

1 This is the peer reviewed version of the following article
2 **Antonio Natalello, Ruggero Menci, Giuseppe Luciano, Frank Monahan, Rufielyn S.**
3 **Gravador, Bernardo Valenti, Mariano Pauselli, Gianni Belvedere, Manuel Scerra,**
4 **Alessandro Priolo, 2023. Effect of dietary pomegranate by-product on lamb flavour.**
5
6 *Meat Science, article 109118. ISSN: 0309-1740*
7 *which has been published in final doi: <https://doi.org/10.1016/j.meatsci.2023.109118>*
8
9 *The terms and conditions for the reuse of this version of the manuscript are specified in the*
10 *publishing policy. For all terms of use and more information see the publisher's website*

11 **Effect of dietary pomegranate by-product on lamb flavour**

12

13 Antonio Natalello^a, Ruggero Menci^a, Giuseppe Luciano^{a*}, Frank Monahan^b, Rufielyn S.
14 Gravador^b, Bernardo Valenti^c, Mariano Pauselli^c, Gianni Belvedere^d, Manuel Scerra^e
15 Alessandro Priolo^a

16

17 ^a University of Catania, Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A), Via
18 Valdisavoia 5, 95123, Catania, Italy

19 ^b School of Agriculture and Food Science, University College Dublin, Dublin 4, Ireland

20 ^c University of Perugia, Dipartimento di Scienze Agrarie, Alimentari e Ambientali (DSA3),
21 Borgo XX Giugno 74, 06123, Perugia, Italy

22 ^d CoRFiLaC, Regione Siciliana, s.p. 25 km 5 Ragusa Mare, 97100 Ragusa, Italy

23 ^e University of Reggio Calabria, Dipartimento di Agraria, Produzioni Animali, Via
24 dell'Università, 25, 89124 Reggio Calabria, Italy

25

26

27 *Corresponding author. *E-mail address:* giuseppe.luciano@unict.it

28

29 **ABSTRACT**

30 This study evaluated the effect of the dietary inclusion of whole pomegranate by-
31 product (WPB) on lamb meat flavour. Seventeen Comisana male lambs (body weight 14.82 kg
32 \pm 2 kg) were assigned to 2 treatments. During 36-day feeding trial, the control group (n=8)
33 received a conventional concentrate diet; the other group (n=9) received a concentrate diet
34 containing 200 g/kg (dry matter) of WPB, replacing part of barley and corn. After slaughter,
35 fatty acids (FA) were determined in raw meat, while volatile organic compounds, SMart nose,
36 and sensory traits in cooked meat. Vaccenic, rumenic, and total polyunsaturated FA
37 concentration was higher in WPB meat. Most of volatile compounds that arise from lipid
38 degradation (i.e., aldehydes, alcohols, ketones, and hydrocarbons) were found at higher
39 concentrations in WPB meat than in control, except for 2-pentanone that was greater in control
40 meat. Although SMart nose clearly discriminated between dietary treatments, consumer panel
41 did not detect differences in meat flavour.

42

43 **KEYWORDS:** volatile aroma compounds; electronic nose; meat odor; PCA, meat quality,
44 sensory evaluation.

45 **1. Introduction**

46 Flavour certainly plays an important role among eating quality attributes of meat,
47 influencing the palatability and acceptability of the meat by consumers. Since raw meat has
48 little or no aroma, the meat flavour is generated by the thermal reactions resulting from
49 cooking (Mottram, 1998). In particular, although numerous thermally induced reactions occur
50 during cooking that generate a large number of new compounds, Maillard reaction and lipid
51 oxidation most contribute to the characteristic flavour of the meat (Mottram, 1998; Sohail et
52 al., 2022). During the Maillard reaction, amino acids and reducing sugar react to form
53 compounds that are mainly responsible for the typical meaty flavour, whereas lipid oxidation
54 gives compounds that provide fatty flavour to cooked meat (Elmore & Mottram, 2009).
55 Different from long-term storage of meat, during which lipid oxidation leads to the
56 development of rancid notes, it is assumed that lipid oxidation during cooking generates
57 pleasant aromas due to the faster degradation of fatty acids compared to refrigerated storage
58 (Mottram, 1998; Resconi, Escudero & Campo, 2013). Indeed, during cooking, fatty acids are
59 quickly degraded forming a wide number of volatile compounds such as aldehydes, ketones,
60 alcohols, hydrocarbons, alkylfurans, and lactones (Elmore & Mottram, 2009). Polyunsaturated
61 fatty acids (PUFA) play a key role in the formation of these compounds due to their higher
62 susceptibility to oxidation, which increases as the number of double bonds increases (Elmore
63 & Mottram, 2009).

64 Due to the crucial role of dietary fatty acids on human health, much ruminant
65 nutrition research has focused on improving the fatty acid composition of meat. In particular,
66 great strides have been made in i) increasing PUFA proportion at the expense of saturated
67 fatty acids (SFA), ii) increasing the beneficial conjugated fatty acids (e.g., conjugated linoleic
68 acid; CLA), and iii) reducing the PUFA n-6/n-3 ratio. For example, promising results have
69 been generally obtained by feeding ruminants with different sources of PUFA, such as

70 sunflower, linseed, and marine oils (Elmore et al., 2005; Bessa, Alves, Jerónimo, Alfaia,
71 Prates & Santos-Silva, 2007; Jerónimo, Alves, Prates, Santos-Silva & Bessa, 2009), or
72 supplementary bioactive substances capable of modulating the ruminal biohydrogenation
73 process (e.g., tannins; Biondi et al., 2019; Frutos et al., 2020; Valenti et al., 2021). However,
74 modifying the lipid composition of meat could have influences on its flavour characteristics
75 (Elmore, Mottram, Enser & Wood, 1999). Indeed, the heating breakdown of each fatty acid
76 could lead to the formation of specific oxidation products, which will give their own
77 contribution to the flavour of meat or may further react with Maillard precursors and
78 intermediates (Elmore & Mottram, 2009).

79 Whole pomegranate by-product (WPB), which contains peels, seeds and residual
80 arils, is generated in large quantities during juice production by agro-food industries and it is
81 considered a waste to be disposed of. However, WPB is rich in PUFA and bioactive
82 compounds, such as tannins and vitamins, and its chemical composition makes it potentially
83 suitable for its use in ruminant diet. In our previous study (Natalello et al., 2019), lambs fed
84 200 g/kg (dry matter basis) pomegranate by-product to replace barley and corn in a
85 concentrate diet showed an improved meat fatty acid profile due to high proportions of
86 PUFA, CLA, vaccenic acid and to a more favourable PUFA/SFA ratio. Moreover, four
87 conjugated linolenic acids [CLnA; i.e., punicic (C18:3 c9 t11 c13), catalpic (C18:3 t9 t11
88 c13), α -eleostearic (C18:3 c9 t11 t13), and β -eleostearic (C18:3 t9 t11 t13) acids], which are
89 well known for their beneficial properties for human health (Yuan, Chen & Li, 2014), were
90 detected only in the muscle of animals fed diet containing pomegranate by-product. It is
91 believed that CLnA in muscle may directly derive from dietary intake, as pomegranate seeds
92 are rich in these fatty acids, especially punicic acid (C18:3 c9 t11 c13), or from isomerization
93 that occurs in rumen during the biohydrogenation processes (Natalello et al., 2020a). To the
94 best of our knowledge, there are no studies in the literature that have explored

95 the effect of feeding pomegranate by-products on meat flavour. Considering the above, we
96 hypothesized that changes in the intramuscular fatty acid profile resulting from feeding
97 pomegranate by-product would influence the flavour development of meat during cooking.
98 Therefore, the aim of the present study was to investigate the effect of dietary inclusion of
99 whole pomegranate by-product on the flavour of cooked lamb in order to have a more
100 complete picture on the influence of this novel feed on the quality of meat.

101

102 **2. Materials and methods**

103 *2.1. By-product, experimental feeding and samplings*

104 The collection and preparation of the pomegranate by-product was described in detail
105 by Natalello et al. (2019). Briefly, pomegranate fruit was mechanically cut in half and squeezed
106 by a local processing company. The processing residue was composed of peels, seeds,
107 membranes and small portion of arils. Then, this wet by-product was dried in a ventilated oven
108 at 40 °C to constant weight. Chemical composition, fatty acid profile, and antioxidant capacity
109 of the dried whole pomegranate by-product (WPB) are shown in Table 1.

