Dietary effects of oregano (*Origanum vulgaris* L.) plant or sweet chestnut (*Castanea sativa* Mill.) wood extracts on microbiological, chemico-physical characteristics and lipid oxidation of cooked ham during storage

David Ranucci,[1] Dino Miraglia,[1] Massimo Trabalza-Marinucci,[1] Gabriele Acuti,[1] Michela Codini,[2] Maria Rachele Ceccarini,[2] Claudio Forte,[1] Raffaella Branciari[1]

[1]Department of Veterinary Medicine, University of Perugia, Perugia; [2]Department of Pharmaceutical Sciences, University of Perugia, Perugia, Italy

Abstract

The aim of this study was to evaluate the dietary effect of feeding pigs with diets enriched with sweet chestnut wood (*Castanea sativa* Mill.) or oregano (*Origanum vulgaris* L.) 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 ham were sampled for analyses at three different storage times (0, 10 and 20 days). At day 0 time, antioxidant capacity of the products ($ORAC_{FL}$ assay) and chemical composition were performed. At each sampling time, from all the samples the following analyses were performed: total microbial count (TMC), lactic acid bacteria count (LAB), *Enterobacteriaceae* count, *Listeria monocytogenes*, pH value, colour coordinates (L*, a*, b*), total basic volatile nitrogen (TBVN) and thiobarbituric reactive substances (TBARs) determinations. No differences in TMC, LAB and *Enterobacteriaceae* count, pH, TBVN, chemical composition and L* values were registered between the three groups at all the sampling times considered. No *Listeria monocytogenes* was detected in the samples tested. Significant differences were registered for $ORAC_{FL}$ at 0 days, a* and b* values and TBARs value at 10 and 20 days of storage, with higher values for $ORAC_{FL}$, a* and b* values and lower values for TBARs in SCW and OR than CTRL. No antimicrobial effect could be recorded for OR and SCW but a higher oxidative stability, also highlighted by the colour maintenance, was observed in both OR and SCW.

Introduction

Antimicrobial and antioxidant activity of different natural plant extracts is well documented (Zheng and Wang, 2001; Burt, 2004; Bozin *et al*., 2006). Recently, the effects of dietary supplementation with natural bioactive compounds on food quality have been considered (Branciari *et al*., 2015). The main effect recorded is a reduction in lipid oxidation in meat (Simitzis *et al.,* 2008; Lauchky *et al*., 2010; Ranucci *et al*., 2015) and meat products (Ranucci *et al*., 2013), but the effect on the meat hygiene was also reported (Nieto *et al*., 2010).

A high content of bioactive polyphenolic compounds is present in sweet chestnut (*Castanea sativa* Mill.) wood extract (SCWE) and oregano (*Origanum vulgaris* L.) extract (Barreira *et al*., 2008; Comandini *et al*., 2014). For this reason, the use of these extracts in animal feed has been proposed for its positive effects on growth performance and meat quality traits (Schiavone *et al.*, 2008; Liu *et al*., 2009). Both oregano and SCWE are considered also for their antioxidant property (Sanchez-Escalante *et al*., 2003; Frankic and Solobir, 2011), as polyphenols owed both radical scavenging and iron binding activities (Lopes *et al*., 1999). Furthermore, the in vitro antimicrobial effects of both extracts are reported. Oregano extract was proved effective against *Listeria monocytogenes*, *Bacillus cereus, Staphylococcus aureus* and *Escherichia coli* (Shan *et al*., 2007). SCWE affects the main pathogens responsible for enteric disorders in different poultry (broilers, turkeys, layer hens), cattle and swine productions (Graziani *et al*., 2006). For oregano, dietary administration shows an inhibitory effect of oregano leaves on the turkey breast fillet microbial load (Botsoglu *et al*., 2010).

No data is yet available, on the effects of the dietary enrichment with oregano extract or SCWE on the microbial, oxidative and quality characteristics of the pork meat products. The aim of the present work is to evaluate the effects of a diet enriched in oregano extract or SCWE on the hygienic characteristics, oxidative stability and some quality traits of cooked pork ham during storage.

