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**Effect of oregano oil and cranberry pulp supplementation in finishing pigs
on the physicochemical quality of fresh loin during storage**

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ABSTRACT

Oregano oil and cranberry pulp supplements were added to the diets of finishing pigs to determine their effects on meat quality of fresh loins during storage. Two and three levels of oregano oil (250 and 500 mg kg⁻¹) and cranberry pulp (5, 10 and 20 g kg⁻¹) were tested, according to a factorial experimental design. The loin meat was vacuum-packed and analysed at 0 (after the 24-h chilling period post slaughter), 23, 45 and 60 days of storage. Samples were repackaged under aerobic conditions after 0 or 23 days and analysed after 4, 8 and 12 days. Oregano and cranberry supplements did not affect lipid oxidation (μ g of MDA equivalent per kg of meat) during anaerobic or aerobic storage. On day 0, the fatty acid profile of the loin samples demonstrated that the addition of cranberries at a dose of 10 g kg⁻¹ was associated with a lower percentage of saturated fatty acids ($P = 0.04$; 42.97% vs. 40.99%) and a trend for a higher percentage of monounsaturated fatty acids ($P = 0.06$; 47.26% vs. 46.09%). Considering the result obtained, feeding pigs with oregano and cranberry supplements had a limited effect on meat quality parameters measured during storage.

Keywords: pork meat, color, oxidation, cranberry pulp, oregano oil, shelf life

RÉSUMÉ

Un supplément d'huile d'origan et de pulpe de canneberge a été ajouté à l'alimentation de porcs en finition afin de déterminer leurs effets sur la qualité de la viande durant l'entreposage. Deux niveaux d'huile d'origan (250 et 500 mg kg⁻¹) et trois niveaux de pulpes de canneberge (5, 10 et 20 g kg⁻¹) ont été évalués selon une distribution factorielle. Les échantillons de viande ont été emballés sous vide et analysés après 0 (24 heures suivant l'abattage), 23, 45 et 60 jours d'entreposage. Des échantillons ont été ré-emballés en aérobiose après 0 ou 23 jours et analysés après 4, 8 et 12 jours d'entreposage. L'ajout d'huile d'origan et de pulpe de canneberge n'affecte pas l'oxydation des lipides (µg kg⁻¹ de viande, MDA équivalent) pendant l'entreposage. Au jour 0, le profil en acides gras des échantillons de la longe a démontré que l'ajout de canneberges à 10 g kg⁻¹ était associé avec un plus faible pourcentage d'acides gras saturés ($P = 0.04$; 42,97% vs 40,99%) et tendait à hausser le pourcentage d'acides gras monoinsaturés ($P = 0,06$; 47,26% vs 46,09%). Compte tenu du résultat obtenu, l'alimentation des porcs avec ces niveaux de suppléments d'origan et de canneberge a eu un effet limité sur les paramètres de qualité de la viande mesurés durant l'entreposage.

Mots clés : qualité de viande, couleur, oxydation, pulpe de canneberge, huile d'origan, durée de vie de tablette.

INTRODUCTION

In meat and meat products, oxidation begins immediately after slaughter and increases during aging. Pork meat is rich in unsaturated fatty acids, proteins, pigments and other oxidizable substances, and is therefore sensitive to oxidation (Jeuge et al. 2012). Oxidation products have a direct or indirect impact on the organoleptic and nutritional properties of meat. Lipid oxidation is one of the main causes of meat quality losses during storage, causing changes in color, flavour, texture and nutritional value among others (Warriss 2000; Mancini and Hunt 2005; Campo et al. 2006). It is favoured by several factors, including high polyunsaturated fatty acids and myoglobin contents, excessive drop in pH, and storage at temperatures above 4°C (Zouari et al. 2010; Jeuge et al. 2012). Processes, such as modified atmosphere packaging and control of ambient lighting and humidity, also influence meat oxidation. Certain additives (nitrite, iron chelators and synthetic antioxidants) added during meat processing can inhibit or reduce oxidation during storage (Morrissey et al. 1994). However, synthetic additives have fallen into disfavour, forcing the industry and the scientific community to develop alternative means of meat preservation. Export markets are particularly demanding in this regard (Stephens et al. 2018).

Essential oils are aromatic products of plant secondary metabolism, formed normally in special cells or groups of cells and found mainly in leaves and stems. Although they are known primarily for their antimicrobial properties (Dussault et al. 2014; Silva Luz et al. 2014; Shekarforoush et al. 2015; Ghabraie et al. 2016), essential oils also appear to have considerable antioxidant effects on

meats, such as turkey (Govaris et al. 2004), pork (Shan et al. 2009) and rabbit (Cardinali et al. 2015). Their exogenous use for controlling oxidation and thereby extending the shelf life of various meats has been investigated (Fasseas et al. 2007; Mohamed et al. 2011; Radha Krishnan et al. 2014). Essential oils are composed of lipophilic and highly volatile secondary plant metabolic. They can cross the cell membrane and degrade the layers of polysaccharides, phospholipids and fatty acids (Dhifi et al. 2016). Oregano oil is particularly well known as a strong antioxidant capable of retarding lipid oxidation (MDA formation) in meat (Florou-Paneri et al. 2006; Simitzis et al. 2008). Govaris et al. (2004) have shown that adding oregano oil to turkey diet is more effective than *post-mortem* addition in reducing lipid oxidation. Botsoglou et al. (2003) concluded that the better oxidative stability of meat samples from turkeys receiving the diets supplemented with oregano oil was probably the result of antioxidant constituents of the oregano oil that entered the circulatory system and were distributed and retained in meat. Also, oregano activity is probably mainly attributed to its main components carvacrol and thymol, substances that react with lipid and hydroxyl radicals converting them into stable products (Yanishlieva-Maslarova 2001).

