http://dx.doi.org/10.1016/j.meatsci.2017.07.003

Accepted Version

Modifications of fatty acids profile, lipid peroxidation and antioxidant capacity in raw and cooked rabbit burgers added with ginger

Simone Mancini[a,*], Giovanna Preziuso[a,b], Alessandro Dal Bosco[c], Valentina Roscini[c], Giuliana Parisi[d], Gisella Paci[a,b]

[a]Department of Veterinary Science, University of Pisa, Viale delle Piagge 2, Pisa 56124, Italy

[b]Interdepartmental Research Center "Nutraceuticals and Food for Health", University of Pisa, Via del Borghetto 80, Pisa 56124, Italy

[c]Department of Agricultural, Food and Environmental Science, University of Perugia, Borgo XX Giugno 74, Perugia 06121, Italy

[d]Department of Agri-Food Production and Environmental Sciences (DISPAA), University of Florence, via delle Cascine 5, Firenze 50144, Italy

*Corresponding author: Dr. Simone Mancini, PhD

Tel: 0039 050 2216803

Fax: 0039 050 2210654

email: simafo@gmail.com

simone.mancini@for.unipi.it

Abstract

Effects of ginger powder were evaluated on fatty acid (FA) profile, lipid peroxidation (TBARS) and antioxidant capacity (ABTS, DPPH and FRAP) of rabbit burgers. Burgers were manufactured as control samples (only meat) and two additions of ginger powder (1% and 2%) and stored raw at 4 °C for 7 days. At day 1, 4 and 7 of storage burgers were analysed both as raw and cooked. Ginger powder affected all the tested parameters; both PUFAω3 and PUFAω6 were incremented in raw and cooked samples leading to decreased atherogenicity and thrombogenicity indexes and increased hypo/hypercholesterolemic index and peroxidability index. Lipid peroxidation values of raw and cooked burgers added with ginger were lower than control burgers, at the same time, ABTS, DPPH and FRAP values were incremented by the addition of ginger powder. The results obtained demonstrate the antioxidant capacity of ginger powder as rabbit meat products additive and highlight the capacity of this spice to maintain its characteristics after burgers' cooking.

Keywords
Ginger, burger, rabbit, antioxidant capacity, lipid peroxidation.

1 Introduction

Ginger (*Zingiber officinale* Roscoe) is widely used as spice in several recipes for its pleasant aroma and pungency taste. Moreover, several ginger medical properties are reported in the traditional herbal medicine, in particular for relieving nausea and indigestion (Tapsell et al., 2006) and are commonly used as eupeptic (stimulate the digestive processes) in several products (Zachariah, 2008).

Ginger products (mainly used as powder or ethyl extract) are rich of biological active compounds such as gingerol, paradol, shogaols, zingerone, zerumbone, terpenoids and other minority molecules as flavonoids and phenols (Rahmani, Al Shabrmi, & Aly, 2014). These molecules, involved in flavour and aroma, are also particularly active as antioxidants and modulator of lipid peroxidation. Several articles reported the efficiency of ginger and plant of the Zingiberaceae family as food additive (Abdel-Naeem & Mohamed, 2016; Cao et al., 2013; Mancini et al., 2017a; Naveena & Mendiratta, 2004) or feed supplementation (Herawati & Marjuki, 2011; Mancini, Paci, Pisseri, & Preziuso, 2017b; Zhao et al., 2011).

The latest data of FAOSTAT report that in 2014 the world annual production of rabbit meat has been estimated in 1.6 million tonnes; interestingly the first three producer countries of rabbit meat represent the 75.87% of the world production. The main producer was China (763 thousand tonnes, 48.89% of the world production) followed by Italy and Democratic People's Republic of Korea (269 and 152 thousand tonnes, respectively) (FAO, 2017). Rabbit meat is characterized by excellent dietetic and nutritive properties due to a low lipid content and a high essential amino acids levels (Dalle Zotte, 2002). As consequence of its high percentage of unsaturated fatty acids, rabbit meat is one of the most susceptible to lipid peroxidation and its employment in processed products is very limited (Dalle Zotte & Szendrő, 2011; Petracci & Cavani, 2013).

Burgers represent one of the main sold meat products both as raw (ready-to-cook) or cooked (ready-to-eat) and could easily meet consumers demands. Burger, as a processed product, could rapidly lose its quality and nutrient values due to deterioration (both chemical and biological). Natural ingredients, widely used as flavouring, could play an important role in the stability of the products (Falowo, Fayemi, & Muchenje, 2014; Mariutti & Bragagnolo, 2017; Overholt et al., 2016; Shah, Bosco, & Mir, 2014).

For all the reasons reported above the aim of this research study was to test the capacity of ginger powder to affect fatty acids profile, lipid peroxidation and antioxidant capacity in burgers formulated with rabbit meat that could rapidly deteriorate. In order to estimate the potential activity of ginger powder two percentages were tested (1% and 2%) and compared to a control formulation (only rabbit

meat) during a storage period of 7 days. Fatty acids profile, lipid peroxidation and antioxidant capacity were also determined on cooked samples in order to quantify these parameters in the ready-to-eat products.

2 Material and methods

2.1 Burger manufacture

Nine experimental units were used, each one consisting of one individual rabbit meat. Meat batches were randomly divided in three formulations (F, 3 batches per F) and meat samples were collected for proximate composition. One formulation was used as control (C, only meat) while the other two F consisted in meat added with ginger powder at the percentage of 1 or 2% (Z1 and Z2). Commercial ginger powder, ready to use, was purchased from 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.

