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Antioxidant Effect of Spent, Ground, and Lyophilized Brew from Roasted Coffee in Frozen Cooked Pork Patties

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ABSTRACT

The ability of light and dark roasted coffee (1 g/kg) in varying application forms (spent ground [SCG], whole ground [WGC], or lyophilized brew [LBC]) to inhibit lipid and protein oxidation in cooked pork patties stored at -18°C was monitored over 3 months. Malondialdehyde (MDA) for the negative control (NC) increased from 0.31 to 1.11 mg MDA/kg pork over 3 months, while pork with coffee or rosemary oleoresin had lower values at month 3 (0.054-0.40 mg MDA/kg pork). The NC had the highest values for hexanal, octanal, and nonanal (2.59, 0.10, and 0.13 mg/kg pork, respectively), while light and dark LBC in pork inhibited hexanal (0.37 and 0.39 mg/kg pork), octanal (0.017 and 0.021 mg/kg pork), and nonanal (0.036 and 0.048 mg/kg pork) to the same extent as rosemary oleoresin at month 3 (0.30, 0.015, 0.036 mg aldehyde/kg pork, respectively). Thiol content for all treatments remained relatively stable from month 0 to 3 (0.56-0.96 to 0.67-1.02), while metmyoglobin slightly increased (49-55% to 55-56%) over 3 months. The results suggest that adding coffee neither inhibited nor promoted protein oxidation in cooked pork patties but inhibited lipid oxidation resulting in comparable values to pork with added rosemary oleoresin.

Key Words: Coffee; Cooked pork; Frozen storage; Oxidation.

1. Introduction

The efficiency and convenience offered by precooking products makes precooked pork appealing in today's fast-paced lifestyle (Nolan, Bowers, & Kropf, 1989). While the convenience appeals to consumers, precooking before packaging and storing can accelerate oxidation, deteriorating product quality (Dai, Lu, Wu, Lu, Han, Liu, et al., 2014; Kingston, Monahan, Buckley, & Lynch, 1998; Salminen, Estevez, Kivikari, & Heinonen, 2006; Sasse, Colindres, & Brewer, 2009). Cooking destroys the integrity of cell membranes releasing phospholipids, and inactivates several protective antioxidants e.g. catalase (Rhee, Anderson, & Sams, 1996). In addition, hemoproteins such as myoglobin are denatured allowing the release of the prooxidant iron, which can result in the warmed-over flavor (WOF). The WOF becomes predominant with precooking and is characterized by undesirable organoleptic qualities (Rojas & Brewer, 2007). These adverse qualities are due to the secondary products of lipid oxidation (LOX) such as hexanal, octanal, and nonanal. Destruction of cell membranes, decreased catalase activity, and the increase in nonheme iron can elevate the rate of LOX in cooked pork, therefore, it is important to apply strategies to prolong the shelf-life of cooked pork products (Fernandez-Lopez, Sevilla, Sayas-Barbera, Navarro, Marin, & Perez-Alvarez, 2003; Rhee, Anderson, & Sams, 1996).

Multiple preservation methods are applied to maintain meat quality, including storage temperature and antioxidants. Frozen storage is an effective method since low temperatures slow down oxidative reactions. However, freezing does not completely inhibit physical and chemical reactions causing discoloration and oxidative rancidity over time. Antioxidants (AOXs) can be added to combat LOX and protein oxidation (POX). Rosemary has become the industry natural AOX based on overall effectiveness in pork and other meat matrices (Georgantelis, Ambrosiadis,

Katikou, Blekas, & Georgakis, 2007; Kim, Cadwallader, Kido, & Watanabe, 2013). The key bioactive antioxidant rosemary compounds are carnosol, carnosic and rosmarinic acid which readily donate their hydrogens acting as free radical scavengers. Recently, roasted coffee was shown to inhibit LOX as effectively as rosemary oleoresin in refrigerated beef with added salt, a known prooxidant of LOX (Lin, Toto, & Were, 2015). The bioactivity of roasted coffee is attributed to a combination of hydroxycinnamic chlorogenic acids (CGAs), and Maillard reaction products (MRPs), which scavenge free radicals and chelate metal catalysts of LOX and POX (Del Pino-Garcia, Gonzalez-SanJose, Rivero-Perez, & Muniz, 2012; Delgado-Andrade, Rufian-Henares, & Morales, 2005; Farah, 2012). Concentrations of AOX compounds differ in whole ground coffee (WGC), lyophilized brew coffee (LBC), or spent coffee ground (SCG) and by roast degrees. Spent coffee, from home or restaurant preparations, are of particular interest since substantial bioactive compounds may remain due to short brewing time and coffeemaker filter (Ludwig, Sanchez, Caemmerer, Kroh, Paz De Pena, & Cid, 2012). Furthermore, SCG are considered waste products hence repurposing into a sustainable preservation method can result in economic gain for the meat and coffee industry, while still producing a safe, quality pork product.

