The aim of this study was to evaluate the dietary effect of feeding pigs with diets enriched with sweet chestnut wood (Castanea sativa Mill.) extract on the microbiological and chemical characteristics of cooked pork ham. Three groups of 10 pigs were fed with a control diet (CTRL), with the CTRL diet enriched with 0.2% of oregano extract (OR) and with the CTRL diet enriched with 0.2% of sweet chestnut wood extract (SCW), respectively. Six cooked hams per group were produced, sliced and packaged under a modified atmosphere (N2:CO2=80:20) and stored at refrigeration temperature (4±1°C). Three packages per cooked hams per group were aseptically sliced after refrigeration period and packaged under a modified atmosphere condition (N2:CO2=80:20; multilayer film composed by polypropylene-ethylene vinyl alcohol-polypropylene; Tecknofood Pack, Castelnuovo, PV, Italy) in 150 g serving packs. Nine packs for each cooked ham were sampled on the turkey breast fillet microbial load and packaged under a modified atmosphere condition (N2:CO2=80:20; multilayer film composed by polypropylene-ethylene vinyl alcohol-polypropylene; Tecknofood Pack, Castelnuovo, PV, Italy) in 150 g serving packs. Nine packs for each cooked ham were sampled on the turkey breast fillet microbial load and packaged under a modified atmosphere condition (N2:CO2=80:20; multilayer film composed by polypropylene-ethylene vinyl alcohol-polypropylene; Tecknofood Pack, Castelnuovo, PV, Italy). Three packages per cooked hams per group were aseptically sliced after refrigeration period and packaged under a modified atmosphere condition (N2:CO2=80:20; multilayer film composed by polypropylene-ethylene vinyl alcohol-polypropylene; Tecknofood Pack, Castelnuovo, PV, Italy). Three packages per cooked hams per group were aseptically sliced after refrigeration period and packaged under a modified atmosphere condition (N2:CO2=80:20; multilayer film composed by polypropylene-ethylene vinyl alcohol-polypropylene; Tecknofood Pack, Castelnuovo, PV, Italy).
days of storage (3 packs/ham/group). Storage was performed at 4±1°C.

After 3 hour (0 day of storage) from packaging the following measurements were performed: chemical composition (AOAC, 1990); oxygen radical absorbance capacity assay (ORACFL) as described by Branciari et al. (2015); pH determination as described by Bendal (1975); colour coordinates (CIE, 1986), total volatile basic nitrogen (TVBN) as described by Pearson (1991); TBARs as described by Tarladgis et al. (1960).

The microbiological analyses were: total microbial count (TMC) on Plate Count Agar (PCA; Oxoid Ltd., Basingstoke, UK) aerobically incubated at 30°C for 72h; Lactobacillus spp. on MRS agar (Oxoid Ltd.) anaerobically incubated at 37°C for 48 h (LAB); Enterobacteriaceae count using Violet Red Bile Glucose Agar (VRBG; Oxoid Ltd.) aerobically incubated at 37°C for 24h. The results were normalized to colony forming unit (cfu) g⁻¹ and converted into Log values. The presence of Listeria monocytogenes was also tested for using the criteria set by ISO 11290-1 (ISO, 1996).

The determination of pH, colour, TVBN and TBARs, and the microbial analyses were repeated at 10 and 20 days of storage at 4°C.

Data were analyzed using an ANOVA model (Statview; SAS Institute Inc., Cary, NC, USA) with diet and time as fix factors. For chemical composition and ORACFL only diet were considered as fixed factor. Tukey’s test was used for post hoc comparisons between groups. Differences were considered to be significant when P<0.05. For microbial analysis the dietary effects on the same sampling time was evaluated using the unpaired T test (Statview) and the significance level was set at a value of P<0.05.

Results

The chemical composition and ORACFL values of the products are reported in Figures 1 and 2. No differences were recorded for chemical composition between the groups. Higher ORACFL mean values were recorded in SCW group (14.20±0.69 standard deviation) and OR (13.03±1.03) than CTRL (9.95±0.96) group (P<0.001).

Microbiological analyses (n=18 for each group at each sampling time) show an increase in TMC and LAB values during storage (Figure 3). No differences were recorded among CTRL, OR and SCW groups at the same storage times considered. Enterobacteriaceae counts were below the detection limit in all the samples tested (with an exception in one SCW sample at 10 days of storage with Log 2.3 cfu g⁻¹ value). No Listeria monocytogenes was isolated from the samples.

