Effects of sous vide vs grilling methods on lamb meat colour and lipid stability during cooking and heated display

Effects of *sous vide vs* grilling methods on lamb meat colour and lipid stability during cooking and heated display

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ABSTRACT

The present study compared the effect of grilling (150°C until 72°C core temperature) and *sous-vide* (SV) cooking (75°C for 35 min in a water bath under vacuum) on lamb patties immediately after cooking and after 4 h display at 65°C. Both methods produced patties with similar (P>0.05) weight loss, and moisture and fat contents. SV-cooking prevented (P<0.05) the formation of thiobarbituric acid reactive substances (TBARS) and oxysterols compared to grilled patties, which showed a larger proportion of highly peroxidisable polyunsaturated fatty acids. Heated display induced dehydration, surface darkening and a reduction in the hexanal/3-methylbutanal ratio, suggesting the progression of Maillard reactions. Moreover, TBARS and some lipid oxidation-derived volatiles increased (P<0.001), while cooked-meat aroma compounds were reduced (P<0.001). SV-cooking inhibited (P<0.05) the formation of malondialdehyde, and 7α- and 7β-hydroxycholesterol, and lowered the cholesterol oxidation ratio during heated display. Overall, SV-cooking may be considered a healthier way of cooking when lamb meat is to be kept warm for considerable periods before consumption.

Keywords

Sous vide cooking, grilling, catering, lipid oxidation, cholesterol oxidation, volatile compounds

1. INTRODUCTION

Modern lifestyles promote food consumption outside the home, and catering represents a relevant subsector in this market (Calderón et al., 2018). Catering offers a broad range of services, including schools, hospitals, hotel buffets, restaurants, take-aways and in-flight meals, thus covering a considerable share of the daily food intake for a large part of the population. Therefore, the nutritional quality of food offered by catering facilities is gaining increasing attention. Traditional catering methods are based on 'making-to-order,' whereby there is no delay between cooking and food consumption, or bulk production, when food may be held warm for considerable periods before being served, as in hospitals and canteens (Smith & West, 2003). In the latter case, according to good hygiene practices, it is recommended that food prepared in advance is kept at a core temperature above 65°C until consumption (UK Food Safety Agency, 2016; Spanish Royal Decree 3484/2000), which may in fact represent a prolonged mild cooking treatment. Although recommendations suggest that this period should be as short as possible to avoid sensory and nutritional losses, heated display times can be extended for a few hours depending on the service. While meat cooking enhances its flavour and improves tenderness, it may result in excessive desiccation and lipid oxidation (Grau et al., 2001), processes that subsequent hot display and exposure to air may aggravate, leading to undesirable odours, rancidity, texture modification, loss of essential fatty acids (FAs) or the formation of toxic compounds, including oxysterols and carbonyl compounds (Domínguez et al., 2014). High plasma concentration of secondary lipid and cholesterol oxidation products (COPs) in humans have been linked to cytotoxic, mutagenic and carcinogenic effects, and are considered to be involved in neurodegenerative disorders, and to act as a primary factor in triggering atherosclerosis (Malaguti et al., 2019; Sousa et al., 2017). Therefore, the use of any strategy able to prevent oxidation in cooked/hot-displayed meat is highly recommended to improve its sensory traits, nutritional value and safety.

Meat cooking conditions strongly determine the resulting transformations that occur in muscle. Prolonged cooking times and temperatures above 140°C promote Maillard reactions, crucial for the development of volatile organic compounds (VOCs), related to the well-appreciated roasty and meaty flavours, and the toasted colour of meat (Mottram, 1998). Nevertheless, higher cooking temperatures are also associated with increased lipid and cholesterol oxidation and the reduction of essential FAs when compared with milder treatments (Rasinska et al., 2019; Rodriguez-Estrada et al., 1997; Sabolová et al., 2017). The traditional methods used for meat cooking, such as grilling or pan-frying, involve high temperatures in an aerobic environment during a limited time to enhance flavour development, while preventing juice losses that may damage the meat texture (Bejerholm & Aaslyng, 2004). *Sous-vide* (SV) is a culinary technique that involves the vacuum-sealing of food inside a plastic bag before cooking at a mild, controlled temperature for a comparatively long time. In Spain, SV has become one of the most common methods for preparing lamb meat in the catering industry, using temperatures of 75-85°C to ensure internal pasteurisation at 65–75°C (Roldán et al., 2015). The SV method enables juicy, tender and flavourful products to be obtained, whereas the removal of air may prevent excessive oxidation in the cooked/hot-displayed meat without need for antioxidants. However, to our knowledge, there is limited information regarding the quality benefits of SV compared with traditional cooking in catering-like systems involving heated display. For this reason, the present work aimed to assess the effects of grilling (G) and *sous-vide* (SV) cooking on the oxidative stability (lean colour, malondialdehyde, COP and VOC formation and FA retention) of cooked/hot-displayed lamb meat.

2. Materials and methods

2.1 Meat sampling and preparation

For the analysis, meat from ten *Segureño* lambs (N=10) was obtained from a local abattoir. After carcass ageing at 2°C for 48 h, the right lamb legs were removed, deboned by a professional butcher,

vacuum packed, frozen and stored at -20°C in darkness until processing. The frozen legs were brought to -5°C and minced in an atmospheric mincer using a 3-mm plate (Mainca PM98, Barcelona, Spain) after the removal of the external fat. Salt (2 g NaCl per 100 g of minced meat) was added and aerobically mixed. From each deboned leg (ca. 1000 g), about forty 25-g patties were prepared and randomly allocated to the different cooking groups: (i) grilling was carried out in a double-sided griddle (Media Liscia, Silanos, Milan, Italy) at 150°C until the meat reached a core temperature of 72°C for 20 s; and (ii) *sous-vide* patties were vacuum-packed in polyamide-polypropylene pouches (200 x 295 mm, thermal resistance -40°C/120°C, oxygen permeability 7 cm^3/m^2 for 24 h at 4°C and 80% R.H., and water steam permeability 0.8 g/m^2 for 24 h) (Wipack, Hamburg, Germany) and cooked at 75°C for 35 min in a water bath to reach a similar final inner temperature. The meat temperature was monitored with a portable T200 thermometer (Digitron Instrumentation Limited, Hertfordshire, UK). Patties were then randomly split into two subsets, one composed of freshly-cooked patties (G0/SV0) and the other of freshly-cooked patties placed on individual covered glass plates and held at 65°C, 800 lux and 80-85% R.H. for 4 h in a climatic cabinet Climacell 404L (MMM Medcenter Einrichtungen GmbH, München, Germany) to simulate catering conditions (G4/SV4). In both cases, patties were allowed to cool at room temperature for 30 min before colour evaluation and further stored at -80°C until lipid and cholesterol oxidation analysis.

2.2 Weight losses, moisture content and CIEL*a*b* colour analysis

The difference in patty weight before and after cooking, and after cooking and catering display was calculated with the following formula: Weight loss (%) = $((W_i - W_0)/W_i) \times 100$; where W_i is the weight of the patty before cooking/immediately after cooking; and W_0 is the weight of the patty immediately after cooking/after 4 h of the heated display. Moisture content was determined by dehydrating the samples (5 g) at 102°C (AOAC method 930.15; 2000). Colour before and after hot display was measured using a CR-200/08 Chroma Meter II (Minolta Ltd., Milton Keynes, UK) with illuminant D65, 2° observer angle, 50-mm aperture size and calibrated against a standard white tile. Reflectance

99 measurements were taken directly on the patty surface. Nine replicate measurements were taken for
100 each sample (three measurements of three different patties). Lightness (L*), redness (a*) and
101 yellowness (b*) were determined and expressed as CIEL*a*b* units.

