; Yang, Lee, Won, & Song, 2016). Antioxidant molecules, as food additives, seem to protect from oxidation and delay microbial growth (Falowo, Fayemi, & Muchenje, 2014) as well as improve or to carry on nutraceutical properties (Decker & Park, 2010).

After the controversial due to the potential adverse effects on health of synthetic antioxidant molecules a growing attention was shown by consumers to prefer products with natural antioxidant, encouraging food industries to research continuously newest natural food additives (Brewer, 2011; Jiang & Xiong, 2016; Shahidi & Ambigaipalan, 2015; Shahidi & Zhong, 2010).

Plant products might be well accepted by the consumers for their natural origin. Several spices, essential oils, extracts, powders and other plant by-products were studied in the last decades in order to assess their activity and their effects on meat products as feed/food supplementation (Burt, 2004; Jiang & Xiong, 2016; Mancini, Preziuso, & Paci, 2016; Mancini, Paci, & Preziuso, 2016; Shah, Bosco, & Mir, 2014).

Ginger (*Zingiber officinale* Roscoe) is one of the most common spice used worldwide, as a condiment for food and beverage. Ginger flavor is a mix of spicy, peppery and sweet with a strong pungent characteristic. *Zingiber officinale* is a species of the *Zingiberaceae* family as well other spices as galangal (*Alpinia galangal*), cardamom (*Elettaria cardamomum*) and turmeric (*Curcuma longa*). Ginger rhizome is generally consumed fresh, dried powder or candy; in some countries, as India and China, ginger is historically used in several food preparation and meat dishes (Zachariah, 2008). Ginger's antioxidant and anticarcinogenic properties have been quantified in several researches (Manju & Nalini, 2005; Mi, Guo, & Li, 2016) and the use of ginger was evaluated both in food (Abdel-Naeem

& Mohamed, 2016; Y. Cao et al., 2013; Naveena & Mendiratta, 2004) and feed (Herawati & Marjuki, 2011; Zhao et al., 2011; Zomrawi, Abdel Atti, Dousa, & Mahala, 2012). Ginger powder contains several antioxidant molecules as gingerol, paradol, shogaols, zingerone, zerumbone, terpenoids as well flavonoids and phenols (Kikuzaki & Nakatani, 1993; Rahmani, Al Shabrmi, & Aly, 2014).

The aim of this research was to evaluate the effect of the addition of two different percentage of ginger powder during a refrigerate storage on pork burger's meat quality (pH, color and water holding capacity), fatty acid profile, lipid oxidation, antioxidant capacity, microbial growth and sensory evaluation.

2. Material and methods

2.1. Meat

Meat was obtained from nine female pigs (Cinta Senese breed, 125 ± 4 kg) reared under pasture system and fed commercial pelleted feed. Pigs were slaughtered after electrical stunning and chilled for 24 h at 4 ± 0.5 °C. *Longissimus lumborum* muscles of the left carcasses were removed and transported to the laboratory (Department of Veterinary Science, Pisa) for the formulation of the burgers.

2.2. Experiment design and preparation of burgers

Each *Longissimus lumborum* muscle was considered as an experimental unit and was analyzed to determine the proximate composition after grinding.

Loins were minced separately and randomly assigned to three different formulations (F, three loins per formulation): control burgers (only meat, B), burgers added with 1% of ginger powder (10 g of ginger for kg of meat, BG1) and burgers added with 2% of ginger powder (20 g of ginger for kg of meat, BG2). Commercial ginger powder, ready to use, was purchased from wholesaler (Drogheria e Alimentari S.p.A., Scarperia e San Piero, Florence, Italy; rhizomes of ginger from India, batch number: L65069N). Proximate composition, antioxidant capacity (ABTS, DPPH and FRAP) and fatty acids profile of ginger powder were reported in Table 1.

From each experimental unit ten burgers of 100 grams were shaped in Petri dishes (85 mm of diameter) for a total of 30 burgers for formulation (a total of 90 burgers). Burgers were placed in single Styrofoam trays and were overwrapped with polyethylene film.

Burgers were stored at 4 ± 0.5 °C and three burgers for experimental unit (9 burgers per formulation) were analyzed after 1, 4 and 7 days (Storage time - ST: D1, D4 and D7) for the determination of the pH, color, water holding capacity (drip loss and cooking loss), fatty acid profile, lipid oxidation (TBARS), antioxidant capacity (ABTS, DPPH, FRAP), microbial growth and sensory.

2.3. Proximate analysis, pH, color and water holding capacity

Proximate composition (moisture, ash, ether extract) were determined on grounding meat derived from each pig (AOAC, 1995).

A pH meter equipped with glass electrode suitable for meat penetration and an automatic temperature compensator was used to determine the pH (Hanna pH 211 equipped with Hanna FC 200B, Hanna Instruments, Padova, Italy), prior to each session pH meter was calibrated with two buffer solutions at pH 4.01 and 7.01 (respectively HI7004L and HI7007L Hanna instruments, Padova, Italy).

