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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 the lipid oxidation (µg 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 (42.97 % vs 40.99%; $P = 0.04$) and a higher percentage of monounsaturated fatty acids (47.26 % vs 46.09 %; $P = 0.06$). 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 des 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 ensuite 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 a démontré que l'ajout de canneberges à une dose de 10 g kg-1 était associé à un pourcentage plus faible d'acides gras saturés (42,97% vs 40,99%; P = 0,04) et un pourcentage plus élevé d'acides gras monoinsaturés (47,26% vs 46,09%; P = 0,06). Compte tenu du résultat obtenu, l'alimentation des porcs avec ces niveaux de suppléments d'origan et de canneberge, durant une période de 6 semaines avant l'abattage, 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 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 losses of meat quality during storage, causing changes in colour, 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 for processed meat 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). Oregano oil is particularly well known as a strong antioxidant effects 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 the 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 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, which adhered to the guidelines of the Canadian Council on Animal Care (2009).

Yorkshire-Landrace x Duroc barrows (n = 42) and gilts (n = 35) 5 months old and weighing about 75 kg 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 essay were determined on the preliminary results of Janz et al. (2007) where authors showed that adding 500 mg kg^{-1} of original 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 document 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). Animals were transported 12 hours before slaughter from the finishing farm to the slaughterhouse pens. During this period, the animals were fasted for a minimum of 10 hours. Animals were moved to the slaughter area and stunning in a CO_2 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

180 (center loin chops) were obtained according to a procedure described previously
181 (Fortier et al. 2012). Briefly, after a chilling period of 24 h (day 0), all *longissimus*
182 *dorsi* (LD) muscles were deboned, sliced to obtain 10 samples (12 cm long) per
183 animal and each sample was immediately placed, upon slicing, into pre-labelled
184 packaging bag. Each sample was vacuum packed (Sealed Air Co, Mississauga,
185 Canada), chilled in a salted water tank and then refrigerated in a commercial
186 cooler set at 2 ± 1^{o}C for up to 60 d (Fig. 1). On day 0 and after 23 days in vacuum
187 packaging, samples were repackaged under aerobic conditions to reflect
188 commercial applications. Samples packaged under aerobic conditions were
189 placed in a styrofoam tray with an absorbent pad and rapped with an oxygen-
190 permeable polyethylene film obtained from a local food equipment distributor
191 (Emballage L. Boucher, Quebec City, Canada). Samples were kept refrigerated in
192 a commercial cooler set at 2 ± 1^{o}C for periods of 4, 8 and 12 d (Fig. 1). The
193 extended period up to 12 days, although not representative of the typical shelf life,
194 was intended to assess the potential for preserving the properties of the meat over
195 a longer period. Color was assessed using a Minolta Chromameter CR 300
196 (Minolta Ltd., Osaka, Japan) with a D65 light source and 0° viewing angle
197 geometry according to the reflectance coordinates (CIE L*, a*, b*) after exposing
198 the muscle surface for 20 min of blooming time (Faucitano et al. 2010). The colour
199 intensity (chroma, C*) was measured using equation 1 and the hue angle (h) with
200 equation 2 (Konika Minolta 2007):
201
202 $$C^* = \sqrt{(a^{*2} + b^{*2})} \quad (1)$$

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

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 *xg* 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 x 10^5 cm^{-1} mol^{-1} 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 1000*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*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 then 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 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

272 and 500 mg kg^{-1} of feed) and sex. For both analyses, time of storage under
273 anaerobic vacuum-sealed condition (0, 23, 45 and 60 days) and under aerobic
274 condition (4, 8, and 12 days from 0 and 23 days) was taken into consideration
275 using the repeated option (SAS 2002).
276

RESULTS

Animal performance

Neither dietary treatment nor sex had any significant effect on pig growth during the finishing phase, during which males and females 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 YY, respectively.

Physicochemical analysis

Meat pH, drip loss and colour

The sex of the animals did not have any effect on meat pH or colour over time or on drip loss on day 0. The dietary treatments did not affect the pH measured 45 min and 24 h after slaughter (Table 1). For all carcasses, the mean value of the $pH_{45'}$ was 5.75 ± 0.08 and the mean value of the pH_{24h} was 5.69 ± 0.05. At 45 min 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, ultimate pH is between 5.5 and 6.0 which indicates normal meat in pigs (Warriss, 2000). Diet had no significant linear effect on drip loss. However, a significant quadratic effect of cranberry pulp was noted, suggesting that feed containing 10 g of pulp per kg was somehow different.

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. Dietary treatment had little effect overall on L* value during storage (Fig. 2), although some effect associated with oregano oil and cranberry pulp did appear on day 4 (Fig. 2a, oregano oil x cranberry pulp, $P = 0.07$) and on day 27 (Fig. 2b, oregano oil x cranberry pulp, $P < 0.05$). 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 (Fig. 2c, $P < 0.001$ for quadratic effect), 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 (Fig. 3a, 6.76 vs 5.46) and 60 (Fig. 3c, 10.15 vs 7.02, $P < 0.05$). 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 versus 6.25 and 5.53 (Fig. 3a; $P < 0.05$). The same variation was observed for vacuum-sealed meat (Fig. 3b): a* = 5.83 on day 23 and 5.19 on day 27 versus 6.69 ($P = 0.067$) and 5.84 ($P = 0.079$). Meat redness measured on day 35 decreased linearly ($P = 0.025$) 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).