102 **2.3 Lipid extraction**

103 Lipids were extracted from the patties following a modified version of the Folch method (Boselli et al.,
104 2005). Briefly, the lipid fraction was extracted using a chloroform:methanol solution (1:1, v/v) followed
105 by the addition of another aliquot of chloroform. After mixing with 1 M KCl, the organic phase was
106 separated and taken to dryness; the fat content was determined gravimetrically. Two independent
107 replicate measurements were made for each sample.

108 **2.4 Determination of TBARS, cholesterol and oxysterols (COPs)**

109 Secondary lipid oxidation was assessed in the patties as thiobarbituric acid reactive substances
110 (TBARS) (mg MDA/kg meat) according to Botsoglou et al. (1994). Sample absorbance was measured
111 at 532 nm using a UV2 (Pye Unicam, Cambridge, UK) spectrophotometer and a malondialdehyde
112 (MDA) standard calibration curve ranging from 0.1 to 10 μM ($y= 0.148x + 0.007$; $R^2=0.999$).

113 Cholesterol and COPs were extracted and purified as described previously by Cardenia et al. (2015).
114 Briefly, the lipid extract containing internal standards (142.8 μg of betulinol (Sigma Chemical, St. Louis,
115 USA) and 12.8 μg of 19-hydroxycholesterol (Steraloids, Newport, Rhode Island, USA) for cholesterol
116 and COPs, respectively) were subjected to cold saponification. One-tenth of the unsaponifiable matter
117 was used to determine the sterol composition, while the remaining 9/10 were purified by SPE-NH_2 for
118 COPs quantification. Both cholesterol and COPs fractions were silylated by adding 1 mL of
119 pyridine:hexamethyldisilazane:trimethylchlorosilane (5:2:1, v/v/v), left to stand at 40°C for 20 min,
120 and then taken to dryness under a nitrogen stream, re-dissolved in *n*-hexane and injected into a Fast
121 gas-chromatograph/mass-spectrometer (GC/MS) (Inchingolo et al., 2014). Cholesterol and COPs were

identified and quantified by comparing their mass spectra and retention times with those of the corresponding chemical standards (Sigma Chemical; Steraloids (Newport, Rhode Island, USA); Avanti Polar Lipids (Alabaster, Alabama, USA)) in the SIM acquisition mode, using calibration curves built for each chemical compound. Cholesterol and COPs were expressed as g/100 g fat and mg/100 g fat, respectively. The proportion of total cholesterol oxidation (%OR) was also determined according to the following formula: %OR = (Total COPs/Total cholesterol) x 100 (Cardenia et al., 2015). Two independent replicate measurements were made for each sample.

2.5 Determination of total FA

FA methyl esters were identified and determined from the extracted lipids as described in Cardenia et al. (2015). About 20 mg of lipid extract were treated with 200 µL of diazomethane, before adding undecanoate methyl ester (CAS 1731-86-8, Sigma-Aldrich) (internal standard, 1 mg/mL) and transmethylated with 40 µL of 2 N KOH in methanol. A 6890N series gas chromatograph (Agilent Technologies, Madrid, Spain) coupled to a flame ionisation detector (GC-FID), an Agilent HP-88 capillary column (60 m x 250 µm x 0.2 µm), an He flow rate of 1.4 mL/min and 1:1 split, were used. The sample injection volume was 1 µL. The working temperatures of the injector and FID detector were 250°C and 260°C, respectively. The oven temperature ramp used was: 125°C initial temperature; 125°C to 145°C at 8°C/min; 145°C for 26 min; 145°C to 220°C at 2°C/min; and 220°C for 1 min. The two standard mixes used were: (i) FAME mix C4–C24 (Supelco, Bellefonte, PA, USA); and (ii) Nº 05632 linoleic acid methyl ester, *cis*/*trans*-isomers mix (Sigma-Aldrich). The results were expressed as relative abundance (g FA/100 FA).

The peroxidation index (PI) of polyunsaturated fatty acids (PUFA) was calculated according to Luciano et al. (2013) using the following equation:

PI = (%dienoic × 1) + (%trienoic × 2) + (%tetraenoic × 3) + (%pentaenoic × 4) + (%hexaenoic × 5)

145 **2.6 Determination of volatile organic compounds (VOCs)**

146 VOCs were determined by headspace-solid phase microextraction (HS–SPME) using an SPME device
147 (Supelco Co., Bellefonte, PA, USA) containing a fibre coated with carboxen-poly(dimethylsiloxane)-
148 divinylbenzene (CAR-PDMS-DVB) (50/30 µm thickness). Prior to analysis, the SPME fibre was
149 preconditioned at 270°C for 60 min in the GC injection port. Semi-frozen muscle samples were ground
150 with a commercial grinder for 20 s with 20 µL of an aqueous solution of 600 mg/L cyclohexanone as
151 internal standard, used to rule out any problems during the sample preparation and injection steps
152 (Rivas-Cañedo et al., 2013). Four grams of meat were weighed into a 20 mL screw-capped amber vial.
153 Sample vials were flushed with helium (99.9%) for 5 s at 275 kPa to minimise lipid oxidation and
154 volatile production by residual oxygen. In order to achieve equilibrium between each sample and its
155 headspace before HS-SPME extraction, vials were kept in a water bath for 10 min at 40°C. The
156 extractions were performed in an autosampler with a water bath thermostated at 40°C for 45 min and
157 with continuous stirring (250 rpm) (Gerstel MPS 2XL; Gerstel, Mülheim an der Ruhr, Germany).
158 Analyses were performed using an Agilent 7890A series gas chromatograph (Agilent, Avondale, AZ,
159 USA) coupled to an IonTrap GC-MS (Agilent). Analytes were separated using a VF-WAXMS (30 m x 0.25
160 mm i.d. x 0.5 µm f.t.) column operating at 45 kPa of column head pressure, resulting in a flow of 1
161 mL/min at 40°C. For analyte separation and identification, the SPME fibre was desorbed in the
162 injection port at 250°C for 5 min. To improve the recovery of the highly volatile components, the
163 injection port was operated in splitless mode for the initial 0.6 min, after which a 50:1 split ratio was
164 established. The initial oven temperature programme was 40°C, which was raised to 130°C at a rate
165 of 2.5°C/min and then raised to 200°C at a rate of 15°C/min, and maintained at this temperature for
166 5 min. The transfer line to the mass spectrometer was held at 280°C. The mass spectra were obtained
167 by electronic impact at 70 eV and a multiplier voltage of 1756 V, collecting data at a rate of 3 scans/s
168 over the *m/z* range 30–300. For identification of the volatile components, *n*-alkanes (Sigma R-8769)
169 (C5-C20) were analysed under the same conditions to calculate the retention indices (RI). Individual

peaks were identified by comparison of their retention indices and mass spectra with those obtained from commercial reference compounds (Acrōs Organics – Geel, Belgium; Sigma-Aldrich – Steinhein, Germany). At the same time, mass spectra from the NIST/EPA/NIH Mass Spectral Database (NIST 11, National Institute of Standards and Technology, Gaithersburg, USA) were used to verify identification. The results are expressed as mean abundance values multiplied by 10^{-5}. Two independent replicate measurements for each sample were made.