110 The experimental design was described in detail by Natalello et al. (2019). In brief, seventeen
111 Comisana male lambs (body weight 14.82 kg \pm 2 kg) were selected in a commercial farm and,
112 at the age of 60 days, were transported to the experimental farm of the University of Catania
113 (37°24'35.3"N 15°03'34.9"E). Lambs were individually penned indoors and assigned to two
114 experimental treatments. After 8 days of adaptation period, the control treatment (CON, 8
115 lambs) was fed a concentrate diet containing (as dry matter basis) corn (22.6%), barley
116 (22.6%), alfalfa hay (19.8%), wheat bran (20%), soybean meal (12%), molasses (0.9%), and a
117 mineral premix (2.1%). While, the other treatment (WPB, 9 lambs) received a concentrate diet
118 containing 20% DM of whole pomegranate by-product to partially replace barley and corn.
119 The ingredients of the experimental diets were ground to pass a 5-mm screen mesh, thoroughly

120 mixed and pelleted to avoid selection. Table 1 shows the chemical composition of the
121 experimental diets and whole pomegranate by-product, which were analysed as detailed in
122 Natalello et al. (2019, 2020b). During the experimental period, lambs had free access to clean
123 drinking water and were fed *ad libitum* with their respective diets. Individual daily intake was
124 determined by manually measuring the feed offered and refused every day, and body weight
125 was recorded weekly.

126 After 36 days of feeding trial, all animals were slaughtered on the same day at a
127 commercial abattoir by captive bolt stunning and exsanguination according to the European
128 Union welfare guidelines. The entire *longissimus thoracis et lumborum* (LTL) muscle was
129 excised from each carcass after 24 h of storage at 4 °C. Muscle samples were aged vacuum-
130 packaged for 3 days at 4 °C, then frozen vacuum-packaged at -80 °C until required for analysis.

131

132 2.2. Fatty acid composition of meat

133 Intramuscular fatty acids were determined as reported in the previous paper (Natalello
134 et al., 2019). In short, intramuscular lipids were extracted from 10 g of muscle using chloroform
135 and methanol (2:1, v/v). Then, 50 mg of extracted fat were methylated using 0.5 M sodium
136 methoxide in methanol to obtain fatty acid methyl esters (FAME). The quantification of FAME
137 was achieved through gas-chromatography analysis (Trace GC with FID; Thermo Fisher
138 Scientific, San Jose, CA) and using nonadecanoic acid methyl ester (C19:0) as internal
139 standard. Fatty acids were expressed as mg/ 100 g of muscle.

140

141 2.3. Meat preparation and cooking procedure

142 A 2-cm thick slice was prepared from each still frozen LTL muscle. Then, the meat slices
143 were denuded of the external visible fat using a scalpel and were thawed inside a plastic bag
144 immersed in a water bath at room temperature for 15 min. The slices were cooked in a domestic

145 pre-heated oven set at 200 °C and in ventilated mode to an internal temperature of 70 °C. Core
146 temperature was continuously monitored by a thermocouple probe (AZ-8856, AZ instrument
147 corp.) inserted at the geometrical centre of the sample.

148

149 2.3.1. Analysis of volatile compounds

150 Volatile compounds were extracted using the solid phase microextraction (SPME)
151 technique as described by Gkarane et al. (2018), with some modifications. Cooked meat was
152 minced by a domestic chopper (Kenwood CH180 Mini Chopper, Kenwood, Hampshire, U.K.)
153 and 2.5 g of sample was placed in a 20-mL glass vial. An equal amount of sodium sulphate
154 anhydrous was added and mixed with a laboratory spatula. Ten µL of 10 ppm bromobenzene
155 was added as internal standard to the sample immediately before capping the vial with a
156 polytetrafluoroethylene (PTFE) septum (VWR, Dublin, Ireland). The vial was equilibrated in
157 a water bath set at 70 ± 2 °C for 20 min, after which a SPME fibre (50/30 µm
158 CAR/DVB/PDMS fibre; 1 cm length; Agilent technologies, Cork, Ireland) was exposed to the
159 headspace over the sample for a further 20 min. The fibre was withdrawn from the vial and
160 immediately inserted into the Varian 3800 GC coupled to a Varian Saturn 2000 ion trap mass
161 spectrometer (Varian Chromatography Systems, Walnut Creek, CA, U.S.A.). Volatile
162 compounds extraction, adsorption, and injection were performed manually. The fibre remained
163 inside the injector, which operated in splitless mode at 250 °C, for 8 min (desorption time).
164 Volatile compounds were separated using a ZB5-MS fused silica capillary column (30 m
165 length × 0.25 mm id × 0.25 µm film thickness, Phenomenex, Cheshire, U.K.) with helium as
166 carrier gas at a flow rate of 1.0 mL/min. The GC oven temperature was programmed as follows:
167 held at 40°C for 5 min; increased to 230°C at 4°C/min and held for 5 min, for a total acquisition
168 program of 57.5 min. The GC/MS interface was heated at 280 °C. Acquisition was performed
169 in electron impact (EI) mode (70 eV) at 10 microscans/s, scanning the mass range m/z 33–230.

170 Compounds were identified by comparing their mass spectra with the National Institute
171 of Standards and Technology (NIST) Mass Spectral Data Centre and confirmed by matching
172 their linear retention indices (LRI) with Kondjoyan and Berdagué (1996) and NIST Mass
173 Spectral Data Centre. Wherever possible, identifications were confirmed by comparison of LRI
174 values and mass spectra with those of authentic compounds. The LRI were calculated by
175 running saturated n-alkane standard from 7 to 30 carbon atoms under the same conditions. The
176 peak area of the volatile compounds was integrated from specific ions for each molecule to
177 avoid overlapping between the compounds. Quantities of the volatile compounds were
178 approximated by comparison of their peak areas with that of the bromobenzene internal
179 standard using a response factor of 1, and expressed as ng/g of cooked meat.

180

181 2.3.2 *SMart Nose® analysis*

182 The analyses of headspace volatile compounds were also performed using a SMart
183 Nose® system (SMart Nose 1.51, LDZ, CH- 2074 Marin-Epagnier, Switzerland), based on
184 mass spectrometry, without separation of the individual organic volatile components
185 (Rapisarda et al., 2013). The SMart Nose system was combined with a Combi Pal autosampler
186 CTC Analytics AG (CTC Combi Pal) with the Cycle Composer software, a high-sensitivity
187 quadrupole mass spectrometer (Inficon AG) with a ionic mass detection ranging from 1 to 200
188 amu equipped with a specific statistical software (SMart Nose 1.51) to apply a multivariate
189 analysis on acquired data. Briefly, a sample (4 g) of cooked meat was placed into a 20-mL vial
190 (adapted for Combi Pal autosampler), closed with a silicone/PTFE septum and a magnetic cap
191 and incubated for 30 min at 60 °C. The vials were randomly placed in the autosampler trays to
192 avoid biases attributable to previous sample and/or external factors. A duplicate sub-sample
193 was also treated similarly. An aliquot of 2.5 mL of the headspace was extracted using a gas-
194 tight syringe and transferred into the mass spectrometer. The syringe and the injector

195 temperatures were set at 100 °C and at 160 °C, respectively. Nitrogen was used as purge gas,
196 to avoid any memory effect, with a purge flow of 200 mL/min. SMart Nose analysis was
197 performed with the following setting: EI mode at 70 eV; mass spectrometer scan speed of 0.5
198 microscan/s; mass range of 10–160 amu; scanning electron microscope voltage at 1160 V; total
199 acquisition time 170 s. Three cycles per injection were measured.

200

201 2.3.3 *Sensory evaluation*

202 Attribute rating and acceptance tests were carried out to elucidate why consumers would like
203 or dislike a sample. Forty-five untrained panellists (aged 22-54 years) who regularly consume
204 lamb meat were recruited. Participants were balanced by gender (51% male and 49% female).
205 Sensory evaluation was performed in five sessions on the same day. Each panelist attended
206 only one session. Meat samples were prepared and cooked as described in Section 2.3. Cooked
207 samples were cut into cubes (approximately 8 cm³), placed in plastic dishes labelled with a
208 random 3-digit code, and served in a complete balanced design to the panellists. Each
209 Consumer received two pieces of meat (one from each treatment) and was asked to score
210 “tenderness”, “juiciness”, “sheep flavour”, “abnormal flavour”, “flavour liking” and “overall
211 liking” on a 10-points scale (1 = extremely tough,

212

213 2.4. *Statistical Analysis*

214 Data for fatty acids, volatile compounds, and sensory parameters were analysed by one-
215 way ANOVA to test the effect of dietary treatment and each lamb was considered as
216 *experimental unit*. When the data did not follow a normal distribution according to the Shapiro-
217 Wilk test ($P < 0.05$), data were logarithmically transformed before ANOVA analysis. Bivariate
218 correlations between muscle fatty acids and volatile compounds were determined by means of
219 Pearson correlation coefficients (r) and “two-tailed” test of significance. Statistical analyses

Commentato [F1]: I would suggest to replace the title with “Consumer test”. Sensory evaluation suggests me that meat properties have been evaluated by expert panelist

Commentato [F2]: Even for the consumer test?

