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Antioxidant activity of grapeseed and chestnut extract in dry-cured sausage “chorizo” during ripening

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18 **Abstract**

19 The effect of natural (grapeseed and chestnut extract) and synthetic
20 antioxidants (buthylatedhydroxytoluene, BHT) on the physico-chemical, lipid
21 oxidation, microbial and sensory characteristics of dry-fermented sausage were
22 investigated during the ripening period. Addition of antioxidants decreased ($P <$
23 0.001) the TBAR'S values, with greater reductions in sausages treated with
24 natural antioxidants than BHT. Addition of antioxidants reduced ($P > 0.05$) the
25 total volatile compounds from lipid oxidation in the following order: chestnut
26 extract > control > BHT > grapeseed, being their mean values 153, 147, 126
27 and 113×10^6 area units, respectively. Regarding hexanal content, significantly
28 higher values were observed in control sausages (16.8×10^6 area units) than in
29 the grapeseed batch (2.27×10^6 area units) and in the BHT group (2.19×10^6
30 area units), while in chestnut treated sausages hexanal was not detect.
31 Sensorial analysis showed the following order of acceptability: grapeseed =
32 BHT > chestnut extract = control group. These results indicated that the most
33 effective antioxidant was found to be grapeseed and that natural antioxidants
34 were more effective than synthetic antioxidants. This study demonstrated that
35 grapeseed and chestnut extracts could be utilized in dry-cured sausages to
36 enhance quality and provide safer products.

38 **Keywords:** natural antioxidant, lipid oxidation, volatile compounds,
39 sensory properties

1. Introduction

The Spanish traditional sausage “Chorizo” is a fermented sausage that undergoes a more or less prolonged process of drying-ripening before consumption. During the manufacture of dry-cured sausages, apart from microbiological changes other chemical modifications occur, mainly dehydration, fermentation, and colour development, lipolysis and proteolysis. Particularly, lipid oxidation is one of the major deteriorative chemical changes that occur during the sausage ripening process. Oxidation of labile double bonds in polyunsaturated fatty acids produces secondary oxidative compounds such as hexanal, pentanal, heptanal and octanal that are responsible for quality deterioration and represent health risks, including carcinogenesis (Grun, Ahn, Clarke, & Lorenzen, 2006; Arabshahi-D, Devi, & Urooj, 2007).

The use of antioxidants is one of the major strategies for preventing lipid oxidation and may be effective in controlling and reducing the oxidation in meat products. Due to the fact that synthetic antioxidants, like BHT and BHA, may constitute a potential health hazard for consumers, interest in natural antioxidants and search on naturally occurring compounds with antioxidant activity has increased dramatically (Moure et al., 2001). Agro-industries generate numerous waste materials that involve environmental problems of pollution and therefore achieving a sustainable agriculture implies the reduction/elimination of residues. In this sense, the possibility of using these residues as natural antioxidants in the food industry could represent a significant step towards maintaining an environment balance. For instance in wineries, where residues account for approximately 30% of the total volume of grapes used for wine production, waste represent serious storage, processing

69 or disposal problems, in ecological and economic terms (Rockenbach et al.,
70 2008). These by-products, such as seeds and peels, are rich in phenolic
71 compounds, which are known to have high antioxidant activity (Guendez,
72 Kallithraka, Makris, & Kefalas, 2005). Furthermore, grape seed extracts have
73 shown both antioxidant and antimicrobial activities in meat (Ahn, Grun, &
74 Fernando, 2002; Jayaprakasha, Selvi, & Sakariah, 2003).

75 Chestnut (*Castanea sativa*) is a traditional crop in Galicia (NW Spain),
76 where around 15,000 t/year of chestnut fruit are produced. Roughly, half the
77 production is destined for fresh consumption and the other half is transformed to
78 obtain several derivatives such as frozen fruit, chestnut purée or flour and
79 chestnut in syrup or marron-glacé. The peeling process generates a waste
80 product, *i.e.* the shell, which represents around 10% of the weight of whole
81 chestnuts, and that is used as fuel. The presence of natural antioxidants, like
82 gallic and ellagic acid, was also found in chestnut nut (Barreira et al., 2008) and
83 has been associated with various positive health effects (Hooper et al., 2008).

84 The aim of this study was to determine the effect of natural antioxidants
85 (chestnut and grape extracts) on physico-chemical and microbiological changes
86 and on lipid oxidation during the ripening process of dry-cured sausages.

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2. Material and methods

2.1. Grape seed extract (GRA)

Three lots of two white grape varieties, *Vitis vinifera* “Albariño” variety and a *Vitis labrusca* hybrid, were used. The grapes were harvested and processed for Albariño wine in 2005 and kindly donated by a local home-grown (Galicia, Spain). Grape seeds were separated, cleaned, washed and frozen at -50 °C until its use. Samples were milled to a final size particle of 3-5 mm. The extraction solvent was a mixture of ethanol/ water (ratio 1/1). An extraction pilot-plant was setup as reported Sánchez, Franco, Sineiro, Magariños, & Núñez, (2009) with the following modifications: batches of 2 kg of grape seed were packed into the column; the extraction solvent was pumped to bed using a flow of 113.0 ± 8.2 mL/min and a temperature of 60 °C; the extract was collected for 2.5 h. Finally, the extraction yielded around 15.25 L, which were evaporated in a rotary evaporator (Büchi R-114, Zurich, Switzerland) at 40 °C. The remaining solid was suspended in 3 L of water. A glass column (7 cm Ø_{in} × 40 cm height) filled with XAD-16 Amberlite was equilibrate with distillate water. To purify the polyphenolic material, 1 L of aqueous suspension extract was poured to the column and washed with 5 L of water. The polyphenolic material was adsorbed on XAD-16 Amberlite and eluted with ethanol (1 L × 3). Finally, the ethanolic extract was evaporated until dryness and later lyophilized using a freeze-dryer (Kinetics EZ-Dryer, Stone Ridge, NY, USA). This raw lyophilized extract was called GRA extract (GRA) and rendered 13.7 ± 0.34 g GRA/ kg grape seed).