322 Yellowness also was higher in the control group, at 9.35 versus 8.21 on
323 average in the experimental diet groups on day 0 and 8.60 versus 7.68 on day 8
324 (Fig. 4a) and 10.55 vs 9.68 ($P < 0.05$) on day 27 (Fig. 4b). The cranberry pulp
325 effect appeared to be quadratic ($P = 0.015$) on day 45, giving b* values of 10.32,
326 9.61 and 10.27, respectively, at 5, 10 and 20 g per kg of finisher diet (Fig. 4c). Low
327 outliers were noted for diets 500/5 and 500/10 on days 4 and 27, whereas the
328 highest value was associated with the 250/5 diet (Fig. 4, a and b, $P = 0.015$ for
329 cranberry pulp x oregano oil). Finally, yellowness measured on day 31 tended to
330 be stronger (10.13 vs 9.62, $P = 0.081$) in association with 250 mg than with 500 mg
331 of oregano oil (Fig. 4b).
332 Significant effects on meat colour intensity were observed only over time
333 ($P < 0.001$) and for the vacuum-sealed condition (Fig. 5c). Cranberry pulp may
334 have affected C* on days 8 ($P = 0.07$) and 12 ($P = 0.08$) in meat under aerobic
335 conditions. The control group tended to have more intense colour than the
336 treatment groups did on day 23 ($P = 0.06$) and 60 ($P = 0.04$).
337 Hue angle measurement suggested an effect ($P = 0.03$) associated with 500 mg
338 of oregano oil, leading to a less red appearance on day 8 (Fig. 6a).
339
340 *Lipid oxidation, fatty acid profile and antioxidant power*
341 Neither oregano oil nor cranberry pulp had any significant effect on meat
342 lipid and protein content (Table 1). Malonaldehyde concentration increased with
343 storage time under both packaging conditions ($P < 0.05$), but pig diet had no
344 impact on this development (Fig. 7). In comparison, such increase in the control

345 group was only a tendency ($P = 0.080$) on day 8 (Fig. 7a). The total phenol and
346 TAC values also tended to be reduced in the control group compared to the dietary
347 treatments (Table 1, $P = 0.069$). The highest TAC and phenol values were
348 observed in association with diets 250/20 and 500/5 ($P < 0.01$ for oregano oil x
349 cranberry pulp).

350 All treatments did affect the fatty acid profile of pork loin ($P < 0.05$), reducing
351 12:0, 14:0 and 16:0 concentrations compared to the control group (Table 1). The
352 20:3-c8-c11-c14(n-6) and 20:4-c5-c8-c11-c14(n-6) fatty acids were more
353 abundant in association with 250 mg of oregano oil than with 500 mg, but these
354 fatty acids represented only 0.07% and 0.23% of the total. Total monosaturated
355 fatty acids and 16:1-c9, 18:1-c9, 18:1-c11 increased linearly with cranberry pulp
356 content ($P < 0.05$). The 20 g per kg of diet was associated with meat containing
357 lower concentrations of 18:3-c9-c12-c15(n-3), 20:3-c11-c14c-17(n-3) and 20:2-
358 c11-c14(n-6) compared to 5 and 10 g per kg ($P < 0.05$ for the quadratic effect).
359 Total omega-3 fatty acids tended to be less abundant ($P = 0.062$). It should be
360 noted that the total saturated fatty acid content was associated inversely with the
361 TAC value $R^2 = 20.04\%$; $P = 0.005$) whereas the total polyunsaturated fatty acid
362 content was positively related with it ($R^2 = 27.73\%$; $P < 0.001$). Malondialdehyde
363 on day 0 was likewise related negatively ($R^2 = 36.96\%$; $P = 0.036$) with saturated
364 fatty acids and positively with polyunsaturated ($R^2 = 50.41\%$; $P < 0.006$) fatty
365 acids. However, no correlation was obtained between concentrations of any type
366 of fatty acid and malondialdehyde measured on any other day of the storage
367 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.

The principal cause of decreases in the quality of stored meat is 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 colour 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 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 concentration was less than 100 ug per kg at 4 days, the usual shelf life of meat. 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

391 vacuum-sealed meat under aerobic conditions on day 23 and observed until day
392 35, the malondialdehyde concentration values increased more rapidly, suggesting
393 that the initial oxidation status of the meat influences the subsequent oxidation
394 rate. Storage conditions aside, it has been reported that adding natural
395 antioxidants, such as oregano oil, to animal feed can slow lipid oxidation in meats
396 such as turkey (Govaris et al. 2004), pork (Cheng et al. 2017) and rabbit (Cardinali
397 et al. 2015). The same has been shown for cranberry extract in studies of turkey
398 (Raghavan and Richards 2006) and cooked ground pork (Lee et al. 2006).

399 Our results are consistent with a previous study in which as much as 1 mL
400 of oregano oil added to pig feed had no effect on malondialdehyde formation in
401 pork (Simitzis et al. 2010). In our trial, 44 IU of vitamin E were added to the basal
402 finisher diet to meet National Research Council recommendations (NRC 2012).
403 This antioxidant might have been sufficient to prevent excessive oxidation in the
404 resulting pork. Another factor that should not be overlooked is the negative
405 correlation between the initial malondialdehyde concentration and the proportion
406 of saturated fatty acid, and the positive correlation with the proportion of mono and
407 polyunsaturated fatty acids. In general, it is unsaturated fatty acids that are
408 oxidized, and any factor that increases their proportion in the fat lowers the
409 oxidative stability and hence the shelf life of the meat (Wood et al. 2003).