220 were performed using IBM® SPSS® version 26 (SPSS Inc, Chicago, Illinois, USA).
221 Differences were considered significant when $P \leq 0.05$ and a trend toward significance when
222 $P \leq 0.10$.

223 Results obtained by SMart Nose analysis were processed using a specific software
224 provided by the SMart Nose system (SMart Nose 1.51) to perform a multivariate analysis.
225 Treated data among the two experimental groups of meat samples allowed the selection of the
226 most discriminant ions used in the principal component analysis (PCA). The statistical software
227 program of the SMart Nose makes group assignment by Euclidean distances in the
228 multidimensional space created by the PCA. For each separation pattern, a new set of
229 parameters was chosen so as to calculate scores of the principal components (PC).

230

231

232 **3. Results and discussion**

233 *3.1. Fatty acids*

234 The intramuscular fatty acid composition of LTL was expressed as mg/100 g of meat (Table
235 2) to better interpret the results on volatile compounds (discussed later). The results of the
236 present study generally confirmed the trends observed in Natalello et al. (2019), where fatty
237 acids (FA) were expressed as a proportion (g/100 g of total FA), although some differences
238 appeared between the two methods of expression. Similar to the previous paper, the inclusion
239 of 200 g/kg DM of WPB did not affect any of the saturated fatty acids ($P > 0.05$) and
240 consequently the total SFA concentration was not affected by the treatment ($P = 0.928$).
241 Comparable concentrations of the total monounsaturated fatty acids (MUFA) and the most
242 abundant meat fatty acid (i.e., oleic acid: C18:1 c9) were observed between the two treatments
243 ($P = 0.702$ and $P = 0.693$, respectively). A double concentration of vaccenic acid (C18:1 t11)
244 was found in the intramuscular fat of lambs fed WPB diet as compared with CON lambs ($P =$

245 0.050). Likewise, the concentration of rumenic acid (C18:2 c9 t11) was nearly threefold higher
246 in the WPB than in the control treatment (P = 0.003). The beneficial effects of rumenic acid on
247 human health have been well documented (Pariza, Park & Cook, 2001) and still it gains much
248 interest in the scientific community due to the anti-diabetic, anti-atherosclerotic, anti-obesity
249 and anti-carcinogenic properties (Vahmani et al., 2020). Vaccenic acid also contributes to the
250 bioactivity and health aspects of ruminant meat lipids, as it is converted to rumenic acid by the
251 activity of Δ -9 desaturase enzyme. Regardless of its conversion into rumenic acid, vaccenic
252 acid may have anti-carcinogenic and anti-inflammatory properties (Vahmani et al., 2020). The
253 sum of PUFA increased by 38% when lambs received the diet containing WPB (P = 0.041),
254 although only a few individual PUFA were significantly affected by the dietary treatment. In
255 fact, apart from rumenic acid, only the concentration of C20:3 n-6 acid was significantly
256 increased by the WPB diet (P = 0.034). The C20:3 n-6 is classified as a long chain n-6 FA and
257 derives from linoleic acid (C18:2 c9 c12) through enzymatic desaturation and elongation
258 processes (Vahmani et al., 2020). Its higher concentration in the muscle of lambs given WPB
259 may be due to the greater availability of its precursor (i.e., linoleic acid), which was numerically
260 more abundant in the WPB group than in the CON group (+24%; P = 0.169). Yet, some
261 possible effects of the WPB diet on the enzymatic processes of elongation and desaturation –
262 that lead to the formation of long-chain n-3 and n-6 FA – cannot be ruled out.

263 The dietary inclusion of WPB significantly reduced the concentration of C17:0
264 anteiso (P = 0.029), C17:0 (P = 0.036) and C17:1 c9 (P = 0.011) and, consequently, affected
265 the total concentration of odd- and branched-chain fatty acids (OBCFA; P = 0.037). The
266 source of these fatty acids is believed to be the cell membranes of bacteria leaving the rumen.
267 In general, changes in OBCFA concentrations in ruminant products may reflect alterations in
268 the rumen microorganism population, as different bacterial species have specific enzymes
269 responsible for de novo synthesis of membrane fatty acids (Torales, Hervás, Della Badia,

270 Gervais & Frutos, 2020). Therefore, it may be speculated that the different chemical
271 composition of the two experimental diets selected different rumen populations. Also, the
272 presence of bioactive substances in the WPB, such as CLnA, tannins, and tocopherols, may
273 have influenced the rumen population and consequently the concentration of OBCFA, which
274 flows from the rumen.

275

276 3.2. Volatile compounds

277

278 The effect of dietary inclusion of WPB on the volatile compound profile of cooked
279 meat is reported in Table 3. A total of 77 volatile compounds were identified and classified
280 according to their chemical nature: 26 aldehydes, 11 alcohols, 8 hydrocarbons, 7 ketones, 5
281 sulfur compounds, 4 benzenoid compounds, 3 phenols, 2 volatiles within lactones, terpenes,
282 branched-chain FAs (BCFA), pyrazines, esters and 1 compound within each class of furans,
283 pyridines and thiazolines. The dietary treatment had a significant effect on the majority of
284 these volatile compounds. Indeed, 38 of these compounds were significantly influenced by
285 WBP diet ($P < 0.05$) and 12 tended to be affected ($P < 0.10$). Specifically, the concentrations
286 of 19 out of 26 aldehydes were increased ($P < 0.05$) by the inclusion of WPB in the lamb diet
287 compared to the control diet. Regarding the 11 alcohols identified, feeding WBP significantly
288 increased the concentration of seven compounds ($P < 0.05$) and two others approached
289 significance ($P < 0.10$). Also, the concentrations of almost all ketones were higher in the
290 cooked meat from WBP lambs compared to CON lambs, with the exception of 2-pentanone
291 which was greater in the control meat ($P = 0.003$). Regarding the class of hydrocarbons, 3-
292 methyl-1-heptene, dodecane and octadecane were found at greater concentration in the
293 cooked meat from lambs given WBP diet than control treatment ($P < 0.05$). Aldehydes,
294 alcohols, hydrocarbons, and ketones are volatile compounds that are mainly produced during

295 the oxidative breakdown of lipid under heating conditions (Elmore & Mottram, 2009).
296 Generally, the lipid oxidation of raw meat during long-term storage leads to the formation of
297 off-flavours linked to rancidity reactions. On the other hand, it is believed that the quickly
298 degradation of lipids – that occurs during cooking – generates volatile compounds, which
299 contribute to a desirable meat flavour (Mottram, 1998; Resconi et al., 2013). It is well known
300 that PUFA undergo oxidation much more readily than saturated lipids, and their susceptibility
301 increases with increasing double bonds (Mottram, 1998; Flores, 2017). Therefore, in the
302 present study, the higher concentration of lipid-derived volatiles observed in the cooked meat
303 from lambs fed the WPB diet may be explained by the greater concentration of PUFA in
304 WPB meat compared to CON. This hypothesis is in line with the amount of highly
305 peroxidizable polyunsaturated fatty acids (HP-PUFA; i.e., sum of fatty acids with
306 unsaturation degree ≥ 3) and the peroxidability index, which were both significantly higher
307 ($P = 0.024$ and $P = 0.042$, respectively) in the WPB meat than in CON. Elmore et al. (1999)
308 suggested that oxidation of highly unsaturated FA produces free radicals, which will
309 propagate the breakdown of other less susceptible fatty acids. This could further explain the
310 amplified formation of lipid-derived volatiles in WPB cooked meat.
311 On the other hand, in our previous study (Natalello et al., 2020b), we observed that
312 feeding lambs with a WPB-containing diet reduced the formation of secondary lipid
313 oxidation products (TBARS) in fresh and cooked meat during refrigerated storage. It was
314 concluded that the higher concentration of vitamin E in muscle of animals fed WBP played a
315 key role in delaying lipid oxidation. This partly contrasts the results of the present study
316 where the formation of lipid-derived volatiles was higher in the dietary treatment containing
317 WPB. However, it should be noted that the vitamin E concentration in WPB meat (< 0.4
318 mg/kg) was much less than the thresholds of 0.61–0.90 and 1.25 mg/kg proposed by others
319 (González-Calvo, Ripoll, Molino, Calvo & Joy, 2015); Kasapidou et al., 2012, respectively),