2.2. Chestnut extract (CHE)

111 Chestnut (*Castanea sativa*) leaves were locally collected in Ribeira Sacra
112 (Ourense, Spain) in 2005. Air-dried and grounded (final moisture 9.68% and
113 less than 1 mm) were stored in sealed plastic bags in a dry dark place before
114 use. Aqueous extraction was realized according to Díaz-Reinoso, Moure,
115 Domínguez & Parajó, 2011. Briefly, leaves (1 kg) were contacted with acidified
116 water (25 L) under conditions leading to maximal radical scavenging activity (25
117 °C, 90 min) in a home-made tank with stirring and temperature control. Solid:
118 liquid separation was accomplished by vacuum filtration, and the liquid phase
119 was processed in a series of two ultrafiltration membranes. Membrane
120 fractionation was performed in an UF lab pilot home-made plant, consisting of a
121 feed tank with 2.5 L, a peristaltic Masterflex pump and a membrane module.
122 Pressure was monitored at the entrance and exit of the membrane module, a
123 needle valve located after the module was used for TMP regulation. The pilot
124 plant was equipped with 5 and 10 kDa Omega membranes (Minisette, Pall
125 Filtron) (0.12 mm × 0.10 mm) having an effective surface area of 0.07 m². The
126 membrane material was modified polyethersulfone and the maximum operation
127 pressure was 4 bar.

128 Finally the antioxidant capacity of GRA and CHE extracts was evaluated *in*
129 *vitro* and subsequently applied as ingredients in the dry-cured “Chorizo”
130 formulation.

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132 2.3. Determination of antioxidant capacity

133 2.3.1. Determination of total phenolic content

134 The total phenolic content was determined based on the method of
135 Singleton et al. (1999), using the Folin–Ciocalteu Reagent (FCR) with gallic acid

as a standard. 50 μ L of sample or blank were added to 3 ml of distilled water in test tubes. A volume of FCR (250 μ L) was placed into the tube and mixed before adding 750 μ L of saturated Na_2CO_3 . The final volume of the reaction mixture was adjusted to 5 ml with distilled water and, after incubation for 2 h at room temperature, the absorbance at 765 nm was read in 1 cm cuvettes. Readings were compared with a standard curve of gallic acid, being the total phenolic content expressed as mg of gallic acid equivalent per g of freeze dried solid (mg GAE /g).

2.3.2. Trolox equivalent antioxidant capacity (TEAC)

This assay is based on the scavenging of ABTS radical (2,2-azinobis-(3-ethyl-benzothiazoline-6-sulfonate)), observed as a decolorization of blue-green color at 734 nm. Solutions of ABTS 7 mM and potassium persulfate 2.45 mM in phosphate buffer saline (PBS, pH 7.4) were allowed to stand overnight to generate the ABTS radical cation ($\text{ABTS}^{\bullet+}$). Then, the $\text{ABTS}^{\bullet+}$ stock solution was diluted with PBS and equilibrated at 30°C to an absorbance of 0.8-0.9 at 734 nm. Triplicate determinations were performed by mixing 100 μ L of the sample with 1.9 mL of radical solution. The decline in absorbance was followed for 10 min. Appropriate solvent blanks were run for each sample. The radical scavenging capacity was compared with that of Trolox (Sigma Aldrich) and results were expressed as g of Trolox equivalent per g of freeze dried solid.

2.3.3. β -carotene bleaching assay

The β -carotene (β C) bleaching assay described by Marco (1968) was modified for use with microplates. Briefly, 4 mg of β C, 0.5 ml of linoleic acid and 4 g of Tween-40 were dissolved in 20 ml of chloroform, the stock solution was

distributed in aliquots of 1 ml and the chloroform was evaporated in a rotary evaporator (50°C/~15 min). The resulting oily residue was washed with N₂ and stored at -18°C. For each microplate, 1 mL of the stock solution was resuspended in 25 ml of *mili-Q* water at the assay temperature (45°C). The absorbance at 470 nm of the reagent thus prepared is ~1.200.

For measuring the antioxidant capacity 50 µl of sample were mixed with 250 µl of reagent in a 96 well microplate. Samples were analysed in triplicate, in a concentration range between 50 and 500 mg/l (final concentrations). Also appropriate solvent blanks were run for each sample. A series in which the sample was replaced by a commercial antioxidant (BHT) at the appropriate concentrations (0.5-5 mM) in the reaction mixture was also included in each microplate.

Absorbance readings (470 nm) were taken at regular intervals in a ThermoFisher Scientific microplate reader until β-carotene was decolored (about 3 h). The antioxidant activity coefficient (AAC) was calculated as follows (Moure et al., 2006):

$$AAC = \frac{(A_{sample})_{t=120} - (A_{control})_{t=120}}{(A_{control})_{t=0} - (A_{control})_{t=120}} \times 1000 \quad [1]$$

where A_{sample} is the Absorbance at 470 nm of the β-carotene in the presence of sample and $A_{control}$ is the Absorbance at 470 nm of the β-carotene in its absence.

The extract concentration providing a 50% β-carotene bleaching inhibition (EC_{50}) was calculated from the plot of AAC versus concentration of antioxidant.

This graphic representation generates dose-response curves that can be adequately described by a sigmoidal model defined by the Weibull equation modified to provide the EC_{50} value in an explicit form (Rial et al., 2011):

$$A = K \left(1 - \exp \left(- \ln 2 \left(\frac{C}{m} \right)^a \right) \right) \quad [2]$$

where, A is antioxidant capacity (dimensionless), K is the maximum activity (dimensionless), C is the concentration of antioxidant (g/L), m is the concentration for semi-maximum response (EC_{50} in g/L) and a is a form parameter related to the maximum slope of the function (dimensionless).

The EC_{50} values for CHE and GRA extracts were determined from the adjustment of the experimental data to equation [2]. The antioxidant activity of GRA and CHE was also expressed as BHT equivalent activity (g BHT/g freeze dried solid), being the equivalent BHT concentration determined from the calibration curve obtained by plotting AAC versus BHT concentrations.

2.3.4. α ,-Diphenyl- β -picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant activity was determined with DPPH as a free radical, using an adaptation to microplate of the method described by Brand-Williams et al, 1995. For the modified procedure, antioxidant solutions (10 μ l) were added to 200 μ l of a 60 μ M solution of DPPH(in 70% ethanol. The decrease in absorbance was followed at 515 nm every 5 min until the reaction reached a plateau (about 2 h). Samples were analysed in triplicate, in a concentration range between 50 and 500 mg/l (final concentrations). Appropriate solvent

blanks were run for each sample and a series where BHT (0-0.5 mM) replaced the in the reaction mixture.

The radical-scavenging activity (RSA) was calculated as a percentage of DPPH decoloration, using the equation (Barreira et al., 2008):

$$RSA = \frac{(A_{control})_{t=60\text{min}} - (A_{sample})_{t=60\text{min}}}{(A_{control})_{t=60\text{min}}} \times 100 \quad [3]$$

where A_{sample} is the Absorbance at 515 nm of the DPPH in the presence of sample and $A_{control}$ is the Absorbance at 515 nm of the β -carotene in its absence.