2.7 Statistical analysis

The normal distribution of the data was tested (P>0.05) using the Shapiro−Wilk method. The results were reported as the mean and standard error of the mean (SEM). An analysis of variance (Repeated Measures Model) was used to ascertain the effect of the cooking method and heated display on the dependent variables. When necessary, the degrees of freedom were adjusted among the repeated measures using the Greenhouse and Geisser correction. The least-square differences (LSD) means test was used to compare the least-square means (LSM), which were considered statistically different at P<0.05. A principal component analysis (PCA) was performed to represent overall data variability. Convex hulls were calculated and displayed for each group (G0/SV0/G4/SV4) in the PCA plots. The data were analysed using the IBM SPSS Statistics 25 software (IBM Software Group, Chicago, IL, USA), while the correlation and PCA were run with PAST 4.01 (Paleontological statistics software package, University of Oslo, Norway) (Hammer et al., 2001).

3. Results and discussion

3.1 Weight loss, selected nutrients and instrumental colour parameters

Table 1 shows the effects of cooking/heated display on the weight loss, moisture content, total fat content and instrumental colour of the lamb patties. Weight losses were similar (P>0.05) for the G0 (26.8 g/100g) and SV0 patties (29.1 g/100g), leading to similar relative percentages of moisture and

fat. Further dehydration occurred in both treatments during heated display, the total lipid content of both G4 and SV4 increasing (P<0.05) as a result of the lower moisture content. Cooking juice loss depends on mass transfer during heating, which is directly related with the meat's characteristics, pre-processing and the cooking conditions applied (Domínguez et al., 2014). Previous studies reported similar weight losses, moisture and fat contents in meat patties and sausages cooked at 72°C by conventional procedures (Naveena et al., 2017; Suleman et al., 2020). In contrast, SV cooking (72.5°C x 2.5 h) was seen to reduce juice loss in rabbit meat compared to roasting (180°C x 60 min) and boiling (100°C x 20 min) (Rasinska et al., 2019). Likewise, Modzelewska-Kapituła et al. (2019) recorded a reduction in cooking loss and shear-force in SV-cooked beef compared with steam-cooked meat, although this might be related to the higher internal core temperature of steaming (75°C) than in SV (60°C). Meat cuts which suffer similar levels of cooking loss lead to similar tenderness and juiciness values (Baldwin, 2012). Subsequent heated display for 4 h dried the meat samples, probably due to the sustained denaturation of sarcoplasmic proteins and the parallel shrinkage of myofibrils (Tornberg, 2005).

As regards colour, the SV0 patties had higher (P<0.05) L* and a* values than their G0 counterparts, while the b* values were similar. Subsequent heated display reduced the L* and b* to reach similar (P>0.05) values in both types of patty, whereas the a* value remained higher (P<0.05) in SV4 patties. The denaturation of proteins that occurs above 60°C reduces the typical red colour of meat (provided by myoglobin) and increases surface light reflectance. However, the final lean colour depends on factors such as a dry/moist environment, oxygen access and the endpoint temperature reached. Dry-heat methods, such as grilling, lead to a darker surface colour than moist methods as a result of the greater dehydration and denaturation caused by direct contact with the heating surface. In the case of SV, the moist environment and the limited oxygen availability produced by the vacuum-packaging seems to prevent surface dehydration, myoglobin oxidation and protein denaturation (Naveena et al., 2017). Meat browning causes a reduction in the L*, a*, and b* values as the cooking temperature and

time increase (Bejerholm & Aaslyng, 2004), which would agree with our findings. The further maintenance of the meat at 65°C intensified the darkening process of the meat surface. Similar reductions in L* and b* values, associated with meat surface darkening, have been described previously during prolonged cooking (Kumar et al., 2006), desiccation probably reducing light reflectance and promoting Maillard reactions (Shahidi et al., 2014).

3.2 Lipid and cholesterol oxidation

Figure 1 represents the effects of cooking/heated display on the levels of malondialdehyde (MDA) (Fig. 1a) and total COPs (Fig. 1b) in lamb patties. The G0 patties had similar MDA values to those mentioned previously in lamb patties grilled under comparable conditions (Serrano, Jordán, et al., 2014; Serrano, Ortuño, et al., 2014). However, SV-cooking led to lower (P<0.05) MDA levels than those obtained after grilling. The increase (P<0.05) of the MDA level during heated display was three times smaller in SV cooked patties (from 0.33 to 0.59 mg MDA/kg meat) than in the grilled patties (from 0.48 to 1.26 mg MDA/kg meat). Thus, the suggested threshold of 1 mg MDA/kg meat for the development of off-flavours (Domínguez et al., 2014) was exceeded in the G4 patties. The effects of heating on meat lipid oxidation are well-known: cooking enhances the disruption of cell membranes, promoting contact between PUFA and pro-oxidant compounds and inducing the protein denaturation that leads to the loss of antioxidant enzyme activity (Grau et al., 2001). TBARS development has been described in both grilled meat (Broncano et al., 2009; Serrano, Jordán, et al., 2014) and SV-cooked meat (Rasinska et al., 2019; Roldán et al., 2014), any difference between the methods generally being attributed to both the heat transfer process and the time-temperature combination. For instance, SV rabbit (72.5°C x 2.5 h) presented intermediate MDA values between boiled (100°C x 20 min) and roasted (180°C x 1 h) meat (Rasinska et al., 2019). Likewise, the MDA concentration increased during SV-cooking at different temperatures up to 6 h (Roldán et al., 2014). Nevertheless, in foods in which haem pigments, free ionic iron or salt are present, as in this case, the most critical factor for lipid oxidation processes to occur is considered to be oxygen availability (Ahn & Kim, 1998). Indeed, lower

MDA levels were observed in cooked meat when oxygen availability during cooking was restricted (Andreo et al., 2003). Moreover, Naveena et al. (2017) found that aerobically boiled pork sausages contained double the MDA of that measured in SV-cooked sausages at the same temperature and time after 20 days under similar storage conditions. Our results seem to agree with the above, and the reduced oxygen availability during SV cooking might have partially limited secondary lipid oxidation during cooking and prevented its further development during heated display.

Six COPs were identified in cooked lamb meat (Table 2): 7α-hydroxycholesterol (7α-HC), 7β-hydroxycholesterol (7β-HC), 5α,6α-epoxycholesterol (α-EC), 5β,6β-epoxycholesterol (β-EC), cholestanetriol (CT) and 7-ketocholesterol (7-KC). After cooking, the most abundant oxysterol in both types of sample was β-EC, followed by 7-KC, 7β-HC α-EC, 7α-HC and CT, this COPs relative abundance pattern remaining unchanged after the heated display period. COPs formation seemed to follow a similar trend to TBARS during cooking, since SV-cooking led to lower (P<0.05) levels of β-EC, 7α-HC, 7β-HC and 7-KC than grilling, which was reflected in a lower total COPs content and lower cholesterol oxidation proportion (%OR). Previous studies made in grilled patties made from light lamb leg identified the same COPs at similar concentrations (Morán et al., 2012; Ortuño et al., 2020). Cooking usually favours COPs formation, but their relative composition and accumulation much depend on the initial oxidative status of the meat, the cooking method applied, and the time-temperature conditions used (Hur et al., 2007). Different cooking methods have been seen to yield different levels of COPs in beef (Rodriguez-Estrada et al., 1997), although Broncano et al. (2009) found a similar COPs content whether chicken meat was fried, roasted, grilled or microwaved. However, to our knowledge, this is the first study to compare the effect of SV and another cooking method on COPs formation. The differences found in COPs development might be explained by the lower level of oxygen available under vacuum-cooking conditions, and/or the higher temperature reached by the outer parts of the patties in contact with the grilling plate (Rodriguez-Estrada et al., 2014). Subsequent heated display hardly increased the total COPs content, even though the %OR increased in both G and SV samples.