Most berries contain large amounts of phenolic compounds, which are known to have antimicrobial and antioxidant properties (Ahmad et al. 2015). Berry extracts added to meat directly, or indirectly through animal feed, appear to reduce lipid oxidation and color deterioration, and thereby extend shelf life (Jia et al. 2012; Lorenzo et al. 2014). Cranberry is rich in polyphenolic compounds,

especially flavonols and anthocyanins, which are also known for their antioxidant activity (Kahkonen et al. 2001; He and Liu 2006). Added exogenously, cranberry pulp or juice extract decreases lipid oxidation in fresh turkey (Raghavan and Richards 2006) and processed pork (Lee et al. 2006). So far, the efficacy of these natural compounds as antioxidants in fresh or processed meats has been tested mainly by exogenous application.

The development of new meat preservation strategies based on feeding natural active compounds to livestock is an attractive approach to improving meat quality and shelf life since it allows clean labelling of the product. Studies in this area are often conducted using a single supplement. Since different compounds can act on different oxidation pathways, we investigated the possibility that oregano oil and ground cranberry pulp added to pig finisher diets have a complementary effect as antioxidants in pork chops. We tested this hypothesis using meat stored as vacuum-packed chops and we examined the effect of opening and subsequently storing the package under aerobic conditions to represent commercial conditions.

MATERIALS AND METHODS

Animals and feeding protocol

The animal use and care committees of Université Laval and Centre de recherche en sciences animales de Deschambault both approved the experimental design and animal handling procedures prior to the study; they both adhere to the guidelines of the Canadian Council on Animal Care (2009).

Yorkshire-Landrace x Duroc barrows ($n = 42$) and gilts ($n = 35$) were 5 months old and weighed about 75 kg. They were housed individually in pens and fed the finisher diet. Pigs were fed following a commercially designed feed program (Nutreco Canada, Ange-Gardien, Canada) based on corn and soybean meal containing 44 IU vitamin E kg^{-1} of feed, served *ad libitum* until reaching an average weight of 120 kg. Six weeks before slaughter, each animal received, once a day as a top dress, 200 g of supplement made from a blend of oregano oil (Regano® C500, Ralco Nutrition, Inc., Marshall, MN), ground dehydrated cranberry pulp (Fruit d'Or, Notre-Dame-de-Lourde, Canada; analyzed values: 9.6% crude protein, 13.4% crude fat, 41% crude fiber) and ground maize. The concentrations of oregano oil chosen in this study were determined on the preliminary results of Janz et al. (2007) where authors showed that adding 500 mg kg^{-1} of oregano oil to the feed of pigs tended to reduce lipid oxidation in meat. For cranberry pulp, the concentrations are established on the fiber content of the pulp which limits its rate of incorporation to a level below 5%.

In Canada, as indicated by guidance documents on classification of veterinary drugs and livestock feeds, oregano essential oil and cranberry are part

of the list of substances currently classified as medicinal ingredients and are allowed in pig feed (Government of Canada 2019). Top dress was distributed daily using one of the six predetermined combination treatments according to the following distribution factor: two doses of oregano oil (250 and 500 mg kg⁻¹ of total feed) and three doses of cranberry pulp (5, 10 and 20 g kg⁻¹ of total feed). Barrows and gilts were assigned randomly to each treatment (5 gilts and 6 barrows per treatment). A control group received the finisher diet without supplements. The six experimental finisher diets were named as follows: 250/5, 250/10, 250/20, 500/5, 500/10 and 500/20, the numbers referring to equivalent mg of oregano oil and g of cranberry pulp per kg of feed.

Muscle sampling and pork quality measurements

Pigs were slaughtered in a federally inspected facility (Canadian Food Inspection Agency 2011). The pigs were not fasted on the farm prior to the 2-h transport journey. Fasting was done at the slaughterhouse to respect the recommended 16 to 24-h fasting period, since the animals were transported the day before in order to be the first on the slaughter line the next morning. Then, they were moved to the stunning CO₂ chamber prior to exsanguination.

An Orion Kniphe pH probe (ThermoFisher, Nepean, Canada) connected to a ROSS Orion 4 Star pH meter equipped with an Orion™ stainless-steel automatic temperature compensation probe (#927007MD, Thermo Scientific, Beverly Hills, CA) was used to measure muscle pH 45 min after slaughter and after a 24-h chilling period. Pork samples (center loin chops) were obtained

according to a procedure described previously (Fortier et al. 2012). Briefly, after a chilling period of 24 h (day 0), all *longissimus dorsi* (LD) muscles were deboned, sliced to obtain 10 samples (12 cm long) per animal and each sample was immediately placed, upon slicing, into pre-labelled packaging bag. Each sample was vacuum packed (Sealed Air Co, Mississauga, Canada), chilled in a salted water tank and then refrigerated in a commercial cooler set at $2 \pm 1^{\circ}\text{C}$ for up to 60 d (Fig. 1). On day 0 and after 23 days in vacuum packaging, samples were repackaged under aerobic conditions to reflect commercial applications. Samples packaged under aerobic conditions were placed in a styrofoam tray with an absorbent pad and rapped with an oxygen-permeable polyethylene film obtained from a local food equipment distributor (Emballage L. Boucher, Quebec City, Canada). Samples were kept refrigerated in a commercial cooler set at $2 \pm 1^{\circ}\text{C}$ for periods of 4, 8 and 12 d (Fig. 1). An extended storage period of 12 d was used since a previous study indicates that the end of shelf life (aerobic mesophilic counts $> 10^6$ cfu/g) was not reached after 4 days in similar conditions (Fortier et al. 2012).