410 Measurement of the total antioxidant capacity (TAC) of biological samples
411 is indicative of their ability to counteract oxidative stress-induced damage in cells
412 (Erel 2004). In association with finisher diets enriched with oregano oil and
413 cranberry pulp, meat TAC and total phenol contents were increased by a mere 5–

10% compared to the control treatment, which did not leave us 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

436 expense of MUFA also in the longissimus lumborum of pigs fed 1 g of rosemary
437 extract per kg of feed (Liotta et al. 2015).

438 All treatments reducing 12:0, 14:0 and 16:0 concentrations compared to the
439 control group, but this magnitude of change is not enough to make a health-related
440 difference on consumption because the values of fatty acids are already low. The
441 significant increase in MUFA content at the expense of saturated fatty acids in
442 association with cranberry pulp in the present study could be due to prevention of
443 MUFA losses to oxidation. However, the MUFA concentration in the longissimus
444 was not related with total antioxidant value (R^2 = 15,58%, P = 0.127) whereas
445 PUFA (R^2 = 26.43%, P < 0.001) was positively associated and SFA (R^2 = 23.29%,
446 P < 0.001) negatively related with this total antioxidant value. The effect on MUFA
447 could be due to delta-9-desaturase activity. This enzyme introduces a double bond
448 between carbon atoms 9 and 10 of stearic acid (18:0) to form oleic acid (18:1). The
449 activities of delta-5 desaturase (Δ5d) and delta-6 desaturase (Δ6d), which are
450 involved in the synthesis of fatty acids 20:5n-3 and 22:6n-3 from de18:3n-3, are
451 increased in the presence of antioxidant compounds such as polyphenols and
452 vitamin E (Infante, 1999; Kühn et al. 2018). However, no such stimulation of delta-
453 9 desaturase was observed in these studies.

454 Colour is an important factor known to influence the consumer's choice of
455 meat (Ngapo and Gariepy, 2008). The colour of meat depends largely on the
456 proportions of the different forms of the pigmenting compounds naturally present
457 in muscle tissue (Mancini and Hunt, 2005; Renerre, 2006), namely myoglobin,
458 oxymyoglobin (oxygenated myoglobin) and metmyoglobin (oxidized myoglobin). In

459 the absence of oxygen, the colour of fresh meat is reddish purple, which
460 corresponds to the color of myoglobin. When myoglobin binds O_2, it forms bright
461 red oxymyoglobin. For obvious physiological reasons, this binding is reversible.
462 Myoglobin in stored meat can be oxidized to metmyoglobin by free radicals or by
463 lipid peroxidation products, changing its colour. The formation of metmyoglobin in
464 meat is irreversible and depends on oxygen partial pressure, temperature, pH,
465 oxidative status and surface microbial growth (Mancini and Hunt 2005; Renerre
466 2006).

467 The greater meat redness and yellowness in the control group suggests that
468 the experimental diets led to reduced redness due either to increased oxidation of
469 myoglobin or to decreased formation of oxymyoglobin. As mentioned above, the
470 oregano and cranberry supplements were associated with an increase in the total
471 antioxidant value (or phenol concentration), but this was not related with redness
472 (R^2 = 3.95% P = 0.208) or yellowness (R^2 = 2,47%, P = 0.887) on day 0. It is
473 possible that other compounds, not associated with the antioxidant properties of
474 oregano or cranberry, affect meat colour (Kumar et al. 2015).

475 The redness of vacuum-sealed meat decreased within one week, and over
476 a longer time in the samples under aerobic conditions on day 23, while yellowness
477 changed much less. This suggests that even limited exposure to the ambient air
478 allows pork colour to remain relatively stable, regardless of pig diet. Under these
479 conditions, the possible effects of oregano oil and cranberry pulp would be barely
480 detectable. In fact, increased redness was associated with 250 mg, but not 500 mg
481 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 colour 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 colour (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 colour 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 colour 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 colour 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%

505 (Monin 1988). In the present study, a weak relation between drip loss and L* value
506 was noted on day 0 (R^2 = 8.20%, P < 0.041), but on no other day. Although many
507 factors contribute to drip loss, changes to muscle protein structure and spatial
508 arrangement are usually involved (Hughes et al. 2014). Changes in pH and redox
509 potential affect the net charge on protein molecules, and thereby affect structure.
510 These effects modify the optical properties of meat, making it more opaque and
511 solid red (Alma et al. 2013). The pH measured between 45 minutes and 24 h after
512 slaughter in the present study appears to be normal, that is, similar to values
513 obtained in other studies of pigs fed a diet supplemented with oregano oil (Alma et
514 al. 2013; Cheng et al. 2017). The drip loss also did not differ between the control
515 group and the experimental diet groups, although 10 g of cranberry pulp per kg
516 was associated with reduced drip loss without affecting the L* value of meat under
517 aerobic conditions and under anaerobic vacuum-sealed meat examined on day 45.
518 As is the case for redness and yellowness, the small variation in reflectance that
519 occurred during storage could be due to the dietary supplements. Antioxidant
520 power is likely the only property that cranberries and oregano essential oil have in
521 common.
522