Indeed, 7α-HC and 7β-HC only increased in the G4 patties, pointing to continued oxysterol formation, unlike in the SV4 patties. Oxysterols in position 7 are formed by a monomolecular mechanism, in which cholesterol hydroperoxides undergo dismutation, generating 7α-HC and 7β-HC, together with 7-KC. Due to its rapid generation and accumulation, 7-KC is often used as a marker of cholesterol oxidation. However, no significant increase in this oxysterol was recorded after heated display, which could be attributed to its dehydration or reaction with an amino group from amino acids, peptides, or proteins to generate Schiff bases (Rodriguez-Estrada et al., 2014). On the other hand, epoxy derivatives, which are formed through a bimolecular reaction mechanism between a hydroperoxyl radical and cholesterol, give rise to CT due to oxirane ring-opening in the presence of H_2O in an acid environment (Rodriguez-Estrada et al., 2014). Only slight amounts of CT were detected in both samples after cooking and heated display, reflecting the usual trend found in meat products (Boselli et al., 2012; Ortuño et al., 2020). Our results suggest that a certain degree of preliminary cholesterol oxidation may occur in raw minced meat, probably as a result of aerobic grinding and the addition of salt (Mariutti & Bragagnolo, 2017), while hydroperoxide dismutation is promoted at 65°C. Total cholesterol was higher in the G0 than in the SV0 patties but only decreased in the G4 patties, in which %OR increased. Studies in model systems indicate that cholesterol thermo-oxidation is maximal at around 150°C, just above its melting point of 147-148°C, when its contact with pro-oxidant agents is promoted (Derewiaka & Molińska, 2015). At lower temperatures, cholesterol seems to be quite stable when it is unaccompanied. However, its autoxidation is enhanced by the presence of unsaturated FA, even when it is not heated. Since the reduction observed in the cholesterol content did not match the quantitative increase in COPs, it is possible that cholesterol and COPs were also dehydrated or broken down at the side-chain site, giving rise to conjugated dienes and volatile compounds, respectively. Moreover, as stated above, a portion of the generated COPs might have reacted with amine-containing compounds and consequently produced Schiff bases, and/or reacted with Maillard reaction products (Rodríguez-Estrada et al., 2014).

3.3 Fatty acid profile

Table 3 shows the major FAs (>5% total FA – TFA) of the cooked/hot-displayed lamb patties. In descending order, the most abundant FAs present in the cooked lamb fat were C18:1 n-9, C18:0, C16:0, C18:2n-6 and C14:0, totalling around 90% of TFA. This profile agrees with previous studies on cooked meat obtained from light lamb legs (Campo et al., 2013; Ortuño et al., 2020). In brief, cooking/heated display did not alter (P>0.05) the proportions of any individual FA, SFA, MUFA or the n6/n3 ratio. However, highly peroxidisable (HP) PUFA and the peroxidation index (PI), which accounts for the increasing susceptibility of PUFA to peroxidation as the unsaturation degree of their molecules increases, were higher in G0 than in SV0 patties. Also, although not significant, a trend (P=0.067) was observed towards an increase in the PUFA concentration in G0. In contrast, no differences were found for any FA or index after heated display. Changes in the FA profile during cooking may occur either through losses of melted adipose lipids or as a result of PUFA oxidation (Gerber et al., 2009). Overall, conventional cooking methods have been reported as having little impact on FA oxidation in ruminant meat (Campo et al., 2013; Rodriguez-Estrada et al., 1997). Moreover, the protective effect of SV against PUFA degradation is not a certainty. In rabbit meat, SV cooking prevented PUFA degradation while roasting did not (Rasinska et al. 2019), although Modzelewska-Kapituła et al. (2019) obtained the opposite results when comparing PUFA levels in SV and steam-cooked beef. HP-PUFA are generally associated to cell membrane phospholipids and thus more restricted in movement during heating. In contrast, triacylglycerides containing SFA and MUFA are more abundant in neutral lipids, and predominant in intermuscular fat (Gerber et al., 2009; Rodriguez-Estrada et al., 1997). Therefore, the heat applied during cooking would promote drip loss of the SFA and MUFA present in adipose tissue rather than of HP-PUFA in a temperature-dependent way. The thermal shock caused by the higher temperatures of grilling seemingly induced a quantitatively higher release of the neutral lipids present in the adipose tissue of the patties than the uniform, but more prolonged, heating applied by SV-cooking. The oxidative degradation of FA involves a free radical mechanism, whose reaction rate is

318 determined by the formation of alkyl radicals from unsaturated FA. Such radicals are formed much
319 more readily from HP-PUFA than from C18:1 and C18:2. Once such radicals are formed, oxidation
320 progresses via a chain reaction that is less dependent on the nature of the unsaturated FA, and in
321 which the more abundant oleic and linoleic acids are involved (Elmore et al., 1999). The findings of the
322 present study seem to agree with this proposed mechanism. During heated display, the higher PI in
323 G0 would lead to the increased formation of free radicals capable of attacking other fatty acids that
324 are less susceptible to oxidation, such as oleic acid, thus justifying the more intense formation of MDA
325 and 7-hydroxy COPs in grilled meat.

326 **3.4 Volatile organic compounds**

327 **Figure 2** shows the different VOCs families present in cooked/hot-displayed patty headspace. This VOC
328 profile agrees with other profiles reported for cooked lamb meat (Almela et al., 2010; Gravador et al.,
329 2014; Nieto et al., 2011; Rivas-Cañedo et al., 2013; Roldán et al., 2015), in which lipid-derived
330 compounds, particularly saturated aldehydes (6-10 carbons), predominated over Maillard-derived
331 compounds. The proportion of alcohols to aliphatic hydrocarbons in our study was high compared
332 with other studies in whole intact cuts (Gravador et al., 2014; Roldán et al., 2015). Since alcohols
333 predominate over alkanes during raw meat oxidation, this fact might be related to the oxidizing effect
334 of aerobic grinding and salt addition (Ortuño et al., 2016). The VOC profile (P>0.05) was similar in the
335 G0 and SV0 patties, while heated display increased (P<0.05) the proportion of aldehydes (from 67 to
336 80% of total area) and furans, and decreased (P<0.05) the proportion of the rest of the VOC families,
337 except alcohols (P>0.05). The formation of aldehydes during heated display was coherent with the
338 progression of the secondary lipid oxidation rate measured by TBARS. Indeed, lipid oxidation
339 promoted by mild cooking (60-70°C) of meat for up to 6 h is generally reflected in higher values of
340 MDA and volatile saturated aldehydes (Del Pulgar et al., 2013; Roldan et al., 2014).