Correspondence: David Ranucci, Department of Veterinary Medicine, University of Perugia, via San Costanzo 4, 06126 Perugia (PG), Italy
Tel: +39.075.5857931 - Fax: +39.075.5857928.
E-mail: david.ranucci@unipg.it

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Materials and Methods

This study was conducted from July to October 2014 on a small farm in Umbria, Italy. Thirty Italian Large White x Landrace x Duroc crossbred male/female hybrid pigs, with an average live weight (LW) of 108.1±11.1 kg at the beginning of the trial, were divided into 3 groups (10 animals/group) and balanced for the LW and sex. The diets (crude protein: 15.86%, ether extract: 4.01%, crude fibre: 5.08%) were isonitrogenous and isoenergetic: i) control (CTRL), a commercial pelleted diet; ii) OR, the CTRL diet with the addition of 0.2% oregano extract; iii) SCW, the CTRL diet with the addition of 0.2% sweet chestnut wood extract. After 68 days of the trial, the animals (final LW: 153.5±12.9 kg) were slaughtered in a local abattoir and submitted to a local meat product industry for the cooked hams production. Carcasses were deboned after refrigeration and cooked hams were produced from each group according to the food producer's procedures. In particular neither ascorbic acid as an antioxidant nor nitrites as preservatives were used. In addition, no final pasteurisation was performed on the products. Six cooked hams/group were aseptically sliced after refrigeration and packaged under a modified atmosphere condition (N2:CO2=80:20; multilayer film composed by polypropylene-ethylene vinil alcohol-polypropylene; Tecknofood Pack, Castelnovetto, PV, Italy) in 150 g serving packs. Nine packs for each cooked ham were sampled for chemical-physical and microbiological analyses. The analyses were performed at 3 hours on 0 day of storage (3 packs/ham/group), at 10 days of storage (3 packs/ham/group) and 20

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 ($ORAC_{FL}$) 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 $ORAC_{FL}$ 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 $ORAC_{FL}$ values of the products are reported in Figures 1 and 2. No differences were recorded for chemical composition between the groups. Higher $ORAC_{FL}$ mean values were recorded in SCW (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 monocytogens* was isolated from the samples.

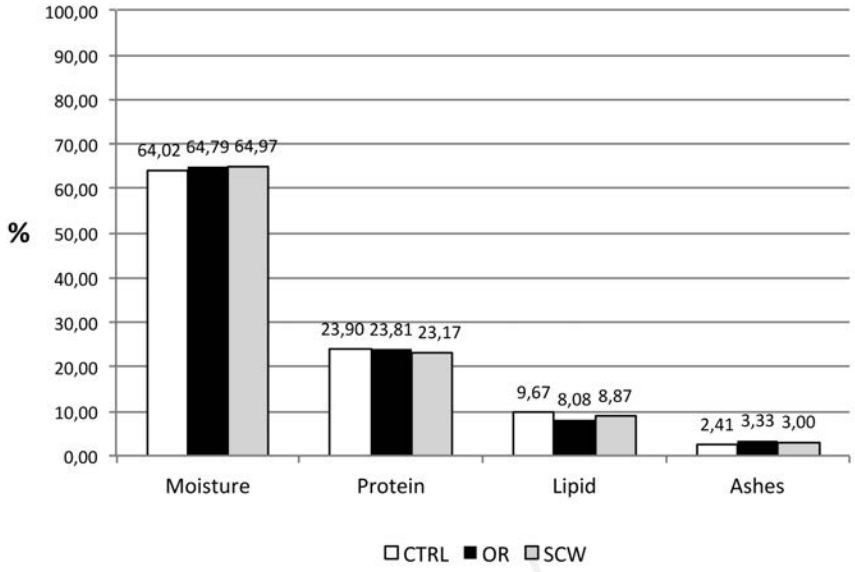

Figure 1. Chemical composition (%) of cooked pork hams produced from pigs fed with oregano extract (OR), with sweet chestnut wood extract (SCW), and with a standard diet (CTRL) (n=6 hams/group).