To our knowledge, no studies have investigated the effectiveness of SCG as an antioxidant in food and limited studies have reported the effects of roasted coffee in food matrices: Nissen, Byrne, Bertelsen, & Skibsted (2004) in cooked frozen pork, Budryn & Nebesny (2013) in cookies and chocolate, and Lin, Toto, & Were (2015) in raw refrigerated beef. The experimental objective was to determine the optimal combination of roast degree (light or dark) with application form (SCG, WGC, LBC) for the greatest AOX effect to preserve shelf life and quality in frozen precooked pork.

2. Material and Methods

2.1. Chemicals

Aldehyde and 4-heptanone standards, bovine albumin serum, biuret reagent, HPLC grade methanol, water, formic acid, acetonitrile, ferrous sulfate heptahydrate and sodium phosphate dibasic anhydrous were purchased from Fisher Scientific (Tustin, CA, USA). Sodium phosphate monobasic was from Spectrum Chemical (Gardena, CA, USA). Ellman's reagent 5, 5'-dithiobis(2-nitrobenzoic acid), EDTA, ferrozine , trichloroacetic acid (TCA), 2-Thiobarbituric acid (TBA), and 1, 1, 3, 3,-tetramethoxypropane (TMP) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of coffee and pork treatments

Green Colombia Primeval coffee beans obtained from Rose Park Roasters (Long Beach, CA, USA) were roasted to produce a light (10 min 32 s to 210°C) and dark (12 min 8 s to 235°C) roast. Whole coffee beans were ground (Cuisinart "Grind Central" Coffee Grinder, East Windsor, NJ, USA) and passed through a 1.0 mm sieve (18-mesh size) resulting in WGC. Lyophilized brewed coffee was prepared following the methods indicated by Budryn & Nebesny (2013) with modifications. Coffee brew was prepared by heating water to 90°C, then adding ground coffee to water at a 1 to 6 ratio. The solution was held at 90°C for five min with constant stirring, filtered with a paper coffee filter to yield liquid brew, which was then lyophilized (Dura-Dry mP manifold lyophilizer, FTS Systems, model #FD2085C0000, Stone Ridge, NY, USA) to yield LBC. The remaining solid grounds from coffee brew extraction was lyophilized and used as SCG. Coffee was stored at < 0°C before use. Before incorporation into minced pork, all coffee forms were passed through a 1.0 mm sieve (18-mesh size).

Meat was prepared in accordance to AOAC Official method 983.18 (AOAC, 2010) with modifications. Minced sirloin pork chops from Butcher hogs, averaging 6 months in age, 95.34 kg live weight at the time of slaughter, was supplied and prepared at Farmer John® facilities (Vernon, CA, USA). Pork meat was minced in an industrial-sized chopper, and refrigerated (<4°C) overnight prior to addition of coffee or rosemary oleoresin the following day. All treatments, negative control (NC), rosemary oleoresin [RO; Herbalox® HT-25 from Kalsec Inc. (Kalamazoo, MI, USA); 2 g/kg)], SCG, WGC, and LBC of light (1 g/kg) and dark (1 g/kg) roasts were mixed using a Hobart Legacy HL200 20 Qt mixer (Troy, OH, USA) for two min resulting in eight different treatment samples with uniform processing. This process was repeated per treatment in order to achieve true duplicates. The pork was transferred to polyethylene plastic bags, placed into cardboard boxes, and transported 56 km from Vernon to Orange, CA, USA.

2.3. Cooked pork preparation

Pork meat was stored at 4°C until ready to be formed into pork patties (~3 h). Pork was prepared and cooked following the Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Fresh Meat (AMSA, 1995) with modifications. Pork patties (100 ± 1 g) were molded (11.5 cm diameter, 1.25 cm thickness) then cooked on two electric griddles (ToastMaster®, Model #TG21W & # TM161GR, St. Louis, MO, USA) set to 205°C for 3.5 min on each side or until internal temperature reached a minimum of 72°C in the center of the patty. Patties were cooled at 22-25°C before being individually placed into oxygen permeable zipper bags (polyethylene, 16.5 cm x 14.9 cm). Patties were stored at -18°C until ready to be analyzed. Patties from each treatment were transferred from -18°C to 4°C to thaw for 12 h, and were hand mixed for 30 s prior to analysis.