341 **Table 4** shows the relative abundance of individual VOCs in the cooked/hot-displayed patty
342 headspace. A total of 32 VOCs were identified, all of them previously detected in cooked lamb meat
343 (Frank et al., 2016; Roldán et al., 2015). The cooking method had a low impact on the VOC profile of
344 freshly cooked patties since the SV0 patties only presented higher (P<0.05) levels of 2-heptenal and
345 1-pentanol and a lower abundance of limonene compared with their G0 counterparts. Hexanal was
346 the most abundant compound at both sampling times, representing up to 50% of total VOCs in the
347 headspace of the cooked patties. However, its content did not increase (P>0.05) during heated display.
348 The increased level (P<0.05) of aldehydes was related to the octanal and 3-methyl-butanal contents,
349 and the neo-formation of benzaldehyde, 2-nonenal and 2,4-decadienal. In this context, the increase
350 in benzaldehyde and decanal was less pronounced in the SV4 patties. Of the alcohols, 1-octen-3-ol
351 was the most abundant in the G0 and SV0 patties, increasing during heated display to reach
352 approximately 10% of total VOCs. The rest of the alcohols, except octanol, decreased (1-pentanol) or
353 disappeared (1-penten-3-ol, 1-pentanol, 1-hexanol, 1-heptanol) in the G4 and SV4 patties. Similarly,
354 most of the alkanes, aromatic hydrocarbons, terpenoids (limonene), ketones and sulphur compounds
355 (carbon disulphide) decreased after cooking (P<0.05) or disappeared (P<0.05) after heated display,
356 with some exceptions (hexane, octane and toluene). The most pronounced decrease was that shown
357 by 2,3-octanedione, from 10.3% in the headspace of freshly cooked patties to 4% after display time.

358 The predominance of hexanal can be attributed to its multiple synthesis pathways, as it can be
359 generated from the oxidation of oleic, linoleic and arachidonic acids. Other VOCs formed in the
360 cooked/hot-displayed meat would be related to more specific oxidation routes: octanal from oleic
361 acid, and benzaldehyde and 2-pentylfuran from linolenic acid, whereas the dialkenals (2-nonenal and
362 decadienal) are by-products of linoleic acid, and 1-octen-3-ol is partly produced by arachidonic acid
363 autoxidation (Elmore et al., 1999; Ortuño et al., 2016). As mentioned above, our results suggest that
364 heating promoted the formation of free radicals from HP-PUFA, which are able to attack other FA that
365 are less susceptible to oxidation, such as oleic acid, in a more advanced stage of the lipid oxidation

process (Elmore et al., 1999). In this sense, octanal and octanol, the most abundant compounds directly derived from lipid oxidation, have been suggested to come from the β-scission of oleic acid from 11- or 10-hydroperoxides. The high content of oleic acid (≈40% TFA) would agree with this mechanism. Also, the higher PI of the FA profile of G4-patties might justify the greater formation of benzaldehyde.

The VOCs profile allows lipid oxidation and Maillard reactions, concomitant reactions during cooking that determine flavour development (Almela et al., 2010; Mottram, 1998; Ortuño et al., 2016), to be assessed in the cooked meat. At low levels, lipid-derived aliphatic aldehydes might play an important role in cooked lamb flavour, especially when the meat is cooked at mild temperatures, such as during boiling or SV-cooking (Roldán et al., 2015). However, if the increase exceeds the relatively high threshold value, unpleasant rancid odours and flavours would be expected (Elmore et al., 1999). At the same time, a prolonged cooking time would involve VOCs formation from amino acids and thiamine even at mild temperatures (Del Pulgar et al., 2013). Branched aldehydes (3-methylbutanal) and alcohols (1-octen-3-ol), compounds formed from the Strecker reaction (Domínguez et al., 2019), increased after heated display. Indeed, the hexanal/3-methyl-butanal ratio (HX/3MB) has been proposed for assessing the balance between lipid oxidative reactions and amino acids degradation in meat products (Del Pulgar et al., 2013). In our study, the HX/3MB ratio was similar ($P>0.05$) in the G0 (206.2) and SV0 (193.5) patties, but substantially decreased during heated display, implying a higher ($P<0.05$) rate of Strecker degradation in G4 (12.6) than in the SV4 (16.3) patties. Despite the fact that the Maillard reaction rate increases with temperature, the activation energy of the different phases may vary depending on other factors such as pH, moisture level, the presence of dicarbonyl compounds and time (van Boekel, 2001). Our results indicate that meat browning and Strecker reactions can progress when meat is kept at mild temperatures after cooking during prolonged time, probably due to surface desiccation and the formation of dicarbonyl compounds (derived from lipid oxidation) (Whitfield & Mottram, 1992). Indeed, the prolonged formation of Strecker aldehydes was

391 already observed in SV-meat kept at similar temperatures (Del Pulgar et al., 2013) and even in long-
392 ripened meat products (Domínguez et al., 2019).

393 In contrast to Strecker-derived products, other desirable odour-active VOCs, such as carbon
394 disulphide, limonene, benzene, 2-heptanone and 2,3-octanedione (Calkins & Hodgen, 2007; Frank et
395 al., 2016; Machiels, 2004), decreased during heated display of the cooked patties, whereas 2-nonenal
396 and 2,4-decadienal, two top odour-active VOCs that may negatively affect cooked lamb flavour (Bueno
397 et al., 2011), were formed. Therefore, the VOC profile, TBARS content and instrumental colour
398 suggested a concomitant progression of both lipid oxidation and Maillard reactions during heated
399 display. Interactions between both pathways are well-documented and may be interconnected by the
400 involvement of carbonyl and α-dicarbonyl compounds from lipid oxidation in the formation of Schiff
401 bases and the Strecker degradation. However, no common VOCs resulting from this interaction
402 (pyrazines, pyridines, pyrroles, oxazoles, thiazoles, thiophenes) were found in our study, probably due
403 to the mild heating applied (Whitfield & Mottram, 1992). A higher degree of protein oxidation, which
404 is positively correlated with lipid oxidation in meat, may increase the amount of amino acid reactive
405 carbonyls. This might participate in the initial attack on the amine group of the amino acids and may
406 explain the differences seen between the SV4 and G4 patties (Gatellier et al., 2010). The increased
407 abundance of aldehydes observed after heated display to the detriment of other aromatic VOCs,
408 suggests a possible loss of meatiness (associated with rancidity), which may be supported by the high
409 level of MDA formation in these samples. Sensory analyses would be necessary to evaluate and
410 confirm these findings.

411 **3.5 Principal component analysis**

412 In order to better understand which parameters were the most relevant for assessing the effect of
413 cooking treatment and heated display on the lamb patties, the data for colour, lipid and cholesterol
414 oxidation, FA and VOCs were subjected to principal component analysis (PCA, Fig. 3). The first two

principal components accounted for 89.5% of the total variance. PC1 (54.7%) seemed to explain the changes produced during heated display, as freshly cooked samples (G0/SV0) were clearly separated from those kept at 65°C for 4 h (Fig. 3b). According to the loadings plot, stearic acid, ketones, alkanes, aromatic alkanes, limonene, carbon disulphide and some aldehydes (pentanal, 2-octenal) and alcohols (1-penten-3-ol, 1-pentanol, 1-hexanol, 1-heptanol) showed a strong negative correlation (>0.80) with PC1, forming a cluster of markers of freshly cooked samples. By contrast, 3-methylbutanal, hexanal, benzaldehyde, octanal, 2-nonenal, 2,4-decadienal, 1-octanol, palmitic acid and b* were among the variables most positively (>0.80) correlated with the changes resulting from heated display. The high positive values in PC1 of both L*, b*, lipid-derived aldehydes and 3-methylbutanal may be an indicator of the joint development of lipid oxidation and Maillard reactions. PC2 (34.8%) best explained mostly the effects derived from cooking treatment, allowing the two groups (G *vs.* SV) to be distinguished at both sampling times (Fig. 3b). All COPs (except CT) and PUFA, HP-PUFA and PI were the most strongly correlated variables with the G-samples, while, at the opposite extreme, SFA, a*, n6/n3 ratio allowed separation of the SV-patties. The PCA results reflected the two different patterns explaining the changes that occurred in the patties: (i) the effect of the cooking treatment, which affected cholesterol oxidation and the relative increase of peroxidisable FA; and (ii) the effect of heated display, which was characterised by the pronounced desiccation the meat, and the subsequent formation of Maillard reaction products, and incipient rancidity to the detriment of the cooked-meat aroma. TBARS was positively correlated with both PC1 (+0.76) and PC2 (+0.53), reflecting the evolution of oxidation during the heated display, probably influenced by the higher peroxidability of G-samples.

