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1   **EFFECT OF GENOTYPE ON RAW AND COOKED POULTRY MEAT**

2   **Fatty acid profile, oxidative status, and content of volatile organic compounds in**

3   **raw and cooked meat of different chicken strains**

4

5   A. Cartoni Mancinelli[*1], E. Silletti[+], S. Mattioli[*], A. Dal Bosco[*], B. Sebastiani[#], L. Menchetti[§], A.

6   Koot[+], S. van Ruth[+] and C. Castellini[*]

7

8   [*]*Department of Agricultural, Environmental and Food Science, University of Perugia, Borgo XX*

9   *Giugno 74, 06124, Perugia, Italy.*

10   [+]*Wageningen Food Safety Research, P.O. Box 230, 6700 AE Wageningen, The Netherlands.*

11   [#]*Department of Chemistry, Biology and Biotechnology University of Perugia, via Elce di Sotto, 8 -*

12   *06123 Perugia, Italy.*

13   [§]*Department of Veterinary Medicine, University of Perugia, Via San Costanzo 74, 06126, Perugia,*

14   *Italy.*

15

16   Declarations of interest: none

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18   [1]Corresponding author: acartonimancinelli@gmail.com

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20

21   **ABSTRACT**

22   Chicken meat is rich in unsaturated fatty acids. Therefore, it is more susceptible to lipid oxidation

23   and production of volatile organic compounds (**VOC**). In this study, we evaluated the fatty acids,

24   antioxidants, and VOC profiles of raw and cooked meat samples derived from four strains of

25   chicken differing in their growth rates, which were as follows: Slow-Growing (**SG**, Leghorn),

26   Medium-Growing (**MG**, Hubbard and Naked Neck) and Fast-Growing (**FG**, Ross). The VOC

27   profile of meat was measured using proton-transfer reaction mass spectrometry (**PTR–MS**). The

28   VOC were identified using PTR–time of flight-MS (**PTR-ToF-MS**). The data were analyzed using

29   both univariate and multivariate models. Twenty main VOC were identified, which were classified

30   into the following chemical categories: aldehydes, alkadienes, alkenes, furans, amides, alcohols,

31   and other compounds. Our results revealed that the chicken genotype and the method of cooking

32   strongly influenced the VOC profile of the meat. Identifying the relationships between these traits

33   allowed us to highlight the trade-off of the main substrates such as n-3 and n-6 Polyunsaturated

34   Fatty Acids (**PUFA**), protective substances (antioxidants) and degradation products (VOC) of the

35   poultry meat produced during cooking. The extent of VOC production and n-3 loss was found to be

36   higher for the SG genotype. Reduction of n-6 was higher in MG whereas small losses in

37   antioxidants and PUFA were observed in the FG genotype, consequently, resulting in the lowest

38   production of VOC.

39   The SG and MG are genotypes more active from a kinetic point of view respect to the FG ones. For

40   this reason, in the FG genotypes the antioxidants are less involved in the oxidative stress induced by

41   the movement thus, they were available to protect the lipid of the meat during the cooking process.

42   These results suggested that the use of SG and MG genotypes requires a specific dietary protocol

43   (e.i. increasing the antioxidants content) in order to counteract the lipid oxidations in all the phases:

44   *in vivo, post-mortem*, and during/after cooking.

45   **Keywords**: genotype, cooking, volatile organic compound, fatty acid profile, antioxidant.

46

47   **INTRODUCTION**

48

49   Humans consume diets with highly unbalanced lipid profiles and there is a consensus on the need to

50   reduce the consumption of saturated fatty acids (**SFA**) and increase that of polyunsaturated fatty

51   acids (**PUFA**), particularly of the n-3 series.

52   Fish is the main source of n-3 long-chain PUFA ($\geq$ 20 carbons) such as eicosapentaenoic (**EPA**) and

53   docosahexaenoic acid (**DHA**). However, increased fishing and aquaculture has resulted in issues of

54   sustainability as they depend on the marine trophic chain and use of wild fish as feed for farmed

55   fish, respectively (Tocher et al., 2019).

56   Thus, it is crucial to find other sustainable food sources containing long chain n-3 (Menchetti. et al.,

57   2018). Consequently, the ability of terrestrial animals to elongate and desaturate α-linolenic acid

58   (**ALA**), the precursor of EPA and DHA, needs to be carefully investigated.

59   Although standard poultry meat contains low levels of EPA and DHA, several factors such as sex,

60   feed, rearing systems, and genotypes may affect their content. Particularly, genotype is an important

61   factor affecting the fatty acid composition of poultry meat, which is as follows: usually, a higher

62   percentage of n-3 PUFA is produced by slow-growing (**SG**) chickens compared to the fast-growing

63   (**FG**) ones (Sirri et al., 2010). Castellini et al., (2002) showed that this content could increase in SG

64   birds raised in free-range and organic systems due to high pasture intake. Simultaneously, intake of

65   grass improves their antioxidant responses and prevents PUFA from oxidation (Dal Bosco et al.,

66   2016, Cartoni Mancinelli et al., 2019).

67   Nevertheless, there is a lack of knowledge on how meat processing (freezing, cooking) can affect its

68   nutritional properties and the development of unpleasant odors and aroma, which are important for

69   consumer acceptability. Volatile organic compounds (**VOC**) (Holm et al., 2013), are some of the

70   molecules responsible for such alteration in the food, the production of which, is influenced by

71   many factors (Leroy et al., 2009). For instance, degradation of nutrients on the surface of cured

72   products, particularly by lactic acid bacteria, can produce unpleasant odours (Holm et al., 2012).

73 However, hygienic conditions and use of low storage temperature, which restrict the growth of

74 spoilage microorganisms, can prevent excessive generation of VOC (Gill, 2007) in raw meat.

75 Additionally, oxidation of lipids during cooking is the main factor responsible for VOC production

76 (Angelo et al., 1987). Warmed-over flavors develop due to the thermal autoxidation of PUFA,

77 although other components such as proteins or carbohydrates can also contribute to the process. A

78 high cooking temperature induces the Maillard reaction, which also results in the development of

79 VOC (Byrne et al., 2001). Therefore, meat is a highly perishable food and its susceptibility to lipid

80 oxidation depends on the species of livestock, which particularly decreases in the order of fish >

81 poultry > pork > beef > lamb. This ranking is attributed to the different levels of PUFA and

82 presence of specific antioxidants, such as tocopherols, in the meat (Rhee et al., 1996).

83 Since the production of VOC is linked to the contents of PUFA and antioxidants, which vary with

84 the species and genotype of the livestock (Sirri et al., 2010), this study evaluates the effect of

85 cooking on lipid oxidation, PUFA, and VOC in different poultry strains. Accordingly, the amount

86 of VOC produced, fatty acid profile, and content of tocols and thiobarbituric acid-reactive

87 substances (**TBARS**) in raw and cooked meat derived from four strains of chicken with different

88 growth rates were analyzed.

MATERIALS AND METHODS

89

90

91 Pure linoleic acid (**LA**) and ALA, linolenic acid methyl ester, and organic solvents were obtained

92 from Sigma-Aldrich (Germany). The methyl ester of stearidonic acid (C18:4n-3) was obtained from

93 Cayman Chem (Ann Arbor, Michigan, USA).

94

95 *Experimental layout and sample preparation*

96 Poultry with 4 different genotypes and growth rates, namely SG (Leghorn), medium growing (**MG**,

97 Naked Neck and Hubbard Red JA), and FG (Ross 308) were reared at the experimental section of

98 the University of Perugia (Perugia, Italy) according to the EU Regulation 834/07 and Directive

99 2010/63/EU and transposed as per Regional Directive n.26 on animal welfare for experimental and

100 other scientific purposes.

101 The animals were reared indoors in equal densities (5 chickens/m^2) and administered the same

102 standard diets (Table S1).

103 Since the growth rate for different genetic strains differs significantly, the birds were slaughtered

104 upon attaining the same stages of maturity (about 60% of the adult weight). Leghorn, Hubbard and

105 naked neck, and Ross chickens were reared for 100, 56, and 42 days, respectively (Table S2). All

106 chickens were slaughtered in a commercial slaughtering house.

107 For each genotype, ten breast samples (five males and females, each) were collected from the

108 carcasses. From every breast sample 3 replicates (3 g of meat each) were analyzed both for raw and

109 cooked meat. From each animal, two aliquots of approximately 100 g of the breast muscle were

110 collected, vacuum-packaged, and frozen at -30 °C. After two weeks of storage, all the samples were

111 thawed at 4 °C for 6 h, of which, half were analyzed immediately, and whereas the other aliquots

112 were cooked by placing them in 250-mL glass bottles and boiling in a water bath at 80 °C for 30

113 min. With this cooking method the muscle samples reach a temperature of 60 °C ± 1. All samples

114　were stored at 5° C until completion of the analysis, which was carried out within 12 h from the

115　time of thawing.

116

117　***Proton-transfer reaction–mass spectrometry (PTR–MS) and PTR–Time-of-Flight (ToF)-MS***

118　***measurements***

119

120　Finely cut raw or cooked meat weighing 3 g was placed in a 250-mL glass bottle and equilibrated in

121　a water bath at 35 °C for 30 min. The samples analyzed on day 1 were randomized across genotypes

122　and replicates.