Chroma meter Minolta CR300 (Minolta, Osaka, Japan) was used to measured the color parameter (aperture size of 8 mm, illuminant D65, incidence angle of 0°). Lightness (L*), redness (a*) and yellowness (b*) indexes were recorded as reported by CIE (1976), after a calibration section using a white tile (L* = 98.14, a* = −0.23 and b* = 1.89). Numerical total color difference (ΔE) was calculated as proposed by Sharma (2002), as well a* and b* indexes were used to calculate the hue (H*) and the chroma (C*) parameters (CIE, 1976). The water holding capacity was calculated as drip loss between D1 and D4 or D1 and D7 (Lundström & Malmfors, 1985) and as cooking loss after a cooking section in a preheated oven at 163 °C to an internal temperature of 71 °C (burgers were turned every 4 min to prevent excess surface crust formation; AMSA, 1995).

2.4. Fatty acids profile

The extraction of intramuscular fat was based on the method of Folch, Lees, & H. G. Stanley (1957). Total lipids were extracted from 5 g of burger and fatty acid composition of meat was determined by gas chromatography. The separation of fatty acid methyl esters (FAME) was performed with an Agilent capillary column (30 m × 0.25 mm I.D., CPS Analitica, Milan, Italy) coated with a DB-Wax stationary phase (film thickness of 0.25 μm). Nonadecanoic acid (C19:0) was used as internal standard. Fatty acid composition was calculated using the peak areas and was expressed on a percentage basis. The average amount of each fatty acid (FA) was used to calculate the sum of the saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) and to calculate the atherogenicity (AI), thrombogenicity (TI), hypocholesterolemic (h), hypercholesterolemic (H) and peroxidisability (PI) indexes as reported below:

AI: (C14:0*2 + C16:0) / (MUFA + PUFAω3 + PUFAω6)

TI: (C14:0 + C16:0 + C18:0) / (MUFA * 0.5 + PUFAω6 * 0.5 + PUFAω3 * 3+ PUFAω3 / PUFAω6)

h: C18:1 + C18:2ω6 + C18:3ω3 + C18:3ω6 + C20:4ω6 + C20:5ω3 + C22:6ω3

H: C14:0 + C16:0

PI: ∑monoenoic * 0.025 + ∑dienoic * 1 + ∑trienoic * 2 + ∑tetraenoic * 4 + ∑pentaenoic * 6 + ∑hexaecoic * 8

137

138 2.5. Thiobarbituric acid reactive substances and antioxidant capacity

139 Thiobarbituric acid reactive substances (TBARS) were evaluated spectrophotometrically following the
140 method modified from Ke, Ackman, Linke, & Nash (1977) by Dal Bosco et al. (2009).

141 Five g sample was homogenized for 45 s at 9000 rpm (Polytron PT 3000, Kinematica AG, Eschbach,
142 Deutschland) with 10 mL of 7.5% trichloroacetic acid (TCA) and 0.1% diethylenetriaminepentaacetic
143 acid (DTPA) in distilled water (final concentration). The homogenized sample was centrifuged at
144 10,000 rpm for 10 min (4235A CWS, ALC International, Milan, Italy) and filtered through Whatman
145 number 1 filter paper. Five mL of the filtrate was mixed with 2.5 mL of 2-thiobarbituric acid (TBA)
146 solution (0.288% in distilled water) in capped test tubes. The tubes were vortexed and placed in a water
147 bath at 95 °C for 45min, then cooled under tap water. The absorbance was determined at 532 nm (V-
148 530 Jasco International, Milan, Italy) against a blank containing TCA/DTPA solution instead of a
149 sample extract. Results were expressed as mg MDA on kg of meat using a calibration curve of TEP
150 (1,1,3,3-tetraethoxypropane, 0-15 μM).

151 In order to assess eventual interferences of ginger powder the same protocol was used to determinate
152 the absorbances of 0.05 and 0.10 g of ginger powder (1% and 2% of the meat samples). As the
153 absorbances of ginger samples were not comparable to meat samples and were close to 0 when
154 expressed as mg equivalent of MDA on kg, no further calibration was taken into account.

155 Antioxidant capacity was measured by quantification of the ability of the burger's ethanol extracts to
156 reduce the radical molecules ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid), DPPH
157 (2,2-diphenyl-1-picrylhydrazyl) and TPTZ-FeCl3 (complex 2,4,6-tris(2-pyridyl)-s-triazine with
158 Iron(III) chloride, FRAP method) as reported in Mancini et al. (2015) and modified respectively from
159 Re et al. (1999), Blois (1958) and Descalzo et al. (2007).

160

161 2.6. Microbial assay

162 For microbial assay 10 g of samples were used. *Enterobacteriaceae* (ISO 21528-2:2004), *Enterococcus*
163 spp. (ISO 7899-2:2000), β-glucuronidase-positive *Escherichia coli* (ISO 16649-2:2001), *Pseudomonas*
164 spp. (ISO 13720:2010), coagulase positive and negative staphylococci (ISO 6888-1:1999) and total
165 aerobic count (ISO 4833:2003) microbial growth were tested in order to evaluate the effect of the main
166 factors on the microbial stability of the burgers. Microbial counts were expressed as log CFU g^{-1}.

167

168 2.8. Sensory evaluation

169 Burgers were cooked with an electrical clamshell grill covered with aluminum foil until they reached
170 an internal temperature of 72 °C measured by a portable thermocouple thermometer (HI 92704C,

Hanna Instruments, Padova, Italy). Each burger was cut in eight wedges; burger pieces were singularly wrapped in aluminum foil and maintained to 60 °C until sensory evaluation.

