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Quality Assessment of Cooked Ham from Medium-Heavy Pigs Fed with Antioxidant Blend

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Abstract

The aim of the study was to evaluate the effect of pig dietary supplementation with an antioxidant mixture (150 mg of Vitamin E and 15 mg of verbascoside from *Verbenaceae* extract) for 38 days before slaughter on the quality parameters of cooked ham. A total of 150 pigs were divided into two experimental groups: one receiving a control diet (C) and one a diet with the antioxidant blend (AO). Twenty pigs per treatment were randomly selected and slaughtered at about 135 kg of live weight. The results showed that cooked ham colour indexes and tenderness were not influenced ($p > 0.05$) by dietary treatment. The nutritional and sensory parameters were unaltered ($p > 0.05$) by the antioxidant supplementation. Dietary treatment positively influenced the oxidative stability ($p < 0.05$) of cooked ham from pigs fed the AO diet compared to the control diet. The present study suggests that dietary supplementation with an antioxidant blend for a short period (38 days) is able to improve the oxidative stability of cooked ham without impacting its chemical and sensory characteristics.

Keywords: pig; antioxidants mixture; cooked ham; quality parameters

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1. Introduction

Cooked ham is a product with a high economic value; in fact, among pork products it is the most consumed in some European countries. In Italy, cooked ham is the most consumed cured meat accounting for 27.1% of total consumption [1]. It is a popular ready-to-eat product worldwide due to its good nutritional value and sensory characteristics [2]. According to the Italian Ministerial Decree 21 September 2005 concerning the production and sale of cured products, cooked ham is defined as a "product obtained from the deboned, defatted pig thigh, without connective tissue and rind, using water, salt, sodium nitrite or potassium nitrite and sodium nitrate or potassium nitrate." This decree also defines three different classes of cooked ham based on the product's quality parameters and the concentration and type of additives and ingredients used: cooked, selected and high-quality ham. The manufacturing process of cooked ham are well described in the review by Lebret et al. [3] and the paper by Moretti et al. [4].

The quality of cooked hams is related to both the raw materials and the meat-processing technologies used. The characteristics of the fresh thighs have a significant

influence on the ham's quality and are affected by pig farming, including genetic type, slaughter weight, feed ingredients and dietary supplements [3]. Also, the processing technologies used in the production of cooked ham, i.e., the brine ingredients and additives, the injection rate and the heat treatment, have a relevant impact on the sensory and nutritional characteristics of the final product, and therefore on consumer acceptance [3,4].

Lipid oxidation is one of the main processes that negatively affects the characteristics of meat and of meat products, as described in the review by Domínguez et al. [5]. Oxidation processes have a negative impact on the products' nutritional parameters, such as vitamins and fatty acids. Moreover, oxidation can lead to changes in colour parameters, development of off-flavours and rancidity, as well as a reduction in sensory shelf-life and consumer acceptability [5]. Lipid oxidation also produces several compounds, such as lipid hydroperoxides, cholesterol oxidation products, aldehydes and oxysterols. These compounds can have detrimental effects on human health, with possible carcinogenic and atherosclerotic outcomes [5,6].

Limiting oxidation processes during meat processing and storage is increasingly important as the demand for cooked and ready-to-eat meat products increases [7]. In addition to the technological processes, pig dietary supplementation with antioxidants is a key factor in reducing oxidative processes and improving the oxidation status and colour stability of meat products [8–11].

Vitamin E is the most important liposoluble antioxidant in the biological system, as it can decrease lipid peroxidation and protect cell membranes from oxidation processes [12]. In the feed formulation, the synthetic forms of Vitamin E, specifically all-rac-α-tocopherol esterified with acetate, are commonly used to stabilize the phenolic group and prevent oxidation [13]. Usually, the use of supra-nutritional dosages of dietary Vitamin E in pigs can prevent oxidating process in fresh meat and pork products [14,15]. Supra-nutritional dosages of Vitamin E in pig diets generally prevent oxidation in both fresh meat and pork products [14,15].