320 below which other factors than vitamin E become more important in determining meat
321 oxidation (e.g., unsaturated lipids, heme pigments, metal catalysts; Bellés, Campo, Roncalés
322 & Beltrán, 2019). Furthermore, it should be stressed that i) TBARS assay and the
323 determination of volatile compounds are completely different analytical procedures, leading
324 to results capable of explaining different mechanisms; ii) the cooking method was different
325 (i.e., boiled vs baked); iii) unlike here, where volatile compounds were analysed immediately
326 after cooking, in the previous study the meat was refrigerated for days to study the shelf life,
327 which obviously has a direct effect on the production of oxidation compounds.
328 In addition to lipid oxidation, the other key pathway in cooked meat flavour formation
329 is the Maillard reaction. Amino acids, peptides and sugars react together during heating,
330 producing a large number of volatile compounds which contribute to the characteristic
331 flavour of cooked meat (Mottram, 1998). Simplifying, this reaction between a reducing sugar
332 – mainly ribose – and an amino acid is favoured by high temperatures and low moisture and
333 initially generates furfural and furanone derivatives, hydroxyketones and dicarbonyl
334 compounds, which further react with amines, amino acids, ammonia, and sulphur compounds
335 to produce heterocyclic molecules such as thiazoles, pyrazines, oxazoles, and thiophenes
336 (Flores, 2017). In the present study, it seems that the dietary inclusion of WPB had no
337 influence on Maillard reaction. Indeed, all the classes of volatile compounds that are
338 generally formed during Maillard reaction (i.e., sulphur compounds, pyrazines, pyridines,
339 thiazoles) were found at comparable concentrations between the two treatments ($P > 0.05$). In
340 line with these findings, we observed no statistical differences between dietary treatments for
341 the concentrations of 2-methyl butanal, 3-methyl-butanal, methional, and 2,3-butanedione (P
342 > 0.05). Although these compounds are classified as aldehydes or ketone, they are formed
343 during Maillard reaction or subsequent reactions such as Strecker degradation (Elmore et al.,
344 2005; Resconi et al., 2013; Flores 2017).

345 One of the most abundant volatile compounds that we detected in the cooked meat
346 from both treatments was hexanal. This finding was in line with previous studies, which
347 found hexanal to be one of the predominant volatile compounds in both lamb (Elmore et al.,
348 2005; Vasta et al., 2013; Del Bianco et al., 2021) and beef (Descalzo et al., 2005; Elmore et
349 al., 2004). It has been reported that hexanal derives from the decomposition of linoleic acid
350 (C18:2 c9 c12; Elmore et al., 2005), however, in the current study, no significant correlation
351 between hexanal and linoleic acid was observed ($r = 0.319$; $P > 0.05$). This was in
352 accordance with the study of Gravador et al. (2015), in which lambs were fed olive cake and
353 linseed. Interestingly, the concentrations of hexanal and CLnA (i.e., puniolic, catalpic, and α -
354 eleostearic acids) were strongly correlated ($r > 0.607$; $P < 0.01$). These correlations seem to
355 suggest that hexanal may also derive from the thermal oxidation of CLnA. Another plausible
356 explanation could be that CLnA – being highly susceptible to oxidation – may catalyse the
357 breakdown of less prone fatty acids and increase the formation of hexanal from linoleic acid,
358 as suggested by Elmore et al. (2005) for meat containing remarkable concentration of highly
359 unsaturated FA. The latter hypothesis may explain why CLnA were statistically correlated
360 with most of the identified aroma compounds. Furthermore, this hypothesis may explain the
361 high concentration of nonanal, which was the predominant volatile compound in WPB
362 treatment. Even though nonanal appears to be formed from oleic acid (Dominguez et al.,
363 2019), no significant correlation was observed between nonanal and its precursor in the
364 present study ($r = 0.226$; $P > 0.05$). Indeed the oleic acid concentration of meat was
365 comparable between the two treatments, but the formation of nonanal was tripled in the WPB
366 treatment. This could mean that the breakdown of oleic acid was speeded up in WPB meat,
367 probably due to the CLnA oxidation.

368 As far as we are aware, there are no studies in literature investigating the effect of
369 dietary WPB – or other sources of CLnA – on the volatile compounds of meat. Therefore, it

370 is not possible to compare our results with previous findings. However, it might be viable to
371 extrapolate useful information from studies that have investigated the volatile profile of
372 pomegranate seed oil or other oils containing punicic acid, α -eleostearic, and catalpic acids.
373 For instance, Costa, Silva and Torres (2019) detected high levels of 2,4-nonadienal in the
374 volatile profile of cold-pressed pomegranate seed oil. Likewise, Jiang, Wu, Zhou and Akoh
375 (2015) reported that 2,4-nonadienal was the predominant aldehyde in samples of cold-pressed
376 *Trichosanthes kirilowii* seed oils, which was abundant in punicic acid. In the present study,
377 2,4-nonadienal was observed at concentrations 100-fold higher in WPB treatment than
378 control one ($P < 0.001$). The strong correlations between (E,E)-2,4-nonadienal and punicic (r
379 = 0.818; $P < 0.01$), α -eleostearic ($r = 0.863$; $P < 0.01$), and catalpic ($r = 0.811$; $P < 0.01$)
380 acids suggest that this aldehyde compound could derive from the breakdown of CLnA.
381 The only volatile compound that was found at higher concentration in the control
382 treatment than the WPB group was 2-pentanone ($P = 0.003$). Interestingly, this ketone was
383 positively correlated only with C18:1 t10 ($r = 0.550$; $P < 0.5$), suggesting that 2-pentanone
384 could derive from the oxidation of C18:1 t10. We are not aware of any published articles
385 where the origin of 2-pentanone has been discussed; therefore it would seem that this is the
386 first report that hypothesizes the origin of this ketone. More in-depth and targeted studies are
387 needed to confirm this finding.

388

389 3.3 SMart nose and sensory evaluation

390 SMart Nose, also known as electronic nose, is a device capable of recognizing volatile
391 compounds through sensors and creating a unique “fingerprint” (Wojnowski, Majchrzak,
392 Dymerski, Gębicki & Namieśnik, 2017). Simulating human olfaction, it provides a global
393 odour perception without separation mechanisms of volatile molecules (Del Bianco et al.,
394 2020). Figure 1 shows the first 2 principal components generated by SMart nose analysis.

395 Meat from each lamb was scored in duplicate, therefore, each point in the biplot is the results
396 of each analysis. Most of the variability was explained by PC 1, which accounted for 95.95%
397 of the total variability. Whereas, PC 2 explained only 1.75% of the variability. The two
398 experimental treatments were perfectly discriminated along PC 1: the CON group was
399 located on the left part of the biplot, while the WPB group was mainly located on the right
400 area. Considering the great influence that the experimental treatment had on volatile
401 compounds (Table 3), such a clear discrimination between the groups was expected.
402 Although the electronic nose was able to distinguish the two treatments, the consumer
403 panellists did not detect differences in “sheep flavour”, “abnormal flavour”, and “flavour
404 liking” (Table 4; $P > 0.10$). This discrepancy between the electronic nose and the sensory
405 assessment results might depend on the odour activity value (OAV) of the various volatile
406 compounds. Indeed, the sensitivity of the human nose varies in relation to the odorous
407 compounds and the OAV is calculated as the ratio between the concentration and the olfactory
408 threshold of each compound (Casaburi, Piombino, Nychas, Villani & Ercolini,
409 2015). Generally, lipid-derived compounds have relatively high odour thresholds, and thus
410 contribute less to overall flavour than sulfuric- and nitrogen-containing heterocyclic
411 molecules, which originate from Maillard reaction (Mottram, 1998). This may explain why
412 the panellists failed to detect differences between the two treatments since, in the current
413 study, the dietary treatment mainly influenced the volatile compounds derived from lipid
414 degradation. While the SMart Nose – based on mass spectrometry – uses the most abundant
415 ions to distinguish samples (Del Bianco et al., 2020), without considering the odour
416 threshold. A further explanation could also lie in the fact that that the consumer test was
417 performed by untrained panellists who are less able to detect specific flavour differences.
418 The effect of dietary inclusion of WPB on other sensory traits of cooked lamb is
419 reported in Table 4. Tenderness and juiciness tended to be slightly enhanced by WPB diet (P

420 = 0.078 and P = 0.082, respectively). Intramuscular fat – also termed as marbling fat – is
421 supposed to have an important role on eating quality and especially on tenderness and
422 juiciness (Wood et al., 2008). Usually, as intramuscular fat increases, juiciness and
423 tenderness increase, even though the strength of the correlation varies noticeably between
424 studies (Wood et al., 2008). In the present study, the intramuscular fat content did not
425 statistically differ between treatments. However, the small numerical difference (1.88 vs 2.01
426 mg/100 g) between the fat content may partially explain the trend observed for the tenderness
427 and juiciness.