Six trained assessors (staff of Department of Veterinary Science of Pisa University) were used as a panel. In each session, corresponding to each sampling time, all samples were evaluated by all panelists and were presented following a balanced design as reported by Macfie, Bratchell, Greenhoff, and Vallis (1989). A 10 cm long unscaled line was used to assess sensory properties of burgers. A total of six parameters were assessed: appearance, aroma intensity (defined as the intensity of the characteristic aroma of pork burgers), off-odors, flavor intensity (defined as the intensity of the characteristic flavor of pork burgers), off-flavors and juiciness. Moreover, the panelist were asked to give a global evaluation to the samples using a 9 point structured scale (1, extremely negative; 5, neither negative nor positive; 9 extremely positive).

2.9. Statistical analysis

The effects of the formulation (F), of the storage time (ST) and their interaction (F × ST) on the burger parameters were analyzed using R software (R Core Team, 2015). Meat quality (pH, a*, b*, L*, C*, H*, drop loss and cooking loss), fatty acids prolife (FA, SFA, MUFA and PUFA), lipid oxidation (TBARS), antioxidant capacity (ABTS, DPPH and FRAP) and microbial growths (*Enterobacteriaceae*, *Enterococcus* spp., β-glucuronidase-positive *Escherichia coli*, *Pseudomonas* spp., coagulase positive and negative staphylococci and total aerobic count) were analyzed with linear model $Yijz = \mu + \alpha i + \beta j + \alpha\beta ij + eijz$, where Yijz is the dependent variable of the z^{th} observation; μ is the overall mean; αi is the effect of the F (i = B, BG1, BG2); βj is the effect of the ST (j = D1, D4, D7); αβij is the effect of the interaction between F and ST, and eijkz is the random error. Moreover, panelist was included as fixed effect (γk, k = 1, 2, 3, 4, 5, 6) in the linear model presented above for analyze sensory evaluation. When the treatments represented a significant ($P<0.05$) source of variation, differences between means for treatment were compared using Tukey's procedure. When the interaction F × ST is not significant the results are reported as the mean of the fixed effects F and T; the variability was expressed as Standard Error of the Mean (SEM).

Proximate compositions (moisture and ether extract) of the meat prior the formulation of the burgers were analyzed via one-way ANOVA as function of the experimental units.

In order to determine the relationships between meat quality (pH, drip loss, cooking loss, L*, a*, b*), fatty acids profile (SFA, MUFA and PUFA), lipid oxidation (TBARS), antioxidant capacity (ABTS, DPPH and FRAP), microbial growth (total aerobic count and *Pseudomonas* spp.) and sensory (appearance, aroma intensity, off-odors, flavor intensity, off-flavor, juiciness and global evaluation) a principal component analysis (PCA) was performed.

205

206 **3. Results and Discussion**

207 3.1. Proximate analysis, pH, color and water holding capacity

208 Moisture and ether extract of the experimental units did not difference statistically between the meat
209 batches (moisture: 71.56 % ± 0.68; ether extract: 2.23% ± 0.45; data not shown).

210 Water holding capacity, color and pH of the burgers are reported in Table 2. No statistical significant
211 interaction was showed between F × ST.

212 Drip loss was influenced by both F and ST (respectively $P<0.05$ and $P<0.01$). Control burger (B)
213 showed greater loss of liquid than BG1 and partially BG2. Drip loss increased constantly during the ST
214 with statistical significance after D4. The increased capacity of retail water by the burgers added with
215 ginger powder could be associated to the capability of the powder to bind the water and to the
216 antioxidant effect against free radicals and reactive oxygen species (ROS) that could affect proteins
217 and leads to modification of the meat structure (Falowo et al., 2014). The capacity to reduce drip loss
218 was also reported in beef burgers added with *Aloe vera* by Soltanizadeh & Ghiasi-Esfahani (2015) but
219 no modification was reported in pork and rabbit burgers added respectively with brown seaweed extract
220 or turmeric powder (Mancini et al., 2015; Moroney, O'Grady, O'Doherty, & Kerry, 2013). Neither F
221 nor ST had the ability to affect cooking loss of the burgers, this lack of variation was previously reported
222 in other meat products added with natural products (Ganhão, Estévez, Armenteros, & Morcuende,
223 2013; Keskekoglu & Uren, 2014).

224 Color indexes were influenced differently by F and ST; The F influenced a*, b*, C* and H*;
225 (respectively with $P<0.05$, $P<0.001$, $P<0.01$ and $P<0.001$), while ST influenced the redness index (a*,
226 $P<0.05$) and the chroma (C*, $P<0.05$). The addition of the ginger powder to pork meat in formulations
227 BG1 and BG2 produced a variation in the color indexes strictly correlated with the natural color of the
228 powder. Indeed, ginger powder conferred to the burgers a yellow color that increased significantly the
229 b* index and decreased significantly the a* index. As consequence of these modifications also the
230 chroma and hue values of BG1 and BG2 were greater than B.