CONCLUSION

Our observations suggest that the effects of oregano oil and cranberry pulp on meat quality parameters such as colour, 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 synergic. 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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841 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					P 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	0.069		0.016[l]	0.009
Phenols (mg 100 g^{-1}) Gallic acid equivalent	1621.50	1465.37	1705.08	1766.74	1314.46	1555.38	1427.74	71.66	0.069		0.015[l]	0.009
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			0.046[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		0.098		
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	0.028			
14:0	1.412	1.289	1.313	1.295	1.434	1.372	1.475	0.044	0.014			
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	0.041			
16:1-c9	1.949	2.040	2.050	1.926	2.285	2.165	2.168	0.122			0.034[l]	
18:0	14.768	14.244	14.340	14.841	13.473	14.479	14.517	0.559			0.021[l]	
18:1-c9	40.495	41.727	42.004	41.483	41.280	41.104	40.546	0.648			0.055[l]	
18:1-c11	2.993	3.169	2.925	2.847	3.248	3.145	3.081	0.128			0.017[l]	
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	0.089			

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			0.023 [q]	
20:2-c11-c14(n-6)	0.408	0.414	0.394	0.410	0.401	0.383	0.391	0.018			0.010 [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		0.049		
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			0.044 [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		0.017		
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			0.086 [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		0.058		
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		0.051		
Saturated	42.614	40.992	41.313	41.996	41.445	42.103	42.973	0.888			0.044 [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			0.017 [l]	
Omega-3	1.409	1.412	1.325	1.383	1.403	1.302	1.418	0.058			0.062 [q]	
Omega-6	10.210	10.341	10.061	10.047	10.020	9.862	9.525	0.448				

[v] Control versus 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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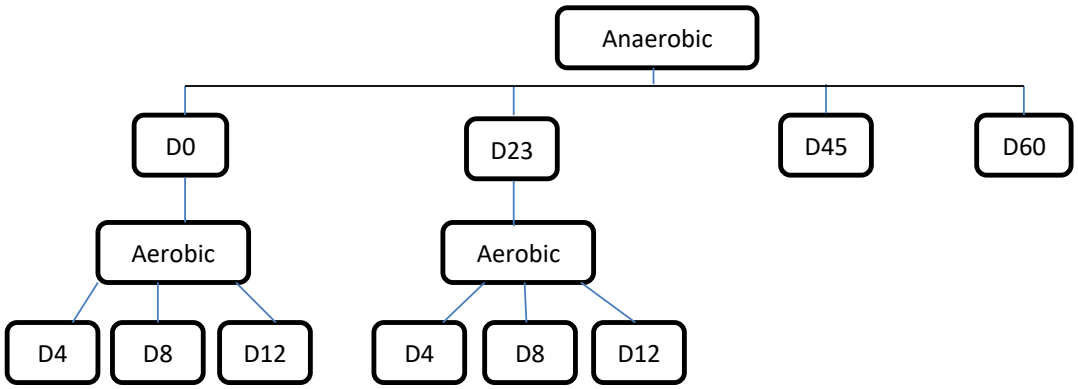

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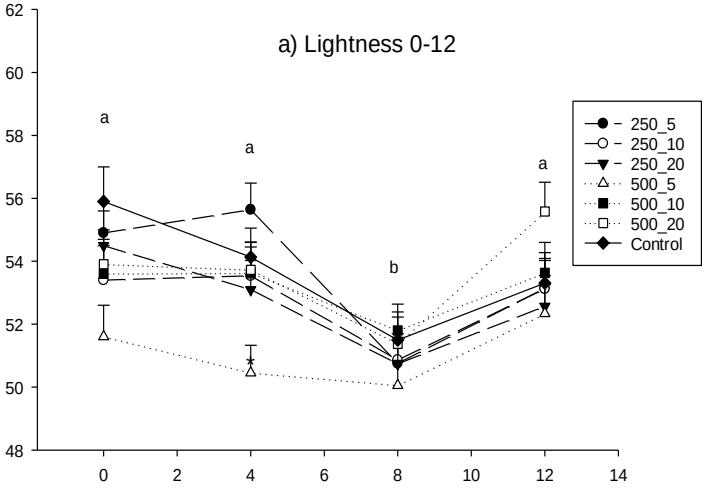

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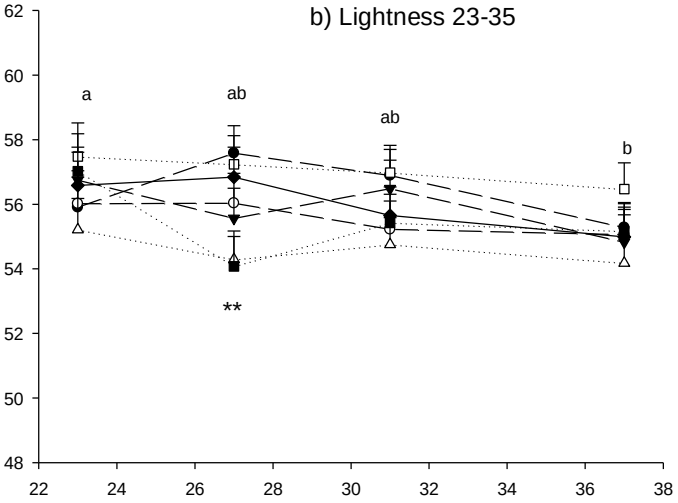