123　After temperature equilibration, the bottles were connected to a PTR–MS inlet, which was heated to

124　60 °C. The headspace flow rate was adjusted to 55 mL/min. A flow of 32 mL/min was directed into

125　the PTR–MS *via* a teflon tubing of diameter 0.25 mm. A constant drift voltage and pressure of 600

126　V and 2.19 ± 0.01 mbar, respectively, were maintained in the reaction chamber. A quadrupole mass

127　spectrometer was used to analyze the masses of the samples, which were detected as the ion counts

128　per second using a secondary electron multiplier. The ion intensities of the masses were converted

129　to volume mixing ratio (ppb) values, based on the method employed by Lindinger et al.,(1998).

130　Mass spectral data were collected over a range of 20–160 *m/z* with a dwell time of 200 ms.

131　Although data of 5 cycles were collected for each measurement, only the average of cycles 2 to 4

132　was used for analysis.

133　Prior to analyzing each meat sample, the PTR-MS spectrum of a blank was acquired by sampling

134　the contents from an empty bottle. This spectrum was subtracted from the sample spectra before

135　data analysis (Aprea et al., 2007).

136　To identify the different molecules in raw and cooked meat, the samples were analyzed using PTR-

137　ToF-MS (Ionicon GmbH, Innsbruck, Austria). Cubes of approximately 3 g were equilibrated at 30

138　°C for 30 min in a 250-mL screw-cap glass bottle, and the sample from the headspace was directed

139　to the inlet of the high-sensitivity (**HS**) PTR-MS system (Ionicon GmbH) with an airflow rate of 75

140   mL/min. The temperatures of the inlet and drift chamber were maintained at 60 °C. The HS PTR-

141   MS was operated at a standard E/N (ratio of the electric field strength across the reaction chamber,

142   E, to the number density of the buffer gas, N, within the chamber) of 138 Td (1 Td = 10-17 cm^2 V

143   molecule^{-1}) and measured in the "mass scan" mode. Thus, a complete mass spectrum in the range

144   of 20–150 atomic mass units (amu) at a mass detection rate of 0.2 s mass^{-1} was obtained in 26 s. the

145   samples were analyzed independently in triplicates and seven mass scan cycles were used to

146   measure each replicate. The background measurements were obtained by alternating seven scan

147   cycles of the sample with seven scan cycles of a blank air sample. The individual components in the

148   fraction were identified by directly comparing the mass spectra obtained from the samples with

149   those available in NIST92 and Wiley5 libraries and of pure standards.

150

151   *Chemical analysis and Fatty Acid determination*

152

153   The proximate composition of raw and cooked meat was determined using the AOAC method

154   (Anastassiades et al., 2003). The fatty acid profile of raw and cooked breast meat was determined

155   by analyzing the lipids extracted from approximately 5 g of the samples in a homogenizer using 20

156   mL of 2:1 chloroform/methanol (Folch et al., 1957), followed by filtration through a Whatman no. 1

157   filter paper. The fatty acids were identified in the form of their methyl esters using a Varian CP-

158   3800 Gas Chromatograph (Milano, Italy) and a DB wax capillary column (25 mm ø, 30 m long).

159   The percentages of fatty acids were calculated using the Chrom-Card software. Individual fatty acid

160   methyl esters (FAME) were identified by referencing against the retention time of authentic FAME

161   standards (Sigma–Aldrich, Bellefonte, PA, USA). The relative quantity of each fatty acid present in

162   the meat was calculated using heneicosanoic acid (C21:0; Sigma–Aldrich) as the internal standard

163   and expressed as mg/g of meat. The contents of the major classes of fatty acids were also calculated

164   (SFA, monounsaturated fatty acid [**MUFA**], and PUFA of n-3 and n-6 series).

165 *Tocols content*

166

167 The content of tocols (α-tocopherol [**α-T**] and its isoforms β+γ [**γ-T**] and δ [**δ-T**]; α, β+γ tocotrienol

168 [**β -T₃, γ-T₃**]) in the meat was quantified using high-performance liquid chromatography (**HPLC**)

169 (Hewavitharana et al., 2004). A single peak for γ-T was detected intermediate to those of α-T and δ-

170 T on the chromatogram, and all three were quantified.

171 Briefly, 5 mL of distilled water and 4 mL of ethanol were added to 2 g of meat and vortexed for 10

172 s. Then, 4 mL of hexane containing butylated hydroxytoluene (**BHT** 200 mg/L) was added, and the

173 mixture was carefully shaken and centrifuged. An aliquot of the supernatant (3 mL) was dried under

174 a nitrogen stream and dissolved using 300 μL of acetonitrile. From this, 50 μL was injected into the

175 HPLC system (Perkin Elmer Series 200) equipped with an autosampler system (model AS 950-10,

176 Jasco, Tokyo, Japan) and a Synergy Hydro-RP column (4 μm, 4.6 × 100 mm; Phenomenex,

177 Bologna, Italy). The peaks were identified using a Jasco FP-1525 FD detector (excitation and

178 emission wavelengths of 295 and 328 nm, respectively) and quantified through external calibration

179 curves obtained using increasing amounts of pure standards (Sigma–Aldrich, Steinheim, Germany)

180 in ethanol.

181

182 *TBARS assay*

183

184 The extent of muscle lipid oxidation was evaluated using a spectrophotometer adjusted to 532 nm

185 (Shimadzu Corp., UV-2550, Kyoto, Japan) according to the modified method of Ke et al., 1984

186 which measures the absorbance of TBARS. The oxidation products were quantified as equivalents

187 of malondialdehyde (mg **MDA**/kg muscle) using a 1,1,3,3-tetraethoxypropane calibration curve.

188

189

190

8

191   *Statistical analysis*

192

193   In addition to analyzing single qualitative traits, the differences in few major traits (n-3 and n-6

194   PUFA, tocols, VOC) between the samples of raw and cooked meat (Δ) were also evaluated. All

195   traits were expressed as dry matter (**DM**) to prevent discrepancies due to changes in the moisture

196   content of the raw and cooked samples.

197   Two different statistical approaches were employed. Firstly, univariate analysis was conducted

198   using a linear model (Muenchen, 2012; StataCorp, 2015; Wang et al., 2016; StataCorp, 2017) in

199   which, the effects of genotype, processing (raw, cooked), and their interactions were considered.

200   The effect of sex was not found to be significant and, hence, omitted from the analysis. The

201   differences were considered significant when $P \leq 0.05$. The statistical significance of values was

202   reported only for major traits for simplification purposes.

203   Secondly, multivariate analysis was performed using an SPSS Statistics version 23. A set of

204   variables was selected by inspecting the R-matrix and communalities (Righi et al., 2019, Twigg,

205   2010), and subjected to principal component analysis (**PCA**). We used the Kaiser–Meyer–Olkin and

206   Bartlett tests to verify the adequacy of sampling and associated correlations. Principal components

207   (**PC**) with eigenvalues > 1 were retained and rotated using the varimax method. Only factor

208   loadings with an absolute value greater than 0.5 were discussed (Menchetti. et al., 2018, Pituch and

209   Stevens, 2015). Regression scores were calculated and additional groups for each PC (PC1 and

210   PC2) were determined.

211

212 **RESULTS**

213

214 *Significance of effects*

215

216 The effects of genotype, processing, and their interactions on chicken meat are reported in Table S3.

217 We found that the genotype of the poultry and the meat processing affected the content of majority

218 of the analyzed compounds except butene, butanol, methyl, metacryl amide, and α-T_3. The

219 interaction of genotype × processing was always found to be significant, except in case of some

220 VOC (butene, butanol, methyl furanone, metacrylic amide, and heptanal), fatty acids (14:0,

221 22:1*cis*9n-9, 20:5n-3, 22:5n-3, 22:6n-3), tocols (δ-T_3, γ-T_3,), and TBARS.

222

223 *VOC development*

224

225 Twenty main VOC were identified and grouped into different chemical categories, which were as

226 follows: aldehydes, alkadiene, alkenes, furans, amides, alcohols, and other compounds (Table 1 and

227 Figure 1). Low amounts of aldehydes, alkadienes, alkenes, furans, amides, and alcohols (2.70, 0.42,

228 0.07, 0.06, 0.04, 0.02% of total VOC, respectively) were detected in the raw meat whereas other

229 compounds (acetic acid, butyramide, methyl pyrrolidine, propionic ether) constituted 96.51%.

230 Cooking had strongly impacted VOC production. The content of VOC detected in cooked samples

231 was 5.5 times that of raw meat (Figure 1). Aldehydes were the most important group of compounds

232 detected in cooked meat of all genotypes (64.85% of the total VOC), of which, ethanal was the

233 principal compound. Alkenes (2.44%, mainly 1,4-hexadiene), furans, amides, and alcohols were the

234 components of VOC detected in minor amounts.

235 We determined that the genotype of the poultry was responsible for the major differences detected

236 in the VOC profiles of their meats. Particularly, the highest increase in VOC (47 times) was

observed in the cooked samples of SG (Leghorn) meat compared to the raw ones. Few changes (3.5

and 3.8 times) were observed in the meats derived from other genotypes such as MG (Hubbard and

Naked Neck, respectively), while the lowest content of VOC was detected in meats from FG (3

times).