4. Conclusions

The heated display of cooked meat is increasingly widespread in catering, even though the practice may impair the cooked meat quality by promoting dehydration, meat surface darkening, lipid oxidation and the loss of desirable aromatic compounds. Compared to grilling, SV cooking at low temperatures and for longer time produces a lighter surface colour, does not prevent juice loss, and

hinders the formation of lipids and cholesterol oxidation compounds. Most of these positive effects of SV remain when the cooked meat is displayed on hot plates for a long time. The SV cooking method was seen to be effective in preventing lipid oxidation during cooking and heated display, as reflected by the TBARS, COPs and VOCs contents, which may be related to the lower oxygen availability and lower release of SFA during cooking. Therefore, SV seems to be a healthier cooking method for catering purposes when food is to be held warm for considerable periods before consumption.

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Table 1. Effects of cooking method and heated display on the moisture (g/100 g meat), total fat (g/100 g meat), weight loss (g/100 g) and CIELab colour (L*a*b*) of cooked lamb patties.

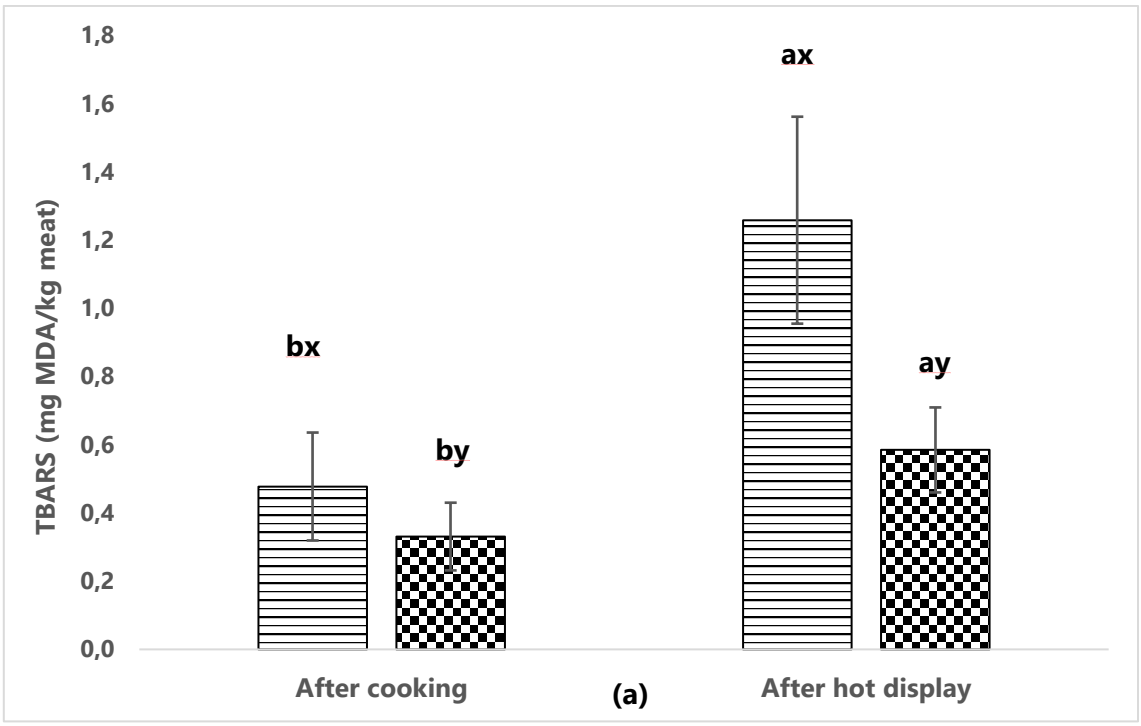
		Treatments		SEM	*P*		
		Cooking	Hot-display		Cooking	Time	CxT
Moisture	G	64.2 ^{a}	53.6 ^{b}	0.706	NS	***	NS
	SV	63.7 ^{a}	54.1 ^{b}				
Total fat	G	8.89 ^{b}	12.7 ^{a}	0.414	NS	***	NS
	SV	9.07 ^{b}	13.3 ^{a}				
Weight loss	G	26.8	24.9	0.771	NS	***	NS
	SV	29.1 ^{b}	23.2 ^{a}				
L*	G	51.2 $^{a\,y}$	37.5 ^{b}	0.983	**	***	*
	SV	55.9 $^{a\,x}$	38.5 ^{b}				
a*	G	8.72 ^{y}	9.25 ^{y}	0.199	***	NS	NS
	SV	10.2 ^{x}	11.0 ^{x}				
b*	G	13.0 ^{a}	10.6 ^{b}	0.777	NS	**	NS
	SV	13.4 ^{a}	10.4 ^{b}				

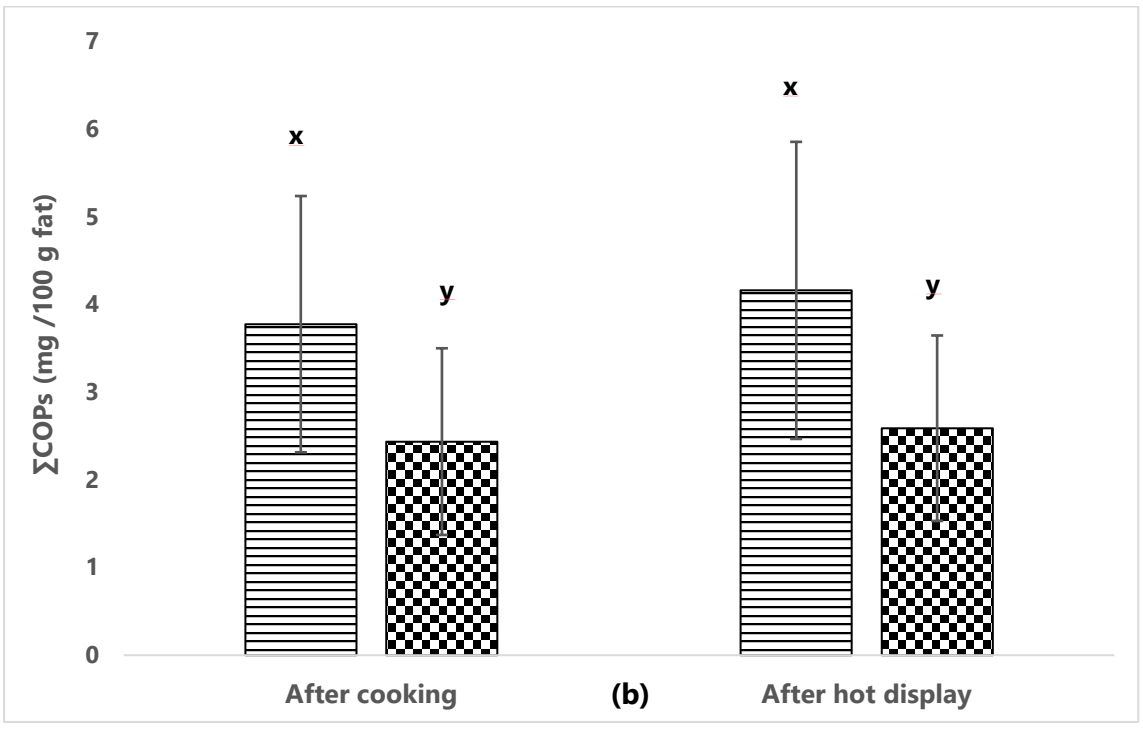
Abbreviations: SV, sous vide; G, grilling; C, cooking; T, time; SEM, standard error of the mean; *P*, probability.