Color was assessed using a Minolta Chromameter CR 300 (Minolta Ltd., Osaka, Japan) with a D65 light source and 0° viewing angle geometry according to the reflectance coordinates (CIE L^* , a^* , b^*) after exposing the muscle surface for 20 min of blooming time (Faucitano et al. 2010). The color intensity (chroma, C^*) was measured using equation 1 and the hue angle (h) with equation 2 (Konika Minolta 2007):

$$C^* = \sqrt{a^2 + b^2} \quad (1)$$

$$h = \tan^{-1} (b/a^*) \quad (2)$$

Drip loss was evaluated on day 0 using the EZ-Driploss method (Rasmussen and Anderson 1996). Briefly, a sample was taken at the centre of the chop using a stainless-steel punch (2.5 cm in diameter) and stored at 4°C for 48 h prior to measuring water loss by weight difference.

Chemical analysis

Proximate composition was determined on day 0. The protein content was measured using 1 g samples in a TruSpec N FP528 device (LECO, St. Joseph, MI) according to the Dumas reference method from AOAC 992.15 (AOAC 2005). Lipid content was measured using a Soxhlet apparatus according to method AOAC 991.36 (AOAC 1996).

Lipid oxidation was evaluated by measuring the malondialdehyde (MDA) content using thiobarbituric acid (Ermis et al. 2005). Briefly, meat (5.0 g) mixed with PBS (10 ml, pH 7.4) and butylated hydroxytoluene solution (0.2 ml, 0.88%, aqueous) was homogenized using an Ultra-Turrax IKA T18 device (IKA Works, Wilmington, USA). The homogenate was mixed with 0.5 ml of trichloroacetic acid (30%), placed on ice for 2 h and centrifuged at 2,000 x g for 15 min. One ml of supernatant was mixed with 0.075 ml of 0.25 M EDTA and 0.1 ml of 1% thiobarbituric acid (in 0.05 N NaOH), placed in boiling water for 15 min and cooled to room temperature. Absorbance at 532 nm was measured.

Malondialdehyde was quantified against a standard curve obtained using thiobarbituric acid reactive substances (TBARS) and calculated using the extinction coefficient $1.56 \times 10^5 \text{ cm}^{-1} \text{ mol}^{-1} \text{ L}^{-1}$. The results were expressed in μg of MDA equivalent per kg of meat.

The total antioxidant capacity (TAC) was measured as described previously (Erel, 2004). About 1 g of meat was homogenized in 10 ml of phosphate buffer (pH = 7.4, 30 mmol/L) then centrifuged at $1,000 \times g$ for 15 min at 25°C . Absorbance at 660 nm was measured after mixing 50 μl of supernatant with 200 μl of acetate buffer 1 (pH 5.8, 0.4 mol/L) and again 5 min after adding 20 μl of acetate buffer 2 (pH 3.6, 30 mmol/L). Trolox standards (Sigma-Aldrich Chemical Co., Milwaukee, WI) were used to create a standard curve and results were expressed in mg of Trolox equivalent per kg of meat.

Total phenol was extracted and quantified as described previously (Jang et al. 2008). Briefly, a homogenate of meat in distilled water (5.0 g in 15 ml) was shaken with chloroform (9.0 ml) then centrifuged at $2,000 \times g$ for 3 min. Supernatant was mixed with Folin-Ciocalteu reagent and 10% sodium carbonate solution (respectively 1.0 ml, 0.05 ml and 1.0 ml). After 1 h at room temperature, absorbance at 610 nm was read. A gallic acid standard curve was used and results were expressed in mg of gallic acid equivalent per 100 g of meat.

The fatty acid profile was analyzed using methods described elsewhere (Faucitano et al. 2008). Briefly, samples composed of muscle and fat (7.0 g) was homogenized in methylene chloride and then mixed with 45 ml of methanol. The mixture was allowed to stand for 5 min then homogenized again with an

additional 45 ml of methanol and left to stand for another 5 min. Chloroform (100 ml) and C19 standard (Sigma-Aldrich Canada, Oakville, Canada) were then added, the homogenization was repeated, and the mixture was again allowed to stand for 5 min then filtered on Whatman no. 1 paper. The filtrate was shaken with NaCl solution (0.1 M, 70 ml) in a separatory funnel. After 24 h of settling, the organic phase was filtered with chloroform on sodium sulfate, collected in a pre-weighed 250-ml round flask and evaporated to dryness. The flask was reweighed, and its contents were processed by base-catalyzed transmethylation as described elsewhere (Chouinard et al. 1997). Fatty acid composition was analyzed on a gas chromatograph (HP 5890A Series II, Hewlett Packard, Palo Alto, CA) equipped with a 100-m CP-Sil 88 capillary column (i.d. 0.25 mm, film thickness 0.20 mm, Chrompack, Middelburg, The Netherlands) and a flame ionization detector. The column temperature profile was as follows: 80°C for 1 min, ramping at 2°C per min to 215°C and holding for 30 min. Inlet and detector temperatures were 220°C and 230°C, respectively. The split ratio was 100:1. The carrier gas was hydrogen at a flow rate of 1 ml per min. Fatty acid peaks were identified and quantified using pure methyl ester standards (Nu Chek Prep., Elysian, MN). C18:1 and C18:2 fatty acid isomers were separated.