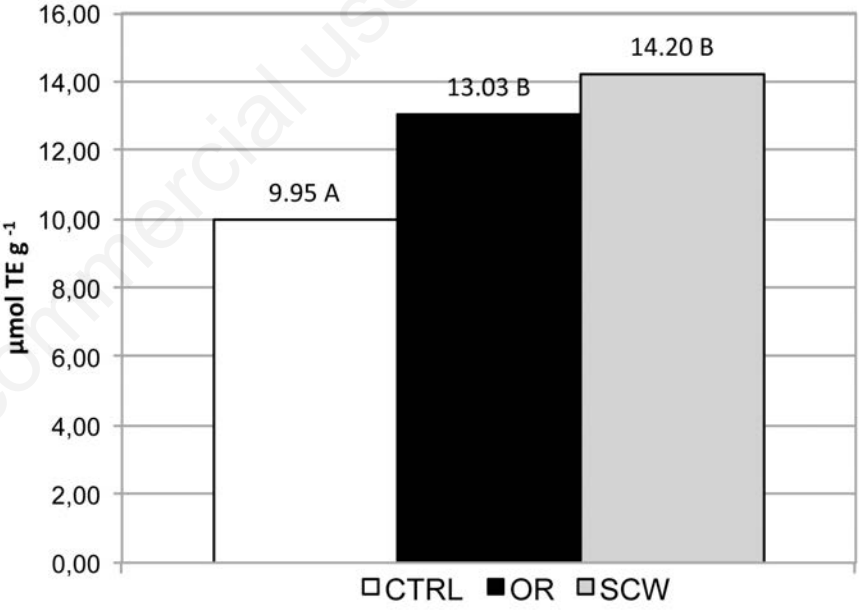

Figure 2. Antioxidant capacity of the products (µmol Trolox© equivalent g^{-1}) of cooked pork hams produced from pigs fed with oregano extract (OR), with sweet chestnut wood extract (SCW), and with a standard diet (CTRL) (n=6 hams/group). Different letters denote statistical differences ($P<0.05$).

For the physical-chemical analyses, pH, L* and TBVN 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 thio-barbituric 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	Storage time									SEM	P		
	0 day			10 days			20 days						
	CTRL	OR	SCW	CTRL	OR	SCW	CTRL	OR	SCW		D	T	D×T
pH	6.14^{c}	6.16^{c}	6.02^{c}	5.79^{b}	5.79^{b}	5.75^{b}	5.35^{a}	5.39^{a}	5.46^{a}	0.041	0.493	<0.001	0.066
L*	62.34^{a}	60.76^{a}	61.53^{a}	63.93^{ab}	65.49^{b}	66.61^{b}	64.84^{b}	66.05^{b}	66.46^{b}	1.084	0.415	<0.001	0.474
a*	12.93^{c}	13.20^{c}	12.73^{c}	10.63^{b}	12.01^{c}	12.48^{c}	9.65^{a}	11.91^{bc}	12.38^{c}	0.570	0.005	0.003	0.140
b*	8.51^{b}	7.89^{ab}	7.69^{ab}	6.92^{a}	7.77^{ab}	7.83^{ab}	6.83^{a}	7.27^{ab}	7.39^{ab}	0.271	0.527	0.001	0.019
TVBN (mg 100 g^{-1})	25.65^{a}	23.37^{a}	23.62^{a}	26.53^{b}	26.96^{b}	27.29^{b}	30.56^{c}	30.60^{c}	31.25^{c}	0.303	0.122	<0.001	0.638
TBARs (mg MDA kg^{-1})	1.48^{a}	1.46^{a}	1.37^{a}	1.80^{c}	1.70^{c}	1.56^{b}	2.12^{f}	2.06^{e}	1.95^{d}	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; DxT, interaction diet and time effects; L*, lightness; a*, redness; b*, yellowness; TVBN, total basic volatile nitrogen; TBARs, thio-barbituric reactive substances; MDA, malondialdehyde. $^{a-f}$Means within a row with different letters are statistically different.

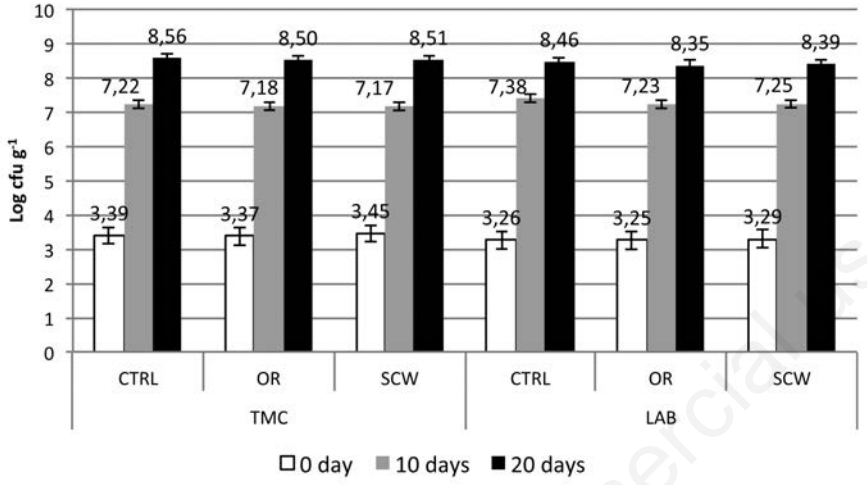

Figure 3. Total microbial count (TMC) and lactic acid bacteria count (LAB) (Log colony forming units g^{-1}) evolution in cooked pork hams produced from pigs fed with oregano extract (OR), with sweet chestnut wood extract (SCW), with a standard diet at 0, 10 and 20 days of storage (CTRL) (n= 6 hams/group).

Discussion

The $ORAC_{FL}$ 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 fed 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-odour and 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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