To minimize the reliance on synthetic compounds in the food production chain, both consumers and producers are increasingly advocating for the use of natural antioxidants. The most widely used natural antioxidants are phenolic compounds from plant sources, including molecules such as phenols, phenolic acids, flavonoids, coumarins, stilbenes, tannins and lignans. These compounds have several health properties, including strong antioxidant activity [16]. Among polyphenols, a water-soluble compound called verbascoside, which is mainly extracted from *Verbascum* spp., has gained attention for its effectiveness as a free radical scavenger and inhibitor of lipid peroxidation. Verbascoside is a natural antioxidant and also has anti-bacterial and anti-inflammatory activities in humans, although its mechanisms of action require further investigation [17]. Our earlier research has shown the application of verbascoside as a dietary antioxidant at the same dosage in pigs, resulting in improved sensory shelf life of the *Longissimus dorsi* (LD) muscle, positively affecting colour parameters and oxidative stability [18,19]. One study of pig slaughter at 110 kg of live weight and receiving long-term supplementation with antioxidants (5 mg/kg diet; 166 days) points out that verbascoside enhance α tocopherol concentration in LD muscle, decreasing oxidative stability in raw meat. Moreover, a reduction in fat odour and rancid flavour intensity was detected in cooked LD muscle stored at 4 °C for 24 h [18]. Moreover, the same antioxidant blend at the present dosage (fed for 38 days) improved oxidative stability and redness colour index in LD muscle packaged in modified atmosphere and stored at 4 °C for 21 days. In particular, the oxidative stability in the group fed the antioxidant blend was lower than the threshold level of fresh meat (0.5 mg MDA/kg muscle) up to 15 d of refrigerated storage. Moreover, the sensory shelf life revealed that at 15 days

of refrigerated storage an improvement in colour and overall smell was detected in LD muscle from pigs fed an antioxidant blend compared to controls [19].

In the literature, so far only one study has shown the effect of a dietary supplement composed of a mixture of liposoluble and water-soluble natural antioxidant compounds on fresh and cured pork products [8]. No previous studies have reported the effect of pig dietary supplementation with an antioxidant mixture containing verbascoside and α-tocopheryl acetate on cooked ham quality.

The aim of the present trial was thus to investigate the quality parameters of cooked ham from pigs fed a control diet and a diet supplemented with a blend of plant extracts rich in verbascoside and α-tocopheryl acetate.

2. Materials and Methods

2.1. Animal and Dietary Treatment

The study involving animals was conducted according to the European Union guidelines (2010/63/EU) and approved by the Italian Ministry of Health (Legislative decree n. 26/2014). One hundred and fifty Duroc × (Landrace × Large White) pigs, half barrows and half females, of an average live weight of 110 ± 5.5 kg were assigned to two dietary treatments: a commercial diet (C) and a diet integrated with a mixture of Vitamin E and verbascoside (AO) from a water-soluble extract of *Verbenaceae* leaves (*Lippia* spp.). The corn-based diets were formulated to meet all the nutrient requirements [20] and contained 20 mg/kg of all-rac-α-tocopheryl acetate. Pigs were rationed on 9% of metabolic weight ($LW^{0.75}$) and the feed intake was about 3 kg/day. The amount of *Verbenacea* extract was chosen according to our previous study [18].

The trial lasted 38 days before slaughter, and the AO blend was fed in a daily amount of 150 mg of Vitamin E and 15 mg of verbascoside. According to the manufacturer, the amount of phenylpropanoid glycosides and benzoic acid in the AO blend is as follows: gallic acid, 1.75 ± 0.07; 3.4-dihydroxybenzoic acid, 0.45 ± 0.04; methyl gallate, 1.91 ± 0.09; isoverbascoside, 0.43 ± 0.04; and verbascoside, 4.47 ± 0.08 g/kg. To prevent oxidation, the AO supplement was microencapsulated in a hydrogenated vegetable lipid matrix, applying spray-cooling (Sintal Zootecnica, Isola Vicentina, Vicenza, Italy).