2.4. Chlorogenic, Maillard reaction products, and iron chelating ability Quantification

Spent, ground, and lyophilized brew of light, medium, and dark roasted coffee were added to deionized water at 1 g/100 mL to test MRPs and 0.1 g/100 mL to test iron chelating ability and CGA, then incubated for 2 hours at 22°C. Quantification of CGA in the various forms of coffee were measured via HPLC following protocols by Lin, Toto, & Were (2015). A C18 column (Kinetex, 2.6u C18 100A, 100 x 4.60 mm, Phenomenx, Torrance, CA, USA) was used at 30°C using a flow rate of 1.5 mL/min with mobile phase (A) 1mg/ mL formic acid in HPLC water and (B) HPLC grade acetonitrile. Sample was injected (5 µL) with starting conditions of A/B, 95/5 held for 10 minutes. Solvent A was linearly decreased to 85% within 1 min and held for 0.5 min before returning back to starting conditions within 2.5 min. A standard curve of chlorogenic acid (0-0.6 mM) was used to quantify chlorogenic acid detected at 330 nm. Quantification of MRPs and ferrous iron chelating ability were measured following protocols by Teets and Were (2008).

2.5. Thiobarbituric acid reactive substances (TBARS) measurement

The TBARS assay was prepared as described by Lin, Toto, & Were (2015) with modifications. The supernatant (5 mL) was reacted with 5 mL of 0.02 M TBA solution in glass test tubes. Recovery values were determined by spiking additional meat samples (randomly chosen each testing day) with 0.5 mL of 0.15 or 0.45 mM TMP solution to achieve final TMP concentration of 0.006 and 0.018 mM after 12.0 mL of TCA had been added. These mixtures were vortexed and centrifuged alongside the other samples. A TMP standard curve (0-7.5 nmol MDA/mL) was used to quantify MDA after 16 h incubation at 22-25°C in the dark. Absorbance

153 was measured at 532 nm and 600 nm, with the latter accounting for any potential turbidity, using
154 a FLUOstar Omega multimode microplate reader (Cary, NC, USA).

155 **2.6. Purge and trap gas chromatography measurement of volatile aldehydes**

156 Volatile compounds from pork samples were extracted via distillation and measured by
157 gas chromatography (GC) using internal standard 4-heptanone (50 µL/L) following protocols by
158 Lin, Toto, & Were (2015). Samples were analyzed in duplicate and hexanal, octanal, and
159 nonanal concentrations were expressed as mg/kg based on internal response factor of the internal
160 standard and each respective aldehyde.

161 **2.7. Free thiol content measurement**

162 Free thiol content was measured following method by Eymard, Baron, & Jacobsen (2009)
163 with modifications. Protein was extracted from pork (5.0 ± 0.1g) and vortexed for 30 s with 25
164 mL of 0.1 M phosphate buffer (pH 7.4) in 50 mL centrifuge tubes. After centrifugation (3,000
165 rpm/127.8 *g*) for 15 min at 4°C, the supernatant was filtered through eight layers of cheesecloth
166 to obtain protein homogenates. For each treatment, 400 µL of homogenate, 600 µL DI water,
167 and 14.3 µL of 0.01 M DTNB in 0.2 M EDTA was added to a microcentrifuge tube and
168 incubated in a water bath (40°C) for 15 min. The Biuret method was used to determine average
169 protein extracted using 0.1 M phosphate buffer. The protein concentration in solution was
170 determined to be 0.037 g/mL. Concentration of free thiol content expressed in µmol thiol/g
171 protein was read in triplicates at 412 nm using a molar extinction coefficient of 13,600 M^{-1} cm^{-1}.

172 **2.8. Quantification of metmyoglobin formation**

173 Metmyoglobin concentration was calculated as described by Tang, Faustman, &
174 Hoagland (2004). Extracted proteins from section 2.7 for each treatment were aliquoted in

triplicate and absorbance was measured at 503, 525, 557, and 582 nm. Percent metmyoglobin was calculated by the equation:

*[% metmyoglobin] = (-0.159 R_1 – 0.085 R_2 + 1.262 R_3 – 0.520) *100*

where $R_1 = A_{582}/A_{525}$, $R_2 = A_{557}/A_{525}$, $R_3 = A_{503}/A_{525}$.

2.9. Statistical analysis

Duplicates frozen cooked pork patties with coffee in pork treatments (1 g/kg of each coffee combination), positive control (2 g/kg Herbalox® HT-25), and negative control (no AOXs added) were monitored over 3 months. General linear model procedure in Statistical Analysis 9.3 Software (SAS Institute, 2011) was used to determine the differences in means between treatments per month for TBARS, GC, free thiol content, and percent metmyoglobin (MetMb). Analysis of variance (ANOVA) and Duncan's multiple range test were performed to determine whether a statistical significance existed. In addition, Pearson's correlation coefficients between each assay were determined. A level of significance of $\alpha = 0.05$ was used throughout analysis.