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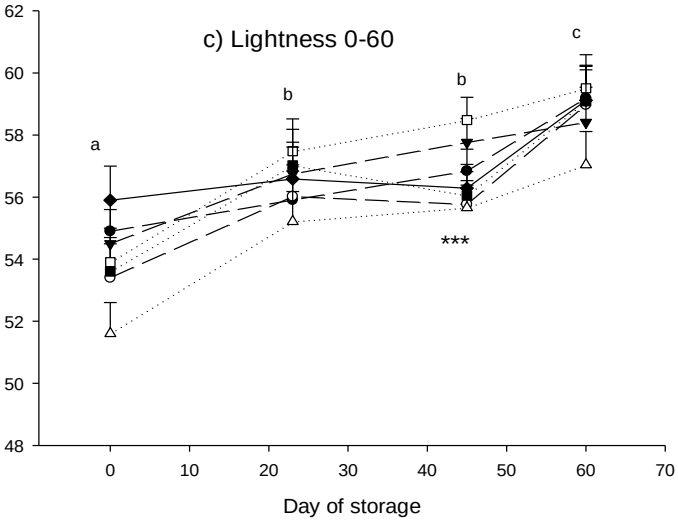

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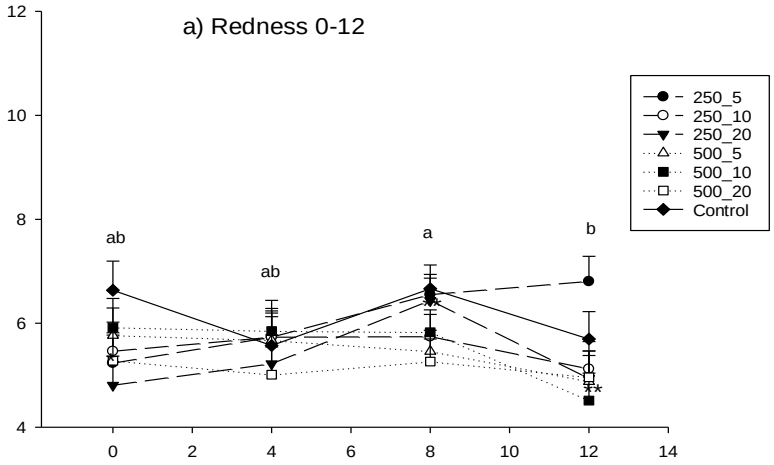

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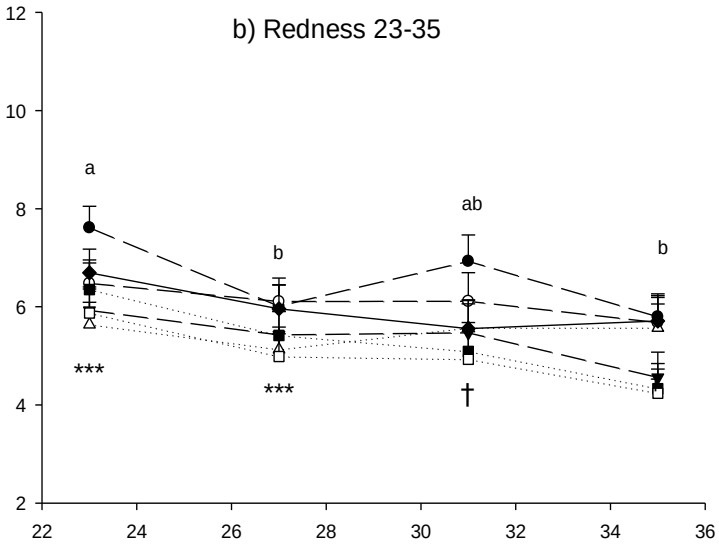

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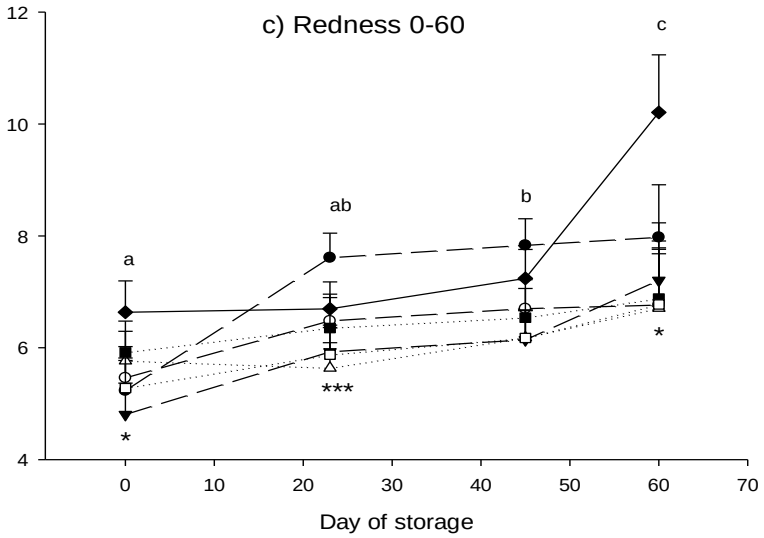