231 Color modifications were expected as ginger powder is normally used in culinary preparations for its
232 taste and its color. Similar changes were be assessed in many studies on meat products added with
233 natural additives which were characterized by its own color such as turmeric, tomato pomace and dog-
234 rose hip (Armenteros, Morcuende, Ventanas, & Estévez, 2013; Mancini et al., 2015; Savadkoohi,
235 Hoogenkamp, Shamsi, & Farahnaky, 2014).

236 The redness index probably decreased during time as a consequence of the natural change in color of
237 the meat linked to the oxidation of the myoglobin to metmyoglobin. As well the a* also C* values
238 decrease during time revealing paler burgers at D7 than D1. Modifications in a* and C* indexes during

ST were not imputable to a variation of pH because no acidification or alkalinization of the burgers were recorded as reported in other studies (Karabagias, Badeka, & Kontominas, 2011; Rodríguez-Calleja, García-López, Santos, & Otero, 2005). The total color difference (ΔE) values are reported in Table 3. As function of the ST only the B presented a variation that might be noticeable by human eyes (value above 2.3 points); B burgers change gradually their color during time, with greater value between D1-D4 than D4-D7 periods, but only the overall evaluation (D1-D7) reported value greater than 2.3. Thus, B burgers presented a noticeable variation during the tested storage time that was not present in BG1 and BG2; as reported before for the a* value, this modification in color could be attributed to the oxidation of myoglobin in B that was delayed or masked by ginger powder in BG1 and BG2.

Calculation of the ΔE at the same storage time between formulations showed that burgers from different groups were recognizable as not equal. The addition of 1% of ginger powder did not modify the color of the burgers immediately from D1 but the differences were expressed (as evident different colors) from D4 (B-BG1). On the contrary the addition of 2% of ginger powder modified instantly the color of the burgers, with evaluated differences maintained during time (B-BG2). Difference between BG1 and BG2 were presented at D1 and D7, at D4 the ΔE value was near to the revealable threshold; at D4 BG1 and BG2 burgers seemed to converge to a common hue of color without maintaining that at D7.

3.2. Fatty acids profile

Fatty acids profile was reported in Table 4. The main fatty acids in the burgers were monounsaturated (MUFA, 40.83%) followed by saturated (SFA, 37.81%) and polyunsaturated (PUFA, 21.33%). The B burgers showed the highest content of C16:0 and C18:0 with a consequent higher percentage of SFA than BG1 and BG2 ($P<0.01$ for C16:0 and C18:0; $P<0.001$ for SFA). The addition of ginger powder in BG1 and BG2 burgers lead to incrementing the polyunsaturated portion (PUFA) with higher percentage of PUFAω3 and PUFAω6 ($P<0.001$). In detail BG1 and BG2 burgers showed higher portion of C18:3ω3, C22:5ω3, C18:2ω6, C20:2ω6 and C22:2ω6 ($P<0.01$ for C18:3ω3 and C22:5ω3; $P<0.001$ for C18:2ω6, C20:2ω6 and C22:2ω6). These modifications in fatty acids profiles as increasing of the percentage of PUFA correlated to the detriment of SFA content were associated to the FA profile of ginger; in fact ginger presents a FA profile rich in PUFA, both ω3 and ω6 (Gur, Turgut-Balik, & Gur, 2006; Zachariah, 2008). The addition of ginger powder produced a reduction of AI and TI indexes and an increase of h/H, PI indexes and of the ratio ω3/ω6 ($P<0.01$ for ratio ω3/ω6; $P<0.001$ for the other calculated indexes). These values reveal an improvement in the healthy characteristics of BG1 and BG2 burgers carried by the addition of ginger. Similar results in positive modification of AI, TI and ω6/ω3 ratio were reported by Selani et al. (2016) in beef burgers added with canola oil and a mix of pineapple by-product and canola oil.

As function of ST total PUFA, PUFAω3, PUFAω6 decreased and showed lower values at D7 (P<0.01); also, oleic acid content (C18:1, P<0.05) decremented during time, without significantly affecting the total MUFA. On the other hand, as consequences of the saturation of MUFA and PUFA occurred during ST, stearic acid and total SFA incremented their concentrations between D4 and D7 (P<0.001 for C18:0; P<0.05 for SFA). Only arachidonic acid content showed an increase during storage time with higher value at D7 than D1 (P<0.05).

As consequence of the degradations of fatty acids during storage time PI, h/H and ω3/ω6 ratios reduced their values with an increase of TI index (P<0.001 for TI and h/H; P<0.01 for ω3/ω6; P<0.05 for PI).

3.3. Thiobarbituric acid reactive substances and antioxidant capacity

Lipid oxidation (TBARS) and antioxidant capacity (ABTS, DPPH and FRAP) values are reported in Table 5. No statistical significance was revealed for the interaction F × ST, while F significantly influenced all the parameters (P<0.001) and ST significantly affected TBARS, ABTS and DPPH (P<0.001, P<0.01 and P<0.05 respectively). Control burgers (B) were more sensitive to oxidation than BG1 and BG2. ABTS and FRAP values determination showed that the antioxidant capacity was directly correlated to the concentration of ginger powder added to the burger (B<BG1<BG2); similarly, DPPH method distinguished between B and burgers added with ginger powder but no statistical difference was found between BG1 and BG2. During storage time the TBARS values increased and showed significant differences between D1 and D4, no additional variation was detected ad D7; on the contrary the antioxidant capacity decreased during time with significance between D1 and D4 for ABTS and between D1 and D7 for DPPH.