Thirty burgers per F were sized in Petri dishes (85 mm of diameter, burger of 100 g, 108 burgers in total) and packaged in single Styrofoam trays, overwrapped with polyethylene film. Burgers were stored raw at 4 ± 0.5 °C to be analysed at day 1, 4 and 7 (Storage time, T; T1, T4 and T7) of storage as raw and cooked. At the fixed storage times from each batch of each formulation, two burgers were used as raw samples and two burgers were cooked (for each F six raw burgers and six cooked burgers were analysed at each T). Burgers derived from the same batch and analysed as raw or cooked samples at a fixed storage time were used as sub-replicates to calculate the experimental unit (batch) value.

Burgers were cooked in a preheated oven at 163 °C to an internal temperature of 71 °C and were turned every 4 min to prevent excess surface crust formation (AMSA, 1995).

Raw and cooked burgers were analysed at T1, T4 and T7 for the determination of fatty acid profile, lipid peroxidation (TBARS) and antioxidant capacity (ABTS, DPPH, FRAP).

2.2 Fatty acids profile

The extraction of intramuscular fat was based on the method of Folch, Lees, & Sloane-Stanley (1957) with chloroform/methanol (2/1); total lipids were extracted from 5 g of burger and fatty acid composition of meat was determined by gas chromatography using a gas chromatograph equipped with a flame ionization detector (Fisons mega 2, Fisons Instruments S.p.A., Rodano, Milano, Italy). 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 DBWax stationary phase (film thickness of 0.25 μm). Nonadecanoic acid (C19:0) was used as internal standard. The fatty acid methyl esters were

identified by retention times compared to the internal standard; the fatty acid profile was calculated using Chrom-Card software and was expressed as percentage of the total fatty acids.

Fatty acid means were used to calculate 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

2.3 Thiobarbituric aid reactive substances (TBARS)

Lipid peroxidation was evaluated with thiobarbituric acid reactive substances (TBARS) method according to Ke, Ackman, Linke, & Nash (1977) and modified by Dal Bosco et al. (2009). Briefly, samples were homogenized with trichloroacetic acid and diethylenetriaminepentaacetic acid and then centrifuged and filtered. Thiobarbituric acid was mixed with the filtrate and tubes were placed in a water bath at 95 °C for 45 min. The absorbance (532 nm) of the samples was recorded and the mg of malondialdehyde (MDA) on 100 g of sample were calculated based on a calibration curve using 1,1,3,3-tetraethoxypropane (TEP).

2.4 Antioxidant capacity (ABTS, DPPH and FRAP)

Five g of samples were homogenized in 10 ml of ethanol at 9000 rpm (Polytron PT 3000, Kinematica AG, Eschbach, Germany) for 45 s in a plastic tube wrapped in aluminium foil. After a centrifugation at 10,000 rpm (4235A CWS, ALC International, Milan, Italy) for 10 min, the supernatant was filtered through Whatman filter paper (N 4). The antioxidant capacity was performed on ethanol extracted samples according the minor modifications reported in Mancini et al. (2015) to the methods of Re et al. (1999) for ABTS reducing activity assay (ABTS, 2,20-azinobis(3-ethylbenzthiazoline-6-sulphonic acid)), of Blois (1958) and Jung et al. (2010) for DPPH scavenging activity (DPPH, 2,2-diphenyl-1-picrylhydrazyl), and Descalzo et al. (2007) for FRAP assay method (ferric reducing ability).

2.5 Statistical analysis

Data of fatty acids profile, lipid peroxidation and antioxidant capacity of both the raw and cooked samples were analysed by applying ANOVA according to a two factorials design with repeated measurements in time. The fixed factors were formulation F (C, Z1, Z2) and storage time T (1, 4, 7 days) and random factors were meat batches. The interaction F × T was also analysed. The two-way

repeated measures ANOVA was conducted separately for raw and cooked samples, and the data are reported as the mean of the fixed effects F and T; the variability was expressed as Root Mean Square Error (RMSE). The significance level was set at 5 % (statistically significant for $P < 0.05$), and if statistical significance was found, the differences were assessed using Tukey's test.

Principal component analysis (PCA) was conducted on fatty acids profile, lipid peroxidation and antioxidant capacity of raw and cooked samples separately and, in order to evaluate the effect of cooking, a PCA was also conducted on the results obtained for both raw and cooked samples mixed.

R free statistical software was used (R Core Team, 2015).

3 Results

No significant differences were observed in proximate analyses on the meat batches between the experimental units (moisture: 72.67 % ± 0.77; ether extract: 2.75% ± 0.67; data not shown).

3.1 Fatty acid profile

Table 2 provides the fatty acids (FAs) profile of raw burgers. The fatty acids of the raw burgers more represented were C16:0 followed by C18:2ω6 and C18:1 (27.85%, 22.44% and 22.14%, respectively). C burgers showed the highest content of C16:0 ($P < 0.001$) and, as a consequence, a higher saturated FAs (SFA) percentage than Z1 and Z2 burgers ($P < 0.001$). Z1 and Z2 burgers were characterized by a higher proportion of total polyunsaturated FAs (PUFA) ($P < 0.001$) due to the contents of ω6-FAs ($P < 0.001$) and ω3-FAs (significant differences between Z2 and the other two F, $P < 0.001$). The differences observed in burgers for total PUFA depended primarily by the significant differences for C18:2ω6 ($P < 0.001$), C18:3ω3 ($P < 0.05$), C20:5ω3 ($P < 0.05$) and C22:6ω3 ($P < 0.01$).