Statistical analysis

Effects of experimental treatment, sex, time and their interactions were detected by analysis of variance (ANOVA) using the SAS MIXED procedure. The overall effect of oregano oil and cranberry pulp was analyzed relative to the

control, then *a priori* contrasts were used to compare the control to all supplemented treatments. The data were then analyzed as a 3 X 2 X 2 factorial experiment in a random design with cranberry pulp (5, 10 and 20 g kg⁻¹ of feed), oregano oil (250 and 500 mg kg⁻¹ of feed) and sex. For both analyses, time of storage under anaerobic vacuum-sealed condition (0, 23, 45 and 60 days) and under aerobic condition (4, 8, and 12 days from 0 and 23 days) was taken into consideration using the repeated option (SAS 2002). A probability level of $P < 0.05$ was chosen as the limit for statistical significance in all tests. Probability levels between $P > 0.05$ and $P < 0.10$ were considered as a tendency.

RESULTS

Animal performance

Neither dietary treatment nor sex had any significant effect ($P > 0.10$) on pig growth during the finishing phase, during which males (XY) and females (XX) gained on average 1.03 kg and 1.04 kg per day, respectively, as they grew from 75 kg to 120 kg. Average daily feed intake was 3.31 kg and 3.75 kg (± 0.17 kg), gain to feed ratio was 0.313 and 0.281 (± 0.011), final weight was 113.0 and 114.5 kg (± 2.5 kg) and carcass weight was 82.1 kg and 83.5 kg (± 2.0 kg) from XX and XY, respectively.

Physicochemical analysis

Meat pH, drip loss and color

The sex of the animals did not have any effect on meat pH or color over time or on drip loss on day 0 ($P > 0.10$). The dietary treatments did not affect the pH measured 45 min and 24 h after slaughter ($P > 0.10$; Table 1). For all carcasses, the mean value of the pH₄₅ was 5.75 ± 0.08 and the mean value of the pH₂₄ was 5.69 ± 0.05 . At pH₄₅ *post-mortem*, pH value lower than 6.0 in the pork muscle is an indicator of abnormal muscle acidification and leads to an increase in the incidence of PSE meat. Despite a rapid drop of pH following slaughter, pH₂₄ is between 5.5 and 6.0 which indicates normal meat in pigs (Warriss, 2000). Diet had no significant linear effect on drip loss ($P > 0.10$).

However, a significant quadratic effect of cranberry pulp was noted, suggesting that feed containing 10 g of pulp per kg has reduced driploss ($P < 0.05$).

Lightness (L^* value), redness (a^* value) and yellowness (b^* value) were affected by time ($P < 0.05$; Fig. 2, 3 and 4), although no significant change in b^* value occurred between days 23 and 35 ($P > 0.10$). Dietary treatment tended to have an effect overall on L^* value during storage (Fig. 2), although some effect associated with oregano oil and cranberry pulp did appear on day 4 ($P = 0.07$; Fig. 2a, oregano oil x cranberry pulp) and on day 27 ($P < 0.05$; Fig. 2b, oregano oil x cranberry pulp). In fact, the lowest lightness values observed were in association with treatments 500/5 and 500/10 whereas the highest value was obtained with treatment 250/5. In vacuum-packaged meat, lightness slightly increased in association with cranberry pulp ($P < 0.001$; Fig. 2c), averaging 56.4 and 55.9 for 5 g and 10 g per kg on day 45 and increasing to 58.2 for 20 g.

There was little difference between the changes in the redness of the meat during storage depending on the treatments. Redness was greater in the control group on days 0 ($P < 0.05$; Fig. 3a, 6.76 vs. 5.46) and 60 ($P < 0.05$; Fig. 3c, 10.15 vs. 7.02). In the case of storage under aerobic conditions, meat lower redness was associated with 500 mg rather than with 250 mg of oregano oil: $a^* = 5.61$ on day 8 and 4.76 on day 12 vs. 6.25 and 5.53 ($P < 0.05$; Fig. 3a). The same pattern of variation, although as just as a tendency, was observed for vacuum-sealed meat (Fig. 3b): $a^* = 5.83$ on day 23 and 5.19 on day 27 vs. 6.69 ($P = 0.07$) and 5.84 ($P = 0.08$). Meat redness measured on day 35 decreased linearly ($P = 0.02$) with the cranberry pulp content of the

finisher diet, averaging 5.68, 5.12, 4.28, respectively, for 5, 10 and 20 g per kg of meat (Fig. 3c; Fig 3b).

Yellowness also was higher ($P < 0.05$) in the control group, at 9.35 vs. 8.21 on average in the experimental diet groups on day 0 and 8.60 vs. 7.68 on day 8 (Fig. 4a) and 10.55 vs. 9.68 on day 27 (Fig. 4b). The cranberry pulp effect appeared to be quadratic ($P = 0.01$) on day 45, giving b^* values of 10.32, 9.61 and 10.27, respectively, at 5, 10 and 20 g per kg of finisher diet (Fig. 4c). Low outliers were noted for diets 500/5 and 500/10 on days 4 and 27, whereas the highest value was associated with the 250/5 diet ($P = 0.01$ for cranberry pulp x oregano oil; Fig. 4, a and b). Finally, yellowness measured on day 31 tended to be stronger ($P = 0.08$; 10.13) in association with 250 mg than with 500 mg of oregano oil (9.62; Fig. 4b).