2.2. Carcass Traits

Pigs were transported to a slaughterhouse (ProSus, s.c.a., Vescovato, Cremona, Italy). The animals were slaughtered at 135 kg of live weight. Animals were electrically stunned and exsanguinated following the Council Directive 93/119/EC of 22 December 1993. Forty thighs, sampled from the left side of the carcasses (20 per treatment), were weighed and processed under commercial guidelines for cooked ham production. Briefly, deboned fresh pig thighs were selected, and the first step of processing was to inject brine (10% raw weight injection level; 10% sodium chloride, 0.2% sodium ascorbate, 0.1% sodium nitrite) into the ham with a multi-needle injector. The brine was a homogeneous mixture of water and sodium chloride, sugars, sodium nitrite, sodium ascorbate and flavourings. Subsequently the ham was placed in a vacuum tumbler for 12 h at 4–5 °C and slow speed (2–4 rpm/min). The raw ham was then put into moulds and cooked for 14 h in a steam oven with a slow heating rate of about 110° C to a core temperature of 69 °C. This was followed by a cooling phase (chill room at 2° C for 24 h) to achieve a core temperature of 4° C. The cooked hams were then packed under vacuum. After processing, the quality parameters of the cooked hams were evaluated.

2.3. Sampling

The cooked hams (N = 40; 20 hams per treatments) were transferred to the laboratories of the University of Milan. The cooked hams were weighed, sectioned perpendicularly and then sampled into slices. At sampling, one slice 1 cm thick was used to determine the colour index of each ham. Samples of 2.54 cm thickness were utilized to determine tenderness using Warner–Bratzler shear force. Two slices (1 cm thick) per ham, sampled for determination of chemical parameters and oxidative stability, were vacuum-packaged and stored at −20 °C pending analyses. The remaining half-hams were vacuum-packaged and stored at 4 °C pending sensory evaluation. Package sealing was performed using a vacuum packaging machine (VM-16 Orved, Venice, Italy).

2.4. Physical Parameters

The International Commission on Illumination (CIE) lightness (L^*), redness (a^*) and yellowness (b^*) colour coordinates were measured using a CR-300 Chroma Meter (Minolta Camera, Co., Osaka, Japan). The instrument was calibrated on the CIE LAB colour space system using the white calibration plate (Calibration Plate CR-A43, Minolta Cameras, New York, NY, USA). The colourimeter had an 8 mm measuring area and was illuminated with a pulsed Xenon arc lamp (illuminated C) at a 0° viewing angle. Reflectance measurements were obtained at a viewing angle of 0°, and the spectral component was included. For each data point, six measurements at the slice surface were performed. The Warner–Bratzler shear force (WBSF) is an instrumental index and is considered a benchmark in the estimation of pork tenderness. The WBSF was determined on cooked ham samples of 2.54 cm thickness. Core samples (Ø 1.25 cm) were prepared, and their peak shear force was assessed using a Warner–Bratzler shear test cell equipped with a texture measurement module (model 4466; Instron Corp., Canton, MA, USA). Each data point is the mean of six measurements and is expressed in Newtons (N).

2.5. Chemical Parameters

Samples of cooked ham, without the external fat, were analyzed for moisture (method 985.41), ash (method 920.153), fat (method 960.39) and crude protein (method 928.08) content. The analyses were performed in duplicate according to the methods recommended by the Association of Analytical Chemists [21].