Storage time significantly affected the proportion of total SFA, total PUFA, PUFAω3 and PUFAω6 ($P < 0.001$ for SFA, PUFAω3 and PUFA; $P < 0.01$ for PUFAω6). At T4 and T7 the burger showed highest content of SFA and lowest portion of PUFA, PUFAω3 and PUFAω6. The significantly highest quantity of total SFA resulted from an increase of C16:0 and C18:0, (respectively $P < 0.05$ and $P < 0.01$). Significant reductions of PUFA, PUFAω3 and PUFAω6 were associated with a significant decrease in the quantity of C20:5ω3, C22:5ω3, C22:6ω3 and C18:2ω6 (respectively, $P < 0.05$, $P < 0.05$, $P < 0.001$ and $P < 0.01$). The interaction F x T was significant only for C18:2ω6 ($P < 0.05$) as the addition of the ginger powder, at both the concentrations, led to an increment of this FA with higher values for Z1 and Z2 than C at all the tested times. Furthermore, the value of C18:2ω6 in C burgers decreased between T1 and T4, instead for Z1 and Z2 a small decrease of content was observed between T4 and T7 (Z1T1 = Z1T4 = Z2T1 = Z2T4 > Z1T7 = Z2T7 > CT1 > CT4 = CT7;

24.44, 23.67, 23.70, 24.21, 23.53, 23.23, 21.44, 18.76 and 18.76%, respectively).

Formulation and storage time had statistical significant effects on all the calculated indexes (ratio ω3/ω6, atherogenicity index, thrombogenicity index, ratio hypocholesterolemic/Hypercholesterolemic indexes and peroxidisability index). Considering the formulation, results of Z1 and Z2 FA profile led to a healthy improvement of atherogenicity, thrombogenicity and peroxidisability indexes and to the hypocholesterolemic over Hypercholesterolemic index ($P < 0.001$). Furthermore, the addition of 2% of ginger powder affected also the ratio ω3/ω6 ($P < 0.05$).

Considering the storage time, from T1 to T4 the values of AI and TI indexes increased while the ratios ω3/ω6, h/H and the PI index decreased ($P < 0.01$ for ω3/ω6; $P = 0.001$ for h/H; $P < 0.001$ for AI, TI and PI); no more modifications were showed between T4 and T7.

Table 3 provides the fatty acids (FAs) profile of cooked burgers. As reported for the raw samples, also in cooked ones the ginger powder addition affected some FA percentages. Both the ginger additions showed to reduce the content of C14:0 and C16:0 and consequently the total SFA content ($P < 0.001$). Moreover, also the percentage of the monounsaturated C16:1 was affected in the same way ($P < 0.05$), but no modification was reported for the total MUFA ($P > 0.05$). Ginger increased total PUFAω3 percentage ($P = 0.05$), while no singular FA differences were detected. The percentages of C18:2ω6, and consequently of PUFAω6, were affected by the F ($P < 0.001$), with higher values in Z1 and Z2 samples than in C.

Considering PUFAω3 and PUFAω6 variations, the total PUFA magnified the differences in polyunsaturated composition between the samples with higher values of Z1 and Z2 than C ($P < 0.001$). Cooked burgers maintained the same trend in the calculated indexes, except for the ratio ω3/ω6 and PI value ($P > 0.05$). Control samples showed higher values of AI and TI and lower values of PI and h/H ratio than Z1 and Z2 burgers ($P < 0.05$ for PI; $P < 0.01$ for TI; $P < 0.001$ for AI and h/H).

As function of storage time few modifications were detected, mainly between T1 and T4; only C18:0 showed an increased content ($P < 0.01$), while at the same time the contents of the polyunsaturated C18:3ω3, C22:6ω3 and C18:2ω6 showed to decrease ($P < 0.05$ for C18:3ω3 and C18:2ω6; $P < 0.001$ for C22:6ω3). These decreases between T1 and T4 affected also the total PUFA ($P < 0.01$), as well as PUFAω3 ($P < 0.01$), PUFAω6 ($P < 0.01$) and the ratio ω3/ω6 ($P < 0.05$). As reported for the raw samples, the indexes AI and TI increased their values during storage time ($P < 0.05$). Moreover, the PI index decreased as function of the T with a significant difference between T1 and T4 ($P < 0.01$).

3.2 Lipid peroxidation and antioxidant capacity

The effects of formulation and storage time on lipid peroxidation and antioxidant capacity of the raw burgers are reported in Table 4. The C burgers showed more susceptibility to lipid peroxidation than Z1 and Z2 burgers ($P < 0.001$), as well as a lower antioxidant capacity ($P < 0.001$). Quantification of

antioxidant capacity by ABTS and FRAP methods showed an increase of antioxidant capacity related to the ginger percentage present in the burgers (Z2>Z1); this difference was not showed in DPPH values as Z1 and Z2 were not statistically different each other.

Storage time (T) affected ABTS, DPPH and FRAP values ($P < 0.001$), but with different timings. A gradual decrease was shown by DPPH and ABTS with statistical differences between all the tested times; FRAP values decreased only at T7, with no differences between T1 and T4.