The EC_{50} (g extract/L) was calculated from the plot of RSA versus concentration of antioxidant using equation [2], and the equivalent activity (g BHT/g freeze dried solid) determined from the calibration curve obtained by plotting RSA versus BHT concentrations.

2.4. Manufacture of dry-cured sausages

Four batches (20 units per batch, 3 per ripening time) of dry-cured sausage “chorizo”: Control (CON), butylhydroxytoluene (BHT), grape seed extract (GRA) and chestnut extract (CHE) were manufactured in the pilot plant of the Meat Technology Center of Galicia. Sausages were manufactured using the primal cuts of shoulder (85%) and pork back fat (15%) from Celta pig breed. The lean and the pork back fat were ground through a 6 mm diameter mincing plate in a refrigerated mincer machine (La Minerva, Bologna, Italy). Mixture was vacuum minced in a vacuum mincer machine (Fuerpla, Valencia, Spain) for 3 minutes with 5 g/kg of NaCl, 20 g/kg of sweet paprika, 3 g/kg of spicy paprika,

0.5 g/kg of garlic and 200 mg/kg of BHT for BHT batch, 1 g/kg of grape seed extract for GRA batch and 1 g/kg of chestnut extract for CHE batches. No starter culture was added. The meat mixture was maintained at 3–5 °C for 24 h and then was stuffed into pig gut (diameter 32-34 mm) to obtain an average final sausage weight of 150 g. After stuffing, the sausages were aconditionated for two days at 7 °C and 85 % of relative humidity. The sausages were transferred to a drying–ripening chamber where they were kept for 48 days at 12 °C and 75–80% relative humidity. Analyses were carried out at 0, 4, 19 and 48 days of ripening time.

2.5. Analytical methods

2.5.1. pH, moisture, water activity, TBARS index and color parameters.

The pH of sausages was measured using a digital pH meter (model 710 A+, Thermo Orion, Cambridgeshire, UK) equipped with a penetration probe. Moisture percentage was determined by oven drying (Memmert UFP 600, Schwabach, Germany) at 105 °C until constant weight (ISO, 1997), and calculated as sample (5 g) weight loss. Water activity (a_w) was determined using a Fast-Lab (GBX, Romans Sur, Isère Códex, France) water activity meter, previously calibrated with sodium chloride. TBARS index method was performed according to Targladis, Watts, Younathan, & Duggan, 1960. TBARS values were calculated against a standard curve and expressed as mg malonaldehyde/kg meat. A portable colorimeter (Konica Minolta CR-600d Osaka, Japan) was used to measure meat colour in the CIELAB space (CIE, 1978). (lightness, L^* ; redness, a^* ; yellowness, b^*).

261 2.5.2. Textural profile analysis

262 Sausage pieces of 1 × 1 × 2.5 cm (height × width × length) were
263 compressed at a crosshead speed of 3.33 mm/s in a texture analyzer
264 (TA.XTplus, Stable Micro Systems, Vienna Court, UK). The test was developed
265 according to the methodology proposed by Bourne (1978). Textural parameters
266 were measured by compressing to 80% using a compression probe with 19.85
267 cm² of surface contact. Between the first and second compressions, the probe
268 waited for 2 s. Hardness, cohesiveness, springiness, gumminess, and
269 chewiness were obtained.

271 2.5.3. Microbial analysis

272 For microbiological analysis, a 10 g sample of sausage was aseptically
273 weighted in a sterile plastic bag, previously removing and discarding the outer
274 plastic. Subsequently samples were homogenized with 90 mL of a sterile
275 solution of 0.1% (w/v) peptone water (Oxoid, Unipath, Basingtoke, UK),
276 containing 0.85% NaCl and 1% Tween 80 as emulsifier, for 2 min at 20-25 °C in
277 a Masticator blender (IUL Instruments, Barcelona, Spain), thus making a 1/10
278 dilution. Serial 10-fold dilutions were prepared by mixing 1 mL of the previous
279 dilution with 9 mL of 0.1% (w/v) sterile peptone water.

280 Total viable counts were enumerated in Plate Count Agar (PCA; Oxoid,
281 Unipath Ltd., Basingstoke, UK) and incubated at 30 °C for 48h; lactic acid
282 bacteria were determined on the Man Rogosa Sharpe medium Agar (Oxoid,
283 Unipath Ltd., Basingstoke, UK) (pH 5.6) after an incubation at 30 °C for 5 days
284 and moulds and yeasts were enumerated using OGYE Agar Base (Merck,
285 Darmstadt, Germany) with OGYE Selective Supplement (Merck, Darmstadt,

Germany), and incubated at 25 °C for 4-5 days. After incubation, plates with 30–300 colonies were counted. The microbiological data were transformed into logarithms of the number of colony forming units (cfu/g).

2.5.4. Analysis of volatile compounds

The extraction of the volatile compounds was performed using solid-phase microextraction (SPME). A SPME device (Supelco, Bellefonte, PA, USA) containing a fused-silica fibre (10 mm length) coated with a 50/30 µm layer of DVD/CAR/PDMS was used and analysis performed as described by Soto et al. (2008). The fibre was conditioned prior to analysis by heating in a gas chromatograph injection port at 270 °C for 60 min. Extraction was performed at 35 °C for 30 min. Before extraction, samples were equilibrated for 15 min at the temperature used for extraction. Once sampling was finished, the fibre was withdrawn into the needle and transferred to the injection port of the gas chromatograph-mass spectrometer (GC-MS) system. Volatiles were analyzed by duplicate in all dry-cured sausages.

Analyses were performed on a Hewlett-Packard 6890N Series GC gas chromatograph fitted with a HP 5973N mass spectrometer and a MSD Chemstation (Hewlett-Packard, Palo Alto, CA, USA). A split/splitless injection port, held at 260 °C, was used to thermally desorb volatiles from the SPME fibre onto the front of DB-624 capillary column (J&W scientific: 30 m×0.25 mm id, 1.4 µm film thickness). The injection port was in splitless mode, the split valve opening after 2 min. Helium was used as a carrier gas with a linear velocity of 36 cm/s. The temperature program used was as follows: 40 °C maintained for 2 min and then raised from 40 to 100 °C at 3 °C/min, then from 100 to 180 °C at 5

311 °C/min, and from 180 to 250 °C at 9 °C/min with a final holding time of 5 min;
312 total run time 50.8 min.