2.6. Oxidative Stability

The oxidative stability was evaluated, on samples stored at −20 °C for four weeks and thawed at 4 °C, following the thiobarbituric acid reactive substances (TBARS) method outlined in Monin et al. [22]. All the analyses were made in duplicate. The absorbance was measured at 532 nm using a UV-VIS spectrophotometer (Cary 100, Varian, Belrose, Australia). Absorbance data were fitted with a calibration curve prepared with 1,1,3,3-tetraethoxypropane (TEP; Sigma-Aldrich, Milan, Italy). Data are expressed as mg of malondialdehyde (MDA) per kg of pork product.

2.7. Sensory Analysis

The cooked hams were cut into 15 mm slices and were offered to panellists on white plastic plates identified by 3-digit codes. A descriptive sensory profile was performed using a selected and trained panel of eight judges [23]. All the evaluation analyses were performed in a sensory laboratory according to ISO recommendations [24]. To establish a common terminology amongst the panel members and to utilize the scale properly, three sessions were planned. During these training sessions the final list of descriptors was set, which consisted of fourteen attributes: five for appearance (pink, colour homogeneity, fat

distribution, slice homogeneity, cohesiveness), two for aroma (typical, cooked meat), three for taste (sweet, salty and umami), two for flavour (typical, cooked meat), and two for texture (tender and fibrous). Attributes were evaluated using a continuous, non-structured scale ranging from absence of sensation (1) on the left to extremely intense (9) on the right. Each sample was evaluated across three sessions. During sampling, the judges had access to water and unsalted crackers. The serving order was randomized across all the sessions. The panel was trained first to evaluate the appearance and aroma, then to take the ham slice (15 mm thickness) and evaluate flavour, taste and texture. In each session the design was balanced to account for order of presentation and any carry-over effects [25].

2.8. Statistical Analyses

The data evaluation was performed using SPSS (SPSS/24 PC Statistics 26.0 IBM). Physical and chemical data regarding the meat were analyzed by one-way ANOVA with dietary treatment as the fixed effect. Cooked ham was the experimental unit for all the meat quality parameters. Data from sensory evaluation were analyzed by three-way ANOVA with samples (C and AO), judges (8), replicates (3) and their interactions as effects [23]. The significance of these effects was tested with F tests. Post hoc pairwise contrasts were evaluated by Duncan's test. Data are presented as means ± SEM, and a value of $p < 0.05$ was used to indicate statistical significance.

3. Results

3.1. Physical Parameters

Figure 1 shows the colour indexes of cooked ham from pigs fed a control diet and a diet containing an antioxidant mixture. The colour indexes—lightness (L^*), redness (a^*) and yellowness (b^*)—were not affected ($p > 0.05$) by AO blend dietary supplementation.