Moreover, ABTS reducing activity and DPPH scavenging activity were affected by the main studied factors and their interaction (F × T, $P < 0.05$ for ABTS and $P < 0.001$ for DPPH). ABTS reducing activity showed the highest value for Z2 at T1 (3.56 mmol of Trolox equivalent per 100 g of fresh meat) followed by Z1 at T1 and T4 and Z2 at T4 and T7 (3.32, 2.87, 3.06 and 2.85 mmol of Trolox equivalent per 100 g of fresh meat, respectively). Control samples at T1 and T4 showed higher reducing activity than Z1 at T7 (2.27 and 2.22 for C at T1 and T4; 1.94 mmol of Trolox equivalent per 100 g of fresh meat for Z1 at T7) then decreased their activity at T7 (1.22) (Z2T1 > Z1T1 = Z1T4 = Z2T4 = Z2T7 > CT1 = CT4 > Z1T7 > CT7).

In DPPH evaluation Z2 burgers showed to maintain the highest level of antioxidant capacity for all the T (1.65, 1.61 and 1.63 mmol of Trolox equivalent per 100 g of fresh meat, respectively at T1, T4 and T7); Z1 burgers showed higher scavenging activity at T1 and T4 than T7 (1.67, 1.64 and 1.44 mmol of Trolox equivalent per 100 g of fresh meat, respectively), whereas C burgers revealed the worst scavenging activity and a decrease over the storage period (1.08, 0.80 and 0.80 mmol of Trolox equivalent per 100 g of fresh meat, respectively at T1, T4 and T7) (Z1T1 = Z1T4 = Z2T1 = Z2T4 = Z2T7 > Z1T7 > CT1 > CT4 = CT7).

Lipid peroxidation (TBARS) and antioxidant capacity (ABTS, DPPH and FRAP) of cooked samples are reported in Table 5. Cooked C burgers showed higher lipid peroxidation and lower antioxidant capacity than both Z1 and Z2 samples ($P < 0.001$). As reported for the raw burgers, a significant increase in antioxidant capacity, evaluated with ABTS and FRAP methods, was reported as function of the percentage of ginger. No difference was found between Z1 and Z2 with DPPH probe. Storage time affected antioxidant capacity between day 4 and day 7 ($P = 0.001$ for ABTS; $P < 0.001$ for FRAP and DPPH). Moreover, a significant interaction F × T was reported for DPPH ($P < 0.05$). The highest value was reported by Z2 at T1 (1.24 mmol of Trolox equivalent per 100 g of fresh meat) followed by Z2 at T4 and Z1 at both T1 and T4 (1.20, 1.15 and 1.19, respectively); at T7, Z1 and Z2 reported their lowest values (both 1.03 mmol of Trolox equivalent per 100 g of fresh meat) but still greater than those of C samples. C cooked samples showed to decrease their DPPH value at T7, with no difference between T1 and T4 (0.94, 1.01 and 0.56 mmol of Trolox equivalent per 100 g of fresh meat, respectively for T1, T4 and T7) (Z2T1 > Z1T1 = Z1T4 = Z2T4 > Z1T7 = Z2T7 > CT1 = CT4 > CT7).

4 Discussion

Fatty acid profiles of burgers added with ginger powder (Z1 and Z2) were modified by the addition of the spice mostly in the PUFA composition. Ginger powder is commonly characterized by high level of PUFA both ω3 and ω6 (Gur, Turgut-Balik, & Gur, 2006; Zachariah, 2008). The main PUFA in ginger, as in other plants, is represented by C18:2ω6 and normally the level is around 23% of the total FAs (Zachariah, 2008). Anyhow, ginger powder employed in this research study showed little higher value of C18:2ω6 (27.35%). Linoleic acid, as an essential fatty acid, cannot be synthesize by human or animals, and must be taken with the diet. Usually rabbit meat presents a high level of linoleic acid when rabbits fed a conventional feed (around 22% of the FA, Hernàndez & Dalle Zotte, 2010). As expected, both raw and cooked Z1 and Z2 burgers showed higher contents of C18:2ω6 than control ones. Particularly in raw samples, burgers added with ginger showed to maintain highest level of this fatty acid also during storage times and to reduce their contents few days later than C burgers. Moreover, the addition of the spice increased the PUFAω3 and decreased the SFA percentages leading to healthier burgers, as confirmed by the calculated indexes and ratios. Similar trends were reported in beef and pork burgers added with plant products (Mancini et al., 2017a; Selani et al., 2016).

Ginger powder also plays a key role as modulator of the chemical stability of the burgers as both lipid peroxidation and antioxidant capacity were affected by the addition of the spice. The results of lipid peroxidation are in agreement with those of Cao et al. (2013), Mansour & Khalil (2000) and Mi, Guo, & Li (2016) who observed high efficacy to prevent lipid peroxidation in meat products supplemented with ginger extracts.

As regard the antioxidant activity, our results confirm those observed by other authors who reported high ABTS and DPPH radical scavenging abilities in meat products added with different concentrations of ginger extract (Mi et al., 2016). Moreover, the antioxidant capacity of ginger remained at high level during storage time in both raw and cooked samples, mainly highlighted by the DPPH probe, indicating that the active antioxidant compounds of ginger are able to maintain their activity over time; similar observation was reported for other natural additives used in meat products (Bañón, Díaz, Rodríguez, Garrido, & Price, 2007; Mancini, Preziuso, & Paci, 2016; Sánchez-Muniz et al., 2012).