Chemical composition and Fatty Acids profile

Significant differences in the total fat content were observed in both raw and cooked samples of

different poultry strains. The highest values for fat content were obtained from the meat samples

belonging to the genotype Ross, followed by those of Hubbard and Naked Neck, while those of

Leghorn were the lowest (Table S4).

It could be elucidated that cooking had affected the chemical composition of the meat mainly due to

a reduction in its water content. However, the percentage of protein and ash was almost the same in

raw and cooked samples whereas lipid loss was approximately 13-17% in cooked meat (data

calculated from table S4).

Upon analyzing the PUFA profiles of cooked meat (Table 2), it was observed that, although values

of total n-6 and n-3 fatty acids were lowest in meat of SG chickens, the loss of n-3 (Δ) was the

highest. Contrarily, their amounts were higher in the meats of MG and FG strains, but their

"absolute" losses were lower.

257   *Antioxidant content and Oxidative stability*

258

259   The TBARS values and the antioxidant content of raw and cooked meat and its variation (Δ) due to

260   the cooking process are reported in Table 3. The Hubbard and Ross chickens showed the highest

261   value of total tocols both in raw and cooked meat, while Leghorn had the lowest ones. The Hubbard

262   and Ross birds exhibited the highest decrease in tocols (35.96 and 30.15 µg/g DM, respectively)

263   after cooking.

264   As expected, the TBARS increased in the cooked meat. The cooked Leghorn meat showed the

265   highest increase in TBARS (about 4 times) and the most decrease in tocols (about 15 times)

266   compared with the other poultry genotypes.

267

268   *Principal Component Analysis*

269

270   Multivariate analysis allowed us to determine the combined relation between the main traits of

271   cooked meat (VOC, Δ n-3, n-6, and content of tocols). PCA revealed two components, which

272   accounted for 76.06% of the total variance (Table 4). The highest loadings of VOC and Δ n-3 were

273   observed in PC1 while PC2 was mainly influenced by Δ n-6 and the content of tocols, and

274   explained 43.04% and 33.02% of the total data variance, respectively.

275   The poultry strains were differentiated based on their PC scores on the factor maps determined

276   through PC1 and PC2, according to their growth rates (Figure 2). The PC scores of SG samples

277   (Leghorn) were concentrated in the fourth quadrant with positive PC1 and negative PC2 scores. The

278   PC1 scores of FG samples (Ross) were negative and mainly located in the first and third quadrants.

279   The scores of meats from Naked Neck and Hubbard, which had similar growth rates, were positive

280   for both PC1 and PC2, since both were located in the second quadrant. Based on their position in

281   the factor map (Figure 2), VOC production and Δ n-3 were higher for the SG genotype whereas MG

282 was characterized by a higher Δ n-6. Furthermore, small antioxidant and PUFA losses and the least

283 production of VOC were observed in meats of the FG genotype.

284

DISCUSSION

286

Volatile Organic Compounds of meat

288

289 Due to its high ratio of unsaturated/saturated fatty acids and low fat content, poultry meat is

290 considered as a healthy food for humans. It is known that PUFA, due to the presence of double

291 bonds, are susceptible to lipid oxidation, which results in the generation of numerous degradation

292 products such as aldehydes, ketones, alcohols, aliphatic hydrocarbons, acids, and esters (Campo et

293 al., 2003, Menchetti et al. 2020), which are responsible for the development of flavors in meat.

294 However, their concentrations depend mainly on the method of cooking because it affects their

295 degree of degradation. For instance, compared to boiling, more compounds such as pyrazines,

296 pyridines, pyrroles, and thiozoles are generated upon roasting, grilling, and frying chicken meat (

297 Shahidi et al., 2020).

298 The present study had focused on the response of poultry genotypes with different growth rates to

299 the cooking procedures based on the generation of VOC and content of fatty acids, tocols and

300 TBARS. To our knowledge, this is one of the few studies to have explored the complex

301 mechanisms, which regulate the relationship between cooking and the dynamics of PUFA, VOC,

302 and antioxidants in meat of different poultry strains.

303 It was observed that the number of VOC in raw meat were few, which increased significantly upon

304 cooking. The main VOC detected in cooked meat belonged to the group of aldehydes (Table 1),

305 which are generally associated with the degradation of PUFA thus, confirming that the majority of

306 VOC produced during cooking originate through lipolysis (Tornberg, 2005).

307   Pentanal and hexanal derive from the β-oxidation of fatty acids, mainly α-linolenic acid (Del Pulgar

308   et al., 2011). Guerrero-Legarreta et al., (2010) have reported that some aldehydes (hexanal,

309   pentanal, heptanal, octanal, and nonanal) are responsible for off-odors. This suggests that the meat

310   from Leghorn chickens was more susceptible to the production of unpleasant odors compared to

311   that of the other genotypes.

312   The main aldehydes detected in our study (butanal, cyclohexadiene, heptanal, hexanal, nonanal,

313   norbornane, octanal, and pentanal) are characterized by the presence of saturated chains of four to

314   nine carbon atoms. These compounds are derived through the oxidation of PUFA and contain more

315   than 20 carbon atoms (C20:4, C20:5, C22:6) whereas C18:2 and MUFA do not contain sufficiently

316   long and saturated alkyl chains. However, the alkyl radicals from C20 PUFA may degrade the other

317   fatty acids (C18:2 and C18:1) as a result of a chain reaction (Tornberg, 2005). Accordingly, our

318   results demonstrated that the content of C20:4n-6 was lower in the meat of genotypes with a higher

319   alkanal content (Leghorn), suggesting the manifestation of a specific oxidation mechanism.

320   Probably, the generation of alkanals was not related to the other C20 PUFA because their content

321   was less than 0.5 mg/g DM compared to C20:4 ($\geq$1 mg/g DM).

322   Despite alcohols being the second most abundant VOC detected in the meat samples, their

323   incidence in the total VOC content was very scarce**.** In raw meat, alcohols are derived largely due to

324   the metabolism of hetero-fermentative lactic acid bacteria. Schuster et al., (2018) have found that

325   the alcohol levels increase in vacuum-packed lamb meat. However, it has been reported that a

326   minor percentage of alcohol production also depends on the content of PUFA (Del Pulgar et al.,

327   2011), thus explaining the presence of alcohols in cooked meat also.

Fat content and Fatty Acid profiles of meat

It is widely known that cooking largely affects the qualitative characteristics of meat. Part of these changes during cooking result from the degradation of substrates (such as PUFA) and concentration of nutrients due to water loss (Badiani et al., 2002).

Our results have confirmed that cooked meats are enriched with nutrients compared to raw ones. Additionally, cooking reduced also the fat content in meat, which mainly affected the content of PUFA and MUFA while that of SFA (Table 2) remained almost stable. This trend was observed in meat samples of birds belonging to all genotypes with variations in the extent of impact.

As mentioned above, chicken meat is highly susceptible to oxidation because it contains long-chain fatty acids with many double bonds. Consequently, losses, mainly in the content of n-3 PUFA, were observed during cooking. Similarly, here, the effect of cooking differed among meats of different genotypes, which is as follows: Δ n-6 PUFA was found to be higher in Hubbard and Naked Neck (MG genotypes) compared to those of Ross (FG) and Leghorn (SG), whereas results contrary to these were observed in SG. It should be noted that, while raw meat of SG was not particularly high in the n-3 content (0.78 mg/g DM), its proportion was the highest (6.3 in LEG *vs*. 4.2, 3.7, and 3.5 % in HUB, NN and ROSS, respectively).

Cortinas et al., (2005) have suggested that a positive correlation exists between the content of PUFA and extent of lipid oxidation in chicken meat.

The PUFA profile of meat could also depends on rearing system, the presence of outdoor runs allow an additional intake of n-3, some authors (Dal Bosco et al., 2012, Castellini et al., 2016) demonstrated a higher percentage of PUFA, in particular of the n-3 series, in SG than FG when birds are reared free-range (Sirri et al., 2011) for the supplementary intake of vegetables, rich of ALA.

352 *Content of Antioxidants and Oxidative stability*

353

354 Although the same diet containing 50 mg/kg tocopheryl acetate was administered to the animals,

355 the content of antioxidants in the raw meat of different genotypes was found to differ. This trend

356 probably depends not only on the fat content of the meat, because tocopherols and retinol are fat-

357 soluble antioxidants, but also on the kinetic behavior of the birds of different genotypes (Mattioli et

358 al., 2017).

359 The same study has revealed that the antioxidant levels in poultry meat depend on the dietary intake

360 and genetic strains of the birds (Mattioli et al., 2017). As stated previously, SG animals are very

361 active, which affects their oxidative metabolism, resulting in high production of reactive oxygen

362 species (ROS) and extensive consumption of antioxidants by the body.

363 In extensive rearing systems, this requirement is supported by the intake of natural antioxidants

364 (tocols, vitamin C, and polyphenols) contained in the pastures (Mugnai et al., 2014). In our study,

365 the absence of outdoor runs prevented the intake of additional antioxidants, probably resulting in

366 the low content of tocopherols in SG meat compared to those of the FG and MG genotypes.

367 Animals of the SG genotype, which demonstrate a superior kinetic behavior and oxidative

368 metabolism, tend to consume more antioxidants enabling them to counteract the oxidative thrust

369 induced by the generation of ROS (Pisoschi and Pop, 2015).