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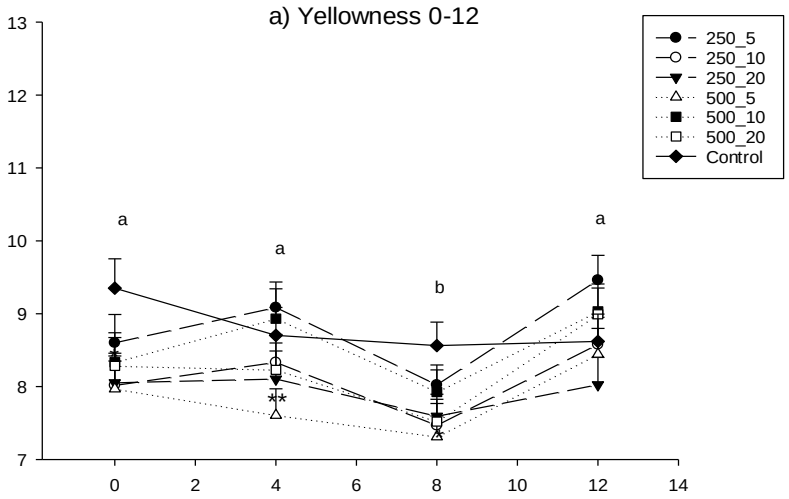

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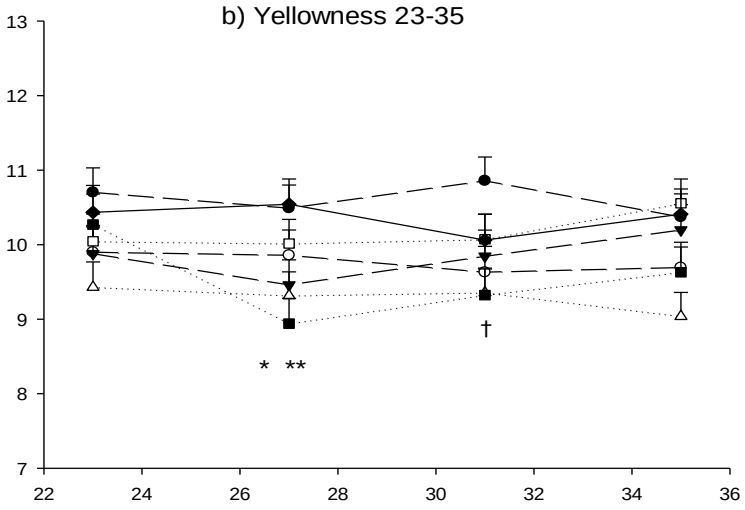

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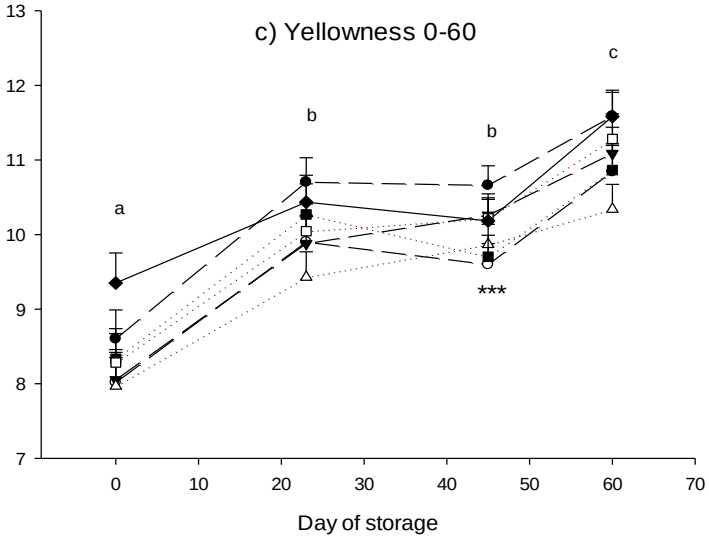

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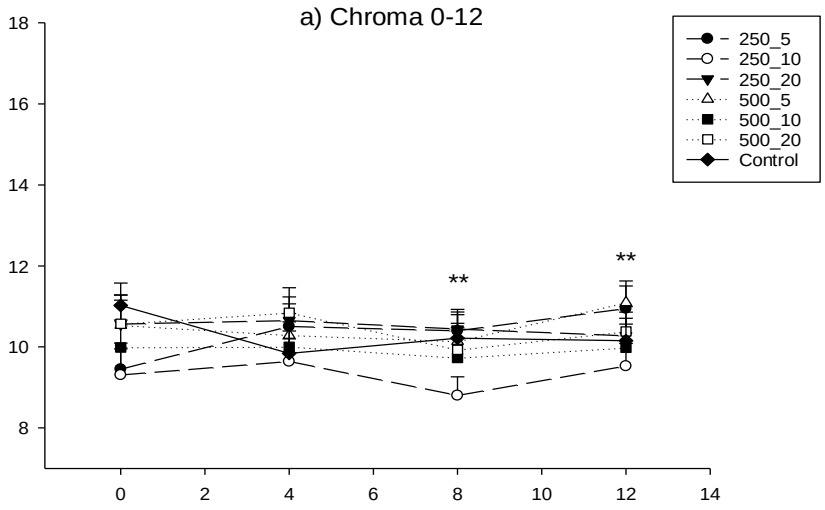