Addition of ginger powder increased the health characteristics of both raw and cooked Z1 and Z2 burgers as the AI decrease represents an increased anti-atherogenic capacity, with lower possibilities of adhesion of lipids to the cells of the immunological and circulatory system, as reported by Nantapo et al. (2015). Moreover, similarly the capacity of form clots in blood vessels might be reduced as also TI was positively affected by ginger addition (Nantapo et al., 2015).

Three principal component analyses (PCA) were conducted, respectively, on raw or cooked samples alone (Fig. 1A and 1B), and on raw and cooked samples mixed (with F, T and raw-cooked as main factors) (Fig. 1C). The first three principal components of each PCA explained the 72.87%, 69.33% and 68.39% of the total variability respectively for the raw samples, the cooked samples and both mixed (Table 6). The PCA of the raw samples (Fig. 1A) shows a complete diversification between C samples (all collocated in the left squares) and the Z1 and Z2 samples. Lipid peroxidation (TBARS) and C16:0 were represented closely to the C samples as more related to their characteristics as well as ABTS, DPPH, FRAP and PUFA were more related and closely represented to the Z1 and Z2 samples. In cooked samples this clustering of samples by F was even more noticeable with the C samples all gathered in the lower left square, while Z1 and Z2 samples were reachable in the diagonal of the graphic (Fig. 1B). As reported for raw samples, TBARS and antioxidant capacities were allocated more closed to C or Z1 and Z2 samples respectively.

Mixed raw and cooked samples analysed as function of F, T and their form (raw or cooked) maintained a well evaluable distinction between C and burgers added with ginger. Furthermore, a lack of difference was shown between raw and cooked samples within the same F. As reported before, lipid peroxidation and antioxidant capacity were linked to the absence or presence of ginger powder (Fig. 1C).

This evaluation highlighted that the characteristics of ginger perpetuate even after cooking, leading to ready-to-eat products with higher antioxidant capacity and lower lipid peroxidation level than burgers of only meat.

From an evaluation of the eigenvectors of the PCAs on the raw and cooked samples analysed alone (Table 6), the PC1 seems to well explain the formulation factor (F). Both PC1 reported eigenvectors values under 0.25 (as absolute value) for all the parameters that did not show to be significant for the F (for raw samples C14:0, C18:0, C16:1, C18:1ω9, C18:3ω3, C22:5ω3 and C20:4ω6; for cooked samples C18:0, C18:1ω9, C18:3ω3, C20:5ω3 C22:5ω3, C22:6ω3 and C20:4ω6), instead a greater eigenvector value than 0.25 (as absolute value) was shown for all the parameters that reached the significant level in the ANOVA analyses. On the other hand, no similarity between the following principal components and the storage time was showed. A partial correspondence seems to be revealed between PC2 and the interaction F × T as in all the plots the trend to separate the tested times within the formulation was shown, but a lack of steadiness is reported.

5 Conclusions

The addition of ginger powder to the rabbit meat could increase the nutritional value of the burgers and lead to the development of nutraceutical products. The antioxidant properties of ginger were highlighted in both raw and cooked samples with a consequent reduction of lipid peroxidation during

storage time. Fatty acid profiles of burgers were affected by ginger's fatty acids composition leading to healthier products. Fatty acids profile, lipid peroxidation and antioxidant capacity of ginger burgers prospective a longer shelf life than only meat burgers.

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413 dietary oxidation stability. *Poultry Science*, *90*(8), 1720–1727. http://doi.org/10.3382/ps.2010-
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415 Table 1 Proximate composition, antioxidant capacity and fatty acid profile of ginger powder.

Proximate composition (%)		Fatty acid 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
Antioxidant capacity		C18.3ω3	2.90
ABTS	118.34	C22:5ω3	2.02
DPPH	10.99	PUFAω3	7.90
FRAP	75.51	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.

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 also detected in lower amounts. All the mentioned fatty acids have been utilized for calculating sum of the fatty acid fractions.