370 Animals belonging to the MG strains (Hubbard and Naked Neck), being less kinetic, were able to

371 preserve their tissue antioxidant stock better, while the highest content of antioxidant compounds

372 was detected in meats of the FG genotype (Ross), which are recognized as more "static" animals

373 (Dal Bosco et al., 2012).

374 The process of cooking increased the level of TBARS and decreased that of α-T because the high

375 temperature of cooking enhances oxidation. Rhee et al. (1996) compared raw and cooked meat of

376 beef, pork, and poultry, and revealed that the TBARS content of cooked chicken drumstick was the

377 highest due to its higher PUFA content. In our study, the lowest level of α-T was detected in the raw

378 meat of the Leghorn genotype and, consequently, the highest levels of TBARS were detected after

379 cooking it compared to that of the other genotypes. Selim et al., (2013) demonstrated the protective

380 effect of a diet supplemented with vitamin E against PUFA autoxidation, which neutralized free

381 radicals in both blood plasma and the muscles. Similarly, Castellini et al., (1998) demonstrated that

382 the supplementation of α-tocopheryl acetate could increase the contents of n-3 fatty acid in both raw

383 and cooked rabbit meat. In agreement, Nardoia et al., (2017) detected lower levels of TBARS in

384 the breast meat of chicken supplemented with vitamin E, grape skin, and grape pomace.

385

386 ***Principal Component Analysis***

387

388 In addition to the trend obtained for single traits such as the content of total fat, fatty acids,

389 antioxidants, TBARS, and VOC in poultry meat, we analyzed the specific relationships among

390 these traits. Previous studies have investigated the effect of cooking on different attributes of meat

391 quality. However, only a handful have evaluated the trade-off of substrates (fatty acids), protective

392 substances (antioxidants), and oxidative and degradation products (TBARS, VOC) in the transition

393 of poultry meat from a raw to cooked state (see Fig. 2).

394 It has been previously reported that raw meat is characterized by the presence of some antioxidants

395 (mainly tocols) and PUFA (n-3 and n-6), which, during cooking, are oxidized easily, consequently

396 producing radicals. Majorly, ROS and reactive nitrogen species (NOS) are the main radicals

397 generated in this process, which, due to the presence of an unpaired electron, trigger an oxidative

398 chain reaction with other compounds (proteins) or PUFA themselves. The effect of these radicals is

399 modulated by the presence of antioxidants (which are primarily fat-soluble vitamins such as tocols)

400 on the muscle enabling it to reduce this pro-oxidant action. If the concentration of tocols is not

401 sufficient to counteract this oxidative process, the content of PUFA decreases due to VOC

402 generation. In our trial, even the presence of PUFA series affected the amount of VOC produced

403 during cooking, the results of which, are as follows: VOC generation was higher when n-3 PUFA

404 were used as substrates instead of n-6 due to a higher degree of unsaturation in the former

405 (Scislowski et al. 2005). This confirmed that n-3 PUFA, due to the presence of many double bonds,

406 were more susceptible to oxidation compared to their n-6 counterparts.

407 A multivariate approach permitted us to highlight the pattern of the aforementioned variables and

408 distinguish the effect of cooking on meat from poultry with different genotypes. Tocols (PC2) were

409 the most stable in meat of FG chickens, with the lowest variation in both n-6 and n-3, and having a

410 low content of VOC. Contrarily, SG meat, which was characterized by low levels of antioxidants

411 and the highest n-3 PUFA content, produced more VOC (PC1) compared to the others. The

412 genotypes for MG, which were characterized by the presence of the highest amount of n-6 PUFA

413 compared to n-3 PUFA, resulted in a lower production of VOC, which was consistent with this

414 assumption.

415 In figure 3, a schematic representation of the mechanisms regulating the relationship between

416 PUFA, VOC, and antioxidants in poultry meat during cooking has been reported.

417 Based on this perspective, more researches should focus on the stability of the PUFA profile during

418 cooking. Our findings have indicated that these compounds undergo significant degradation in the

419 meat after cooking, producing VOC and other substances (TBARS) with potential concerns for

420 human health. Accordingly, greater attention should be paid to protect against PUFA, particularly in

421 case of meats with high nutraceutical values.

CONCLUSIONS

422

423 VOC such as alkanes, alkenes, aldehydes, ketones, alcohols, represent the principal products of the

424 lipid oxidation and consequently are the main responsible of the reduction of the meat quality. A

425 drastic increase in the content of VOC (aldehydes and alkanals) was observed in cooked chicken

426 meat derived mainly due to the oxidation of PUFA, which was significantly correlated with the

427 genotype of the organism. Maximum changes in the VOC content due to cooking were observed in

428 the meat of breeds which could be defined as "more vulnerable", with greater susceptibility to

429 PUFA oxidation and associated with a lower content of antioxidants (Leghorn), resulting in a

430 remarkable loss of n-3. Contrarily, losses of n-3 PUFA from the cooked meat of the FG genotypes

431 were lower. Moreover, due to their lower activity, dietary antioxidants were rendered available for

432 protecting the lipids in their meats.

433 Nowadays, increasing attention towards animal welfare has promoted the use of SG and MG

434 genotypes, particularly in alternative rearing systems (organic, free-range) because these strains are

435 more adaptable with a better welfare status in outdoor conditions. This adaptation is strictly related

436 to the kinetic behavior of chickens. Hence, the relationship between activity (oxidative burst) and

437 the nutritional quality of meat (PUFA profile) needs to be better defined. Our results have suggested

438 that the use of these genotypes (MG and SG) requires a specific dietary protocol to balance the

439 oxidative status of chickens *in vivo*, post-mortem, and, possibly during/after cooking. Accordingly,

440 additional studies are needed to understand the mechanism for protecting n-3 PUFA from oxidation.

441 For example, using dietary antioxidants (natural as per organic systems or synthetic, for the

442 conventional) and/or finding more appropriate cooking methods to preserve the meat quality.

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449

450   **REFERENCE**

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564 **TABLES**

565 Table 1. Influence of genetic strain and processing (raw *vs.* cooked) on VOC composition (ppb) of

566 chicken meat

Compounds	RAW				COOKED				Pooled SE[1]
Genotypes[2]	LEG	HUB	NN	ROSS	LEG	HUB	NN	ROSS	
Aldehydes									
2-Butenal	0.9	1.9	1.0	1.0	10.5	6.5	6.9	4.2	2.1
3-Ciano-propanal	0.3	0.3	0.4	0.3	38.9	13.2	17.3	9.0	4.8
Butanal	8.1	20.4	5.5	45.6	64.1	79.2	63.9	92.5	25.8
Butene	9.7	8.3	8.2	11.7	27.3	24.7	37.8	32.6	300.2
Cyclohexadiene	0.2	0.7	0.2	0.4	3.9	1.5	1.6	1.1	0.6
Ethanal	24.2	13.1	18.4	9.0	7736.2	5359.2	5613.1	3090.8	1493.9
Heptanal	0.1	0.2	0.2	0.4	2.0	1.2	1.3	0.9	0.6
Hexanal	0.3	0.4	0.4	0.1	46.8	12.7	22.2	9.0	11.9
Nonanal	0.2	0.3	0.5	0.2	14.3	3.3	5.0	1.9	3.0
Norbornane	0.2	0.3	0.3	0.2	9.5	6.1	8.0	5.1	4.3
Octanal	0.4	0.4	0.4	0.4	2.8	1.4	1.7	1.1	0.7
Pentanal	0.7	1.4	1.7	1.5	19.9	10.5	13.2	9.5	4.3
Xylenes	0.4	0.9	0.4	0.3	9.5	10.5	8.4	9.9	5.7
Alkadiene									
1,2-Butadiene	3.9	7.8	16	2.1	557.3	122.3	222.8	73.2	81.8
Alkenes									
1,4-Hexadiene	0.7	1.3	1.7	1.1	504.7	114.7	195.5	71.8	71.5
Furanas									
Methyl furanone	0.8	0.8	0.9	1.0	12.5	12.5	10.7	10.5	4.0
2-Pentyl Furan	0.2	0.2	0.1	0.2	1.7	1.1	1.4	1.0	0.8
Ammides									
Butyramide	0.1	0.1	0.1	0.1	1.1	0.1	1.1	1.5	0.3
Methachrylic amide	0.2	0.2	0.3	0.2	3.8	4.3	3.3	3.3	1.3

Alcohols

Butanol	0.5	1.3	0.2	0.5	0.01	23.8	1.5	2.7	72.2
Σ Aldehydes	45.7	48.6	37.6	71.1	7985.7	5530.0	5800.4	3267.6	693.7
Σ Alkadiene	3.9	7.8	16	2.1	557.3	122.3	222.8	73.2	81.8
Σ Alkenes	0.7	1.3	1.7	1.1	504.7	114.7	195.5	71.8	71.5
Σ Furanas	1	1	1	1.2	14.2	13.6	12.1	11.5	3.9
Σ Ammides	0.3	0.3	0.4	0.3	4.9	4.4	4.4	4.8	1.1
Σ Alcohols	0.5	1.3	0.2	0.5	0.01	23.8	1.5	2.7	72.2
Other compounds	192.0	2417.0	2494.0	1695.0	2421.	2967.0	3462.0	1958.3	1120.2
Total VOC[3]	244.1	2477.3	2550.9	1771.3	11487.8	8775.8	9698.7	5389.9	3210.0