Significant effects on meat color intensity were observed only over time ($P < 0.001$) and for the vacuum-sealed condition (Fig. 5c). Cranberry pulp tended to have an effect on C^* on days 8 ($P = 0.07$) and 12 ($P = 0.08$) in meat under aerobic conditions. The control group tended to have more intense color than the treatment groups on day 23 ($P = 0.06$). Also, the effect on color intensity is significant on day 60 ($P = 0.04$).

Hue angle measurement suggested an effect associated with 500 mg of oregano oil, leading to a less red appearance on day 8 ($P = 0.03$; Fig. 6a).

Lipid oxidation, fatty acid profile and antioxidant properties

Neither oregano oil nor cranberry pulp had any significant effect on meat lipid and protein content ($P > 0.10$; Table 1). Malonaldehyde equivalent concentration increased with storage time under both packaging conditions ($P < 0.05$), but pig diet had no impact on this development (Fig. 7). In comparison, such increase in the control group was only a tendency on day 8 ($P = 0.08$; Fig. 7a). The total phenol and TAC values also tended to be reduced in the control group compared to the dietary treatments ($P = 0.07$; Table 1). The highest TAC and phenol values were observed in association with diets 250/20 and 500/5 ($P < 0.01$ for oregano oil x cranberry pulp).

All treatments did affect the fatty acid profile of pork loin ($P < 0.05$), reducing 12:0, 14:0 and 16:0 concentrations compared to the control group (Table 1). The 20:3-c8-c11-c14(n-6) and 20:4-c5-c8-c11-c14(n-6) fatty acids were more abundant in association with 250 mg of oregano oil than with 500 mg, but these fatty acids represented only 0.07% and 0.23% of the total. Total monosaturated fatty acids and 16:1-c9, 18:1-c9, 18:1-c11 increased linearly with cranberry pulp content ($P < 0.05$). The 20 g per kg of diet was associated with meat containing lower concentrations of 18:3-c9-c12-c15(n-3), 20:3-c11-c14-c17(n-3) and 20:2-c11-c14(n-6) compared to 5 and 10 g per kg ($P < 0.05$ for the quadratic effect). Total omega-3 fatty acids tended to be less abundant ($P = 0.06$). It should be noted that the total saturated fatty acid content was associated inversely with the TAC value ($P < 0.01$; $R^2 = 20.04\%$), whereas the total polyunsaturated fatty acid content was positively related with it

($P < 0.001$; $R^2 = 27.73\%$). Malondialdehyde equivalent concentration on day 0 was likewise related negatively ($P = 0.04$; $R^2 = 36.96\%$) with saturated fatty acids and positively with polyunsaturated ($P < 0.01$; $R^2 = 50.41\%$) fatty acids. However, no correlation was obtained between concentrations of any type of fatty acid and malondialdehyde equivalent measured on any other day of the storage period.

DISCUSSION

The apparent lack of effect of feed enriched with cranberry extract has been noted previously with broiler chickens (Leusink et al. 2010), as has the effect of oregano oil diets on the growth and carcass weight of pigs (Simitzis et al. 2010) and rabbits (Soultos et al. 2009). Based on the present study, conventional indicators of livestock performance do not appear to be sensitive to the presence of essential oils or cranberry supplements in a pig finisher diet.

It is known that microbial contamination is a cause of degradation of organoleptic characteristics and quality loss (Aminzare et al. 2016). Also, quality of stored meat can be affected by lipid oxidation, which causes rancidity and other off-flavours, losses of nutritional value and textural quality and reduced product safety (Cheng et al. 2017). In general, oxidation increases with storage time. However, packaging can also influence oxidation and meat shelf life. Packaging pork sausages in the absence of oxygen is reported to reduce oxidation and extend shelf life, based on color and odour stability (Martinez et al. 2006). The packaging procedures used in the present study provided a comparison of anaerobic and aerobic conditions. Vacuum sealing clearly limited

oxidation for up to 60 days, allowing malondialdehyde equivalent concentrations to increase by about 1 µg per kg per day compared to about 5 µg for 12 days in samples under aerobic conditions. Under both conditions, the increase appeared to be more or less linear and the concentration reached nearly 110 µg per kg at 12 or 60 days. It can be noted that the malondialdehyde equivalent concentration was less than 100 µg per kg at 4 days, the usual shelf life of meat when packaged under aerobic conditions. A product containing less than 1 mg equivalent MDA kg⁻¹ is generally accepted as being unoxidized, so this value is acceptable (Jeuge et al. 2012). However, when meat was vacuum-sealed under anaerobic conditions for 23 days and then stored under aerobic conditions for 35 days, the malondialdehyde equivalent concentration values increased more rapidly, suggesting that the initial oxidation status of the meat influences the subsequent oxidation rate. Storage conditions aside, it has been reported that adding natural antioxidants, such as oregano oil, to animal feed can slow lipid oxidation in meats such as turkey (Govaris et al. 2004), pork (Cheng et al. 2017) and rabbit (Cardinali et al. 2015). The same has been shown for cranberry extract in studies of turkey (Raghavan and Richards 2006) and cooked ground pork (Lee et al. 2006).