313 Mass spectra were obtained using a mass selective detector working in
314 electronic impact at 70 eV, with a multiplier voltage of 1953 V and collecting
315 data at a rate of 6.34 scans/s over the range m/z 40–300. Compounds were
316 identified comparing their mass spectra with those contained in the NIST05
317 (National Institute of Standards and Technology, Gaithersburg) library and/or by
318 calculation of retention index relative to a series of standard alkanes (C_5 – C_{14})
319 (for calculating Kovats indexes, Supelco 44585-U, Bellefonte, PA, USA) and
320 matching them with data reported in literature. Results were reported as relative
321 abundance expressed as total area counts ($AU \times 10^6$).

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323 2.5.5. Sensorial analysis

324 The sensory panel evaluation was conducted with ten panellists (3 men
325 and 7 females) selected among members of the Meat Technology Centre of
326 Galicia. Panellists were trained according to the methodology proposed by UNE
327 regulations (UNE 87-024-95) during three months, with the attributes and the
328 scale to be used. The casing was removed and then, sausages were cut in
329 slices of approximately 4 mm thickness and finally served at room temperature
330 on white plastic dishes. Samples were individually labelled with three-digit
331 random numbers. A quantitative descriptive analysis (QDA) was used for
332 evaluating colour, typical sausage aroma, rancid taste, textural acceptability,
333 abnormal taste and overall acceptability. The intensity of every attribute was
334 expressed on a structured scale from 0 (sensation not perceived) to 9
335 (maximum of the sensation). Panellists evaluated the samples in three sessions

(two samples per session). During sensory evaluation, the panellists were situated in private cabinets illuminated with red light. Water was used to clean the palates and remove residual flavours, at the beginning of the session and in between samples.

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2.5.6. Statistical analysis

Mean, standard deviation and standard error were calculated for all quantified variables. Two-way analysis of variance (ANOVA) was carried out to analyse the effect of ripening time and antioxidant extracts on traits studied. The least squares means (LSM) were separated using Duncan's test. All statistical tests of LSM were performed for a significance level of 0.05. Correlations between variables ($P < 0.05$) were determined by correlation analyses using Pearson's linear correlation coefficient.

To evaluate the relationship between variables, a factorial analysis of the traits studied with significant differences ($P < 0.05$) among the four groups was carried out. Principal components analysis (PCA) was used as extraction method and performed on the correlation matrix. All statistical analyses were carried out using the SPSS 18.0 for Windows (SPSS, Chicago, IL, USA) software package.

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3. Results and discussion

3.1. Antioxidant activity of extracts

The results of several *in vitro* models for the evaluation of antioxidant activity of GRA (grapeseed) and CHE (chestnut) extracts are shown in Table 1. The polyphenols content in GRA (373.0 ± 6.10 mgGAE/g GRA) was very high

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361 compared to those values reported by Guendez, R., Kallithraka, S., Makris, D.
362 P., & Kefalas, P. 2005 (85 mg GAE/g grapeseed) and Negro, C., Tommasi, L.,
363 & Miceli, A. 2003 (35 mg GAE/g grapeseed). CHE extract showed a
364 polyphenols content significantly lower than GRA extract. Consistently, TEAC
365 assay provided a 10-fold higher value for GRA extract, being both parameters
366 (TEAC and polyphenols values) positively correlated, the higher polyphenols
367 content the higher antioxidant activity.

368 Also the scavenging activity of the extracts was determined following the
369 DPPH method, showing that GRA equivalent activity was almost twice the
370 antioxidant power of BHT standard and nearly 4-fold higher than that of CHE
371 extract (Table 1). The EC_{50} values obtained for scavenging effects on DPPH
372 revealed a poor antioxidant activity of CHE extracts (more than 1 g/L), although
373 radical-scavenging activity did not show a sigmoidal profile with increasing
374 concentrations of CHE (data not shown) and therefore EC_{50} parameter on the
375 Weibull model was non significant ($p > 0.05$). Nevertheless, GRA extract
376 showed a best EC_{50} value (0.16 g/L) than CHE extract, although lower than
377 those previously reported for seeds of red grapes (*Vitis vinifera* L.) varieties,
378 which ranged between 2.71 and 4.62 μ g/mL (Bozan et al., 2008). On the
379 contrary, similar EC_{50} values were found in ethanolic extracts of Italian grape
380 seeds extracts (Mandic et al. 2008)

381 In addition, β -carotene bleaching results for GRA, CHE extracts and BHT
382 standard are shown in Table 1, being EC_{50} values better than those obtained for
383 scavenging on DPPH radical. Chestnut EC_{50} values (100 μ g/mL) are lower than
384 those obtained by Barreira et al. (2008) for chestnut flower, outer and inner skin
385 and fruits, which varied from 161 to 3632 μ g/mL, respectively. The grapeseed

386 EC_{50} value was 50 $\mu\text{g/mL}$, which is 2-fold lower than that of CHE extract.
387 Regarding the BHT equivalent activity, the CHE extract required twice the mass
388 of BHT to provide equivalent β -carotene bleaching inhibition, while GRA extract
389 had slightly higher antioxidant activity than BHT.

390 Overall, between both extracts, GRA extract showed the highest
391 polyphenols content and antioxidant activity as determined by three methods
392 (TEAC, β -carotene bleaching assay and scavenging of DPPH radical).

394 3.2. Physico-chemical analyses

395 Changes in sausage pH values during ripening are given in Fig. 1 A.
396 During the first 19 days of ripening, pH values decreased ($P < 0.001$) from 5.60
397 to approximately 5.50, which could be due to the production of organic acids by
398 bacteria (Lücke, 1994). Then, during dry-ripening period, pH values increased
399 significantly ($P < 0.05$) probably due to the liberation of peptides, amino acids
400 and ammonia from proteolytic reactions (Spaziani, Del Torre, & Stecchini,
401 2009). At the end of process, statistical analysis indicated that pH values of
402 sausages was not affect ($P > 0.05$) by the addition of antioxidants although the
403 highest pH values were observed by CON group followed by BHT and CHE
404 ones. Our final pH values were similar to those found in other varieties of
405 sausages (Lorenzo, Michinel, López, & Carballo, 2000; Franco, Prieto, Cruz,
406 López, & Carballo, 2002; Roseiro, Gomes, Gonçalves, Sol, Cercas, & Santos,
407 2010). Contrarily, other authors (Salgado, García-Fontán, Franco, López, &
408 Carballo, 2005; Van Schalkwyk, McMillin, Booyse, Witthuhn & Hoffman, 2011)
409 have reported pH values lower than ours (below 5) at the end of the ripening
410 process.