For the physical-chemical analyses, pH, L* and TVBN values did not differ between the groups considered (Table 1). For ham redness (a* value) and yellowishness (b* value) no difference at 0 day of storage was registered between the groups but at 10 and 20 days of storage. Significant decreases were recorded only in CTRL samples (a* value: from 12.93 at 0 day to 9.65 at 20 days; for b* value from 8.51 at 0 days to 6.83 at 20 days). TBARs values followed the same trend as higher values were recorded in CTRL samples than in OR and SCW after 10 and 20 days of storage. At 20 days TBARs value in SCW were also lower than OR (1.95 vs 2.06 mg MDA/kg respectively). The effect of time was evident for all the parameters considered (Table 1).
Table 1. pH, colour coordinates, total basic volatile nitrogen and thiobarbituric reactive substances values of cooked pork ham produced from pigs fed with oregano extract, sweet chestnut wood extract and with a standard diet at 0, 10 and 20 days of storage (n=6 hams/group).

| Parameters                     | CTRL 0 day | OR 0 day | SCW 0 day | CTRL 10 days | OR 10 days | SCW 10 days | CTRL 20 days | OR 20 days | SCW 20 days | SEM | D | T | D×T |
|-------------------------------|------------|----------|-----------|--------------|------------|-------------|--------------|------------|-------------|-----|---|---|-----|
| pH                            | 6.14       | 6.16     | 6.02      | 5.79         | 5.79       | 5.79        | 5.75         | 5.75       | 5.35        | 5.46 | 0.041 | 0.483 | <0.001 | 0.066 |
| L*                            | 62.34      | 60.76    | 61.53     | 63.93        | 65.49      | 66.61       | 64.84        | 66.05      | 66.46       | 1.084 | 0.415 | <0.001 | 0.474 |
| a*                            | 12.93      | 13.20    | 12.73     | 10.63        | 12.01      | 12.48       | 9.65         | 11.91      | 12.38       | 0.570 | 0.005 | 0.003 | 0.140 |
| b*                            | 8.51       | 7.89     | 7.65      | 6.92         | 7.77       | 7.83        | 6.83         | 7.27       | 7.39        | 0.271 | 0.527 | 0.001 | 0.019 |
| TBARS (mg MDA kg⁻¹)           | 1.48       | 1.46     | 1.37      | 1.80         | 1.70       | 1.59        | 2.12         | 2.06       | 1.95        | 0.034 | <0.001 | <0.001 | 0.437 |

SEM, standard error of mean; CTRL, control diet; OR, oregano extract; SCW, sweet chestnut wood extract; D, diet effect; T, time effect; D×T, interaction diet and time effects; L*, lightness; a*, redness; b*, yellowness; TBARS, thiobarbituric reactive substances; MDA, malondialdehyde. * Means within a row with different letters are statistically different.

Discussion

The ORAC₉₀ results confirm that bioactive substances could reach meat and meat products through enriched diets (Moñino et al., 2008). These molecules are responsible for the different effects on the products but in this study only antioxidant effects were registered. Furthermore the hygienic characteristics of meat products are strongly affected by several factor including food processing, packaging and storage conditions. The addition of natural preservatives directly to the products could affect microbial growth only if a certain amount of extract is used (Zhang et al., 2009). Probably, in this study, the amount of bioactive substances used could not affect the hygienic characteristics of the products during storage. This consideration is in contrast to other author who found dietary antimicrobial effects of natural preservative in meat (Govaris et al., 2007; Nieto et al., 2010; Bañón et al., 2012; Serrano et al., 2014), including oregano leaves (Botsoglou et al., 2010).

The dietary effects on lipid oxidation of OR and SCW was observed in cooked pork hams. This effects in meat exerted by oregano is reported by several authors (Simitzis et al., 2008), even though Janz et al. (2007) observed only a tendency towards a reduced lipid oxidation (oregano essential oil added to the diet at a dose of 0.05%) and Simitzis et al. (2010) (feed supplemented with oregano essential oil at concentrations of 0.25, 0.5 and 1 ml/kg of feed diet) did not find any effect on stored pig meat when different concentrations of oregano essential oils were added to the diet. SCW extract was proved effective against lipid oxidation in rabbit meat (Liu et al., 2009) but not in poultry meat (Schiavone et al., 2008). An antioxidant effect of a combination of the two extract was found in pork meat when it was used in the diet of outdoor reared pigs (Ranucci et al., 2015).

Oxidation is a relevant factor affecting shelf life of the products (i.e. development of off-flavour) (Nieto et al., 2011) and colour stability (Luciano et al., 2011). This effects was registered in SCW ham, where a* value remained stable during storage time even when pH fell under acceptable conditions (pH<5.5). The SCW samples showed a higher antioxidant capacity and subsequently a lower oxidation than OR.

Conclusions

The dietary supplementation with SCWE and oregano extract affected the oxidative status of cooked pork ham but no antimicrobial effects were detected on the products. More relevant information could be provided by the evaluation of the antimicrobial effects on specific bacterial that inhabit the animal intestine and are responsible for foodborne disease and are possibly present in meat. Nonetheless the cooking process performed on the hams considered, that reach 72°C at the core of the product, is responsible for the elimination of such pathogens and only post process intervention (i.e. slicing) could contaminate the products. Strategies could nevertheless be considered with the use of such diet enrichments and other processing interventions able to promote food hygiene and improve shelf-life through a combination of antibacterial and antioxidant effects.

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