3. Results and Discussion

3.1. Antioxidants in coffee

The LBC form for all roast degrees had the highest CGA amongst all application forms, while SCG had the least (Table 1), attributed to the higher solubility of low molecular weight compounds extracted from coffee grounds into the brew. The majority of CGAs remained in the brew, however, extraction of all CGA was incomplete as noted by the amount left in the SCG. Ludwig, Sanchez, Caemmerer, Kroh, Paz De Pena, & Cid (2012) reported that brew time and temperature affects the final concentration of CGA extracted. The incomplete removal of

197 phenolic compounds into the brew supports the hypothesis that spent coffee could be utilized as
198 an AOX. Increasing roast degree decreased concentrations of CGA as expected since roasting
199 degrades CGAs or incorporates them into MRPs (Nicoli, Anese, Manzocco, & Lerici, 1997).
200 The MRPs in lyophilized brew increased by 23% from light to medium roast coffee,
201 whereas dark roasted coffee showed similar values of MRPs to the medium roast (Table 1).
202 With the exception of light SCG, all roasts of SCG and WGC forms reported similar values of
203 MRPs, since the majority of water soluble MRPs are also extracted into the brew form (Bravo,
204 Juaniz, Monente, Caemmerer, Kroh, Paz De Pena, et al., 2012). Due to the extraction of water
205 soluble MRPs into the brew, this also resulted in higher levels of metal chelation values for LBC
206 compared to the spent and ground form. However, there was a decreasing trend with increasing
207 roast degree in regards to LBC, which was consistent with Lin, Toto, & Were (2015). In regards
208 to the other application forms, there was an increase in metal chelation with increasing roast
209 degree for SCG, while WGC maintained similar values with increasing roast degree.

210 **3.2. Extent of lipid oxidation measured by thiobarbituric acid reactive substances**

211 The TBARS values reached the highest levels in month 2, increasing from 0.031-0.31 mg
212 MDA/kg pork in month 0 to 0.070-1.15 mg MDA/kg pork in month 2 (Fig 1). In month 3
213 amongst treatments, TBARS levels remained the same (light and dark LBC, light and dark
214 WGC, and negative control) or decreased (light and dark SCG, rosemary oleoresin) from month
215 2. Despite the decrease observed from month 2 to 3 for some treatments, there was an overall
216 increase from initial to end of storage with increases ranging from 43-270%. This trend was
217 similar to the results reported by Sasse, Colindres, & Brewer (2009) and Abd El-Alim, Lugasi,
218 Hovari, & Dworschak (1999). While oxidative reactions are significantly inhibited in frozen
219 storage, LOX reactions can still occur, although at slower rates as noted by the overall increase

during storage (Rhee, Anderson, & Sams, 1996; Sasse, Colindres, & Brewer, 2009). The decrease from month 2 to 3 could be attributed to (1) MDA decomposition to other organic by-products such as alcohols and acids, or (2) decomposition by bacteria such as *Pseudomonas*, which attack carbonyl compounds like MDA (Georgantelis, Ambrosiadis, Katikou, Blekas, & Georgakis, 2007). The former is the most probably pathway since growth of *Pseudomonas*, a psychrotrophic bacteria, would be negligible at frozen conditions. Furthermore, secondary products of LOX, including MDA, can react with free amino groups from proteins. Since the TBARS assay measures only free MDA, the MDA-protein interaction can result in lower TBARS values (Viljanen, Kivikari, & Heinonen, 2004).

Negative control exhibited higher TBARS values throughout storage compared with other treatments ($p \leq 0.05$), suggesting that AOX compounds from rosemary oleoresin and coffee inhibited MDA formation. By month 3, all pork samples with added coffee presented similar TBARS values (0.054-0.22 mg MDA/kg pork) to rosemary oleoresin (0.070 mg MDA/kg pork), with the exception of light WGC which reported slightly higher MDA concentrations by the end of storage (0.40 mg MDA/kg pork). Despite the overall increase over time in TBARS, all coffee treated pork, with the exception of light SCG, never exceeded the threshold of when rancidity can be detected; 0.5 mg MDA/kg of pork (Gray & Pearson, 1987; Sheard, Enser, Wood, Nute, Gill, & Richardson, 2000). Negative control surpassed the threshold value after the first month of storage with TBARS value of 0.660 mg MDA/kg pork. In month 2, light SCG (0.55 mg MDA/kg pork) reached the highest value of TBARS for all coffee treated pork for the entirety of testing.

The results in the present study contradict those by Nissen, Byrne, Bertelsen, & Skibsted (2004) who reported that coffee provided little to no protection against LOX in comparison to

243 rosemary extracts. This may be due to the 20-fold difference between coffee extract
244 concentration in the previous study (0.05 g/kg) compared with the present study (1 g/kg), and use
245 of a different rosemary extract. The present study shows that all coffee combinations were as
246 effective as rosemary oleoresin at inhibiting MDA production.