Changes in water content of sausages during ripening are given in Fig. 1 B. Statistical analysis indicated that moisture content was significantly affected ($P < 0.001$) by ripening time and addition of antioxidants. During the drying period, moisture content decreased as a result of moisture loss at high ripening temperature and low percentage of relative humidity. At the end of process, the GRA and CHE batches had higher water content than the CON group (27.99 and 27.16 vs. 20.58%, $P < 0.001$). Pearson correlation test indicated that moisture contents were positively ($P < 0.01$) related to instrumental colour attributes of L^* ($r = 0.91$), a^* ($r = 0.65$) and b^* values ($r = 0.62$). By contrast, Gimeno, Ansorena, Astiasarán, & Bello (2000) found that L^* values were not affected by moisture content of Spanish dry-fermented sausages.

In the same line, water activity (a_w) gradually declined over the curing period from an initial value of 0.96 to 0.82 due to the drying process. Statistical analysis indicated that sausage a_w values were affected ($P > 0.001$) by the addition of antioxidants. Sausages with CHE and GRA extract showed higher a_w values than CON and BHT groups at the end of process (Fig. 1 C). The observed a_w decrease is largely due to the water loss, in fact values showed a positive correlation with moisture content ($r = 0.93$, $P < 0.01$).

3.3. Colour parameters

Changes in colour parameters (L^* , a^* , and b^* values) of sausages during the ripening period are given in Fig. 2 A, B and C, respectively. In relation to lightness (L^*), a decrease was observed ($P < 0.001$) during the ripening period, with values ranging from 43.8 to 30.5 (Fig 2 A). This result is in agreement with those reported by Bozkurt & Bayram (2006) and Lorenzo et al. (2012) who

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436 observed that L* values decreased during the ripening period. The decrease in
437 L* values during the dry-curing process could be attributed to moisture losses,
438 as expected in dry-cured sausages. This decrease was significantly correlated
439 with a_w ($r = 0.93$, $P < 0.01$) and moisture content ($r = 0.91$, $P < 0.01$).

440 Statistical analysis indicated that L* values of sausages were affected ($P <$
441 0.001) by the addition of antioxidants, because sausages with CHE, GRA and
442 BHT showed higher L* values than CON at the end of process (see Fig. 2 A).
443 This result is in disagreement whit those showed by Karabacak et al. (2008),
444 who observed that L*values were not affected ($P > 0.05$) by ripening time and
445 addition of antioxidants in Turkish dry-fermented sausages.

446 Redness (a^* values) decreased ($P < 0.01$) from 27.3 to 21.2 during the
447 whole process. The possible reason for decreasing a^* values might be partial or
448 total denaturation of nitrosomyoglobin, because of the production of lactic acid
449 (Pérez-Alvarez, Sayas-Barberá, Fernández-López, & Aranda-Catalá, 1999).

450 The addition of natural antioxidant extract had a significant effect ($P <$
451 0.05) on redness since the lower a^* values were obtained in CON group
452 compared to the other ones (Fig. 2 B). These results contrast with those
453 reported by Bozkurt (2006), who found that redness was not significantly
454 affected by the addition of green tea extract, *Thymbre spicata* oil or BHT in
455 Turkish dry-cured sausages. Colour is one of the most important quality
456 attributes of sausages that attract the preference of the consumers (brick-red
457 colour). The attraction of sausages is attained when desired brick-red colour is
458 formed during the ripening period, and therefore the addition of natural
459 antioxidants can provide more attractive sausages.

On the other hand, statistical analysis indicated that yellowness (b^* values) values were significantly affected ($P < 0.05$) by ripening time and addition of antioxidants (Fig 2 C). During ripening period, b^* values decreased significantly ($P < 0.05$) and is in agreement with those found by Pérez-Alvarez et al. (1999), who observed that b^* values of Spanish sausages decreased during the fermentation and ripening periods. The decrease in b^* values indicated that sausage colour turned to blue rather than yellow, likely due to browning reactions that occur when reducing sugars react with amino acids or proteins.

Statistical analysis indicated that b^* values of sausages were affected ($P < 0.05$) by the addition of antioxidants because sausages with chestnut and grape extract and BHT showed higher b^* values than the control (Figure 2 C). This result is in disagreement whit those reported by Bozkurt (2006) who found that yellowness was not significantly affected by the addition of green tea extract, *Thymbre spicata* oil or BHT in Turkish dry-cured sausages.

3.4. Thiobarbituric acid reactive substances (TBARS)

Changes in sausage TBARS were followed during ripening, being the results given in Fig. 3. Statistical analysis indicated that TBARS were significantly affected ($P < 0.05$) by ripening time and addition of antioxidants. TBARS increased gradually ($P < 0.001$) from 0.24 mg MDA/kg to around 0.78 mg MDA/kg during the whole process and are in agreement with those reported by Lorenzo, Temperán, Bermúdez, Cobas and Purriños (2012) who observed an increase of TBARS values during drying process. Our final TBARS values were similar to those observed by other authors (Franco et al., 2002; Soyer,

2005), who found values below 1.5. On the contrary, after 35 days of ripening, Domínguez (1988) and Domínguez Fernández, & Zumalacárregui Rodríguez (1991) reported much higher values of malonaldehyde (2.21 mg/kg of “chorizo”) than the average values observed in the present work, at the end of the ripening process. Taking into account that TBARS values higher than 1 mg MDA/kg are reported to produce “off-odours” and are considered as the threshold of organoleptic perception of lipid oxidation (Wu, Rule, Busboom, Field, & Ray, 1991), final TBARS values obtained in this study show suitable values from an organoleptic viewpoint.

Statistical analysis showed that the addition of antioxidant decreased ($P < 0.001$) TBARS values. At the end of the process TBARS content decreased in the order: CON > BHT > GRA > CHE, being their mean values of 0.78, 0.26, 0.23 and 0.22 mg MDA/kg, respectively. These results indicated that natural antioxidants were more effective against TBARS formation than BHT, in agreement with results reported by several authors [Bozkurt (2007), Wanasundara & Shahidi (1998), Zandi & Gondon (1999), Yanishlieva & Marinova (2001), and Tang et al. (2001)]. Contrarily, Ockerman, and Sun, (2002) reported that the addition of *H. sabdariffa* less effective ($P < 0.05$) in the reduction of TBARS in suck sausages than other antioxidants because of its low phenolic content. It is well known that phenolic compounds in herbs and spices have antioxidative properties and consequently, the presence of these compounds may have retarded ($P < 0.001$) the lipid oxidation during ripening process. Thus, the addition of antioxidants might have reduced lipid deterioration (TBARS) through the inhibition of malonaldehyde formation.