428

429 4. Conclusions

430 In the present study we have investigated the effect of dietary inclusion of 200 g/kg
431 DM of WBP on cooked lamb flavour, and its relation with intramuscular fatty acids. The
432 concentration of PUFA, vaccenic and rumenic acids were higher in the meat from lambs
433 given WPB. CLnA from pomegranate were deposited in lamb muscle and their high
434 susceptibility to lipid oxidation may have catalysed the breakdown of other FA. Indeed, most
435 of the lipid-derived volatiles in cooked meat were increased by WBP dietary inclusion.
436 Whereas, aroma compounds that are formed through Maillard reaction or Strecker
437 degradation were not affected by dietary treatment. As expected from the volatile compound
438 results, SMart nose analysis clearly discriminated the two treatments. However, no
439 differences in flavour attributes were distinguished between treatments by untrained
440 panellists. Overall, the inclusion of WPB improved the acid profile from a healthy point of
441 view without worsening the eating quality of the lamb.

442

443

444

445 **Acknowledgements**

446 Authors would like to thank Dr. Vasiliki Gkarane (University College Dublin) for her
447 invaluable help with the analysis of volatile compounds. This research was conducted under
448 the 2-year collaborative research program (2016-2018) officially established between the
449 Departments Di3A (University of Catania) and DSA3 (University of Perugia). Moreover, the
450 University of Catania funded part of the research activities conducted (project “QUALIGEN”;
451 Linea 2 – Piano di Incentivi per la Ricerca di Ateneo 2020/2022; principal investigator:
452 Giuseppe Luciano). A. Nataello benefits from PON “RICERCA E INNOVAZIONE” 2014—
453 2020 research contract (Azione IV.6—CUP E61B21004280005) supported by Ministero
454 dell’Università e della Ricerca.

455

456 **References**

- 457 Bellés, M., del Mar Campo, M., Roncalés, P., & Beltrán, J. A. (2019). Supranutritional doses
458 of vitamin E to improve lamb meat quality. *Meat Science*, *149*, 14-23.
- 459 Bessa, R. J., Alves, S. P., Jerónimo, E., Alfaia, C. M., Prates, J. A., & Santos-Silva, J. (2007).
460 Effect of lipid supplements on ruminal biohydrogenation intermediates and muscle
461 fatty acids in lambs. *European Journal of Lipid Science and Technology*, *109*(8), 868-
462 878.
- 463 Casaburi, A., Piombino, P., Nychas, G. J., Villani, F., & Ercolini, D. (2015). Bacterial
464 populations and the volatilome associated to meat spoilage. *Food Microbiology*, *45*, 83-
465 102.
- 466 Costa, A. M. M., Silva, L. O., & Torres, A. G. (2019). Chemical composition of commercial
467 cold-pressed pomegranate (*Punica granatum*) seed oil from Turkey and Israel, and the
468 use of bioactive compounds for samples’ origin preliminary discrimination. *Journal of*
469 *Food Composition and Analysis*, *75*, 8-16.

470 Del Bianco, S., Natalello, A., Luciano, G., Valenti, B., Campidonico, L., Gkarane, V., ... &
471 Piasentier, E. (2021). Influence of dietary inclusion of tannin extracts from mimosa,
472 chestnut and tara on volatile compounds and flavour in lamb meat. *Meat Science*, *172*,
473 108336.

474 Del Bianco, S., Natalello, A., Luciano, G., Valenti, B., Monahan, F., Gkarane, V., ... &
475 Piasentier, E. (2020). Influence of dietary cardoon meal on volatile compounds and
476 flavour in lamb meat. *Meat Science*, *163*, 108086.

477 Descalzo, A. M., Insani, E. M., Biolatto, A., Sancho, A. M., García, P. T., Pensel, N. A., &
478 Josifovich, J. A. (2005). Influence of pasture or grain-based diets supplemented with
479 vitamin E on antioxidant/oxidative balance of Argentine beef. *Meat Science*, *70*(1), 35-
480 44.

481 Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019).
482 A comprehensive review on lipid oxidation in meat and meat products. *Antioxidants*,
483 *8*(10), 429

484 Elmore, J. S., & Mottram, D. S. (2009). Flavour development in meat. *Improving the sensory*
485 *and nutritional quality of fresh meat*, 111-146.

486 Elmore, J. S., Cooper, S. L., Enser, M., Mottram, D. S., Sinclair, L. A., Wilkinson, R. G., &
487 Wood, J. D. (2005). Dietary manipulation of fatty acid composition in lamb meat and
488 its effect on the volatile aroma compounds of grilled lamb. *Meat Science*, *69*(2), 233-
489 242.

490 Elmore, J. S., Mottram, D. S., Enser, M., & Wood, J. D. (1999). Effect of the polyunsaturated
491 fatty acid composition of beef muscle on the profile of aroma volatiles. *Journal of*
492 *Agricultural and Food Chemistry*, *47*(4), 1619-1625.

493 Elmore, J. S., Warren, H. E., Mottram, D. S., Scollan, N. D., Enser, M., Richardson, R. I., &
494 Wood, J. D. (2004). A comparison of the aroma volatiles and fatty acid compositions

495 of grilled beef muscle from Aberdeen Angus and Holstein-Friesian steers fed diets
496 based on silage or concentrates. *Meat Science*, 68(1), 27-33.

497 Flores, M. (2017). The eating quality of meat: III—Flavor. In *Lawrie's Meat Science* (pp. 383-
498 417). Woodhead Publishing.

499 Frutos, P., Hervás, G., Natalello, A., Luciano, G., Fondevila, M., Priolo, A., & Toral, P. G.
500 (2020). Ability of tannins to modulate ruminal lipid metabolism and milk and meat fatty
501 acid profiles. *Animal Feed Science and Technology*, 269, 114623.

502 Gkarane, V., Brunton, N. P., Harrison, S. M., Gravador, R. S., Allen, P., Claffey, N. A., . . .
503 Moloney, A. (2018). Volatile Profile of Grilled Lamb as Affected by Castration and
504 Age at Slaughter in Two Breeds. *Journal of Food Science*, 83(10), 2466-2477.

505 González-Calvo, L., Ripoll, G., Molino, F., Calvo, J. H., & Joy, M. (2015). The relationship
506 between muscle α -tocopherol concentration and meat oxidation in light lambs fed
507 vitamin E supplements prior to slaughter. *Journal of the Science of Food and*
508 *Agriculture*, 95(1), 103-110.

509 Gravador, R. S., Serra, A., Luciano, G., Pennisi, P., Vasta, V., Mele, M., ... & Priolo, A. (2015).
510 Volatiles in raw and cooked meat from lambs fed olive cake and linseed. *Animal*, 9(4),
511 715-722.

512 Jerónimo, E., Alves, S. P., Prates, J. A., Santos-Silva, J., & Bessa, R. J. (2009). Effect of dietary
513 replacement of sunflower oil with linseed oil on intramuscular fatty acids of lamb
514 meat. *Meat Science*, 83(3), 499-505.

515 Jiang, X., Wu, S., Zhou, Z., & Akoh, C. C. (2016). Physicochemical properties and volatile
516 profiles of cold-pressed *Trichosanthes kirilowii* maxim seed oils. *International Journal*
517 *of Food Properties*, 19(8), 1765-1775.

518 Kasapidou, E., Wood, J. D., Richardson, R. I., Sinclair, L. A., Wilkinson, R. G., & Enser, M.
519 (2012). Effect of vitamin E supplementation and diet on fatty acid composition and on

520 meat colour and lipid oxidation of lamb leg steaks displayed in modified atmosphere
521 packs. *Meat Science*, 90(4), 908-916.

522 Kondjoyan, N., & Berdagué, J.-L. (1996). *A compilation of relative retention indices for the*
523 *analysis of aromatic compounds*: Ed. du Laboratoire Flaveur.

524 Mottram, D.S., (1998). Flavour formation in meat and meat products: a review. *Food*
525 *Chemistry*, 62(4), pp.415-424.

526 Natalello, A., Hervás, G., Toral, P. G., Luciano, G., Valenti, B., Mendoza, A. G., ... & Frutos,
527 P. (2020a). Bioactive compounds from pomegranate by-products increase the in vitro
528 ruminal accumulation of potentially health promoting fatty acids. *Animal Feed Science*
529 *and Technology*, 259, 114355.