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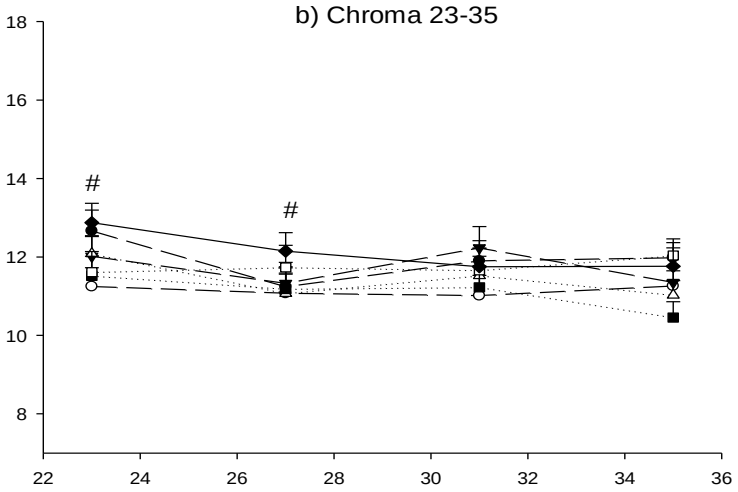

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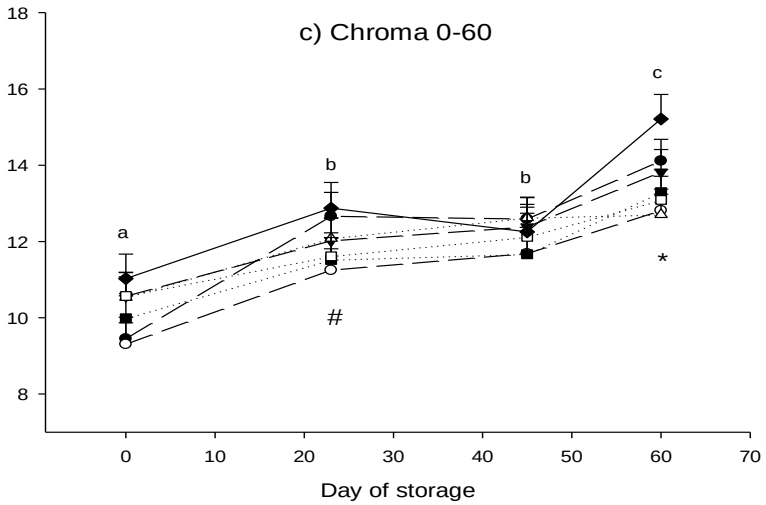

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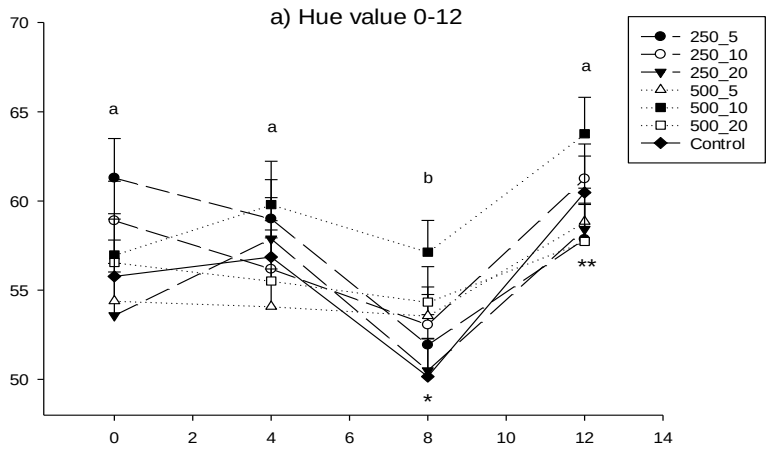
a) Hue value 0-12
250_5
250_10
250_20
500_5
500_10
500_20
Control
a
a
b
a
*
**
70
65
60
55
50
0
2
4
6
8
10
12
14

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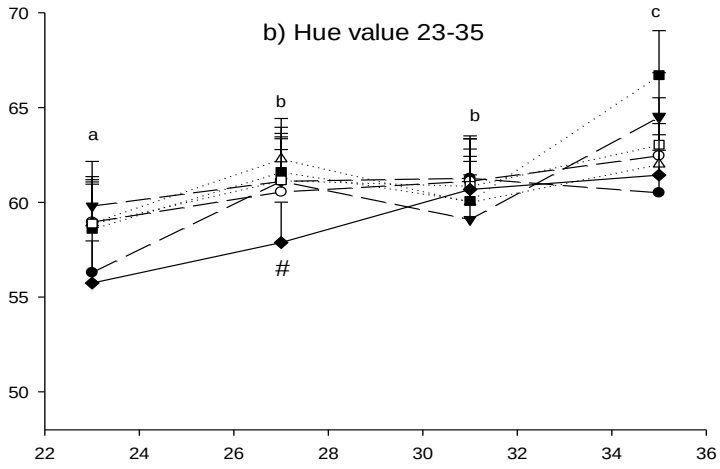
b) Hue value 23-35
a
b
#
b
c
70
65
60
55
50
22
24
26
28
30
32
34
36