3.5. TPA analysis

Sausage texture profile (hardness, springiness, chewiness, gumminess and cohesiveness) was followed during the ripening period and results are given in Table 2. Values recorded in the texture analysis were different among batches. Generally, the major changes in fermented sausage structure take place during fermentation when the pH declines and the myofibrillar proteins aggregate to form a gel. After fermentation, drying is a major factor affecting binding and rheological properties (González-Fernández, Santos, Rovira, & Jaime 2006). Hardness increased ($P < 0.001$) during the ripening process from 2.04 to 17.49 kg/cm² at 48 days of ripening, due to the decrease in moisture content. It was found from Pearson correlation test that hardness was negatively related ($P < 0.01$) to moisture content ($r = -0.88$) and aw content ($r = -0.93$). It is very noticeable that statistical analysis showed that the addition of antioxidants decreased ($P < 0.001$) hardness. At the end of the process, hardness varied in the following order: CON > GRA > BHT > CHE, being their mean values 17.49, 10.29, 9.84 and 7.58 kg/cm², respectively.

Gumminess and chewiness values increased ($P < 0.001$) during the whole process and are in agreement with those found by Szczesniak (2002) who reported that gumminess changed from short to pasty gummy along ripening, thus producing pasty gummy sausages as gumminess increased. Increasing chewiness values indicated that sausages became tougher during the ripening period. The addition of natural antioxidant extract had a significant effect ($P < 0.001$) on gumminess and chewiness values, showing CON group higher values (4.26 kg and 1.28 kg*mm for gumminess and chewiness, respectively) than the other assayed treatments.

Finally, cohesiveness and springiness values decreased during the ripening period, although no significant differences ($P > 0.05$) were observed (Table 2). Springiness values have been related to the elastic properties of sausages (Bozkurt, & Bayram, 2006). In fact, our results indicated a lost of sausage elasticity along with springiness decrease, probably due to water removal during the ripening period. Pearson correlation tests indicated that cohesiveness and springiness were significantly related ($P < 0.01$) to moisture content ($r = -0.78$ and $r = 0.79$) and a_w content ($r = -0.72$ and $r = 0.67$), respectively. Statistical analysis indicated that cohesiveness and springiness values were not affected ($P > 0.05$) by the addition of antioxidants although sausages from control groups presented the highest values of springiness (0.31 mm) and the lowest values of cohesiveness (0.24).

3.6. Microbial profile

Changes in the microbial populations, TVC, LAB and mould and yeasts, during dry-cured sausage “chorizo” process are shown in Figures 4 A, B and C, respectively. The initial TVC, LAB and mould and yeasts counts ranged from 10^3 to 10^5 CFU/g and no differences in microbial counts were detected among batches. TVC counts increased from 5.17 to 8.2 $\log_{10}$ CFU/g ($P < 0.001$) during the first 19 days of ripening, remaining stable until the end of process. Pearson correlation tests indicated that TVC was significantly related ($P < 0.01$) to LAB ($r = 0.89$) and mould and yeasts counts ($r = 0.71$).

The raw mixture showed a sufficient presence of LAB, which is important for a correct fermentation process. LAB counts increased during the first 19 days of fermentation from 4.7 to 7.7 $\log_{10}$ CFU/g and remained practically

constant for the rest of the fermentation and ripening period in all sausage groups, probably due to the absence of fermentable carbohydrates. At the end of the ripening process, statistical analysis indicated that LAB counts were affected ($P < 0.001$) by the addition of antioxidants because the higher counts were obtained in sausages from CHE group ($8.2 \log_{10}$ CFU/g) and control batch ($8.1 \log_{10}$ CFU/g). At the end of the drying period (48 days of ripening), LAB were well established and had grown to 10^8 CFU/g. These results are in agreement with those of Moretti et al. (2004) who reported that this microbial group was the microbiota typical of fermented sausages. Also high LAB counts have been described in other meat products such as traditional sausages from retail markets (Papadima, Arvanitoyannis, Bloukas, & Fournitzis (1999)) where LAB counts of $8-9 \log_{10}$ CFU/g were reported, and in fermented sausages (Flores, & Bermell, 1996). The presence of high LAB populations may inhibit the growth of pathogenic bacteria, particularly *Staphylococcus aureus* (Geisen, Luecke, & Kroeckel, 1992), and also can have a positive effect on human health (Incze, 1992).

Mould and yeasts counts increased rapidly during the first 4 days of ripening, from 3.4 to $7.6 \log_{10}$ CFU/g in the grape extract group and to a lesser extent in the other groups (from 6.1 to $6.5 \log_{10}$ CFU/g). At the end of process, statistical analysis indicated that mould and yeast counts were also affected ($P < 0.01$) by the addition of antioxidants since higher counts were obtained in sausages from CON and GRA extracts batches ($6.2 \log_{10}$ CFU/g) than in BHT ($5.6 \log_{10}$ CFU/g) and CHE ($5.7 \log_{10}$ CFU/g) treated sausages.

3.7. Volatile compounds from lipid oxidation

Changes in volatile compounds from lipid oxidation of dry-cured sausages during the ripening period are summarized in Table 3. Statistical analysis indicated that volatile compounds from lipid oxidation were significantly affected ($P < 0.05$) by ripening time and addition of antioxidants. Total volatile compounds increased from 265×10^6 area units to 316×10^6 area units after 4 days of ripening process. From these maximum values, a significant ($P < 0.001$) drop was observed until the end of process, reaching final average values of 146×10^6 area units. Production of octenal and 2,4-decadienal was not detected in any of the samples, suggesting a good product sensory quality because these compounds are known to have a low threshold off-odour. However, small amounts of hexanal, a typical indicator from linoleic acid oxidation, were detected in all batches, except CHE treated sausages. Besides, higher hexanal values were observed in CON sausages (16.8×10^6 area units) than in GRA (2.27×10^6 area units) and BHT (2.19×10^6 area units) batches.

On the other hand, statistical analysis showed that the addition of antioxidants decreased ($P > 0.05$) total volatile compounds from lipid oxidation. At the end of process, volatile compounds contents were found in the following order: CHE > CON > BHT > GRA and their mean values were 153, 147, 126 and 113×10^6 area units, respectively. These results indicated that the addition of GRA extract improved the control of lipidic oxidation, compared to the control and sausages treated with CHE extract. These results are consistent with the polyphenol content and the *in vitro* evaluation of antioxidant activity of the GRA and CHE extracts (Table 1).