416

417 Table 2 Fatty acid composition (%) and calculated indexes of raw burgers.

	Formulation (F)			Storage time (T)			*P* value			RMSE
	C	Z1	Z2	T1	T4	T7	F	T	F × T	
C14:0	2.74	2.66	3.18	2.51	3.13	2.95	0.157	0.096	0.559	0.573
C16:0	31.54^{a}	26.65^{b}	25.35^{b}	26.89^{y}	28.34^{x}	28.31^{x}	<0.001	0.037	0.383	1.218
C18:0	10.29	9.77	9.60	8.79^{y}	10.25^{x}	10.62^{x}	0.451	0.011	0.489	1.176
SFA	46.62^{a}	40.76^{b}	40.38^{b}	40.16^{y}	43.63^{x}	43.97^{x}	<0.001	0.002	0.591	2.015
C16:1	2.72	2.44	2.33	2.45	2.61	2.42	0.085	0.488	0.977	0.352
C18:1	21.66	22.69	22.08	22.74	21.70	21.98	0.119	0.101	0.739	0.986
MUFA	25.19	26.16	25.49	26.19	25.23	25.42	0.106	0.096	0.794	0.913
C18:3ω3	2.30^{b}	2.56^{ab}	2.82^{a}	2.70	2.55	2.43	0.045	0.391	0.980	0.394
C20:5ω3	0.47^{b}	0.68^{ab}	0.80^{a}	0.80	0.53	0.62	0.022	0.065	0.632	0.221
C22:5ω3	0.58	0.62	0.84	0.86^{x}	0.67^{xy}	0.52^{y}	0.105	0.049	0.788	0.262
C22:6ω3	0.41^{b}	0.54^{b}	0.75^{a}	0.87^{x}	0.42^{y}	0.41^{y}	0.002	<0.001	0.235	0.167
PUFAω3	4.26^{b}	4.94^{b}	5.84^{a}	5.90^{x}	4.70^{y}	4.45^{y}	<0.001	<0.001	0.762	0.601
C18:2ω6	19.72^{b}	23.88^{a}	23.71^{a}	23.20^{x}	22.21^{y}	21.90^{y}	<0.001	0.004	0.020	0.708
C20:4ω6	3.06	3.42	3.58	3.69	3.31	3.06	0.153	0.081	0.246	0.551
PUFAω6	23.46^{b}	27.82^{a}	28.09^{a}	27.56^{x}	26.18^{y}	25.64^{y}	<0.001	<0.001	0.078	0.782
PUFA	27.72^{b}	32.76^{a}	33.94^{a}	33.45^{x}	30.88^{y}	30.09^{y}	<0.001	<0.001	0.312	1.118
ω3/ω6	0.18^{b}	0.18^{b}	0.21^{a}	0.21^{x}	0.18^{y}	0.17^{y}	0.025	0.003	0.749	0.022
AI	1.35^{a}	0.98^{b}	0.94^{b}	0.96^{y}	1.14^{x}	1.16^{x}	<0.001	<0.001	0.119	0.081
TI	1.21^{a}	0.94^{b}	0.87^{b}	0.86^{y}	1.06^{x}	1.09^{x}	<0.001	<0.001	0.547	0.105
h/H	1.40^{b}	1.84^{a}	1.90^{a}	1.87^{x}	1.64^{y}	1.64^{y}	<0.001	0.001	0.329	0.119
PI	49.02^{b}	54.48^{a}	62.91^{a}	63.49^{x}	53.98^{y}	51.94^{y}	<0.001	<0.001	0.747	4.510

C: only meat; Z1: meat + 1% ginger powder; Z2: meat + 2% ginger powder.

C15:0, C17:0, C20:0, C21:0, C22:0, C23:0, C24:0, C14:1, C15:1, C17:1, C20:1, C22:1, C24:1, C20:3ω3, C18:3ω6, C20:2ω6, C20:3ω6, C22:2ω6 were detected but not listed in the table. All the mentioned fatty acids have been utilised for calculating the sums of the fatty acid fractions.

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

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

418

419 Table 3 Fatty acid composition (%) and calculated indexes of cooked burgers.

	Formulation (F)			Storage time (T)			*P* value			RMSE
	C	Z1	Z2	T1	T4	T7	F	T	F × T	
C14:0	3.30^{a}	2.59^{b}	2.72^{b}	2.78	2.87	2.95	<0.001	0.631	0.185	0.370
C16:0	30.24^{a}	27.91^{b}	27.88^{b}	28.44	28.99	28.61	<0.001	0.550	0.898	1.068
C18:0	9.81	10.07	10.09	9.15^{y}	10.26^{x}	10.56^{x}	0.692	0.003	0.331	0.753
SFA	46.10^{a}	42.34^{b}	42.61^{b}	42.42	44.13	44.50	0.001	0.078	0.643	1.915
C16:1	2.97^{a}	2.43^{b}	2.50^{b}	2.48	2.85	2.56	0.017	0.124	0.426	0.381
C18:1	21.38	22.26	21.70	22.12	21.31	21.91	0.155	0.182	0.397	0.913
MUFA	25.72	25.87	25.30	25.89	25.50	25.51	0.486	0.657	0.865	1.013
C18:3ω3	2.20	2.08	2.24	2.34^{x}	2.11^{y}	2.07^{y}	0.236	0.021	0.372	0.195
C20:5ω3	0.48	0.60	0.56	0.65	0.45	0.54	0.561	0.188	0.601	0.228
C22:5ω3	0.39	0.38	0.52	0.47	0.41	0.41	0.219	0.709	0.710	0.180
C22:6ω3	0.31	0.39	0.40	0.50^{x}	0.33^{y}	0.27^{y}	0.078	<0.001	0.090	0.090
PUFAω3	3.67^{b}	4.01^{ab}	4.25^{a}	4.46^{x}	3.74^{y}	3.74^{y}	0.050	0.005	0.851	0.453
C18:2ω6	20.52^{b}	23.32^{a}	23.23^{a}	23.20^{x}	22.18^{xy}	21.69^{y}	<0.001	0.031	0.439	1.101
C20:4ω6	2.95	2.93	3.05	3.47	2.68	2.78	0.927	0.062	0.972	0.699
PUFAω6	24.02^{b}	26.95^{a}	27.11^{a}	27.42^{x}	25.54^{y}	25.13^{y}	<0.001	0.007	0.699	1.389
PUFA	27.70^{b}	30.96^{a}	31.37^{a}	31.87^{x}	29.28^{y}	28.87^{y}	<0.001	0.005	0.760	1.779
ω3/ω6	0.15	0.15	0.16	0.17^{x}	0.15^{y}	0.15^{y}	0.359	0.018	0.807	0.012
AI	1.34^{a}	1.07^{b}	1.07^{b}	1.08^{y}	1.20^{xy}	1.21^{x}	<0.001	0.040	0.713	0.111
TI	1.21^{a}	1.06^{b}	1.05^{b}	1.01^{y}	1.15^{x}	1.16^{x}	0.007	0.015	0.997	0.103
h/H	1.44^{b}	1.70^{a}	1.68^{a}	1.69	1.55	1.58	<0.001	0.094	0.954	0.131
PI	46.63	51.25	52.78	55.50^{x}	47.65^{y}	47.50^{y}	0.065	0.008	0.949	5.292

C: only meat; Z1: meat + 1% ginger powder; Z2: meat + 2% ginger powder.