567

568   Abbreviations:[1]SE, standard error;

569   [2] HUB, Hubbard; NN, Naked Neck; LEG, Leghorn; ROSS, Ross 308;

570   [3]VOC, Volatile organic compounds;

571 Table 2. Influence of genetic strains and processing (raw *vs.* cooked) on the fatty acid profiles (mg/g

572 DM) of poultry meat

Compounds[1]	RAW				COOKED				Pooled SE[3]
Genotypes[2]	LEG	HUB	NN	ROSS	LEG	HUB	NN	ROSS	
14:0	0.17	0.90	0.52	0.35	0.12	0.24	0.22	0.31	0.10
16:0	2.45	5.43	4.45	6.89	2.36	6.27	5.00	6.81	1.62
18:0	1.55	2.8	2.35	3.31	1.80	2.46	1.91	2.18	1.15
Σ SFA	4.17[a]	9.13[c]	7.32[b]	10.55[c]	4.28[a]	8.97[bc]	7.13[b]	9.30[c]	0.25
16:1cis9	0.85	2.21	1.94	2.86	0.58	1.61	1.32	2.3	0.20
18:1cis9 n-9	2.62	6.42	4.94	7.73	2.36	5.36	4.17	6.83	1.35
22:1cis9	0.15	0.31	0.34	0.30	0.17	0.35	0.35	0.37	0.01
Σ MUFA	3.73[a]	9.14[bc]	7.46[b]	10.92[c]	3.38[a]	7.83[b]	6.28[b]	9.89[c]	0.20
18:2n-6 (LA)	2.5	5.58	4.68	5.72	2.17	4.18	3.36	5.20	5.58
20:4n-6 (AA)	1.1	1.72	1.32	1.08	0.91	1.20	1.07	0.89	1.72
Σ n-6 PUFA	3.60[a]	7.30[b]	6.00[b]	6.80[b]	3.08[a]	5.38[b]	4.43[ab]	6.09[b]	1.38
Δ n-6 PUFA					0.52[a]	1.92[b]	1.57[b]	0.71[a]	0.10
18:3n-3 (ALA)	0.35	0.57	0.36	0.40	0.15	0.32	0.27	0.30	0.57
20:5n-3 (EPA)	0.22	0.35	0.28	0.39	0.15	0.30	0.22	0.34	0.35
22:5n-3 (DPA)	0.11	0.11	0.09	0.16	0.08	0.08	0.07	0.13	0.11
22:6n-3 (DHA)	0.10	0.11	0.09	0.08	0.06	0.09	0.07	0.05	0.11
Σ n-3 PUFA	0.78[a]	1.14[b]	0.82[a]	1.03[b]	0.44[a]	0.79[b]	0.63[ab]	0.82[b]	0.13
Δ n-3 PUFA					0.43[b]	0.30[ab]	0.23[a]	0.20[a]	0.08

573

574 Abbrevations:[1]SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; AA, arachidonic acid; LA,

575 linolenic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; LCP, long chain

576 PUFA;

577 [2]HUB, Hubbard; NN, Naked Neck 1; LEG: Leghorn; ROSS: Ross 308;

578 [3]SE: standard error;

579 [a, b, c] on the same row indicate the same type of processing (raw, cooked), with means of P < 0.05;

580

581 Table 3. Influence of genetic strains and processing (raw *vs*. cooked) on the content of antioxidants

582 (µg/g DM) and oxidative stability (mg/kg DM) of poultry meat

Compounds[1]	RAW				COOKED				Pooled SE[3]
Genotypes[2]	LEG	HUB	NN	ROSS	LEG	HUB	NN	ROSS	
α-T	10.73	34.46	16.01	32.98	0.98	4.22	2.93	3.35	1.73
δ-T	1.09	0.50	0.63	0.99	0.03	0.23	0.17	0.16	0.02
γ-T	0.13	0.37	0.17	0.41	0.01	0.05	0.02	0.06	0.04
α-T$_3$	5.28	6.03	8.65	6.07	0.24	1.23	0.74	0.52	0.65
δ-T$_3$	1.13	0.17	1.38	0.87	0.30	0.03	0.17	0.20	0.14
γ-T$_3$	1.00	0.35	0.85	0.89	0.27	0.03	0.12	0.18	0.09
TBARS	0.34[a]	0.70[b]	0.54[a]	1.12[c]	1.28[c]	0.95[b]	0.65[a]	1.55[c]	0.14
Σ–T	19.50[a]	41.76[c]	28.22[b]	42.19[c]	1.28[a]	5.80[b]	4.20[b]	12.04[c]	1.82
Δ tocols (raw-cooked)					18.22[a]	35.96[c]	24.02[b]	30.15[c]	1.11

583

584 Abbreviations:[1]α-T, α-tocopherol; δ-T, δ-tocopherol; γ-T, γ-tocopherol; α-T3, α-tocotrienol; δ-T3, δ-tocotrienol; γ-T3, γ-tocotrienol; TBARS,

585 Substances Reactive to Thiobarbituric acid;

586 [2]HUB, Hubbard; NN: Naked Neck 1; LEG: Leghorn; ROSS: Ross 308;

587 [3] SE, standard error;

588 [a, b, c] on the same row indicates the same type of processing (raw, cooked), with means of P < 0.05;

589

28

590   Table 4. Principal components loadings, eigenvalue and variance

	PC1	PC2
VOC	0.71	-0.11
Δ n-3	0.59	0.05
Δ n-6	0.31	0.64
Δ tocols	0.20	0.75
Eigenvalue	1.72	1.32
% variance explained	43.04	33.02
Cumulative variance explained	76.06	

591
592   Abbreviations: VOC, Volatile organic compounds;

593   Loadings ≥0.50 or ≤–0.50 are presented in bold;

594 **FIGURES**

595 Figure 1. Main VOC classes (%) in raw (a) and cooked (b) chicken meat
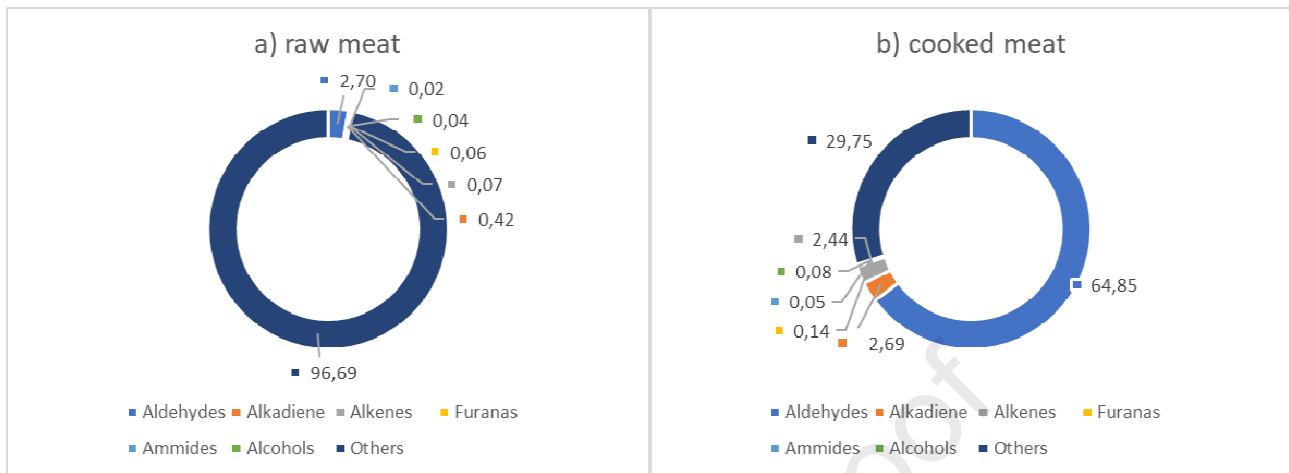
597 Abbreviations: VOC, Volatile Organic Compound;