The presence of ginger powder, as an antioxidant product, protected the burgers from lipid oxidation and enhanced the antioxidant capacity of the burgers. As reported by Yeh et al. (2014) the main antioxidant molecules of ginger powder are 6-gingerol, 6-shogaol, 8-gingerol,10-gingerol and curcumin. The presence of these molecules, and other minor components, could by correlated with the strong antioxidant activity of ginger. Cao et al. (1993) reported the activity against superoxide anion and hydroxyl radicals of ginger extract.

Similar results were reported by other authors in research studies on meat products added with natural antioxidant additives (Bañón, Díaz, Rodríguez, Garrido, & Price, 2007; Cao et al., 2013; Mancini et al., 2015; Mansour & Khalil, 2000; Mi et al., 2016; Sánchez-Muniz et al., 2012).

3.4. Microbiological growth

Microbiological growth reported the absence of *Enterobacteriaceae*, *Enterococcus* spp., *Escherichia coli* and staphylococci (Table 6).

307 The total aerobic count and *Pseudomonas* spp. growth were affected by the interaction F × ST
308 (P<0.001). The total aerobic count reflected the *Pseudomonas* spp. growth, with lowest log CFU g^{-1} at
309 D1 and a constant increment of bacteria growth during ST for all the burgers. In any case BG1 and
310 BG2 presented lower value than B at all the tested times. Antibacterial activity of ginger and its extracts
311 were reported against several microorganisms such as *Salmonella typhi, Escherichia coli, Enterobacter*
312 spp., *Klebsiella* spp. and *Pseudomonas aeruginosa* (Ekwenye & Elegalam, 2005; Karuppiah &
313 Rajaram, 2012; Park, Bae, & Lee, 2008). The results obtained in this study showed that ginger powder
314 delayed bacterial growth in meat burgers thus BG1 and BG2 burgers presented a contamination of
315 *Pseudomonas* spp. at D7 comparable to B burgers at D4; this statement could be useful for further
316 evaluation on the commercial shelf life of meat products added with ginger powder.
317
318 3.5. Sensory evaluation
319 Sensory analysis reported no difference between the F for the tested parameter (Figure 1A). Trends to
320 increment the valuation of juiciness and the global evaluation for BG1 and BG2 were showed but not
321 statistical significances were determined (P>0.05). Also, ginger powder did not modify the appearance,
322 the aroma and the flavor intensities of the burger. Even if ginger has a strong pungent flavor at
323 percentages of 1 and 2% did not modified the burgers characteristics.
324 Contrariwise the storage time influenced the typical characteristics of the burgers (aroma, flavor and
325 appearance; P<0.01) and the global evaluation (P<0.05) (Figure 1B). Sensory evaluation showed that
326 between D1 and D4 the exterior aspect, the odor and the taste of the burger decrease their evaluations.
327 No further decrease was detected between D4 and D7. While the burgers decreased their intrinsic
328 characteristics between D1 and D4 global evaluation did not show statistical difference; a following
329 reduction of the overall sensory evaluation was showed at D7.
330 Mansour and Khalil (2000) reported that beef patties added with ginger extract showed lower rancid
331 odor after twelve days of storage at 5°C than patties with potato peel or fenugreek extract.
332
333 3.6. Principal component analysis
334 Principal component analysis (Figure 2) showed that the first three principle components (PCs) explain
335 63.63% of the total variability. The first two principal components (PC 1: 36.59% and PC 2: 18.27%)
336 differentiate well the burgers of group B from the burgers with ginger (BG1 and BG2); diversification
337 between the samples was presented also for D1 and D4-D7, with less difference between the last two
338 tested storage times (Figure 2A). From Figure 2A and 2B emerge that aroma and flavor as well
339 appearance of the burgers were most evaluated at D1 and most correlated with B burgers (represented
340 also by an higher a* value than BG1 and BG2). In the bottom-right square of the loading plot (Figure

2B) is well represented as ginger addition incremented b* index (as reported in Table 2) as well antioxidant capacity (Table 5) and PUFA (Table 4). TBARS values reported to be associated with the presence of SFA and to be situated in the diagonal opposite square than antioxidant capacity (FRAP, ABTS and DPPH values) and PUFA (Figure 2B) as most related to control burgers (Figure 2A). Total aerobic count and *Pseudomonas* spp. growth were plotted as opposite of appearance, aroma, flavor and global evaluation of the burgers and closely to TBARS, as the presence of bacterial growth enhanced lipid oxidation and decreased sensory evaluation.

Moreover, antioxidant capacity was plotted on PC 1 axis as opposite to microbial growth, this statement highlighted the antimicrobial potency of ginger powder as reported in Table 6.

4. Conclusions

Ginger powder seems to enhance shelf-life of pork burger and to increase both nutritional and functional properties. The concomitance of the highest value of PUFA content and the lowest vale of lipid oxidation, as reported in both the formulation with ginger powder, highlights the potency of this spice as important food ingredient and additive. Furthermore, the antioxidant capacity showed by burgers added with ginger leads to the formulation of new meat product with an incremented health value.