C15:0, C17:0, C20:0, C21:0, C22:0, C23:0, C24:0, C14:1, C15:1, C17:1, C20:1, C22:1, C24:1, C20:3ω3, C18:3ω6, C20:2ω6, C20:3ω6, C22:2ω6 were detected but not listed in the table. All the mentioned fatty acids have been utilised for calculating the sum of the fatty acid fractions.

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

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

420

421 Table 4 Lipid peroxidation (TBARS) and antioxidant capacity of the raw burgers (ABTS, DPPH and
422 FRAP).

	Formulation (F)			Storage time (T)			*P* value			RMSE
	C	Z1	Z2	T1	T4	T7	F	T	F × T	
TBARS	1.02^{a}	0.53^{b}	0.35^{b}	0.54	0.74	0.63	<0.001	0.208	0.682	0.221
ABTS	19.03^{c}	27.07^{b}	31.55^{a}	30.49^{x}	27.16^{y}	20.00^{z}	<0.001	<0.001	0.046	2.396
DPPH	0.90^{b}	1.58^{a}	1.63^{a}	1.47^{x}	1.40^{y}	1.30^{z}	<0.001	<0.001	<0.001	0.039
FRAP	2.70^{c}	9.43^{b}	14.30^{a}	9.96^{x}	9.59^{x}	6.88^{y}	<0.001	<0.001	0.134	0.447

C: only meat; Z1: meat + 1% ginger powder; Z2: meat + 2% ginger powder.

Results expressed as: mg of MDA per 100 g of fresh meat for TBARS; as mmol of Trolox equivalent per 100 g of fresh meat for ABTS and DPPH; as mmol of Fe^{II} equivalent per 100 g of fresh meat for FRAP.

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

423

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

	Formulation (F)			Storage time (T)			*P* value			RMSE
	C	Z1	Z2	T1	T4	T7	F	T	F × T	
TBARS	1.94^{a}	1.00^{b}	1.19^{b}	1.35	1.34	1.43	<0.001	0.820	0.834	0.328
ABTS	13.98^{c}	21.10^{b}	27.21^{a}	22.79^{x}	23.14^{x}	16.36^{y}	<0.001	0.001	0.773	3.409
DPPH	0.84^{b}	1.12^{a}	1.16^{a}	1.11^{x}	1.13^{x}	0.87^{y}	<0.001	<0.001	0.008	0.066
FRAP	2.40^{c}	9.04^{b}	12.68^{a}	9.00^{x}	8.64^{x}	6.48^{y}	<0.001	<0.001	0.541	0.798

C: only meat; Z1: meat + 1% ginger powder; Z2: meat + 2% ginger powder.

Results expressed as: mg of MDA per 100 g of fresh meat for TBARS; as mmol of Trolox equivalent per 100 g of fresh meat for ABTS and DPPH; as mmol of Fe^{II} equivalent per 100 g of fresh meat for FRAP.