Our results are consistent with a previous study in which as much as 1 mL of oregano oil added to pig feed had no effect on malondialdehyde formation in pork (Simitzis et al. 2010). In our trial, 44 IU of vitamin E were added to the basal finisher diet to meet National Research Council recommendations (NRC 2012). This antioxidant might have been sufficient to prevent excessive oxidation in the

resulting pork. Another factor that should not be overlooked is the negative correlation between the initial malondialdehyde equivalent concentration and the proportion of saturated fatty acid, and the positive correlation with the proportion of mono and polyunsaturated fatty acids. In general, it is unsaturated fatty acids that are oxidized, and any factor that increases their proportion in the fat lowers the oxidative stability and hence the shelf life of the meat (Wood et al. 2003).

Measurement of the total antioxidant capacity (TAC) of biological samples is indicative of their ability to counteract oxidative stress-induced damage in cells (Erel 2004). In association with finisher diets enriched with oregano oil and cranberry pulp, meat TAC and total phenol contents were increased by a mere 5–10% compared to the control treatment, which did not leave much room to observe the effect of higher antioxidant capacity on lipid oxidation. The limited accumulation of antioxidant compounds in meat is due to the limited capacity of muscle tissue to concentrate them. We also cannot exclude that pigs simply do not absorb antioxidants from oregano oil and cranberry pulp. Polyphenols are not easily absorbed by the small intestine, and the amount found in muscle is not related directly to the dietary content (Manach et al. 2004; Fotina et al. 2013; Surai 2014). Based on a review, absorption of polyphenols in the small intestine and subsequent metabolization has been estimated at 5–10% (Chiva Blanch and Visioli, 2012), the rest reaching the colon along with deconjugated polyphenols in bile, where they are metabolized by colonic microbiota before being either eliminated or reabsorbed.

Along with the limited increase in TAC values, a slight increase in the percentage of monounsaturated fatty acid at the expense of saturated fatty acid in pork loin was associated with increasing cranberry pulp content of the finisher diet. The cranberry and oregano supplements reduced 16:0 fatty acid specifically but had no effect on polyunsaturated fatty acids. This is in contrast with previous findings that MUFA decreased in the longissimus thoracis of pigs fed 250 mg of oregano oil per kg of diet (Cheng et al. 2017). In a broiler chicken study, the percentages of saturated and monounsaturated fatty acid were found to decrease in backfat while PUFA increased in response to 135 mg of ethoxyquin and propyl gallate added per kg of diet (Lu et al. 2014). PUFA was found to increase at the expense of MUFA also in the longissimus lumborum of pigs fed 1 g of rosemary extract per kg of feed (Liotta et al. 2015).

All treatments were associated with lower 12:0, 14:0 and 16:0 fatty acids concentrations, but this magnitude of change is not enough to make a health-related difference on consumption because the values of fatty acids are already low. The significant increase in MUFA content at the expense of saturated fatty acids in association with cranberry pulp in the present study could be due to prevention of MUFA losses to oxidation. However, the MUFA concentration in the longissimus was not related with total antioxidant value ($P > 0.10$; $R^2 = 15.58\%$) whereas PUFA was positively associated ($P < 0.001$; $R^2 = 26.43\%$) and SFA ($P < 0.001$; $R^2 = 23.29\%$) negatively related with this total antioxidant value. The effect on MUFA could be due to delta-9-desaturase activity. This enzyme introduces a double bond between carbon atoms 9 and 10 of stearic acid (18:0)

to form oleic acid (18:1). The activities of delta-5 desaturase ($\Delta 5d$) and delta-6 desaturase ($\Delta 6d$), which are involved in the synthesis of fatty acids 20:5n-3 and 22:6n-3 from de18:3n-3, are increased in the presence of antioxidant compounds such as polyphenols and vitamin E (Infante 1999; Kühn et al. 2018). However, no such stimulation of delta-9 desaturase was observed in these studies.

Color is an important factor known to influence the consumer's choice of meat (Ngapo and Gariépy, 2008). The color of meat depends largely on the proportions of the different forms of the pigmenting compounds naturally present in muscle tissue (Mancini and Hunt 2005; Renerre 2006), namely myoglobin, oxymyoglobin (oxygenated myoglobin) and metmyoglobin (oxidized myoglobin). In the absence of oxygen, the color of fresh meat is reddish purple, which corresponds to the color of myoglobin. When myoglobin binds O_2 , it forms bright red oxymyoglobin. For obvious physiological reasons, this binding is reversible. Myoglobin in stored meat can be oxidized to metmyoglobin by free radicals or by lipid peroxidation products, changing its color. The formation of metmyoglobin in meat is irreversible and depends on oxygen partial pressure, temperature, pH, oxidative status and surface microbial growth (Mancini and Hunt 2005; Renerre 2006).

The greater meat redness and yellowness in the control group suggests that the experimental diets led to reduced redness due either to increased oxidation of myoglobin or to decreased formation of oxymyoglobin. As mentioned above, the oregano and cranberry supplements were associated with an increase in the total antioxidant value (or phenol concentration), but this was not related

with redness ($P > 0.10$; $R^2 = 3.95\%$) or yellowness ($P > 0.10$; $R^2 = 2.47\%$) on day 0. It is possible that other compounds, not associated with the antioxidant properties of oregano or cranberry, affect meat color (Kumar et al. 2015).

The redness of vacuum-sealed meat decreased within one week, and over a longer time in the samples under aerobic conditions on day 23, while yellowness changed much less. This suggests that even limited exposure to the ambient air allows pork color to remain relatively stable, regardless of pig diet. Under these conditions, the possible effects of oregano oil and cranberry pulp would be barely detectable. In fact, increased redness was associated with 250 mg, but not 500 mg of oregano oil and only on days 8, 12, and 27. Meanwhile, yellowness in the experimental diet groups never reached the highest value observed in the control group (observed on day 27). For vacuum-sealed meat, although both color intensities increased over time, the effects of the supplemented diets were limited to small variations of yellowness on day 45 and of redness on day 60.