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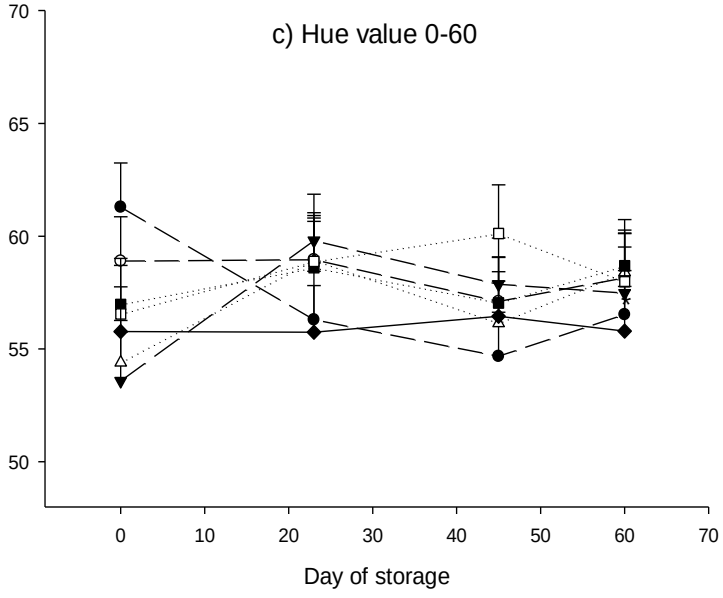
c) Hue value 0-60
70
65
60
55
50
0
10
20
30
40
50
60
70
Day of storage

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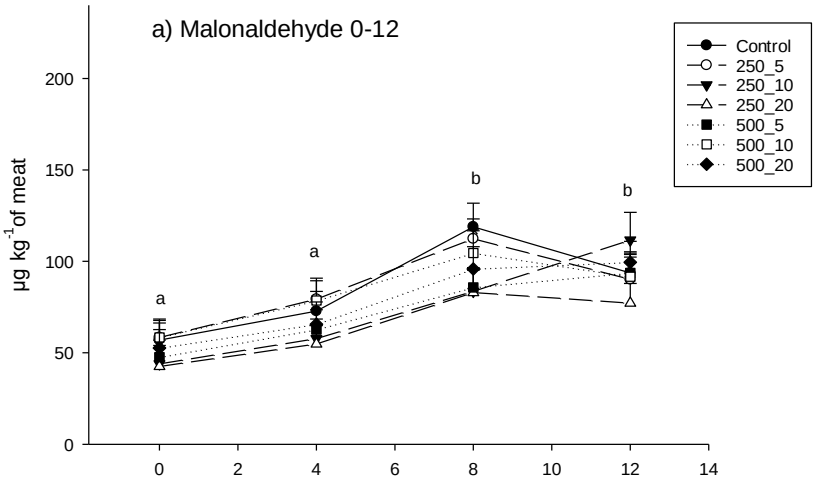

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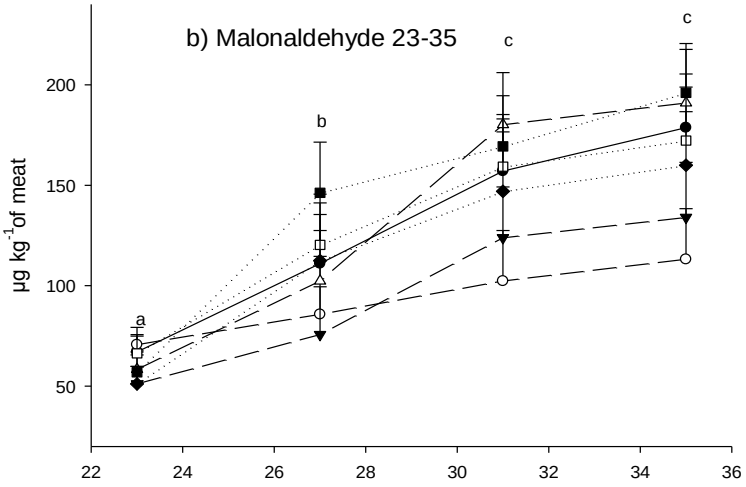

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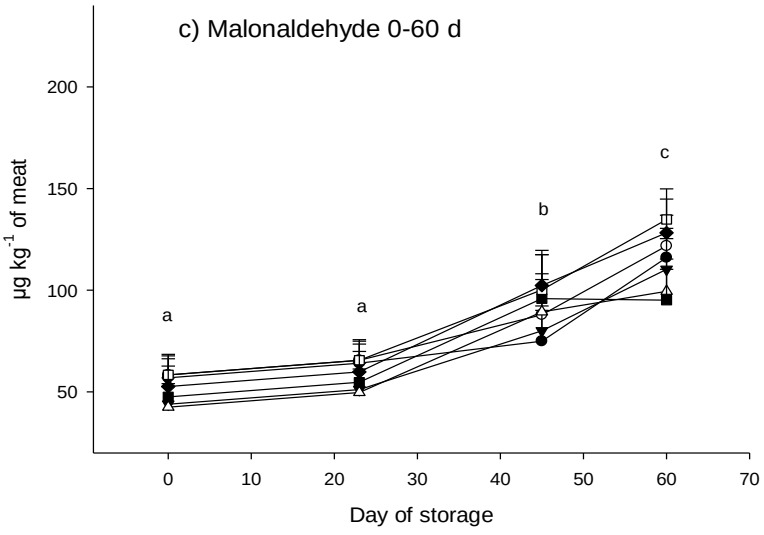

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Figure 1. Distribution of analyses over sampling days (D) and meat storage conditions (anaerobic and aerobic).

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 (lipid oxidation index). Control vs experimental diets, * $p = 0.080$.