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543 Table 1. Ginger powder proximate composition, antioxidant capacity evaluations and fatty acids
544 profile.

Proximate composition (%)	
Moisture	6.47
Fat	6.51
Protein	13.80
Ash	8.02
Antioxidant capacity	
ABTS	118.34
DPPH	10.99
FRAP	75.51

Fatty acids profile (%)	
C16:0	20.49
C18:0	10.08
SFA	37.52
C18:1	15.85
MUFA	21.23
C18.3ω3	2.90
C22:5ω3	2.02
PUFAω3	7.90
C18.2ω6	27.35
C20:2ω6	2.03
C22:2ω6	2.00
PUFAω6	33.35
PUFA	41.25

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, C20:5ω3, C22:6ω3, C18:3ω6 and C20:4ω6 were detected in lower amounts. All the mentioned fatty acids have been utilized for calculating sum of lipid fractions.

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547 Table 2. Burgers quality evaluations (water holding capacity, color and pH).

		Formulation (F)			Storage time (ST)			*P* value		SEM
		B	BG1	BG2	D1	D4	D7	F	ST	
Drip loss	%	3.33^{a}	2.00^{b}	2.44^{ab}	1.56^{y}	2.89^{x}	3.33^{x}	0.013	0.001	0.928
Cooking loss	%	21.50	21.55	20.08	21.36	20.51	21.27	0.208	0.595	1.381
L*		23.45	24.01	24.53	24.58	23.93	23.48	0.080	0.069	0.971
a*		13.50^{a}	13.26^{ab}	11.95^{b}	13.83^{x}	12.58^{xy}	12.30^{y}	0.018	0.021	1.055
b*		9.68^{c}	12.52^{b}	14.45^{a}	12.57	12.26	11.81	<0.001	0.356	1.051
C*		17.09^{b}	18.26^{a}	18.79^{a}	18.77^{x}	17.65^{y}	17.71^{xy}	0.002	0.026	0.944
H*		37.83^{c}	43.41^{b}	50.54^{a}	42.12	44.14	45.53	<0.001	0.116	1.815
pH		6.15	6.15	6.18	6.10	6.23	6.15	0.746	0.099	0.338

B: burgers of only meat; BG1: burgers of meat added with 1% (w/w) of ginger powder; BG2: burgers of meat added with 2% (w/w) of ginger powder; $^{a, b, c}$ in the same row indicate significant differences for F; $^{x, y}$ in the same row indicate significant differences for ST.

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549

550 Table 3. Total color difference (ΔE) during storage time the same formulation and between different
551 formulations at the same storage time.

	ΔE Storage time (ST)		
Formulation (F)	D1-D4	D4-D7	D1-D7
B	2.24	1.85	2.82*
BG1	1.13	0.85	1.39
BG2	1.28	1.01	2.02

	ΔE Formulation (F)		
Storage time (ST)	B-BG1	B-BG2	BG1-BG2
D1	1.66	4.28*	2.69*
D4	2.74*	4.21*	2.14
D7	2.94*	5.01*	2.53*

B: burgers of only meat; BG1: burgers of meat added with 1% (w/w) of ginger powder; BG2: burgers of meat added with 2% (w/w) of ginger powder.

*: value over the threshold (2.3 points) with a noticeable difference in color between the samples.

552

553

554 Table 4. Fatty acids profile (%) and correlated indexes of burgers.

	Formulation (F)			Storage Time (ST)			*P* value		SEM
	B	BG1	BG2	D1	D4	D7	F	ST	
C14:0	1.23	1.10	1.11	1.11	1.15	1.18	0.096	0.586	0.369
C16:0	23.72^{a}	23.11^{b}	22.90^{b}	23.45	23.32	22.95	0.003	0.064	0.664
C18:0	13.15^{a}	12.36^{b}	12.55^{b}	12.15^{y}	12.32^{y}	13.58^{x}	0.008	<0.001	0.698
SFA	38.88^{a}	37.25^{b}	37.31^{b}	37.45^{y}	37.51^{y}	38.48^{x}	<0.001	0.012	0.852
C16:1	2.22	1.95	2.31	2.04	2.18	2.26	0.071	0.356	0.569
C18:1	38.41	38.16	38.00	38.67^{x}	38.03^{xy}	37.86^{y}	0.386	0.029	0.786
MUFA	41.22	40.52	40.75	41.18	40.75	40.57	0.117	0.184	0.830
C18:3ω3	0.97^{b}	1.20^{a}	1.27^{a}	1.22	1.14	1.07	0.001	0.139	0.389
C20:5ω3	0.18	0.22	0.21	0.22	0.20	0.19	0.088	0.199	0.200
C22:5ω3	0.17^{b}	0.34^{a}	0.30^{a}	0.34^{x}	0.29^{x}	0.18^{y}	0.001	0.002	0.285
C22:6ω3	0.17	0.20	0.19	0.22	0.19	0.16	0.516	0.078	0.224
PUFAω3	1.49^{b}	1.96^{a}	1.97^{a}	2.00^{x}	1.82^{xy}	1.60^{y}	<0.001	0.002	0.444
C18:2ω6	14.86^{b}	16.58^{a}	16.51^{a}	16.71^{x}	15.50^{y}	15.74^{y}	<0.001	0.001	0.780
C18:3ω6	0.73	0.75	0.72	0.76	0.72	0.71	0.817	0.507	0.315
C20:2ω6	0.16^{b}	0.22^{a}	0.22^{a}	0.20	0.20	0.20	<0.001	0.835	0.152
C20:4ω6	2.32	2.43	2.32	2.25^{y}	2.31^{xy}	2.50^{x}	0.324	0.014	0.411
C22:2ω6	0.19^{b}	0.29^{a}	0.28^{a}	0.26	0.25	0.25	<0.001	0.514	0.173
PUFAω6	18.62^{b}	20.26^{a}	20.05^{a}	20.18^{x}	19.41^{xy}	18.98^{y}	<0.001	0.005	0.822
PUFA	19.75^{b}	22.23^{a}	22.01^{a}	22.18^{x}	21.01^{y}	20.81^{y}	<0.001	0.003	0.881
AI	0.43^{a}	0.40^{b}	0.40^{b}	0.40	0.41	0.42	<0.001	0.130	0.100
TI	1.11^{a}	1.00^{b}	1.00^{b}	1.00^{y}	1.06^{x}	1.06^{x}	<0.001	<0.001	0.161
h/H	2.28^{b}	2.43^{a}	2.44^{a}	2.45^{x}	2.38^{y}	2.31^{y}	<0.001	<0.001	0.224
PI	32.42^{b}	36.66^{a}	35.85^{a}	36.27^{x}	34.39^{xy}	34.27^{y}	<0.001	0.028	1.266
ω3/ω6	0.08^{b}	0.10^{a}	0.10^{a}	0.10^{x}	0.10^{x}	0.08^{y}	0.002	0.002	0.095