Redness and chroma values of bacon made from pigs fed cranberry juice powder are reportedly stable, but this improved stability was not observed in the loin chops (Larrain et al. 2008). The redness and yellowness of longissimus thoracis of lambs fed a diet supplemented with oregano essential oil were higher than in the control group (Simitzis et al. 2008). Similar results were obtained in chickens fed oregano supplements (Al-Hijazeen et al. 2016). However, other authors report that oregano oil in the animal diet has little influence on meat color (Khaled and Marii 2016). Neither 0.05% oregano oil (Janz et al. 2007) nor 40 mg

of rosemary oil per kg (Haak et al. 2008) was found to have any effect on the color stability of pork. These inconsistencies could be due to differences in animal weight, genetics or livestock management and the number of animals in the study (Khaled and Marii 2016).

The lightness of a meat surface color is related to the drip loss or the water-holding capacity of the mass. As this capacity decreases, water collects on the surface and increases reflectance (L^* value) of the incident light, giving the meat color a paleness (Cheng and Sun 2008; Hutto 2017). The pH of muscle tissue generally drops from about 7 at slaughter to the 5.3–6.0 range. As this occurs, myofilaments release water, which migrates to the surface. This drop in water content appears to lead to reduction of the myofibrillary volume by about 20% (Monin 1988). In the present study, a weak relation between drip loss and L^* value was noted on day 0 ($P = 0.04$; $R^2 = 8.20\%$), but on no other day. Although many factors contribute to drip loss, changes to muscle protein structure and spatial arrangement are usually involved (Hughes et al. 2014). Changes in pH and redox potential affect the net charge on protein molecules, and thereby affect structure. These effects modify the optical properties of meat, making it more opaque and solid red (Alma et al. 2013). The pH measured between 45 minutes and 24 h after slaughter in the present study appears to be normal, that is, similar to values obtained in other studies of pigs fed a diet supplemented with oregano oil (Alma et al. 2013; Cheng et al. 2017). The drip loss also did not differ between the control group and the experimental diet groups, although 10 g of cranberry pulp per kg was associated with reduced drip loss without affecting the L^* value

of meat under aerobic conditions and under anaerobic vacuum-sealed meat examined on day 45. This effect of the cranberry pulp at 10 g/kg is difficult to explain since this treatment had no effect on the pH₄₅ and the pH_{24h} and a limited effect on the antioxidant capacity. Improving antioxidant capacity and reducing lipid oxidation would be associated with maintaining the integrity of cell membranes and therefore the water reduction capacity of meat (Cheng et al. 2016). As is the case for redness and yellowness, the small variation in reflectance that occurred during storage could be due to the dietary supplements. Antioxidant properties is likely the only property that cranberries and oregano essential oil have in common.

CONCLUSIONS

Our observations suggest that the effects of oregano oil and cranberry pulp on meat quality parameters such as color, lipid oxidation, water loss in association with the pig finisher diets were present but were of limited magnitude. The effects of these two sources of antioxidants do not appear to be complementary or synergistic. Considering the result obtained, feeding pigs with oregano oil and cranberry pulp supplements had a limited effect on meat quality parameters measured during storage and does not significantly improve shelf life. To promote the use of essential oils and polyphenols in pig feed more research is needed to assess the dose-cost benefits. Additional research is also needed to identify and quantify the main antioxidant constituents of oregano oil and cranberry pulp deposited in the pig muscle tissues.

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For Review Only

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Table 1. Physicochemical characteristics of fresh pork on day 0 (after a chilling period of 24 h)

Dietary supplement												
Oregano oil (mg kg ⁻¹)	250			500					<i>P</i> value			
Cranberry pulp (g kg ⁻¹)	5	10	20	5	10	20	Control	SEM	C vs. S ^y	Or ^x	P ^w	Or x P ^v
Lipids (g 100 g ⁻¹)	14.76	16.72	15.60	15.67	15.57	17.11	15.03	0.72				
Antioxidant (mg kg ⁻¹) Trolox equivalent	0.80	0.76	0.82	0.83	0.72	0.78	0.75	0.02	0.07		0.02 ¹	0.01
Phenols (mg 100 g ⁻¹) Gallic acid equivalent	1621.50	1465.37	1705.08	1766.74	1314.46	1555.38	1427.74	71.66	0.07		0.02 ¹	0.01
Protein (g 100 g ⁻¹)	18.51	17.13	18.67	17.77	18.68	17.00	18.06	0.69				
Drip loss (%)	4.09	3.50	4.53	3.82	3.05	4.50	4.65	0.56			0.05 ^q	
pH _{45'}	5.69	5.73	5.80	5.78	5.79	5.73	5.74	0.05				
pH _{24h}	5.66	5.69	5.74	5.74	5.70	5.67	5.71	0.05				
Fatty acids (% of total)												
8:0	0.013	0.014	0.012	0.015	0.014	0.015	0.013	0.001		0.10		
10:0	0.085	0.086	0.088	0.081	0.092	0.093	0.094	0.005				
12:0	0.079	0.073	0.074	0.072	0.081	0.080	0.084	0.003	0.03			
14:0	1.412	1.289	1.313	1.295	1.434	1.372	1.475	0.044	0.01			
14:1-c9	0.026	0.026	0.026	0.026	0.029	0.028	0.028	0.002				
16:0	25.664	24.703	24.898	25.107	25.812	25.501	26.249	0.426	0.04			
16:1-c9	1.949	2.040	2.050	1.926	2.285	2.165	2.168	0.122			0.03 ¹	
18:0	14.768	14.244	14.340	14.841	13.473	14.479	14.517	0.559			0.02 ¹	
18:1-c9	40.495	41.727	42.004	41.483	41.280	41.104	40.546	0.648			0.06 ¹	
18:1-c11	2.993	3.169	2.925	2.847	3.248	3.145	3.081	0.128			0.02 ¹	
18:2-c9-c12(n-6)	9.408	9.511	9.227	9.296	9.257	9.096	8.778	0.404				
20:0	0.234	0.246	0.237	0.246	0.219	0.223	0.240	0.014				
18:3-c6-c9-c12(n-6)	0.018	0.020	0.020	0.020	0.018	0.021	0.016	0.002	0.09			