530 Natalello, A., Luciano, G., Morbidini, L., Valenti, B., Pauselli, M., Frutos, P., . . . Priolo, A.
531 (2019). Effect of Feeding Pomegranate Byproduct on Fatty Acid Composition of
532 Ruminal Digesta, Liver, and Muscle in Lambs. *Journal of Agricultural and Food*
533 *Chemistry*, 67(16), 4472-4482.

534 Natalello, A., Priolo, A., Valenti, B., Codini, M., Mattioli, S., Pauselli, M., ... & Luciano, G.
535 (2020b). Dietary pomegranate by-product improves oxidative stability of lamb
536 meat. *Meat Science*, 162, 108037.

537 Pariza, M. W., Park, Y., & Cook, M. E. (2001). The biologically active isomers of conjugated
538 linoleic acid. *Progress in lipid research*, 40(4), 283-298.

539 Rapisarda, T., Pasta, C., Belvedere, G., Schadt, I., La Terra, F., Licitra, G., & Carpino, S.
540 (2013). Variability of volatile profiles in milk from the PDO Ragusano cheese
541 production zone. *Dairy Science & Technology*, 93(2), 117-134.

542 Resconi, V. C., Escudero, A., & Campo, M. M. (2013). The development of aromas in ruminant
543 meat. *Molecules*, 18(6), 6748-6781.

544 Toral, P. G., Hervás, G., Della Badia, A., Gervais, R., & Frutos, P. (2020). Effect of dietary
545 lipids and other nutrients on milk odd-and branched-chain fatty acid composition in
546 dairy ewes. *Journal of Dairy Science*, *103*(12), 11413-11423.

547 Vahmani, P., Ponnampalam, E. N., Kraft, J., Mapiye, C., Bermingham, E. N., Watkins, P. J.,
548 ... & Dugan, M. E. (2020). Bioactivity and health effects of ruminant meat lipids.
549 Invited Review. *Meat Science*, *165*, 108114.

550 Valenti, B., Campidonico, L., Natalello, A., Lanza, M., Salami, S. A., Priolo, A., ... & Luciano,
551 G. (2021). Fatty acid metabolism in lambs supplemented with different condensed and
552 hydrolysable tannin extracts. *Plos One*, *16*(10), e0258265.

553 Vasta, V., Aouadi, D., Brogna, D. M., Scerra, M., Luciano, G., Priolo, A., & Salem, H. B.
554 (2013). Effect of the dietary supplementation of essential oils from rosemary and
555 artemisia on muscle fatty acids and volatile compound profiles in Barbarine
556 lambs. *Meat Science*, *95*(2), 235-241.

557 Wojnowski, W., Majchrzak, T., Dymerski, T., Gębicki, J., & Namieśnik, J. (2017). Electronic
558 noses: Powerful tools in meat quality assessment. *Meat Science*, *131*, 119-131.

559 Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., ... &
560 Whittington, F. M. (2008). Fat deposition, fatty acid composition and meat quality: A
561 review. *Meat Science*, *78*(4), 343-358.

562 Yuan, G. F., Chen, X. E., & Li, D. (2014). Conjugated linolenic acids and their bioactivities: a
563 review. *Food & Function*, *5*(7), 1360-1368.

564

Table 1. Chemical composition and antioxidant capacity of whole pomegranate by-product and experimental diets.

	Whole pomegranate by-product	Experimental diet ^a	
		CON	WPB
Chemical composition, g/100 g DM			
Dry matter (DM), g/100 as fed	90.0	88.7	89.2
Crude Protein	6.52	17.6	17.8
NDF ^b	28.8	23.3	26.3
ADF ^b	20.7	12.9	15.5
ADL ^b	5.52	2.98	2.70
Ash	3.52	5.87	4.40
Crude Fat	3.99	2.11	2.51
Individual fatty acids, g/kg DM			
C16:0	1.52	2.29	2.16
C18:0	0.64	0.31	0.33
C18:1 <i>c</i> 9	2.08	2.32	1.93
C18:2 <i>c</i> 9 <i>c</i> 12	2.31	5.55	5.03
C18:3 <i>c</i> 9 <i>c</i> 12 <i>c</i> 15	0.17	0.67	0.79
C18:3 <i>c</i> 9 <i>t</i> 11 <i>c</i> 13	19.20	-	1.72
C18:3 <i>c</i> 9 <i>t</i> 11 <i>t</i> 13	0.69	-	0.16
C18:3 <i>t</i> 9 <i>t</i> 11 <i>c</i> 13	1.12	-	0.21
C18:3 <i>t</i> 9 <i>t</i> 11 <i>t</i> 13	0.75	-	0.10
Phenolic compounds, g/100g DM			
Total phenols ^c	9.51	0.30	1.89
Total tannins ^c	9.34	0.14	1.70
Condensed tannins ^d	0.80	0.10	0.20
Tocopherols, mg/kg DM			
γ -Tocopherol	11.1	0.74	2.04
α -Tocopherol	48.3	7.82	16.8
Antioxidant capacity (ORAC), μ mol TE/g DM ^e			
Hydrophilic fraction	684	103	342
Lipophilic fraction	27.3	21.1	31.1

^a CON: control barley-corn based concentrate diet. WPB: diet including 20% of whole pomegranate by-product.

^b NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin.

^c Expressed as tannic acid equivalents.

^d Expressed as taxifolin equivalents.

^e ORAC: oxygen radical absorbance capacity; TE: trolox equivalents.

Table 2. Effect of the dietary treatment on muscle fatty acids (mg/100 g of muscle).

	Dietary treatment ^a		SEM ^b	P-value
	CON	WPB		
Intramuscular fat (g/100 g)	1.88	2.01	0.156	0.690
C10:0	2.39	2.64	0.355	0.735
C12:0	1.77	1.63	0.250	0.799
C14:0	34.0	31.7	4.236	0.795
C14:1 <i>c</i> 9	0.98	0.94	0.136	0.889
C15:0	6.29	3.76	0.659	0.058
C15:0 <i>iso</i>	1.08	0.78	0.104	0.172
C15:0 <i>anteiso</i>	1.57	1.03	0.160	0.103
C16:0	349	335	34.61	0.850
C16:0 <i>iso</i>	1.93	1.93	0.197	0.987
C16:1 <i>c</i> 7	3.90	3.46	0.342	0.543
C16:1 <i>c</i> 9	20.5	17.9	1.885	0.530
C17:0	27.0	14.6	2.953	0.036
C17:0 <i>iso</i>	5.27	4.61	0.418	0.456
C17:0 <i>anteiso</i>	8.89	5.15	0.858	0.029
C17:1 <i>c</i> 9	14.5	6.96	1.533	0.011
C18:0	250	253	25.90	0.946
C18:1 <i>c</i> 6	5.27	7.07	0.746	0.253
C18:1 <i>c</i> 9	564	523	48.86	0.693
C18:1 <i>c</i> 11	16.9	13.5	1.100	0.138
C18:1 <i>c</i> 12	6.76	6.74	0.825	0.992
C18:1 <i>c</i> 13	1.53	1.55	0.146	0.940
C18:1 <i>c</i> 14	2.13	3.16	0.291	0.086
C18:1 <i>t</i> 5	0.88	0.42	0.125	0.074
C18:1 <i>t</i> 6 + <i>t</i> 7 + <i>t</i> 8	2.41	1.88	0.258	0.329
C18:1 <i>t</i> 9	4.49	5.20	0.457	0.463
C18:1 <i>t</i> 10	17.8	6.01	1.963	0.001
C18:1 <i>t</i> 11	10.8	20.8	2.547	0.050
C18:2 <i>c</i> 9 <i>c</i> 12	80.5	99.9	6.750	0.169
C18:2 <i>c</i> 9 <i>t</i> 11	5.18	13.78	1.530	0.003
C18:3 <i>c</i> 6 <i>c</i> 9 <i>c</i> 12	0.95	1.06	0.073	0.468
C18:3 <i>c</i> 9 <i>c</i> 12 <i>c</i> 15	5.59	7.35	0.602	0.160
C18:3 <i>c</i> 9 <i>t</i> 11 <i>c</i> 13 (PA)	n.d.	6.34	1.031	-
C18:3 <i>c</i> 9 <i>t</i> 11 <i>t</i> 13 (α -ESO)	n.d.	0.92	0.128	-
C18:3 <i>t</i> 9 <i>t</i> 11 <i>c</i> 13 (CA)	n.d.	0.48	0.076	-
C18:3 <i>t</i> 9 <i>t</i> 11 <i>t</i> 13 (β -ESO)	n.d.	0.07	0.018	-
C20:0	1.87	1.94	0.223	0.879
C20:1 <i>c</i> 11	2.03	1.98	0.150	0.876
C20:2 <i>n</i> -6	0.89	1.12	0.099	0.273