598   Figure 2. Principal component analysis plot depicting the loadings and variables

599
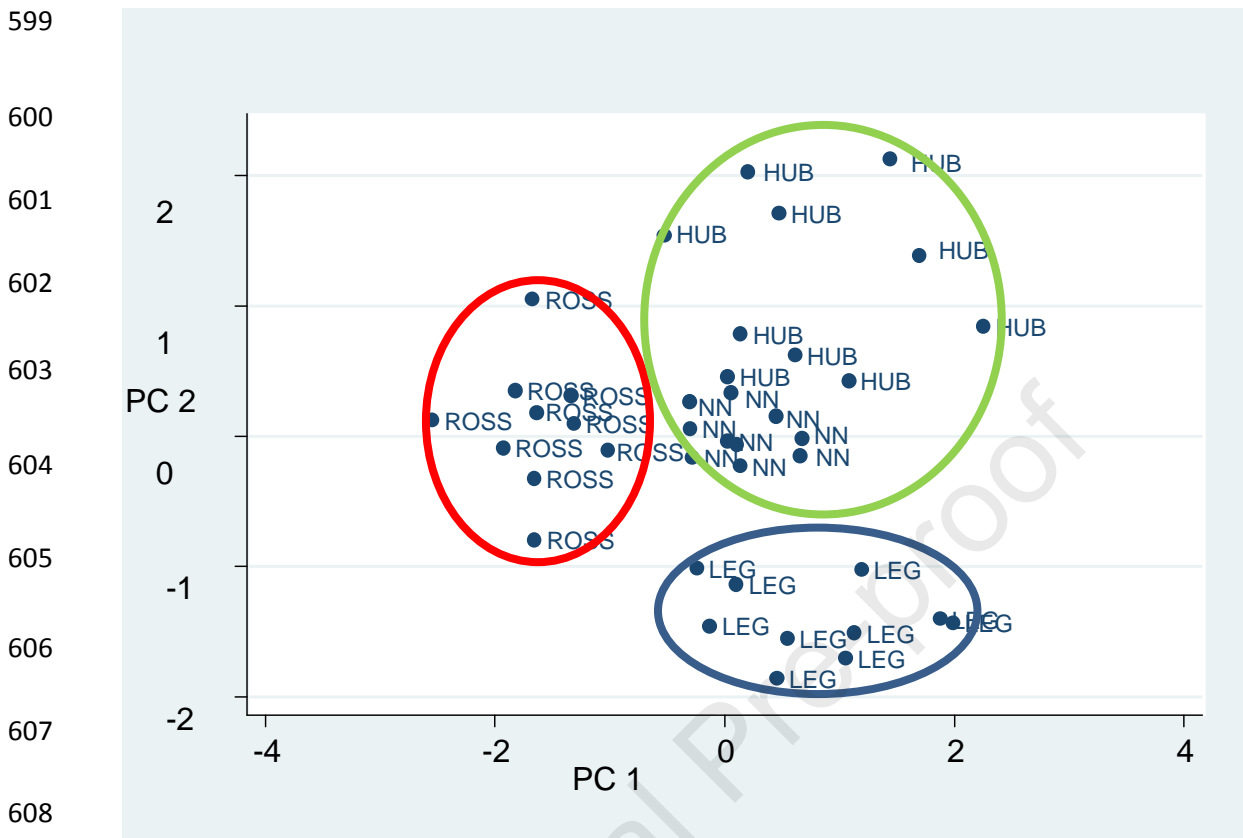
609   Abbreviations: HUB, Hubbard; NN, Naked Neck; LEG, Leghorn; ROSS, Ross 308;

610

611   Figure 3. Scheme of PUFA, VOC and antioxidant dynamics in poultry meat during cooking process

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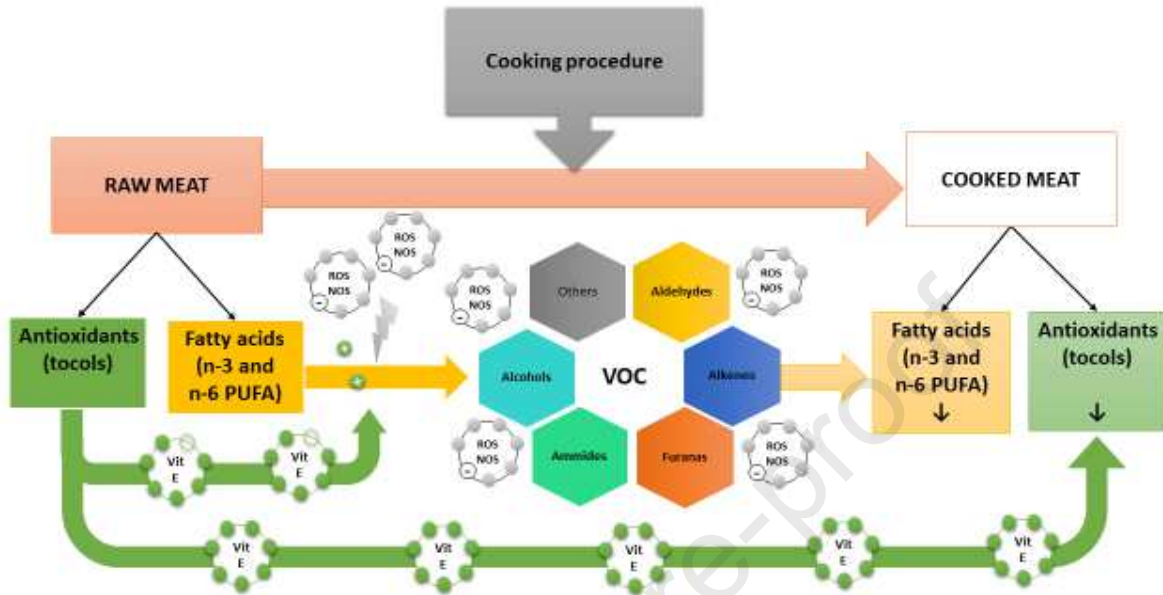
625   Abbreviations: VOC, volatile organic compound; PUFA, polyunsaturated fatty acid; NOS, reactive nitrogen species; ROS, reactive oxygen species;

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627 **SUPPLEMENTARY MATERIAL**

628 Table S1. Formulation and chemical analysis of chicken feed

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		Starter	**Grower**	**Finisher**
Ingredients				
Corn	%	53.92	55.95	53.11
soybean meal 48%	%	30.23	24.67	15.69
Extruded corn flour	%	5.08	8.90	11.45
wheat	%	5.00	5.00	15.00
Dicalcium phosphate	%	1.71	1.58	1.21
Calcium carbonate	%	1.23	1.16	1.29
Corn gluten 70	%	1.00	1.00	/
Soybean oil	%	0.62	0.54	1.15
Vitamin supplement	%	0.40	0.40	0.40
salt	%	0.20	0.18	0.23
Mineral supplement	%	0.16	0.16	0.11
Sodium bicarbonate	%	0.15	0.15	0.15
Chemical composition				
Dry Matter (DM)	%	87.80	87.89	88
Crude protein	% DM	24.01	22.16	18.41
Lipids	% DM	3.99	3.98	4.55
Crude fiber	% DM	3.48	3.58	3.60
Ashes	% DM	6.92	6.43	5.78
Metabolizable energy	kcal/kg	3245	3242	3295
Vitamin A	U.I.	11385	11377	11364
Vitamin E	Mg	36.43	36.41	36.37

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647 Table S2. Productive performance of the four genotypes (slaughtered at about 60% adult weight)

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Genotypes	slaughter age (d)	slaughter weight (kg)	daily gain (g)
Leghorn	100	1.3	13
Hubbard	56	2.0	35
Naked Neck	56	2.4	42
Ross	42	3.0	71

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654   Table S3. Significance of the effects on volatile compounds (A) total lipids and fatty acids (B) and

655   oxidative parameters (C)

A) Volatile Organic Compaunds[1]	Genotype - G	Processing – P (raw *vs.* cooked)	GxP
Ethanal	***	***	***
Butadiene	***	***	***
Butene	ns	*	ns
Butenal	***	***	***
Butanal	***	***	**
Butanol	ns	ns	ns
Cyclohexadiene	***	***	***
Hexadiene	***	***	***
Cyano-propanal	***	***	***
Methyl furanone	ns	***	ns
Metacrylic amide	ns	***	ns
Pentanal	***	***	***
Butyramide	***	***	***
Norbomane	**	***	**
Hexanal	***	***	***
Xylenes	***	***	***
Heptanal	***	***	ns
Octanal	***	***	***
2-Pentyl furan	***	***	***
Nonanal	**	***	***
Σ Aldheydes	***	***	***
Σ Alchool	**	***	**
Σ Alkanales	***	***	***
Σ Ammine	***	***	***
Others	***	***	ns
Σ VOC	***	***	***

656   Abbreviations: [1]VOC: volatile organic compounds; *** P <0.001; ** P< 0.01; P< 0.05. ns: not significant.

B) Fatty Acids[2]	Genotype – G	Processing – P (raw *vs.* cooked)	GxP
16:0	***	***	***
18:0	***	**	***
ΣSFA	**	**	***
16:1cis9	***	***	***
18:1cis9 n-9	***	***	***
22:1cis9	***	***	ns
ΣMUFA	***	***	***
20:4n-6 (AA)	***	***	ns
18:2n-6 (LA)	***	**	***
Σn-6	***	***	***
18:3n-3 (ALA)	***	***	***
20:5n-3 (EPA)	***	***	ns
22:5n-3 (DPA)	***	***	ns
22:6n-3 (DHA)	***	**	ns
Σn-3	***	**	***
n-3/n-6	***	***	***
ΣPUFA	***	***	***
ΣLCP	***	***	**

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658 [2]SFA, saturated fatty acids; MUFA; monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; AA, arachidonic acid; LA, linolenic acid; ALA,

659 α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; LCP, long chain polyunsaturated fatty

660 acids;

661 *** $P < 0.001$; ** $P < 0.01$; $P < 0.05$. ns: not significant;

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C) Oxidative parameters [3]	Genotype - G	Processing – P (raw *vs.* cooked)	GxP
α-T	***	***	***
δ-T	***	***	**
γ-T	***	***	*
α-T$_3$	ns	***	*
δ-T$_3$	**	***	ns
γ-T$_3$	***	***	ns
TBARs	**	**	ns

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664 Abbreviations:[3]α-T, α-tocopherol; γ-T, γ-tocopherol; δ-T, δ-tocopherol; α-T3, α-tocotrienol; γ-T3, γ-tocotrienol; δ-T3, δ-tocotrienol; TBARs,

665 substances reactive to the thiobarbituric acid;

666 *** P <0.001; ** P< 0.01; P< 0.05. ns: not significant.