3.8. Sensory characteristics

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610 In order to assess the acceptability of both natural extracts in dry-cured
611 sausages, a hedonic sensorial test was carried out. Mean scores given by the
612 panellists for the four manufactured batches are shown in Figure 5. No
613 significant differences ($P > 0.05$) were observed for colour, aroma, rancid and
614 abnormal taste compared to the control batch. Despite colour showed a slightly
615 lower score for CHE and GRA groups (Fig. 5), textural acceptability provided
616 significantly ($P < 0.001$) higher values for CHE and GRA treatments compared
617 to the CON batch (7.6 and 7.1 vs. 4.2, respectively).

618 On the other hand, panellists did not find significant differences ($P > 0.05$)
619 among groups for the overall acceptability, which was found to be GRA = BHT >
620 CHE = CON. These results indicated that the addition of GRA extract as natural
621 antioxidant improved the acceptability of dry-cured sausages.

622 623 3.9. Principal components analysis

624 Principal component analysis allows obtaining a better overall idea of
625 relationships between variables. Results for PCA applied to the mean values of
626 the evaluated parameters are summarized in Figure 6. The PCA showed that
627 about 85.6% of the variability was explained by two main principal components.
628 The correlation matrix for this model has a determinant close to zero indicating
629 the existence of significant correlations among variables and the suitability of
630 this type of analysis (Table 4). Principal component 1 (PC1) was the most
631 important variable in terms of differences among sausages as it accounted for
632 56.86% of the total variability. PC1 was positively related with moisture content,
633 a_w , colour parameters (L^* and a^*) and acetic acid concentration. In addition,
634 PC1 was inversely related with TBARS and TPA (hardness, gumminess and

chewiness). CHE sausage group had the greatest component 1 value therefore; they were related to moisture content, a_w and colour parameters. As can be seen in Figure 6, CHE sausages groups are in the positive side of both PC1 and PC2, GRA and BHT sausages batches are in the positive side of PC1 and in the negative side of PC2, while CON sausages are in the negative side of PC1 and in the positive side of PC2. In contrast, CON sausages were inversely correlated to moisture content, a_w and colour parameters while BHT and GRA sausages were intermediate.

On the other hand, principal component 2 (28.72%) was positively related to microbial counts (TVC and BAL) and butanoic acid methyl ester and pentanoic acid methyl ester contents. BHT and GRA sausages were located on the negative PC2 axis, while CON and CHE sausages were on the positive PC2 axis.

In conclusion, PC1 differentiated the sausages with antioxidant that the CON group. Natural antioxidant was related to moisture content, a_w and colour parameters, which were more abundant in the CHE sausages, followed by GRA and BHT sausages.

CONCLUSIONS

The effect of natural (grapeseed and chestnut extract) antioxidants on the safety and quality of dry-fermented sausages were investigated during the ripening process. The results of our study indicated that addition of grapeseed and chestnut extracts were more effective against TBAR'S formation than the BHT.

On the other hand, pH, colour, and overall sensory quality were also affected by the addition of these antioxidants. The results of this study pointed out that the most effective antioxidant was found to be grapeseed, being both grapeseed and chestnut extracts more effective than BHT.

Overall, our results indicated that natural antioxidants could be used to improve the safety and quality of dry-fermented sausages and also to obtain more attractive products for the consumers. Furthermore, the extracts used in this study were obtained from by-products of the food industry and so this work describes the valorization of agri-waste products to produce natural antioxidants with potential application in processed meat products.

Acknowledgements

Authors are grateful to Galician government (Xunta Galicia 09TAL006CT) for the financial support. Special thanks to ASOPORCEL (Lugo, Spain) for the pork samples supplied for this research.

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842 **CAPTION TO TABLES AND FIGURES**

843 **Table 1.** Total polyphenols and evaluation of antioxidant activity *in vitro* models
844 for characterization of grapeseed and chestnut extracts.

845 **Table 2.** Evolution of textural properties of dry-cured sausages treated with
846 BHT, GRA and CHE during ripening.

847 **Table 3.** Evolution of the main volatile compound from dry-cured sausages
848 treated with BHT, GRA and CHE during ripening.

849 **Table 4.** Factor loadings for each parameter studied on the first two principal
850 components obtained

851 **Figure 1.** Evolution of pH values, moisture content and water activity in dry-
852 cured sausages treated with BHT, GRA and CHE during ripening.

853 **Figure 2.** Evolution of CIE parameters (L*, a* and b*) in dry-cured sausages
854 treated with BHT, GRA and CHE during ripening.

855 **Figure 3.** Effect of BHT, GRA and CHE extract on TBARS index in dry-cured
856 sausages during ripening.

857 **Figure 4.** Effect of BHT, GRA and CHE extract on TVC, BAL, mould and yeast
858 in dry-cured sausages during ripening.

859 **Figure 5.** Mean values of sensory properties of dry-cured sausages treated with
860 BHT, GRA and CHE in the final point.

861 **Figure 6.** Relationships among dry-cured sausages significant variables in the
862 last point obtained by PCA. Projection of the variables and dry-cured sausages
863 groups in the plane defined by the first two principal components

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Table 1.

	Total polyphenols	TEAC	DPPH		β-carotene	
	(mg GAE/g extract)	(g Trolox/g extract)	EC ₅₀ (g extract/L)	ratio (g Eq BHT/g extract)	EC ₅₀ (g extract/L)	ratio (g Eq BHT /g extract)
GRA	373,0 ± 6,10	2,93 ± 0,03	0,16 ± 0,019	1,80 ± 0,28	0,05 ± 0,008	1,28 ± 0,22
CHE	89,01 ± 0,34	0,27 ± 0,02	1,04 (NS)	0,48 ± 0,11	0,10 ± 0,013	0,53 ± 0,11

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12	Days	0	4				19				48			
13			CON	BHT	CHE	GRA	SEM	SIG	CON	BHT	CHE	GRA	SEM	SIG
14														
15														
16	Hardness (kg)	2.04	2.26 ^a	2.09 ^a	1.96 ^a	3.97 ^b	0.33	*	8.65 ^b	7.77 ^b	2.49 ^a	8.72 ^b	0.99	**
17														
18	Springiness (mm)	0.41	0.42 ^b	0.38 ^b	0.31 ^a	0.29 ^a	0.02	**	0.31	0.35	0.32	0.34	0.01	n.s.
19														
20														
21	Chewiness (kg*mm)	0.18	0.18 ^{bc}	0.13 ^{ab}	0.09 ^a	0.20 ^c	0.02	*	0.96 ^c	0.62 ^b	0.17 ^a	0.86 ^{bc}	0.12	**
22														
23														
24	Gumminess (kg)	0.39	0.40 ^a	0.34 ^a	0.27 ^a	0.66 ^b	0.06	**	2.99 ^c	1.79 ^b	0.53 ^a	2.40 ^{bc}	0.35	**
25														
26	Cohesiveness	0.18	0.19	0.16	0.16	0.16	0.07	n.s.	0.34 ^b	0.23 ^a	0.22 ^a	0.27 ^{ab}	0.02	*
27														
28	870													
29														
30	871													
31														
32	872													
33														
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Table 3.