247 **3.3. Extent of lipid oxidation measured by gas chromatography**

248 Pork samples in the last 2 months of analysis exhibited the highest concentrations of
249 hexanal with values ranging from 0.30-2.59 mg hexanal/kg pork, compared to octanal (0.015-
250 0.12 mg octanal/kg pork) and nonanal (0.036-0.13 mg nonanal/kg pork) (Fig 2). Hexanal, the
251 main aldehyde formed, was consistent with literature (Meynier, Genot, & Gandemer, 1998), as it
252 forms from oxidation of n-6 fatty acids, specifically linoleic acid, the predominant
253 polyunsaturated fatty acid in pork (14.3 g/100 g). By month 3, the negative control (2.59 mg/kg
254 pork) had the highest hexanal, while both light and dark roasts of LBC (0.37 and 0.39 mg/kg
255 pork, respectively), inhibited hexanal production to the same extent as rosemary oleoresin (0.30
256 mg/kg pork) ($p \leq 0.05$).

257 There was no significant difference for both octanal and nonanal from month 2 to 3,
258 which was consistent with a study done on beef by Lin, Toto, & Were (2015). Despite the
259 differences in study parameters (muscle types investigated and storage temperatures), octanal
260 and nonanal displayed similar trends in both studies. Nonanal and octanal forms from oxidation
261 of n-9 fatty acids, such as oleic acid. Although oleic acid (34.3 g/100 g) is present in higher
262 quantities in pork than linoleic acid (14.3 g/100 g), monounsaturated fatty acids are more stable
263 against oxidation than polyunsaturated fatty acids (Meynier, Genot, & Gandemer, 1998).
264 Amongst the coffee in pork treatments, there was no significant difference observed with the
265 exception of month 3 for octanal. In month 3, the negative control displayed the highest octanal

(0.10 mg/kg pork), while light LBC and rosemary oleoresin inhibited octanal formation (0.017 and 0.015 mg/kg pork, respectively). Volatile aldehydes are considered to be one of the most important products of LOX since they have such low threshold values for detection (Ladikos & Lougovois, 1990). The rancidity thresholds for hexanal reported by Shahidi & Pegg (1994) can range from 0.0045-0.15 mg/kg cooked pork. Although MDA flavor thresholds for rancidity were not surpassed (section 3.2), hexanal thresholds were exceeded by month 2 of storage by all treatments, with the lowest value for hexanal being observed by dark brew (0.65 mg/kg pork).

Based on results from section 3.1, differing CGA levels had no effect on MDA formation since all added coffee in pork treatments produced similar results to rosemary oleoresin. However, CGA and MRP levels correlated with greater AOX effect against volatile aldehydes in frozen cooked pork. The ranking for application form effectiveness against hexanal, octanal, and nonanal was as follows: LBC > WGC > SCG, corresponding with CGA and MRP concentrations found in Table 1**.**

3.4. Protein oxidation measured by thiol content

Thiol content increased from month 0 to 1 ($p \leq 0.05$) ranging in values from 0.56-0.96 to 0.82-1.10 µmol/g protein, respectively. In month 2, all treatments decreased in thiol content from month 1 ranging in values from 0.59-0.90 µmol/g protein. From month 2 to 3, thiol content for negative control, rosemary oleoresin, and dark WGC increased, light LBC, dark SCG and LBC decreased, while light SCG and WGC remained relatively the same (Fig. 3). Thiol content was expected to gradually decrease or remain stable over the frozen storage period (Lund, Hviid, & Skibsted, 2007; Nieto, Jongberg, Andersen, & Skibsted, 2013). Despite unexpected increases in certain coffee in pork treatments, no significant difference amongst treatments were observed

288 across the storage period ($p \geq 0.05$), indicating added coffee and Herbalox® HT-25 may not
289 have a significant effect on POX.

290 Haak, Raes, & De Smet (2009) found that 0.005-0.02% rosemary extract, tocopherols,
291 and green tea extract did not influence POX in frozen cooked pork until day 8 of frozen storage.
292 Whereas, Nieto, Jongberg, Andersen, & Skibsted (2013) found that 0.05% and 0.4% of rosemary
293 essential oil was able to protect against thiol loss in chilled raw pork throughout the 9 day
294 storage. Lara, Gutierrez, Timon, & Andres (2011) found that 0.03% rosemary and 0.1% lemon
295 balm extracts in cooked meat exerted a protective effect against POX throughout storage. While
296 these studies differ in design, which influences the extent of effectiveness of AOXs, our results
297 correspond to other studies which indicate that rosemary oleoresin does not have an effect in
298 inhibiting the loss of sulfhydryl content.