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668   Table S4. Proximate composition of raw and cooked samples in the different poultry genotypes

Raw		LEG	HUB	NN	ROSS	Pooled SE
DM	%	23.85	24.23	23.92	24.20	1.82
Protein	g/100 g DM	93.92	92.28	92.39	92.31	8.23
Ash	"	4.61	4.62	5.02	4.17	0.32
Lipids	"	1.47[a]	3.10[b]	2.59[b]	3.51[b]	0.10
Cooked						
DM	%	33.71	34.82	35.18	34.30	3.20
Protein	g/100 g DM	93.89	92.48	92.61	92.71	9.02
Ash	"	4.89	4.88	5.12	4.37	0.40
Lipid	"	1.22[a]	2.64[b]	2.27[b]	2.9[b]	0.12

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670   Abbreviations: HUB, Hubbard; NN, Naked Neck; LEG, Leghorn; ROSS, Ross 308;

671   a..b on the same row: $P \leq 0.05$.

672

1   **TABLES**

2   Table 1. Influence of genetic strain and processing (raw *vs.* cooked) on VOC composition (ppb) of

3   chicken meat

Compounds	RAW				COOKED				Pooled SE[1]
Genotypes[2]	LEG	HUB	NN	ROSS	LEG	HUB	NN	ROSS	
Aldehydes									
2-Butenal	0.9	1.9	1.0	1.0	10.5	6.5	6.9	4.2	2.1
3-Ciano-propanal	0.3	0.3	0.4	0.3	38.9	13.2	17.3	9.0	4.8
Butanal	8.1	20.4	5.5	45.6	64.1	79.2	63.9	92.5	25.8
Butene	9.7	8.3	8.2	11.7	27.3	24.7	37.8	32.6	300.2
Cyclohexadiene	0.2	0.7	0.2	0.4	3.9	1.5	1.6	1.1	0.6
Ethanal	24.2	13.1	18.4	9.0	7736.2	5359.2	5613.1	3090.8	1493.9
Heptanal	0.1	0.2	0.2	0.4	2.0	1.2	1.3	0.9	0.6
Hexanal	0.3	0.4	0.4	0.1	46.8	12.7	22.2	9.0	11.9
Nonanal	0.2	0.3	0.5	0.2	14.3	3.3	5.0	1.9	3.0
Norbornane	0.2	0.3	0.3	0.2	9.5	6.1	8.0	5.1	4.3
Octanal	0.4	0.4	0.4	0.4	2.8	1.4	1.7	1.1	0.7
Pentanal	0.7	1.4	1.7	1.5	19.9	10.5	13.2	9.5	4.3
Xylenes	0.4	0.9	0.4	0.3	9.5	10.5	8.4	9.9	5.7
Alkadiene									
1,2-Butadiene	3.9	7.8	16	2.1	557.3	122.3	222.8	73.2	81.8
Alkenes									
1,4-Hexadiene	0.7	1.3	1.7	1.1	504.7	114.7	195.5	71.8	71.5
Furanas									
Methyl furanone	0.8	0.8	0.9	1.0	12.5	12.5	10.7	10.5	4.0
2-Pentyl Furan	0.2	0.2	0.1	0.2	1.7	1.1	1.4	1.0	0.8
Ammides									
Butyramide	0.1	0.1	0.1	0.1	1.1	0.1	1.1	1.5	0.3
Methachrylic amide	0.2	0.2	0.3	0.2	3.8	4.3	3.3	3.3	1.3

Alcohols

Butanol	0.5	1.3	0.2	0.5	0.01	23.8	1.5	2.7	72.2
Σ Aldehydes	45.7	48.6	37.6	71.1	7985.7	5530.0	5800.4	3267.6	693.7
Σ Alkadiene	3.9	7.8	16	2.1	557.3	122.3	222.8	73.2	81.8
Σ Alkenes	0.7	1.3	1.7	1.1	504.7	114.7	195.5	71.8	71.5
Σ Furanas	1	1	1	1.2	14.2	13.6	12.1	11.5	3.9
Σ Ammides	0.3	0.3	0.4	0.3	4.9	4.4	4.4	4.8	1.1
Σ Alcohols	0.5	1.3	0.2	0.5	0.01	23.8	1.5	2.7	72.2
Other compounds	192.0	2417.0	2494.0	1695.0	2421.	2967.0	3462.0	1958.3	1120.2
Total VOC[3]	244.1	2477.3	2550.9	1771.3	11487.8	8775.8	9698.7	5389.9	3210.0

8 Table 2. Influence of genetic strains and processing (raw *vs.* cooked) on the fatty acid profiles (mg/g

9 DM) of poultry meat

Compounds[1]	RAW				COOKED				Pooled SE[3]
Genotypes[2]	LEG	HUB	NN	ROSS	LEG	HUB	NN	ROSS	
14:0	0.17	0.90	0.52	0.35	0.12	0.24	0.22	0.31	0.10
16:0	2.45	5.43	4.45	6.89	2.36	6.27	5.00	6.81	1.62
18:0	1.55	2.8	2.35	3.31	1.80	2.46	1.91	2.18	1.15
Σ SFA	4.17^a	9.13^c	7.32^b	10.55^c	4.28^a	8.97^{bc}	7.13^b	9.30^c	0.25
16:1cis9	0.85	2.21	1.94	2.86	0.58	1.61	1.32	2.3	0.20
18:1cis9 n-9	2.62	6.42	4.94	7.73	2.36	5.36	4.17	6.83	1.35
22:1cis9	0.15	0.31	0.34	0.30	0.17	0.35	0.35	0.37	0.01
Σ MUFA	3.73^a	9.14^{bc}	7.46^b	10.92^c	3.38^a	7.83^b	6.28^b	9.89^c	0.20
18:2n-6 (LA)	2.5	5.58	4.68	5.72	2.17	4.18	3.36	5.20	5.58
20:4n-6 (AA)	1.1	1.72	1.32	1.08	0.91	1.20	1.07	0.89	1.72
Σ n-6 PUFA	3.60^a	7.30^b	6.00^b	6.80^b	3.08^a	5.38^b	4.43^{ab}	6.09^b	1.38
Δ n-6 PUFA					0.52^a	1.92^b	1.57^b	0.71^a	0.10
18:3n-3 (ALA)	0.35	0.57	0.36	0.40	0.15	0.32	0.27	0.30	0.57
20:5n-3 (EPA)	0.22	0.35	0.28	0.39	0.15	0.30	0.22	0.34	0.35
22:5n-3 (DPA)	0.11	0.11	0.09	0.16	0.08	0.08	0.07	0.13	0.11
22:6n-3 (DHA)	0.10	0.11	0.09	0.08	0.06	0.09	0.07	0.05	0.11
Σ n-3 PUFA	0.78^a	1.14^b	0.82^a	1.03^b	0.44^a	0.79^b	0.63^{ab}	0.82^b	0.13
Δ n-3 PUFA					0.43^b	0.30^{ab}	0.23^a	0.20^a	0.08

10

11 Abbrevations:[1]SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; AA, arachidonic acid; LA,

12 linolenic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; LCP, long chain

13 PUFA;

14 [2]HUB, Hubbard; NN, Naked Neck 1; LEG: Leghorn; ROSS: Ross 308;

15 [3]SE: standard error;

16 [a, b, c] on the same row indicate the same type of processing (raw, cooked), with means of $P < 0.05$;

17

18 Table 3. Influence of genetic strains and processing (raw *vs*. cooked) on the content of antioxidants

19 (μg/g DM) and oxidative stability (mg/kg DM) of poultry meat

Compounds[1]	RAW				COOKED				Pooled SE[3]
Genotypes[2]	LEG	HUB	NN	ROSS	LEG	HUB	NN	ROSS	
α-T	10.73	34.46	16.01	32.98	0.98	4.22	2.93	3.35	1.73
δ-T	1.09	0.50	0.63	0.99	0.03	0.23	0.17	0.16	0.02
γ-T	0.13	0.37	0.17	0.41	0.01	0.05	0.02	0.06	0.04
α-T$_3$	5.28	6.03	8.65	6.07	0.24	1.23	0.74	0.52	0.65
δ-T$_3$	1.13	0.17	1.38	0.87	0.30	0.03	0.17	0.20	0.14
γ-T$_3$	1.00	0.35	0.85	0.89	0.27	0.03	0.12	0.18	0.09
TBARS	0.34[a]	0.70[b]	0.54[a]	1.12[c]	1.28[c]	0.95[b]	0.65[a]	1.55[c]	0.14
Σ–T	19.50[a]	41.76[c]	28.22[b]	42.19[c]	1.28[a]	5.80[b]	4.20[b]	12.04[c]	1.82
Δ tocols (raw-cooked)					18.22[a]	35.96[c]	24.02[b]	30.15[c]	1.11

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21 Abbreviations:[1]α-T, α-tocopherol; δ-T, δ-tocopherol; γ-T, γ-tocopherol; α-T3, α-tocotrienol; δ-T3, δ-tocotrienol; γ-T3, γ-tocotrienol; TBARS,