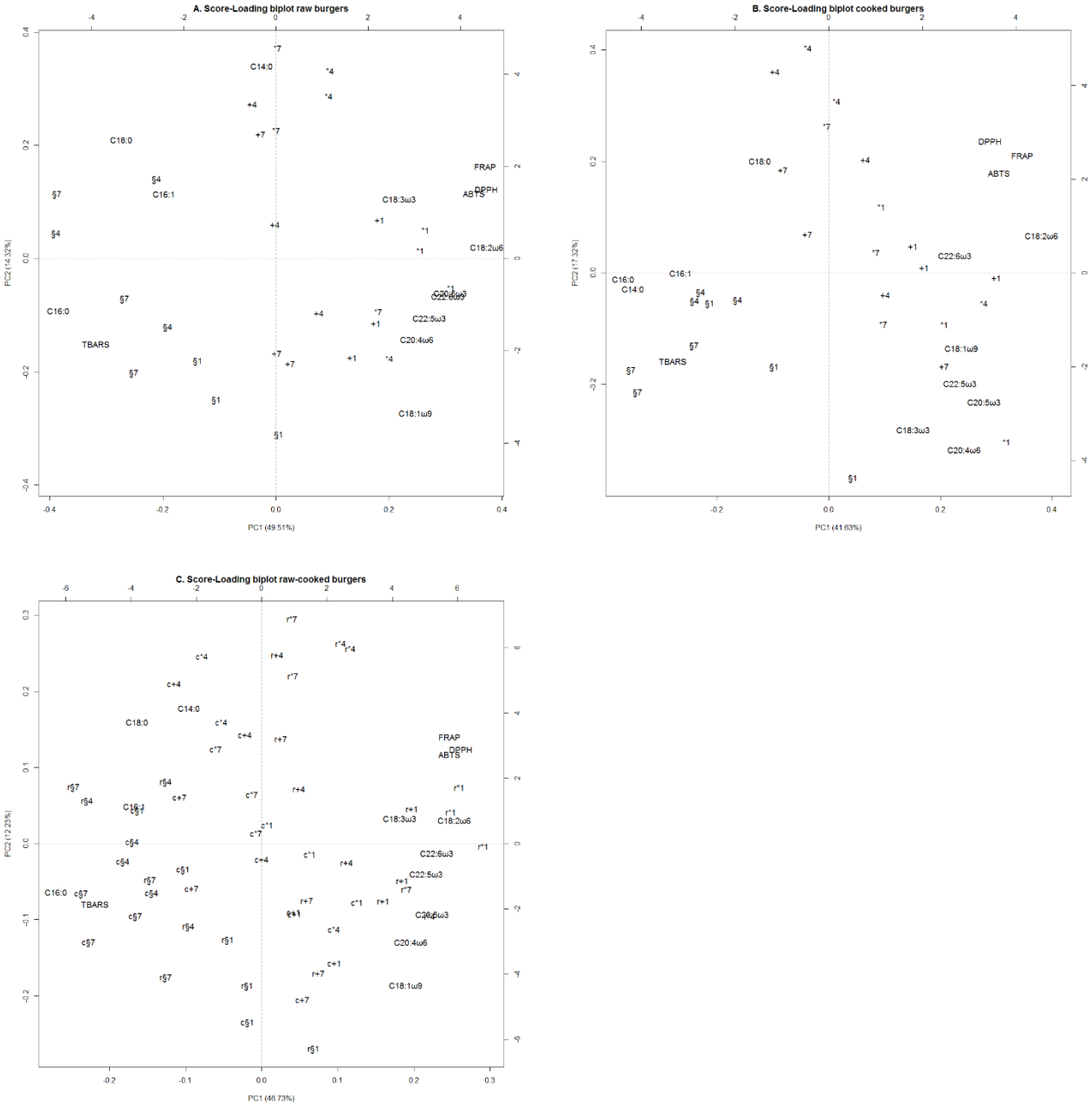
$^{a, b, c}$ in the same row indicate significant differences for F; $^{x, y}$ in the same row indicate significant differences for T.

427 Table 6 Eigenvalues and eigenvectors of the first three principal components of PCA analyses
428 conducted on raw and cooked samples alone and mixed.

	Raw samples			Cooked samples			Raw-Cooked samples		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
Eigenvalues	7.4304	2.1512	1.3733	6.2724	2.6010	1.5705	7.0192	1.8391	1.4156
Eigenvectors									
TBARS	-0.2772	-0.2512	0.1774	-0.2632	-0.2164	0.1577	-0.2653	-0.1926	0.0896
FRAP	0.3209	0.2613	-0.0428	0.3196	0.3032	0.0806	0.2962	0.3279	-0.1822
ABTS	0.3032	0.1848	0.1270	0.2829	0.2633	0.1550	0.2962	0.2745	0.0241
DPPH	0.3222	0.1933	-0.2082	0.2690	0.3382	0.1258	0.3140	0.2890	-0.0642
C14:0	-0.0218	0.5485	0.1325	-0.3214	-0.0416	-0.1218	-0.1145	0.4143	0.3888
C16:0	-0.3333	-0.1477	0.0564	-0.3375	-0.0153	-0.1226	-0.3236	-0.1470	0.0377
C18:0	-0.2369	0.3394	-0.2206	-0.1100	0.3003	0.5183	-0.1954	0.3774	-0.1797
C16:1	-0.1734	0.1772	0.5415	-0.2596	0.0152	0.2936	-0.2004	0.1157	0.4149
C18:1	0.2137	-0.4384	-0.1574	0.2162	-0.1975	-0.2863	0.2261	-0.4347	-0.1986
C18:3ω3	0.1886	0.1643	0.4574	0.1373	-0.3988	0.2009	0.2173	0.0746	0.4716
C20:5ω3	0.2666	-0.1015	0.0549	0.2448	-0.3263	0.1399	0.2680	-0.2188	0.0912
C22:5ω3	0.2341	-0.1725	0.2563	0.2169	-0.2713	0.3085	0.2587	-0.0911	0.3266
C22:6ω3	0.2620	-0.1114	0.2460	0.2034	0.0300	-0.5310	0.2739	-0.0341	0.1872
C18:2ω6	0.3235	0.0363	-0.3089	0.3482	0.0894	-0.0289	0.3033	0.0693	-0.3072
C20:4ω6	0.2150	-0.2342	0.2932	0.2223	-0.4453	0.1556	0.2350	-0.3015	0.3015
Cumulative %	49.51	63.83	72.87	41.63	58.95	69.33	46.73	58.96	68.39

429

Fig. 1 Biplots of loading and scores of the principal component analysis (PCA) on the raw samples (A), on the cooked samples (B) and on the raw and cooked samples mixed (C).



PCAs were performed with fatty acids profile, lipid peroxidation values (TBARS), antioxidant capacity values (FRAP, ABTS and DPPH).

Formulations are reported as: control (C), only meat, §; meat + 1% ginger powder (Z1), +; meat + 2% ginger powder (Z2), *. Storage times are reported as number of days (1, 4 and 7).