Days	0						4						19						48					
	CON	BHT	CHE	GRA	SEM	SIG	CON	BHT	CHE	GRA	SEM	SIG	CON	BHT	CHE	GRA	SEM	SIG	CON	BHT	CHE	GRA	SEM	SIG
Nonane	2.5	2.22	3.12	3.37	4.36	0.46	n.s.	3.47	2.24	2.39	3.69	0.40	n.s.	1.48	1.29	1.08	1.79	0.24	n.s.					
Dodecane	2.26	2.17	n.d.	n.d.	n.d.	0.35	***	2.32 ^b	n.d.	1.54 ^a	n.d.	0.38	**	1.51 ^a	1.11 ^a	1.91 ^a	2.79 ^b	0.24	*					
Hexanal	14.22	15.40 ^a	9.00 ^a	48.93 ^c	28.65 ^b	5.82	***	2.44 ^a	n.d.	n.d.	2.95 ^b	0.51	***	16.76 ^b	2.19 ^a	n.d.	2.27 ^a	2.52	***					
Decanal	2.64	3.39	n.d.	n.d.	n.d.	0.55	***	3.29 ^b	0.98 ^a	n.d.	n.d.	0.54	*	n.d.	4.51	n.d.	4.83	0.89	**					
Heptanone	0.00	n.d.	n.d.	n.d.	n.d.	0.00	n.s.	5.03 ^b	4.28 ^{ab}	2.98 ^{ab}	2.22 ^a	0.48	n.s.	2.51	2.20	0.99	1.93	0.27	n.s.					
Nonanone	0.00	n.d.	n.d.	n.d.	0.00	0.00	n.s.	n.d.	6.21 ^b	6.56 ^b	1.72 ^a	1.08	**	4.60	4.06	4.76	3.21	0.38	n.s.					
Hexanol	2.07	3.13 ^a	n.d.	n.d.	5.48 ^b	0.87	***	2.30 ^b	n.d.	1.21 ^a	1.33 ^a	0.31	**	0.93	n.d.	n.d.	n.d.	0.15	**					
Acetyl acid	0.00	n.d.	13.94 ^a	26.16 ^b	n.d.	4.18	**	7.37 ^a	20.72 ^b	48.30 ^c	31.70 ^b	5.79	**	14.57 ^a	22.14 ^b	28.01 ^b	40.12 ^c	3.57	**					
Butanoic acid methyl ester	84.09	96.50	103.30	103.70	60.98	8.03	n.s.	84.80 ^b	78.98 ^b	139.06 ^c	37.27 ^a	13.91	**	20.12 ^a	22.44 ^a	48.58 ^b	12.12 ^a	5.28	**					
Pentanoic acid methyl ester	17.61	17.71	13.27	13.38	13.37	1.17	n.s.	10.24 ^{ab}	8.47 ^a	11.90 ^b	8.80 ^a	0.54	*	5.26 ^b	3.37 ^a	5.23 ^b	3.25 ^a	0.37	**					
Hexanoic acid methyl ester	113.87	133.11	100.13	115.38	154.57	9.99	n.s.	52.40 ^a	48.51 ^a	56.06 ^a	76.92 ^b	4.49	*	64.16	39.72	37.97	30.41	5.86	n.s.					
Heptanoic acid methyl ester	3.24	5.01 ^b	n.d.	2.75 ^a	n.d.	0.80	**	2.51	2.66	2.98	n.d.	0.46	**	n.d.	n.d.	1.42	n.d.	0.23	*					
Octanoic acid methyl ester	18.54	25.51	32.76	31.83	17.56	3.55	n.s.	11.39 ^b	14.06 ^b	15.53 ^b	6.65 ^a	1.35	*	9.96 ^b	13.20 ^{bc}	14.72 ^c	5.49 ^a	1.40	n.s.					
Decanoic acid methyl ester	1.03	0.96 ^a	2.11 ^{ab}	2.48 ^b	1.16 ^{ab}	0.27	*	0.98	1.41	2.78	0.73	0.42	n.s.	1.79	2.81	2.55	1.01	0.35	*					
Tetradecanoic acid	1.33	7.28	n.d.	6.25	n.d.	1.32	**	0.21 ^a	0.61 ^a	7.50 ^b	n.d.	1.19	**	0.65 ^a	4.05 ^b	1.94 ^a	n.d.	0.61	n.s.					
p-xylene	2.11	3.72	4.46	1.84	4.88	0.60	n.s.	3.97	3.09	4.80	2.84	0.38	n.s.	2.34	3.11	3.38	3.48	0.22	n.s.					
Total volatile	265.5	316.2	282.2	356.1	291.1	17.7	n.s.	192.8 ^a	192.3 ^a	303.7 ^b	176.9 ^a	19.7	**	146.7 ^a	126.3 ^{ab}	152.6 ^c	112.8 ^a	7.07	*					

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Variables	PC1	PC2	Communality
L*	0.981	-0.098	0.979
a*	0.763	-0.326	0.810
Water activity	0.888	0.047	0.967
Moisture	0.878	-0.102	0.960
TBAR'S	-0.942	0.273	0.971
Hardness	-0.993	0.040	0.989
Gumminess	-0.966	0.003	0.946
Chewiness	-0.909	0.055	0.851
TVC	-0.121	0.958	0.993
BAL	-0.232	0.956	0.972
Acetic acid	0.635	-0.292	0.979
Butanoic acid methyl ester	0.525	0.779	0.991
Pentanoic acid methyl ester	-0.291	0.931	0.971
Octanoic acid methyl ester	0.344	0.476	0.976
Eigenvalue	7.960	4.021	
Percent of variance	56.859	28.722	
Acumulative percentage	56.859	85.581	

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Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.