C B: burgers of only meat; BG1: burgers of meat added with 1% (w/w) of ginger powder; BG2: 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, C20:1, C22:1, C24:1 were detected but not summarized. All the mentioned fatty acids have been utilized for calculating sum of lipid fraction.

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

$^{a, b}$ in the same row indicate significant differences for F; $^{x, y}$ in the same row indicate significant differences for ST.

555

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

	Formulation (F)			Storage time (ST)			*P* value		SEM
	B	BG1	BG2	D1	D4	D7	F	ST	
TBARS	0.09^{a}	0.04^{b}	0.04^{b}	0.03^{y}	0.06^{x}	0.07^{x}	<0.001	<0.001	0.089
ABTS	1.82^{c}	2.47^{b}	3.11^{a}	2.96^{x}	2.42^{y}	2.03^{y}	<0.001	0.001	0.663
DPPH	0.14^{b}	0.18^{a}	0.18^{a}	0.18^{x}	0.17^{xy}	0.16^{y}	<0.001	0.037	0.130
FRAP	0.06^{c}	1.92^{b}	3.07^{a}	1.72	1.75	1.58	<0.001	0.593	0.621

B: burgers of only meat; BG1: burgers of meat added with 1% (w/w) of ginger powder; BG2: burgers of meat added with 2% (w/w) of ginger powder; $^{a, b, c}$ in the same row indicate significant differences for F; $^{x, y}$ in the same row indicate significant differences for ST.

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.

558

559 Table 6. Microbial analysis on the burgers (log CFU g^{-1}).

Formulation (F)	B			BG1			BG2			*P* value			SEM
Storage time (ST)	D1	D4	D7	D1	D4	D7	D1	D4	D7	F	ST	F × ST	
Total aerobic count	7.40^{e}	9.07^{b}	9.41^{a}	7.26^{f}	8.96^{c}	8.98^{c}	7.31^{f}	8.15^{d}	9.04^{b}	<0.001	<0.001	<0.001	0.145
Pseudomonas spp.	5.76^{d}	7.04^{b}	7.60^{a}	5.92^{d}	6.51^{c}	7.23^{b}	5.05^{d}	6.01^{c}	7.07^{b}	<0.001	<0.001	<0.001	0.176
Enterobacteriaceae	0	0	0	0	0	0	0	0	0	-	-	-	-
Enterococcus spp.	0	0	0	0	0	0	0	0	0	-	-	-	-
Escherichia coli	0	0	0	0	0	0	0	0	0	-	-	-	-
Staphylococci	0	0	0	0	0	0	0	0	0	-	-	-	-

B: burgers of only meat; BG1: burgers of meat added with 1% (w/w) of ginger powder; BG2: burgers of meat added with 2% (w/w) of ginger powder; $^{a, b, c, d, e, f}$ in the same row indicate significant differences for F × ST.