C20:3 <i>n</i> -6	2.23	3.22	0.233	0.034
C20:4 <i>n</i> -6	19.9	25.3	2.187	0.245
C20:5 <i>n</i> -3	1.85	2.61	0.203	0.065
C22:0	0.37	0.44	0.035	0.371
C22:4 <i>n</i> -6	2.27	2.39	0.169	0.737
C22:5 <i>n</i> -6	0.65	0.72	0.061	0.569
C22:5 <i>n</i> -3	3.78	4.89	0.379	0.160
C22:6 <i>n</i> -3	1.25	1.46	0.160	0.550
SFA ^c	639	627	63.70	0.928
MUFA ^c	662	615	56.64	0.702
PUFA ^c	130	180	12.11	0.041
OBCFA ^c	70.7	42.1	6.834	0.037
<i>n</i> -3 PUFA	12.7	16.5	1.101	0.091
<i>n</i> -6 PUFA	108	134	8.555	0.137
<i>n</i> -6/ <i>n</i> -3 PUFA	8.53	8.20	0.138	0.247
PUFA/SFA	0.22	0.32	0.025	0.051
HP-PUFA ^d	38.7	57.0	0.041	0.024
Peroxidability index ^e	207	285	0.191	0.042

^a CON: control barley-corn based concentrate diet. WPB: diet including 20% of whole pomegranate by-product.

^b SEM, standard error of the mean.

^c SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; OBCFA, odd- and branched-chain fatty acids.

^d Highly peroxidizable-PUFA, calculated as the sum of PUFA with three or more unsaturated bonds.

^e Calculated according to Valenti et al. (2019). Peroxidability index = (Σ dienoic \times 1) + (Σ trienoic \times 2) + (Σ tetraenoic \times 3) + (Σ pentaenoic \times 4) + (Σ hexaenoic \times 5).

Table 3. Effect of the dietary treatment on the volatile compounds of cooked lamb (ng/g of cooked meat).

Compound	LRI ^a	Ions used ^b	Method of identification ^c	Dietary treatment ^d		SEM ^e	P-value
				CON	WPB		
<i>Aldehydes</i>							
2-methyl Butanal		39, 41, 57	MS, Std	0.50	0.48	0.072	0.657
3-methyl Butanal		41, 43, 58	MS, Std	0.44	0.64	0.096	0.180
Pentanal	704	43, 44, 58	MS, Std, LRI	2.63	15.40	2.215	<0.001
(E)-2-Hexenal	854	39, 41, 55	MS, Std, LRI	0.10	0.49	0.085	0.009
Hexanal	804	39, 41, 56	MS, Std, LRI	30.67	56.91	5.610	0.021
(Z)-4-Heptenal	901	41, 55, 67	MS, Std, LRI	0.09	0.16	0.027	0.158
Heptanal	903	39, 41, 70	MS, Std, LRI	9.30	34.25	4.462	0.001
Methional	907	48, 104, 47	MS, Std, LRI	0.30	0.33	0.040	0.656
Octanal	1003	41, 67, 69	MS, Std, LRI	11.57	24.46	3.117	0.022
(E,E)-2,4-Heptadienal	1013	81, 53	MS, Std, LRI	0.06	0.19	0.031	0.004
(E)-2-Octenal	1058	39, 55, 83	MS, Std, LRI	1.14	3.84	0.620	0.017
4-Nonenal	1097	41, 39, 55	MS	0.21	0.85	0.138	0.002
Nonanal	1105	69, 81, 57	MS, Std, LRI	22.87	64.22	8.887	0.006
(E,Z)-2,6-Nonadienal	1153	41, 69, 70	MS, Std, LRI	0.06	0.12	0.020	0.067
(E)-2-Nonenal	1160	43, 55, 70	MS, Std, LRI	1.25	10.84	2.027	<0.001
Decanal	1205	41, 67, 55	MS, Std, LRI	5.47	7.87	0.977	0.320
(E,E)-2,4-Nonadienal	1215	81, 39, 41	MS, LRI	0.09	9.55	2.188	<0.001
(E)-2-Decenal	1261	39, 81, 55	MS, Std, LRI	1.01	3.76	0.627	0.011
Undecanal	1306	41, 67, 81	MS, Std, LRI	0.77	1.37	0.137	0.027
(E,E)-2,4-Decadienal	1317	81, 67	MS, Std, LRI	0.44	1.41	0.237	0.014
(E)-2-Undecenal	1364	41, 70, 55	MS, LRI	2.03	3.71	0.467	0.022
Dodecanal	1408	67, 41, 81	MS, Std, LRI	0.72	1.34	0.132	0.007
Tridecanal	1510	67, 41, 81	MS, LRI	0.51	1.19	0.166	0.007
Tetradecanal	1612	67, 41, 81	MS, LRI	1.47	2.79	0.302	0.004
Pentadecanal	1714	67, 41, 81	MS, LRI	1.70	3.08	0.340	0.011
Hexadecanal	1816	67, 41, 81	MS, LRI	2.12	3.38	0.316	0.059
<i>Alcohols</i>							
1-Pentanol	766	41, 55, 70	MS, Std, LRI	1.43	2.47	0.267	0.076
1-Hexanol	872	56, 41, 39	MS, Std, LRI	1.26	3.93	0.457	<0.001
2-Heptanol	907	45, 27, 55	MS, Std, LRI	0.14	0.13	0.024	0.863
1-Heptanol	973	41, 55, 70	MS, Std, LRI	1.74	3.57	0.448	0.033
1-Octen-3-ol	982	57, 69, 43	MS, Std, LRI	4.73	12.08	1.660	0.016
2-Ethyl-1-hexanol	1030	57, 41, 55	MS, Std, LRI	5.59	6.32	0.336	0.363
4-Ethylcyclohexanol	1036	81, 43, 57	MS	0.06	0.10	0.012	0.046
2-Octen-1-ol	1069	41, 57, 67	MS, Std, LRI	0.82	1.82	0.253	0.026
1-Octanol	1073	69, 41, 55	MS, Std, LRI	3.49	6.99	0.817	0.018
α -Terpineol	1194	93, 59, 121	MS, Std, LRI	0.25	0.32	0.016	0.084
1-Pentadecanol	1779	69, 83, 97	MS, Std, LRI	0.03	0.05	0.005	0.042
<i>Hydrocarbons</i>							

3-methyl-1-Heptene	783	41, 45, 56	MS, LRI	0.03	0.07	0.009	0.004
Nonane	901	43, 57, 71	MS, LRI	0.06	0.06	0.009	0.482
Dodecane	1200	57, 41, 71	MS, Std, LRI	0.23	0.37	0.039	0.030
Tridecane	1300	57, 71, 41	MS, Std, LRI	0.79	1.20	0.108	0.056
Tetradecane	1400	57, 71, 41	MS, Std, LRI	1.12	1.55	0.121	0.073
Pentadecane	1500	57, 71, 41	MS, Std, LRI	0.91	1.19	0.086	0.106
Hexadecane	1600	57, 71, 41	MS, Std, LRI	0.30	0.38	0.022	0.056
Octadecane	1800	57, 71, 85	MS, Std, LRI	0.16	0.36	0.045	0.016
<i>Ketones</i>							
2,3-Butanedione		43	MS, Std	1.37	1.40	0.167	0.979
2-Pentanone		43, 86, 71	MS, Std	0.67	0.20	0.091	0.003
2-Heptanone	890	43, 58	MS, Std, LRI	0.67	1.34	0.161	0.029
1-Octen-3-one	977	55, 70, 27	MS, LRI	0.56	1.50	0.222	0.025
6-Methyl-5-hepten-2-one	986	43, 59, 41	MS, LRI	2.94	8.38	1.554	0.050
2,3-Octanedione	986	43, 30, 41	MS, LRI	4.08	12.47	2.065	0.022
2-Nonanone	1090	43, 58	MS, Std, LRI	0.12	0.17	0.013	0.026
<i>Sulphur compounds</i>							
Dimethyl sulphide		62, 47, 45	MS, Std	0.04	0.03	0.003	0.854
Carbon disulphide		76, 44, 78	MS	2.65	2.51	0.215	0.564
Dimethyl disulphide	739	94, 79	MS, Std, LRI	0.01	0.01	0.002	0.100
Dimethyl trisulfide	967	126, 45, 79	MS, Std, LRI	1.17	1.59	0.135	0.071
Hexathiane	2038	64, 128, 192	MS	2.35	3.34	0.308	0.073
<i>Benzenoid compounds</i>							
Toluene	762	91, 92	MS, Std, LRI	1.96	2.11	0.127	0.517
Ethylbenzene	858	91, 106, 65	MS, LRI	0.29	0.26	0.044	0.917
Benzaldehyde	960	105, 77, 106	MS, Std, LRI	29.64	34.81	1.739	0.102
Benzeneacetaldehyde	1042	91, 92	MS, Std, LRI	0.87	1.13	0.077	0.079
<i>Phenols</i>							
p-Cresol	1076	107, 108	MS, Std, LRI	0.25	0.17	0.086	0.335
2-Isopropylphenol	1194	121, 136	MS, Std, LRI	0.15	0.18	0.009	0.062
2,4-Di-tert-butylphenol	1506	191, 57	MS, LRI	0.75	0.74	0.046	0.741
<i>Lactones</i>							
γ -Octalactone	1254	85, 57	MS, Std, LRI	0.02	0.04	0.005	<0.001
γ -Nonalactone	1359	85, 29	MS, Std, LRI	0.07	0.10	0.015	0.271
<i>Terpenes</i>							
p-Cymene	1023	119, 91	MS, Std, LRI	0.03	0.03	0.002	0.688
Limonene	1028	67, 68, 93	MS, Std, LRI	0.50	1.28	0.199	0.050
<i>BCFAs</i>							
4-Methyl octanoic acid	1236	57, 55, 73	MS, Std, LRI	0.49	0.54	0.034	0.251
4-Ethyl octanoic acid	1316	55, 57, 71	MS, Std, LRI	0.05	0.08	0.025	0.428
<i>Pyrazines</i>							
2,5-Dimethyl pyrazine	915	42, 108	MS, Std, LRI	0.33	0.28	0.050	0.763
2-Ethyl-3,6-dimethyl-pyrazine	1082	135, 136	MS, Std, LRI	0.74	0.73	0.095	0.889
<i>Esters</i>							