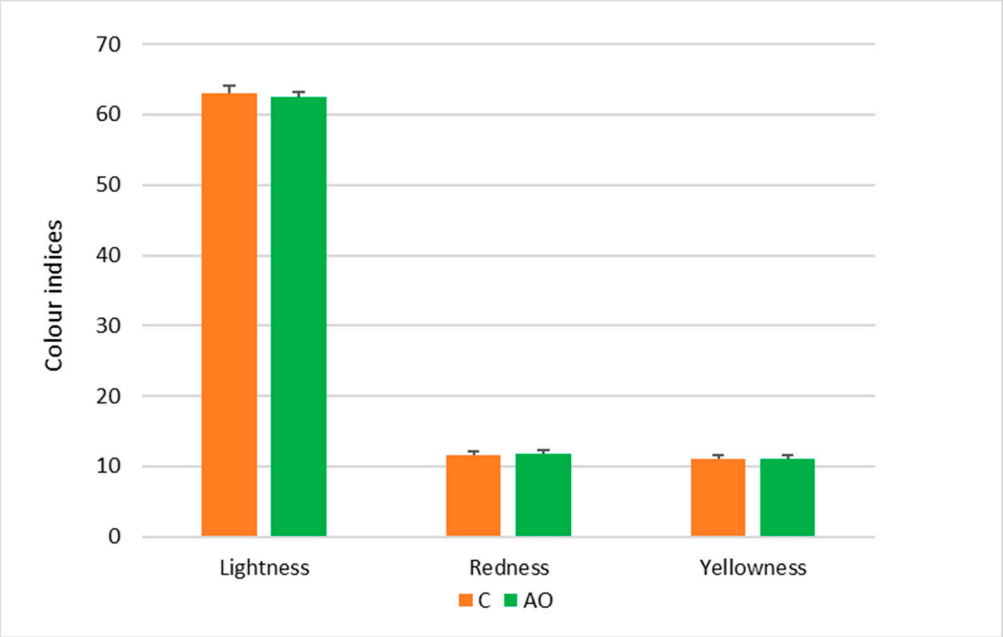

Figure 1. Colour indices of cooked ham from pigs fed a control diet (C) and a diet containing an antioxidant blend (AO). $N = 20$; data are reported as mean ± SEM. Lightness (L^*) values: Effects of treatment, $p = 0.406$; redness (a^*) values: effects of treatment, $p = 0.880$; yellowness (b^*) values: effects of treatment, $p = 0.309$.

The WBSF is an instrumental index of pork tenderness. The data showed that cooked ham tenderness, measured with WBSF, did not reveal any significant difference ($p > 0.05$)

between dietary treatments. The mean values were 10.19 ± 0.294 N for ham from the C group and 9.90 ± 0.098 N for ham from the AO group.

3.2. Chemical Parameters

Table 1 reports the chemical parameters of the cooked ham in relation to the dietary treatments of the pigs. Dry matter, protein, fat content and ash were unaffected ($p > 0.05$) by dietary supplementation with the AO blend.

Table 1. Chemical characteristics of cooked ham from pig fed a control diet (C) and diet containing an antioxidant blend (AO).

Item [1]	C	AO	*p* Value
Dry matter %	31.94 ± 0.26	31.99 ± 0.22	0.955
Crude protein, % [2]	20.25 ± 0.25	19.78 ± 0.29	0.401
Crude fat, % [2]	7.49 ± 0.40	7.71 ± 0.20	0.801
Ash, % [2]	2.55 ± 0.01	2.56 ± 0.03	0.375

[1] Data are reported as mean values ± SEM, $N = 20$. [2] Data are reported as percentages of wet weight.

3.3. Oxidative Stability

Figure 2 shows the oxidative stability of the cooked ham from pigs fed a control diet and a diet containing an antioxidant blend. The TBARS values of cooked ham were affected ($p < 0.05$) by dietary treatments; a higher value was observed in the cooked ham from pigs fed a C diet rather than the diet containing the AO blend.