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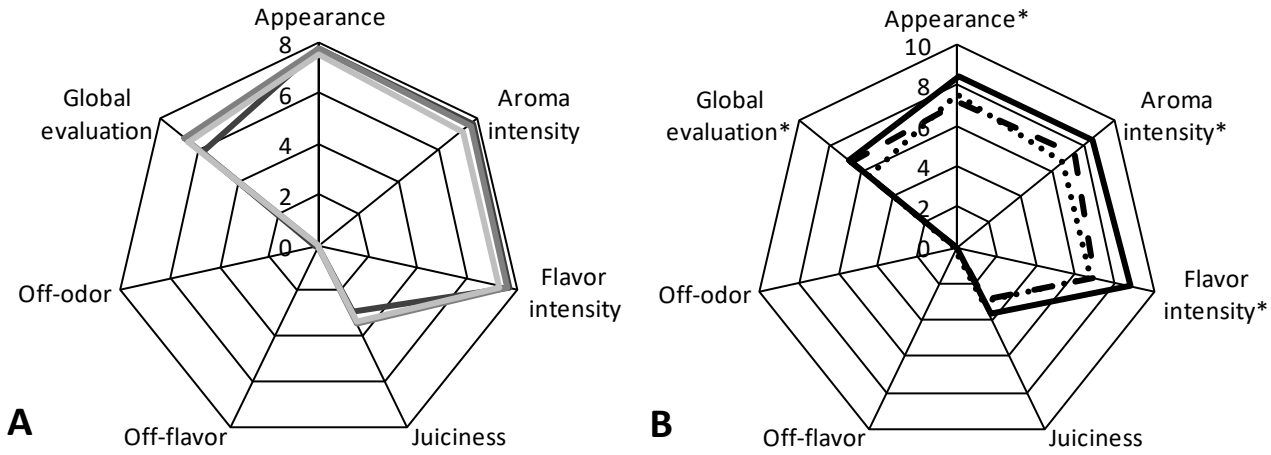
Figure 1. Sensory evaluations.

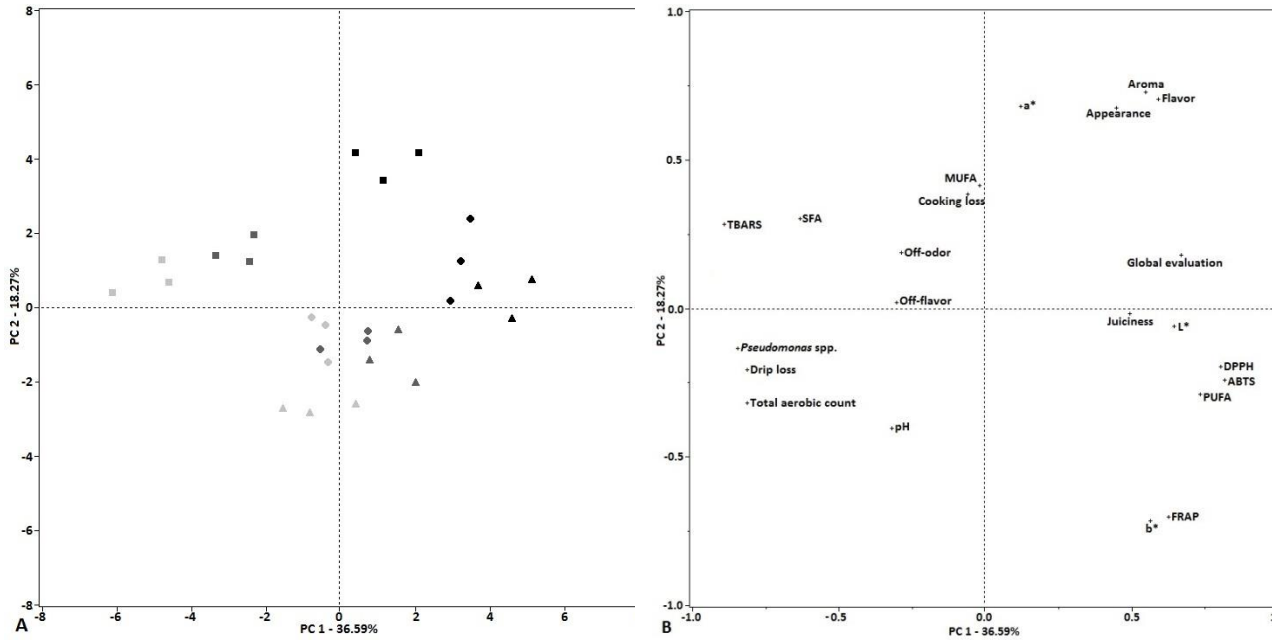


A. Sensory evaluations as function of the formulation (F): B, burgers of only meat (■); BG1, burgers of meat added with 1% (w/w) of ginger powder (■); BG2, burgers of meat added with 2% (w/w) of ginger powder (■).

B. Sensory evaluations as function of the storage time (ST): D1, solid line; D4, dashed line; D7, dotted line.

* indicate significant differences between the F or the ST (P<0.05).

Figure 2. Loading and score plots of the principal component analysis (PCA).



PCA performed on the physical-chemical characteristics (pH, drip loss, cooking loss, L*, a*, b*), fatty acids (SFA, MUFA and PUFA), lipid oxidation (TBARS), antioxidant capacity (FRAP, ABTS and DPPH), microbial growth (total aerobic count and *Pseudomonas* spp.) and sensory (appearance, aroma, off-odors, flavor, off-flavor, juiciness and global evaluation).

A. Score plot: B, burgers of only meat (square); BG1, burgers of meat added with 1% (w/w) of ginger powder (circle); BG2, burgers of meat added with 2% (w/w) of ginger powder (triangle). Colors indicate Storage time: black as D1, dark gray as D4 and light gray as D7.

B. Loading plot.