Propanoic acid, 2-methyl-, 2-ethyl-3hydroxyhexyl ester	1371	43, 41, 71	MS, LRI	0.49	0.82	0.166	0.307
Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	1586	43, 71, 28	MS, LRI	8.45	7.38	0.849	0.640
<i>Furan</i>							
2-Pentyl-furan	989	81, 138, 53	MS, Std, LRI	1.47	5.50	0.897	0.005
<i>Pyridine</i>							
2-Pentyl-pyridine	1194	93, 106, 120	MS, Std, LRI	0.10	0.10	0.017	0.148
<i>Thiazole</i>							
2-Acetyl-2-thiazoline	1101	43, 129, 60	MS, LRI	2.05	1.50	0.154	0.086

^a Linear retention indices (LRI)

^b Specific ions used for identification and peak area integration

^c Method of identification: NIST, NIST library; Std, authentic standard; LRI, linear retention indices

^d CON: control barley-corn based concentrate diet. WPB: diet including 20% of whole pomegranate by-product

^e SEM, standard error of the mean

Table 4. Effect of the dietary treatment on the sensory parameters of cooked lamb.

	Dietary treatment ^a		SEM ^b	P-value
	CON	WPB		
Tenderness	5.23	5.64	0.117	0.081
Juiciness	5.61	6.00	0.115	0.094
Sheep flavour	5.26	5.21	0.121	0.852
Abnormal flavour	0.74	0.67	0.079	0.639
Flavour liking	5.90	6.12	0.116	0.355
Overall liking	6.35	6.55	0.119	0.431

^a CON: control barley-corn based concentrate diet. WPB: diet including 20% of whole pomegranate by-product.

^b SEM, standard error of the mean.

Table 5. Pearson correlation coefficient (*r*) between selected fatty acids and volatile compounds in cooked lamb.

	C18:0	C18:1 <i>t</i> 10	C18:1 <i>t</i> 11	C18:1 <i>c</i> 9	C18:2 <i>c</i> 9 <i>c</i> 12	C18:3 <i>c</i> 9 <i>c</i> 12 <i>c</i> 15	C18:2 <i>c</i> 9 <i>t</i> 11	C18:3 <i>c</i> 9 <i>t</i> 11 <i>c</i> 13	C18:3 <i>c</i> 9 <i>t</i> 11 <i>t</i> 13	C18:3 <i>t</i> 9 <i>t</i> 11 <i>c</i> 13
2-methyl Butanal	-0.042	-0.071	-0.032	-0.180	-0.410	-0.208	-0.043	0.155	0.142	0.086
3-methyl Butanal	-0.112	-0.258	-0.064	-0.197	0.016	0.032	0.066	0.241	0.254	0.216
Pentanal	0.027	-0.453	0.381	0.034	0.349	0.415	0.578*	0.763**	0.777**	0.733**
(E)-2-Hexenal	0.068	-0.302	0.320	0.145	0.383	0.445	0.515*	0.713**	0.686**	0.687**
Hexanal	0.053	-0.336	0.260	0.104	0.319	0.393	0.425	0.648**	0.610**	0.607**
Heptanal	0.034	-0.426	0.386	0.061	0.369	0.421	0.566*	0.735**	0.740**	0.709**
Methional	-0.389	-0.025	-0.171	-0.478*	-0.362	-0.392	-0.183	-0.153	-0.088	-0.120
Octanal	0.152	-0.286	0.328	0.215	0.414	0.494*	0.476*	0.676**	0.617**	0.637**
(E)-2-Octenal	0.091	-0.285	0.302	0.181	0.394	0.471*	0.484*	0.690**	0.645**	0.655**
Nonanal	0.192	-0.353	0.407	0.226	0.457	0.542*	0.566*	0.761**	0.707**	0.729**
(E)-2-Nonenal	0.092	-0.421	0.462	0.118	0.429	0.489*	0.645**	0.799**	0.796**	0.777**
(E,E)-2,4-Nonadienal	0.061	-0.563*	0.473*	0.046	0.439	0.475*	0.687**	0.818**	0.863**	0.811**
1-Hexanol	0.153	-0.404	0.512*	0.158	0.468	0.517*	0.684**	0.800**	0.813**	0.783**
1-Octanol	0.212	-0.229	0.361	0.285	0.462	0.550*	0.516*	0.708**	0.650**	0.675**
2-Pentanone	-0.199	0.550*	-0.480*	-0.074	-0.408	-0.370	-0.581*	-0.535*	-0.571*	-0.579*

* = Correlation is significant at the 0.05 level ($P < 0.05$).

** = Correlation is significant at the 0.01 level ($P < 0.01$).

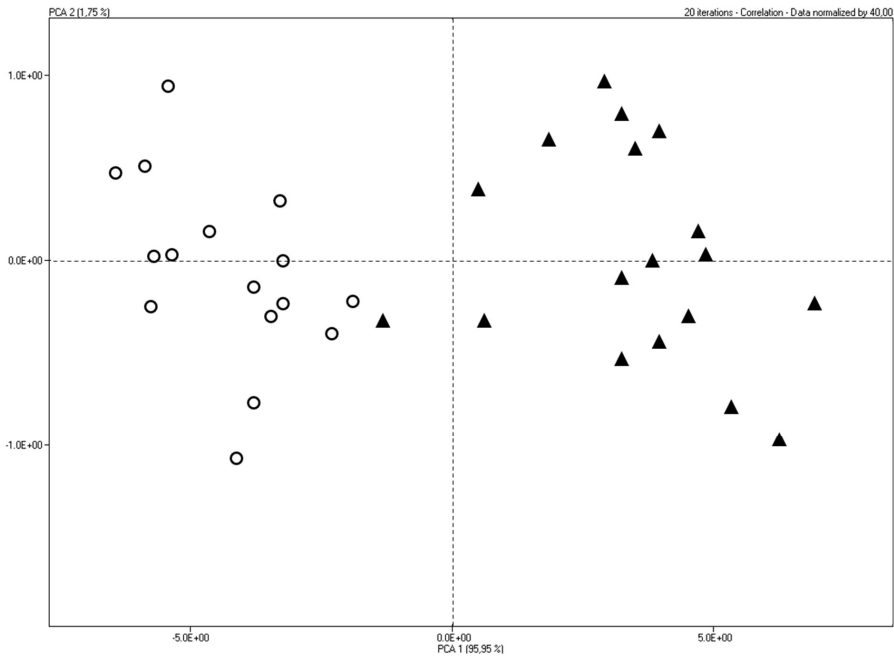


Figure 1. Principal component analysis (PCA) of the effect of dietary treatment from SMart Nose analysis of cooked lamb (analysed in duplicate). The treatments were: CON (control barley-corn based concentrate diet; empty circles) and WPB (diet including 20% of whole pomegranate by-product; solid triangles).