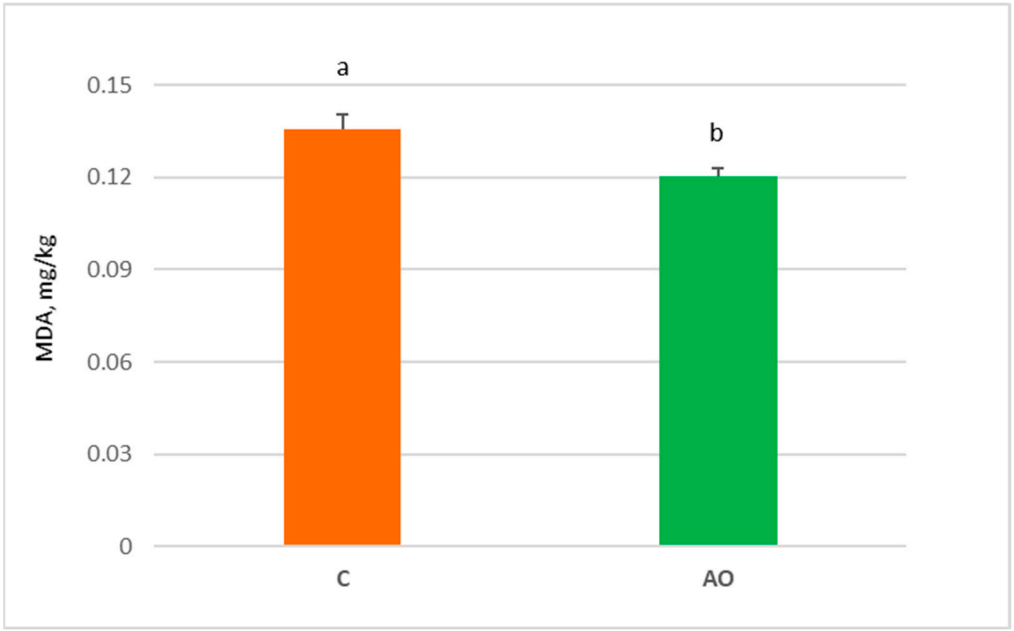

Figure 2. Oxidative stability of cooked ham from pigs fed a control diet (C) and a diet containing an antioxidant blend (AO). MDA = malondialdehyde; $N = 20$; data are reported as means ± SEM. a, b indicate $p < 0.05$.

3.4. Sensory Profile

Table 2 reports the F values for the appearance, aroma, taste, flavour and texture parameters of the cooked ham sensory profile. Sensory analysis revealed no significant difference ($p < 0.05$) between the control and antioxidant-treated cooked hams. For all the sensory parameters, the judges had different opinions and there was a significant difference ($p < 0.001$). This difference is common in sensory analysis [26]. No significant difference

was observed ($p > 0.05$) between samples, replicates or their interaction. This suggests that the score for each parameter can be assumed to be correct for the sensory profile of the ham.

Table 2. Sensory profile: F values and statistical significance of samples (C and AO), judges ($N = 8$), replicates ($N = 3$) and their interaction for each sensory parameter.

	F Value					
Descriptor	**Samples**	**Judges**	**Replicates**	**S × J ***	**S × R ***	**J × R ***
Appearance						
Pink colour	0.01	14.22 *	0.69	1.39	1.52	0.82
Colour homogeneity	1.12	8.47 *	1.20	1.54	2.99	0.48
Fat distribution	0.53	4.39 *	0.58	1.14	0.09	0.59
Slice homogeneity	0.35	4.72 *	3.04	0.88	2.59	0.52
Cohesiveness	0.55	4.69 *	0.33	0.33	0.18	0.67
Aroma						
Typical	3.36	43.99 *	0.33	1.41	0.13	1.37
Cooked meat	2.18	16.54 *	0.23	0.50	3.08	1.16
Taste						
Sweet	0.001	13.87 *	1.15	0.65	1.97	0.78
Salty	0.81	2.91 *	0.74	0.65	1.36	0.48
Umami	0.60	30.71 *	2.38	0.60	0.32	0.76
Flavour						
Typical	1.10	25.62 *	1.66	1.14	0.20	1.78
Cooked meat	0.28	9.41 *	2.23	0.47	0.38	1.44
Texture						
Tender	2.34	22.64 *	0.78	1.41	0.86	0.62
Fibrous	0.18	28.01 *	1.89	1.31	0.77	1.27

S × J, treatment × judges; S × R, treatment × replicates; J × R, judges × replicates. * Significant at $p < 0.001$.

A spider plot of cooked ham sensory attributes is shown in Figure 3. The attributes related to the appearance, aroma, flavour and texture were comparable in both experimental groups and revealed no significant differences ($p > 0.05$) between the control and antioxidant-treated groups.