CIELab coordinates = L* (lightness), a* (redness), b* (yellowness).

$^{a, b}$ Heated display time effects ($P<0.05$); $^{x, y}$ Cooking method effect ($P<0.05$). P: probability values. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; NS $p > 0.05$.

Fig. 1. Effect of cooking method and hot display on (a) secondary lipid oxidation (TBARS; mg MDA/kg meat) and (b) total cholesterol oxidation products (∑COPs; mg/100 g fat) in grilled (lines) and *sous vide* (grid) lamb patties. Mean value ± standard deviation.





[a, b] Heated display time effects (P<0.05); [x, y] Cooking method effects (P<0.05).

Table 2. Effects of cooking and heated display time on the mean values of cholesterol oxidation products (COPs) (mg/100 g fat), total cholesterol (g/100 g fat) and cholesterol oxidation proportion (%OR) in lamb patties.

		Treatment				SEM	*P*		
		Cooking		Hot-display			Cooking	Time	CxT
7α-HC	G	0.49	b x	0.60	a x	0.072	*	*	NS
	SV	0.28	y	0.33	y				
7β-HC	G	0.80	b x	0.95	a x	0.107	*	*	NS
	SV	0.49	y	0.55	y				
α-EC	G	0.50		0.47		0.079	NS	NS	NS
	SV	0.42		0.34					
β-EC	G	1.10	x	1.01	x	0.117	*	NS	NS
	SV	0.65	y	0.67	y				
CT	G	0.14		0.10		0.029	NS	NS	NS
	SV	0.10		0.15					
7-KC	G	0.83	x	0.93	x	0.104	*	NS	NS
	SV	0.52	y	0.54	y				
Total cholesterol	G	1.52	a x	1.29	b	0.070	*	*	NS
	SV	1.23	y	1.19					
OR	G	0.25	b	0.32	a x	0.029	*	*	NS
	SV	0.19		0.21	y				

Abbreviations: SV, sous vide; G, grilling; C, cooking; T, time; SEM, standard error of the mean; 7α-HC, 7α-hydroxycholesterol; 7β-HC, 7β-hydroxycholesterol; α-EC, 5α,6α-epoxycholesterol; β-EC, 5β,6β-epoxycholesterol; CT, cholestanetriol; 7-KC, 7-ketocholesterol.

[a, b] Heated display time effects ($P<0.05$); [x, y] Cooking method effect ($P<0.05$). P: probability values. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; NS $p > 0.05$.

Table 3. Effects of the cooking and heated display time on the mean values of the major fatty acid profile (g/100 g FA) and technological indexes of cooked lamb patties.

		Treatment			SEM	P		
		Cooking		Hot-display		Cooking	Time	CxT
C14:0	G	5.02		5.34	0.303	NS	NS	NS
	SV	5.01		5.34				
C16:0	G	25.0		25.7	0.354	NS	NS	NS
	SV	25.0		25.7				
C18:0	G	12.5		12.0	0.598	NS	NS	NS
	SV	12.6		12.2				
C18:1 n-9	G	40.1		40.3	0.813	NS	NS	NS
	SV	41.2		40.2				
C18:2 n-6	G	6.17		6.17	0.280	NS	NS	NS
	SV	5.90		5.90				
∑ SFA	G	46.2		46.3	0.851	NS	NS	NS
	SV	46.3		47.1				
∑ MUFA	G	43.6		44.2	0.765	NS	NS	NS
	SV	44.7		43.9				
∑ PUFA	G	9.55		9.49	0.311	NS	NS	NS
	SV	8.93		8.99				
∑ HP-PUFA	G	2.56	x	2.69	0.103	*	NS	NS
	SV	2.31	y	2.39				
n6/n3	G	7.81		7.62	0.809	NS	NS	NS
	SV	8.31		8.35				
PI	G	14.6	x	14.8	0.464	*	NS	NS
	SV	13.4	y	13.6				

Abbreviations: SV, sous vide; G, grilling; C, cooking; T, time; SEM, standard error of the mean; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; HP-PUFA, highly peroxidizable PUFA; PI, peroxidation index.

∑ SFA= Sum of C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, and C22:0.

∑ MUFA = Sum of cis and trans isomers of C14:1, C16:1, C17:1, C18:1, and C20:1.

∑ PUFA = Sum of cis and trans isomers of C18:2, C18:3, C20:4, C20:5, C22:4, C22:5, and C22:6.

∑HP-PUFA: Sum of PUFA with three or more unsaturated bonds.

n-6/n-3 = Σn-6 PUFA/Σn-3 PUFA.

PI = (%dienoic×1) + (%trienoic×2) + (%tetraenoic×3) + (%pentaenoic×4) + (%hexaenoic×5)

[a, b] Hot-display time effects (P<0.05); [x, y] Cooking method effect (P<0.05). P: probability values. *** p < 0.001; ** p < 0.01; * p < 0.05; NS p> 0.05.