299 **3.5. Extent of protein oxidation measured by metmyoglobin formation**

300 An increase in metmyoglobin (MetMb) formation by 1-14% over 3 months was observed
301 (Fig 4). Significant differences were found amongst treatments for all testing months with the
302 exception of month 1, which had % MetMb range of 55.25-56.24%. By month 3, rosemary
303 oleoresin had lower MetMb (54.47%) ($p \leq 0.05$), while pork with added coffee were more
304 similar to the negative control and ranged in values from 56.20-56.37%, which indicates that
305 coffee may not inhibit MetMb to the same extent as Herbalox® HT-25 in frozen cooked pork
306 patties.

307 The overall range for MetMb for the entire study was 49.33-57.18%. Fernandez-Lopez,
308 Sevilla, Sayas-Barbera, Navarro, Marin, & Perez-Alvarez (2003) found with fresh meat, values
309 for MetMb initially ranged from 25-30%. After cooking, these values increased to 32-45%. At
310 the end of the 8 day testing of chilled storage, Fernandez-Lopez, Sevilla, Sayas-Barbera,

311 Navarro, Marin, & Perez-Alvarez (2003) detected a distinct difference between control samples
312 and added AOX samples with MetMb ranging from 70-75% and 42-48%, respectively. In the
313 present study, MetMb values for all treatments were higher on the initial day of testing (49.33-
314 55.36%). However, the range of MetMb formed stayed relatively stable throughout frozen
315 storage. The difference in results could have been due to frozen storage versus refrigerated
316 storage.

317 Myoglobin oxidation in pork meat is predominantly affected by temperature and time
318 (Faustman, Sun, Mancini, & Suman, 2010). The pork in the present study was cooked prior to -
319 18°C storage. The cooking could explain why MetMb values were initially higher. Greene,
320 Hsin, & Zipser (1971) found that MetMb levels exceeding 40% were rejected by consumers as a
321 poor quality raw product, however, since these pork products are cooked, there is a wider range
322 of acceptance since browning is expected with cooking. Denaturation of myoglobin unravels
323 intact proteins, exposes heme iron. Oxidation of the centrally located iron, accounts for the
324 higher MetMb values observed initially, which is expected to increase with extended storage
325 periods. The reactive products of LOX, such as peroxides, promote myoglobin oxidation
326 (Fernandez-Lopez, Sevilla, Sayas-Barbera, Navarro, Marin, & Perez-Alvarez, 2003; Rhee,
327 Anderson, & Sams, 1996). The stable MetMb values could be explained by the slowing of
328 oxidative reactions due to frozen storage.

329 **4. Conclusion**

330 The use of light and dark roasted coffee in spent ground, whole ground, and lyophilized
331 brewed form at 1g coffee/kg pork lowered MDA and hexanal in frozen cooked pork patties to the
332 same extent as rosemary oleoresin, showing potential as an alternative AOX for LOX. In regards
333 to POX, coffee AOXs neither had a positive nor negative impact on free thiol content or MetMb

334 formation. The results show that all forms of both roast degrees could extend the shelf life of
335 frozen cooked pork patties.

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1 ***Tables***

2 Table 1: Maillard reaction products, metal chelation and chlorogenic acid concentration for spent
3 ground, whole ground, and lyophilized brew form coffee solutions.

4

Table 1

	MRPs[a] (Absorbance intensity units)	Metal chelation[b] (µmol EDTA/g coffee equivalents)	3-CGA[c] (mg/g coffee)	Total 3-CGA[c] (mg/g coffee)
Light				21.00
Spent	0.73 ± 0.012	14.05 ± 1.48	1.98	
Ground	1.23 ± 0.012	24.00 ± 6.96	4.70	
Lyophilized Brew	4.80 ± 0.14	58.38 ± 2.29	14.32	
Medium				16.13
Spent	1.42 ± 0.024	21.43 ± 0.70	2.01	
Ground	1.47 ± 0.017	24.23 ± 0.76	6.83	
Lyophilized Brew	5.91 ± 0.15	43.67 ± 2.83	7.28	
Dark				12.16
Spent	1.75 ± 0.019	30.97 ± 1.65	1.86	
Ground	2.22 ± 0.013	26.79 ± 1.95	4.40	
Lyophilized Brew	5.83 ± 0.47	35.94 ± 1.02	5.90	

[a] Brown nitrogenous Maillard reaction products from hot brewed coffee at 1 g coffee/100 mL measured at 420 nm

[b] Metal chelation by ferrozine assay for hot brewed coffee at 0.1 g coffee/100 mL measured at 562 nm

[c] Chlorogenic acid content by HPLC analysis for cold brewed coffee at 0.1 g/100 mL

Figures

Fig. 1. Thiobarbituric acid reactive substances/TBARS (mg MDA/kg pork) ± standard deviation with negative control (no antioxidants added), coffee in pork treatments (1 g/kg light and dark roasts of spent, whole, lyophilized brewed) and rosemary oleoresin (2 g/kg) in cooked pork patties analyzed monthly over 3 months of frozen storage. Means followed by the same letter between samples for each month are not significantly different (Duncan's multiple range test $p \leq 0.05$).