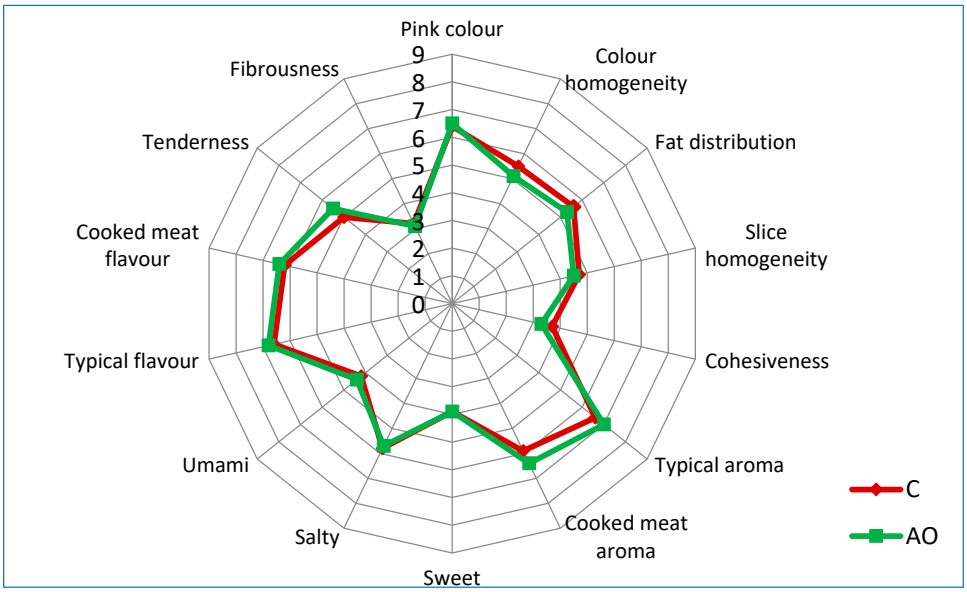

Figure 3. Spider plot of the sensory profile parameters of cooked ham from pigs fed a control diet (C) and a diet containing an antioxidant blend (AO). No difference ($p > 0.05$) in appearance, aroma, taste, flavour or texture parameters was observed.

4. Discussion

Only few prior papers have reported the effects of pig dietary integration with antioxidant molecules on the quality parameters of cooked ham [8,9,27,28]. In our study, no effect of dietary treatment with antioxidants on the colour parameters of cooked ham was appreciated. The colour parameters related to lightness, redness and yellowness are comparable with data reported by other authors [4,15,27]. Colour is one of the principal quality parameters of cooked ham that positively affects consumer preference [3,29]. In fact, oxidative processes have an unfavourable impact on the appearance of pork products as they oxidize myoglobin to oxymyoglobin and metmyoglobin, thus generating a brown colour [30].

Our previous study reported that dietary supplementation with an antioxidant mixture (AO) positively affected colour lightness and yellowness indexes in the *Longissimus dorsi* muscle during refrigerated storage, indicating persistent colour stability [19]. A stabilizing effect on meat colour indexes has also been observed with dietary antioxidants, such as Vitamin E and polyphenols [31,32]. In cooked ham, the processing technique may affect colour stability. In fact, the brine contains additives that have a colour-stabilizing effect [3]. Therefore, it is possible that the cooked hams were more stable products than the raw meat due to the brine components, which obscure the protective effects of the antioxidant mixture on ham colour parameters. Alternatively, in relation to current dosage and length of dietary supplementation, the components of the AO blend are not able to positively affect colour parameters, in line with Haack et al. [8].

Texture parameters, in particular instrumental tenderness, are very important quality traits of cooked ham. The present data showed that tenderness did not differ between dietary treatments. Our data fall within the tenderness range reported by Valkova et al. [33] for thirteen commercial brands of cooked ham. Our data are lower than the mean value reported in Valkova's study, and this may be related to the high fat content of the cooked ham due to the different pig slaughter weight and genetic types [30,33]. Our previous study reported no differences in the instrumental tenderness of fresh Longissimus dorsi muscle in relation to pig dietary supplementation with plant extract containing verbascoside [18].