668 **Table 4**. Effects of cooking method and heated display on the relative abundance (as AUx10^5) of lipid
669 oxidation volatiles in lamb patties.
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Compound		Treatment				SEM	C	T	CxT
		Cooking		Hot-display			P		
Aldehydes									
Pentanal	G	12.2	a	9.32	b x	0.545	*	***	NS
	SV	11.5	a	8.29	b y				
3-Methylbutanal	G	1.04	b	9.44	a	0.412	NS	***	NS
	SV	1.32	b	8.29	a				
Hexanal	G	115		120		6.948	NS	NS	NS
	SV	114		136					
Heptanal	G	14.3		16.4		1.370	NS	NS	NS
	SV	15.4	a	12.2	b				
2-Heptenal(E)	G	0.05	y	0.03		0.018	***	**	*
	SV	0.16	a x	0.06	b				
Octanal	G	4.68	b	63.9	a	5.991	NS	***	NS
	SV	5.08	b	63.9	a				
2-Octenal(E)	G	0.03	a	nd	b	0.008	NS	***	NS
	SV	0.04	a	nd	b				
Nonanal	G	9.04	a	0.97	b	0.596	NS	***	NS
	SV	9.59	a	0.86	b				
Benzaldehyde	G	nd	b	1.02	ax	0.049	***	***	**
	SV	nd	b	0.54	ay				
2-Nonenal(E)	G	nd	b	0.03	a	0.004	NS	***	NS
	SV	nd	b	0.04	a				
Decanal	G	<0.01	b	0.01	a	0.001	NS	*	NS
	SV	<0.01		<0.01					
2.4-Decadienal(E)	G	nd	b	<0.01	a	<0.01	NS	***	NS
	SV	nd	b	<0.01	a				
Alcohols									
1-Penten-3-ol	G	2.13	a	nd	b	0.063	NS	***	NS
	SV	2.54	a	nd	b				
1-Pentanol	G	5.79	a y	1.52	b	0.177	*	***	NS
	SV	7.76	a x	1.17	b				
1-Hexanol	G	1.73	a	nd	b	0.075	NS	***	NS
	SV	1.96	a	nd	b				
1-Heptanol	G	0.50	a	nd	b	0.025	NS	***	NS
	SV	0.60	a	nd	b				
1-Octen-3-ol	G	17.6		25.4		1.111	NS	**	NS
	SV	20.8	b	35.2	a				
1-Octanol	G	<0.01	b	0.24	a	0.009	NS	***	NS
	SV	<0.01	b	0.23	a				
Furans									
2-Pentylfuran	G	1.22	b	1.88	a	0.285	NS	**	NS
	SV	1.12	b	1.54	a				
Linear alkanes									
Hexane	G	nd	b	1.17	a	0.118	NS	***	NS
	SV	nd	b	1.54	a				
Heptane	G	9.63	a	1.30	b	0.184	NS	***	NS
	SV	9.44	a	1.27	b				
Octane	G	3.66		4.11		0.223	NS	NS	NS
	SV	3.92		4.34					

Decane	G	0.82	a	nd	b	0.083	NS	***	NS
	SV	1.08	a	nd	b				
Dodecane	G	0.02	a	nd	b	0.001	NS	NS	NS
	SV	0.01	a	nd	b				
Aromatic hydrocarbons									
Benzene	G	0.40	a	nd	b	0.015	NS	***	NS
	SV	0.48	a	nd	b				
Toluene	G	1.18	a	1.56	b	0.096	NS	***	NS
	SV	0.89	a	1.22	b				
Ethylbenzene	G	0.12	a	nd	b	0.008	NS	***	NS
	SV	0.11	a	nd	b				
o-Xylene	G	0.12	a	nd	b	0.008	NS	***	NS
	SV	0.11	a	nd	b				
Terpenoids									
Limonene	G	0.08	a x	nd	b	0.006	*	***	NS
	SV	0.04	a y	nd	b				
Ketones									
2-Heptanone	G	1.00	a	nd	b	0.021	NS	***	NS
	SV	0.97	a	nd	b				
2.3-Octanedione	G	24.5	a	11.9	b	0.917	NS	***	NS
	SV	25.2	a	11.9	b				
Sulphur compounds									
Carbon disulphide	SV	2.69	a	0.62	b	0.205	NS	***	NS
	G	3.52	a	0.82	b				

Abbreviations: SV, sous vide; G, grilling; C, cooking; T, time; M: Mean; SEM, standard error of the mean.

[a, b] Heated display time effects ($P<0.05$); [x, y] Cooking method effects ($P<0.05$). P: probability values. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; NS $p > 0.05$.

Fig. 2. Relative abundance of VOC families (% of total area) in the headspace of grilled (G) and sous vide (SV) lamb patties after cooking (0) and heated display (4).

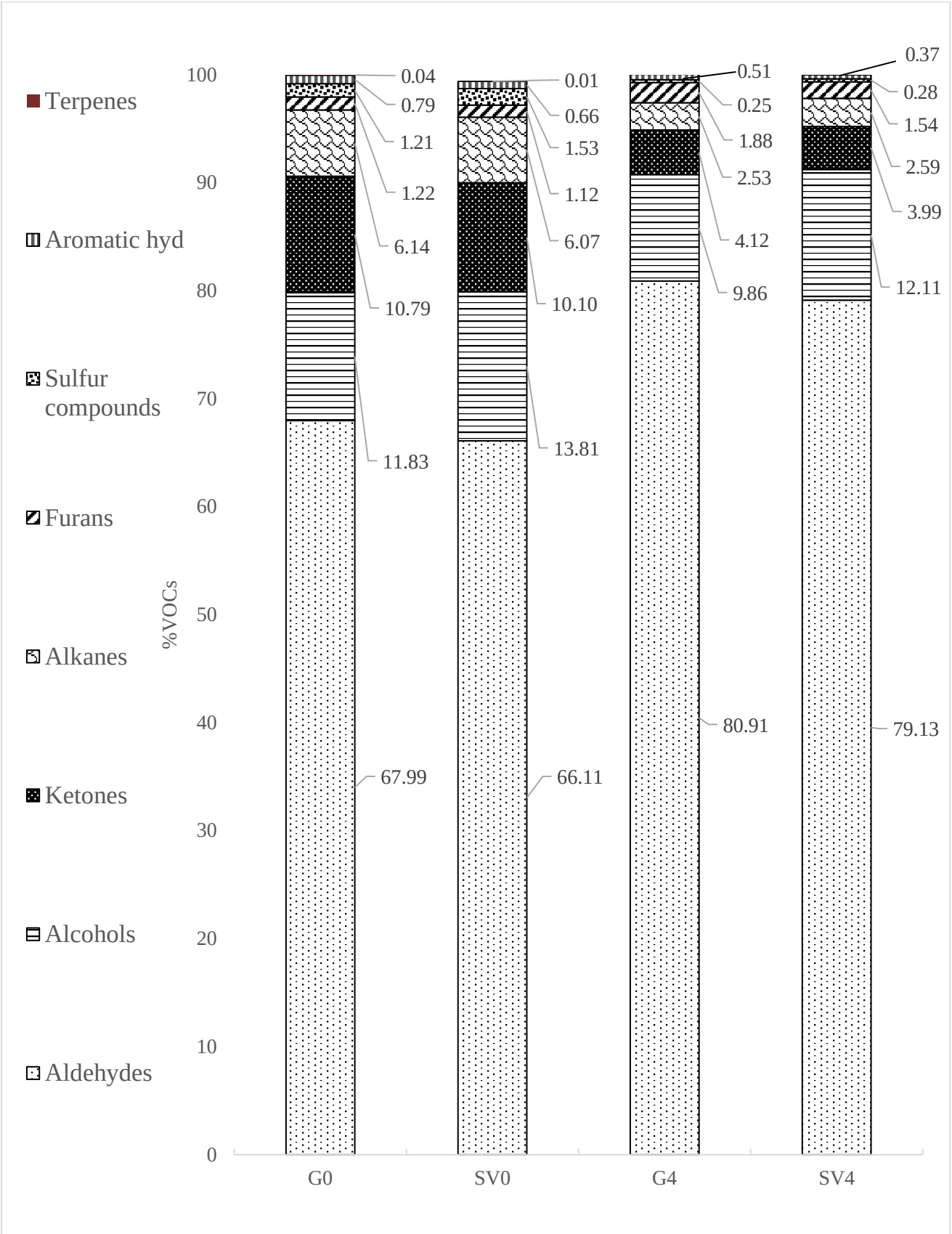

Fig 3. Graphical representation of the loadings (a) and scores (b) of the first two Principal Components (PC).