20:1-c9	0.025	0.020	0.024	0.021	0.021	0.021	0.021	0.002				
18:3-c9-c-12-c15(n-3)	1.270	1.273	1.176	1.256	1.277	1.178	1.291	0.053			0.02 ^q	
20:2-c11-c14(n-6)	0.408	0.414	0.394	0.410	0.401	0.383	0.391	0.018			0.01 ^q	
22:0	0.018	0.016	0.023	0.017	0.019	0.020	0.020	0.002				
20:3-c8-c11-c14(n-6)	0.065	0.072	0.072	0.064	0.066	0.067	0.062	0.006		0.05		
22:1-c13	0.013	0.006	0.011	0.008	0.010	0.008	0.008	0.005				
20:3-c11-c14-c17(n-3)	0.064	0.066	0.059	0.066	0.065	0.059	0.065	0.004			0.04 ^q	
20:4-c5-c8-c11-c14(n-6)	0.246	0.257	0.277	0.197	0.214	0.230	0.213	0.035		0.02		
22:2-c13-c16(n-6)	0.007	0.007	0.007	0.006	0.004	0.006	0.005	0.001				
20:5-c8-c11-c14-c17(n-3)	0.020	0.016	0.029	0.011	0.009	0.013	0.011	0.004			0.09 ^q	
24:0	0.009	0.004	0.015	0.002	0.002	0.004	0.001	0.003				
24:1-c15	0.004	0.005	0.004	0.004	0.003	0.005	0.004	0.001				
22:4-c7-c10-c13-c16(n-6)	0.059	0.061	0.064	0.054	0.060	0.060	0.060	0.006				
22:5-c7-c10-c13-c16-c19(n-3)	0.039	0.042	0.043	0.039	0.038	0.037	0.037	0.004		0.06		
22:6-c4-c7-c10-c13-c16-c19(n-3)	0.016	0.015	0.017	0.011	0.014	0.015	0.014	0.003		0.05		
Saturated	42.614	40.992	41.313	41.996	41.445	42.103	42.973	0.888			0.04 ^l	
Polyunsaturated	11.620	11.754	11.386	11.430	11.422	11.165	10.943	0.472				
Monounsaturated	45.767	47.255	47.302	46.574	47.133	46.733	46.085	0.741			0.02 ^l	
Omega-3	1.409	1.412	1.325	1.383	1.403	1.302	1.418	0.058			0.06 ^q	
Omega-6	10.210	10.341	10.061	10.047	10.020	9.862	9.525	0.448				

^v Control vs. all supplemented treatments; ^w effect of oregano oil level; ^x effect of cranberry pulp level; ^y interaction of oregano oil and cranberry pulp levels; l = linear effect; q = quadratic effect.

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Figure 1. Distribution of analyses over sampling days (D) and meat storage conditions (anaerobic and aerobic).

Figure 2. Surface lightness (L^*) of pork chops from pigs fed finisher diets supplemented or not with oregano oil and cranberry pulp; days 0 to 60 refer to samples vacuum-sealed only, 0 to 12 and 23 to 35 to samples opened and rewrapped respectively on days 0 and 23. Different letters indicate significant inter-day differences. For cranberry x oregano, * $P = 0.07$ and ** $p < 0.05$; for cranberry quadratic effect, *** $p < 0.001$.

Figure 3. Scheme as in Figure 2, for redness (a^*). Control vs. experimental diets, * $p < 0.05$; oregano oil effect, ** $p < 0.05$ and *** $p < 0.01$; for cranberry quadratic effect, † $p < 0.05$.

Figure 4. Scheme as in Figure 2, for yellowness (b^*). Control vs. experimental diets, * $p < 0.05$; cranberry x oregano interaction, ** $p < 0.05$; cranberry pulp quadratic effect, *** $p < 0.05$; oregano oil effect, † $p < 0.10$.

Figure 5. Scheme as in Figure 2, for chroma value (C^*). Cranberry quadratic effect, * $p < 0.07$ and ** $p < 0.08$; control vs. experimental diets, # $p < 0.10$, * $p < 0.05$.

Figure 6. Scheme as in Figure 2, for hue angle (h). Oregano oil effect, * $p < 0.05$; cranberry quadratic effect, ** $p < 0.05$; control vs. experimental diets, # $P = 0.07$.

Figure 7. Scheme as in Figure 2, for malondialdehyde equivalent concentration (lipid oxidation index). Control vs. experimental diets, * $P = 0.080$.