As expected, the nutritional parameters of the cooked ham, such as protein, fat and ash content, were not affected by dietary treatments [8,9,34]. The fat content of the cooked ham in our study was different from other studies in the literature, possibly due to the high pig slaughter weight and different genetic type. In our previous studies, the chemical composition of raw meat for fresh consumption was not affected by pig antioxidant supplementation [18,19]. Similarly, Ranucci et al. [9] found that pig dietary supplementation with 0.2% oregano extract or 0.2% sweet chestnut wood extract for 68 days did not affect the nutritional composition of cooked ham.

Oxidative processes are the main causes of the decline in quality of pork products and are related to anti- and pro-oxidant balance and the composition of oxidation substrates. Livestock dietary supplementation with antioxidant substances, meat incorporation of suitable antioxidant molecules and active packaging techniques are employed to enhance meat and meat-product shelf life [8,18,35,36].

In our study, pig dietary supplementation with a blend containing Vitamin E and verbascoside from *Verbenacea* extract was able to increase the oxidative stability of cooked ham. The low TBARS values found in cooked hams, which indicate a more stable product than raw meat, may be due to the heat treatment and the different additives with stabilizing effects added to the brine. Our results are comparable with the data reported in previous studies [8,15,28,29].

This result is likely due to the synergic effects of liposoluble and water-soluble molecules contained in the AO supplement. The antioxidant activity of *Verbenaceae* extract

is related to the bioavailability of its metabolites (verbascoside, isoverbascoside and some minor compounds such as hydroxytyrosol, caffeic acid, ferulic acid and its glucuronide and homoprotocatechuic acids) [17]. It has been reported that these are absorbed into the intestinal tract and by the circulatory system. They are subsequently transported to several tissues, where they exert their antioxidant effects, but their concentration in various tissues is not yet understood [37]. Although not measured in the present study, it can be hypothesized that verbascoside in the diet protects α-tocopherol from oxidative degradation, thereby enhancing the oxidative stability of cooked ham. A previous study found that dietary verbascoside increased the α-tocopherol content of the longissimus dorsi muscle [18].

It has also been reported that Vitamin E is a highly functional antioxidant that counteracts damage caused by free radicals of polyunsaturated fatty acids at the cell membrane level, with a stabilizing effect [37]. In contrast, another study reported that dietary supplementation during the fattening period with 200 mg/kg of α-tocopheryl acetate failed to reveal differences in cooked ham oxidative stability [15]. Additionally, pig dietary supplementation with 0.2% oregano extract or 0.2% sweet chestnut wood extract did not show any difference in cooked ham oxidative stability [9].

The cooked ham's sensory profile from control and AO groups did not reveal differences in all the attributes related to appearance, aroma, flavour and texture. The present data agree with a previous study that described no effects of dietary α-tocopheryl acetate on cooked ham sensory attributes [15]. Another study reported that the flavour of cooked ham after 16 days of refrigerated storage was positively affected by pig dietary α-tocopheryl acetate supplementation [28].

5. Conclusions

Our results show that pig dietary supplementation, for 38 days, with a mixture of Vitamin E and verbascoside, from *Verbenaceae* extract, is able to improve cooked ham oxidative stability. No differences in colour indexes, nutritional parameters and instrumental tenderness were observed. The sensory profile did not reveal any changes in the appearance, aroma, flavour or texture of cooked ham. Our data reveal the potential of this antioxidant mixture in decreasing oxidative processes in cooked ham. Further studies are needed to validate the optimal amount and length of supplementation of the antioxidant mixture and to evaluate its usefulness in extending the shelf life of cooked ham.

Author Contributions: Conceptualization, C.C. and R.R.; methodology, C.C., R.R., S.R. and F.V.; investigation R.R., F.V. and S.R.; data curation, R.R., S.R., E.M. and F.V.; writing—original draft preparation, C.C., R.R., F.V., E.M. and S.R.; writing—review and editing, C.C., R.R. and F.V.; project administration, C.C. and R.R.; funding acquisition, C.C. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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