22 Substances Reactive to Thiobarbituric acid;

23 [2]HUB, Hubbard; NN: Naked Neck 1; LEG: Leghorn; ROSS: Ross 308;

24 [3] SE, standard error;

25 [a, b, c] on the same row indicates the same type of processing (raw, cooked), with means of $P < 0.05$;
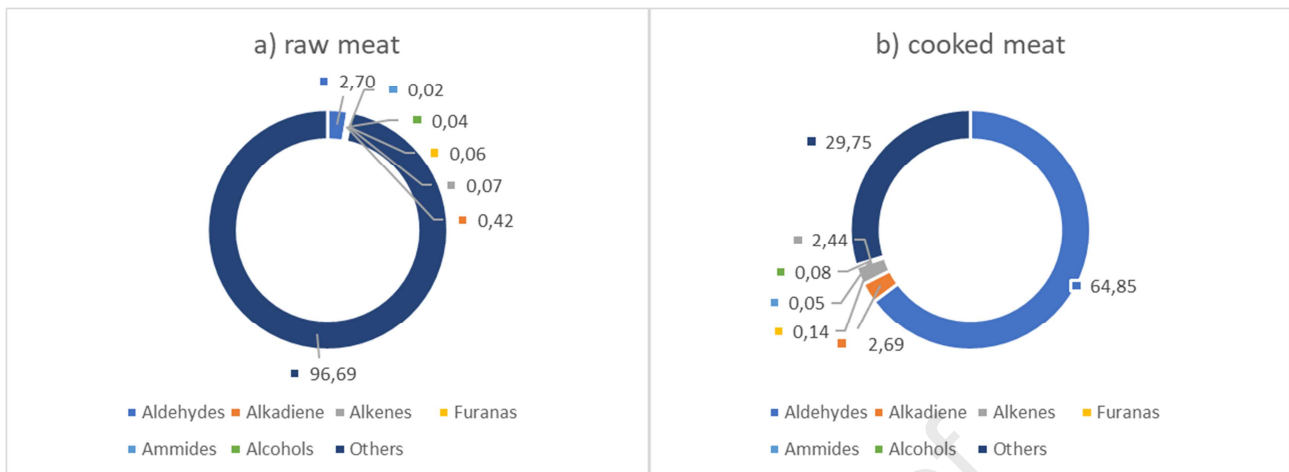
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Table 4. Principal components loadings, eigenvalue and variance

|	PC1	PC2
VOC	0.71	-0.11
Δ n-3	0.59	0.05
Δ n-6	0.31	0.64
Δ tocols	0.20	0.75
Eigenvalue	1.72	1.32
% variance explained	43.04	33.02
Cumulative variance explained	76.06

Abbreviations: VOC, Volatile organic compounds;

Loadings ≥0.50 or ≤−0.50 are presented in bold;

Figure 1. Main VOC classes (%) in raw (a) and cooked (b) chicken meat

a) raw meat
- 2,70
- 0,02
- 0,04
- 0,06
- 0,07
- 0,42
- 96,69

■ Aldehydes ■ Alkadiene ■ Alkenes ■ Furanas
■ Ammides ■ Alcohols ■ Others

b) cooked meat
- 29,75
- 2,44
- 0,08
- 0,05
- 0,14
- 2,69
- 64,85

■ Aldehydes ■ Alkadiene ■ Alkenes ■ Furanas
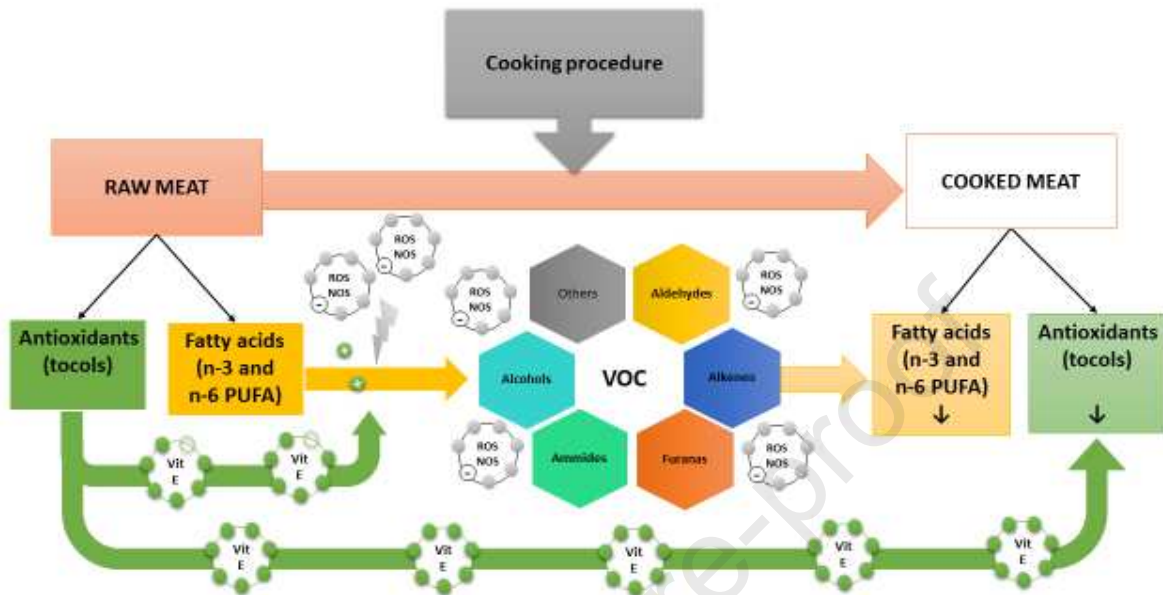■ Ammides ■ Alcohols ■ Others

Abbreviations: VOC, Volatile Organic Compound;

Figure 2. Principal component analysis plot depicting the loadings and variables
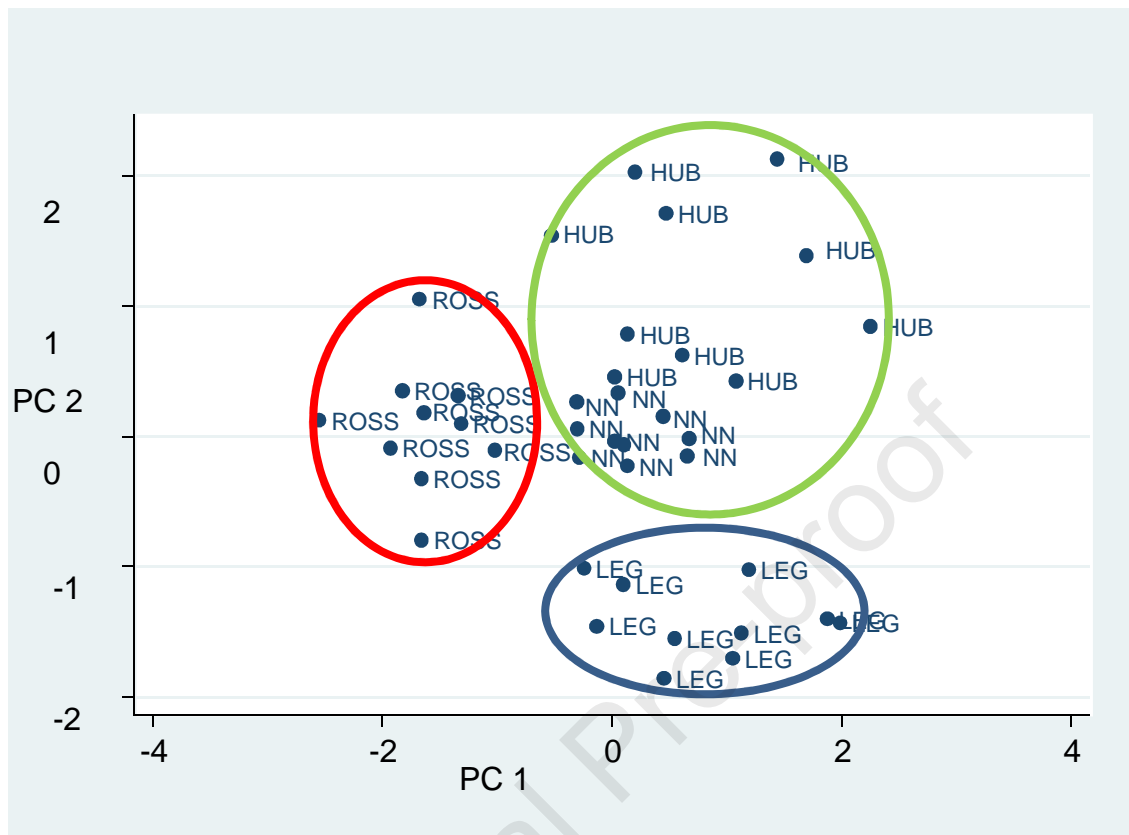
Abbreviations: HUB, Hubbard; NN, Naked Neck; LEG, Leghorn; ROSS, Ross 308;

Figure 3. Scheme of PUFA, VOC and antioxidant dynamics in poultry meat during cooking process

Abbreviations: VOC, volatile organic compound; PUFA, polyunsaturated fatty acid; NOS, reactive nitrogen species; ROS, reactive oxygen species;