Fig. 2. Key volatile aldehyde (mg/kg cooked pork) for negative control (no antioxidants added), coffee in pork treatments (1 g/kg light and dark roasts of spent, whole, lyophilized brewed), and rosemary oleoresin (2 g/kg) in cooked pork patties analyzed after 2 months of frozen storage.

Fig.3. Thiol content (µmol thiol/g pork) ± standard deviation for negative control (no antioxidants added), coffee in pork treatments (1 g/kg light and dark roasts of spent, whole, lyophilized brewed), and rosemary oleoresin (2 g/kg) in cooked pork patties analyzed monthly over 3 months of frozen storage.

Fig. 4. Percent metmyoglobin for negative control (no antioxidants added), coffee in pork treatments (1 g/kg light and dark roasts of spent, whole, lyophilized brewed), and rosemary oleoresin (2 g/kg) in cooked pork patties analyzed monthly over 3 months of frozen storage. Means followed by the same letter between the samples for each month are not significantly different (Duncan's multiple range test $p \leq 0.05$). Error bars represent standard deviation.

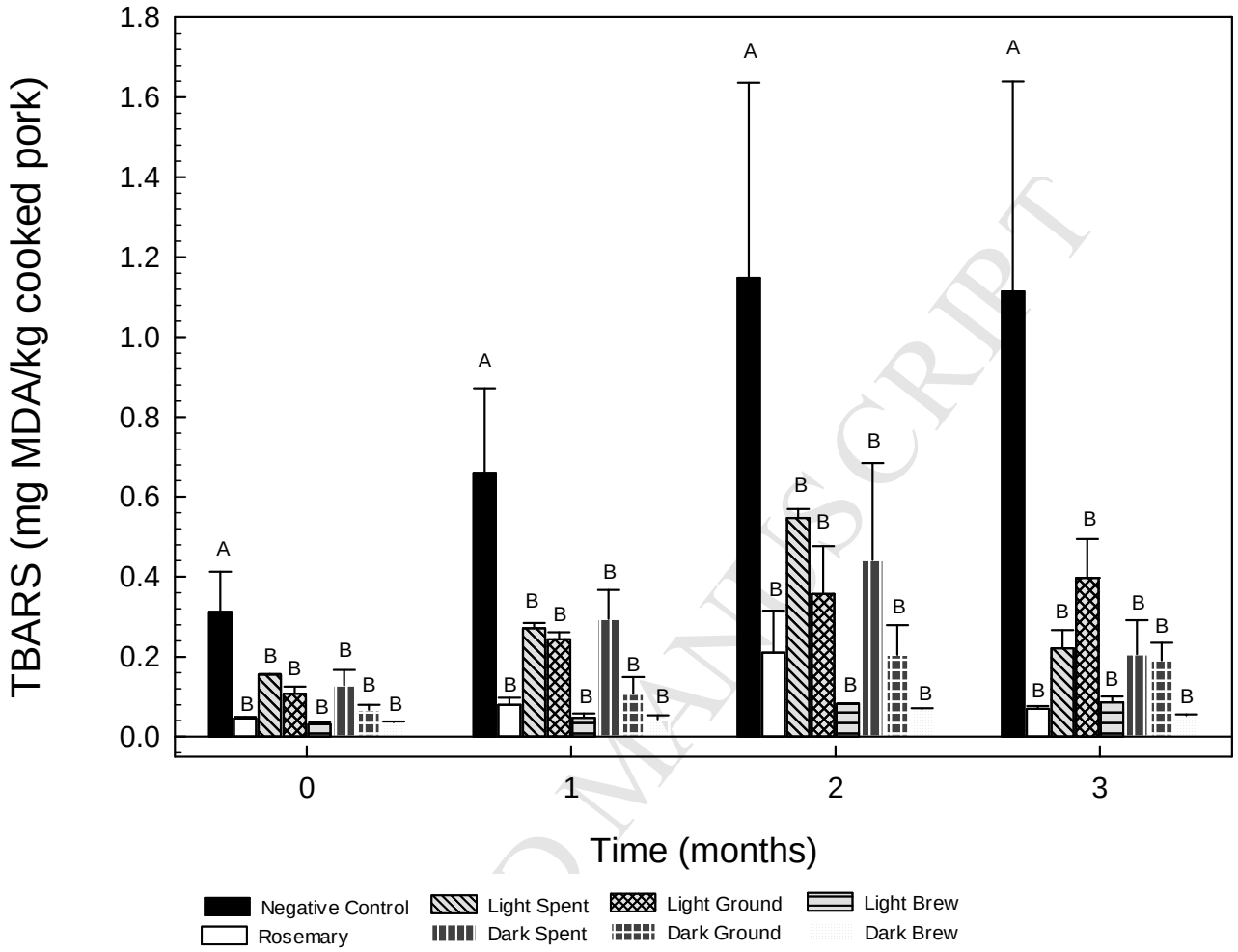
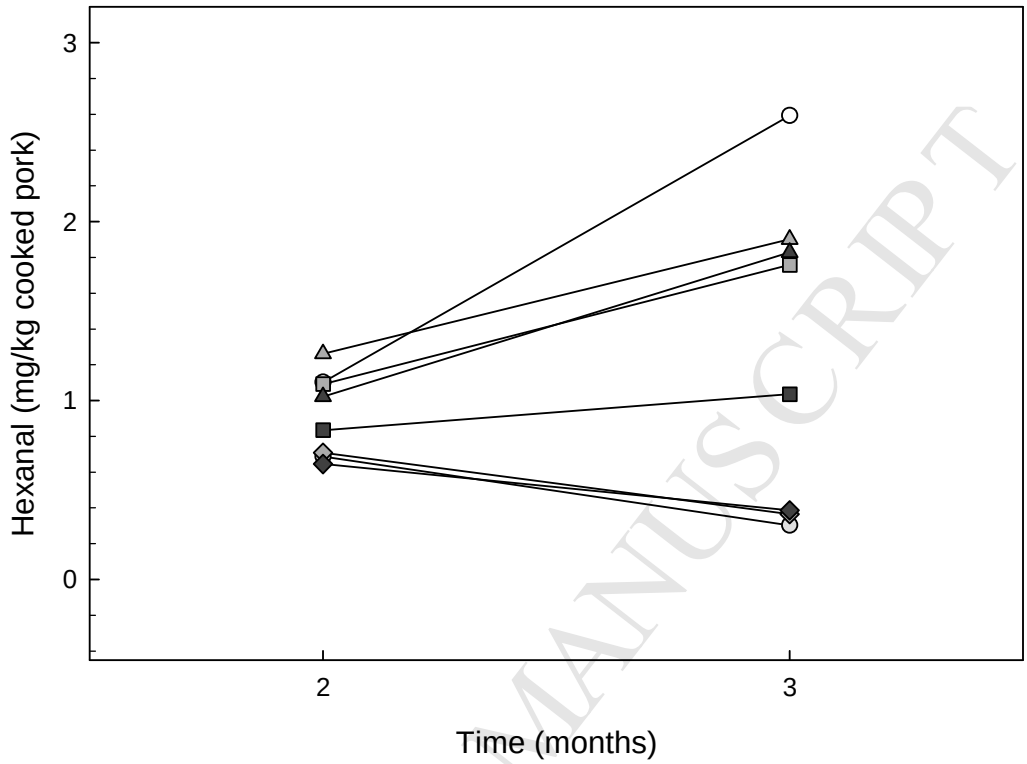
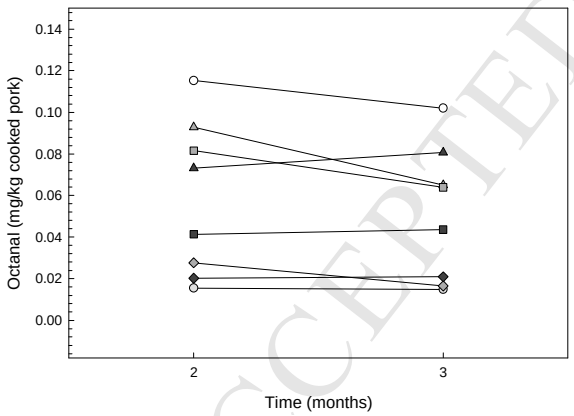


Fig. 1.

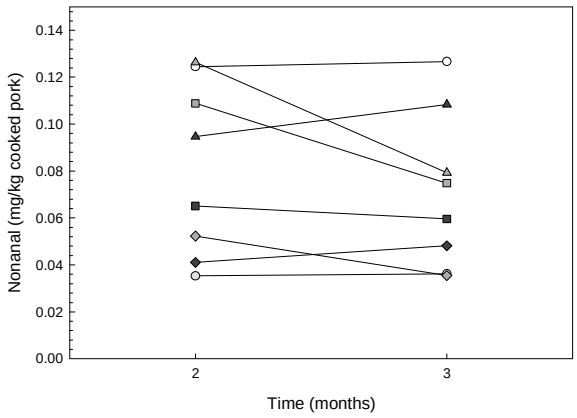
A) Hexanal



B) Octanal



C) Nonanal



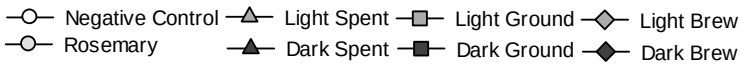


Fig. 2.

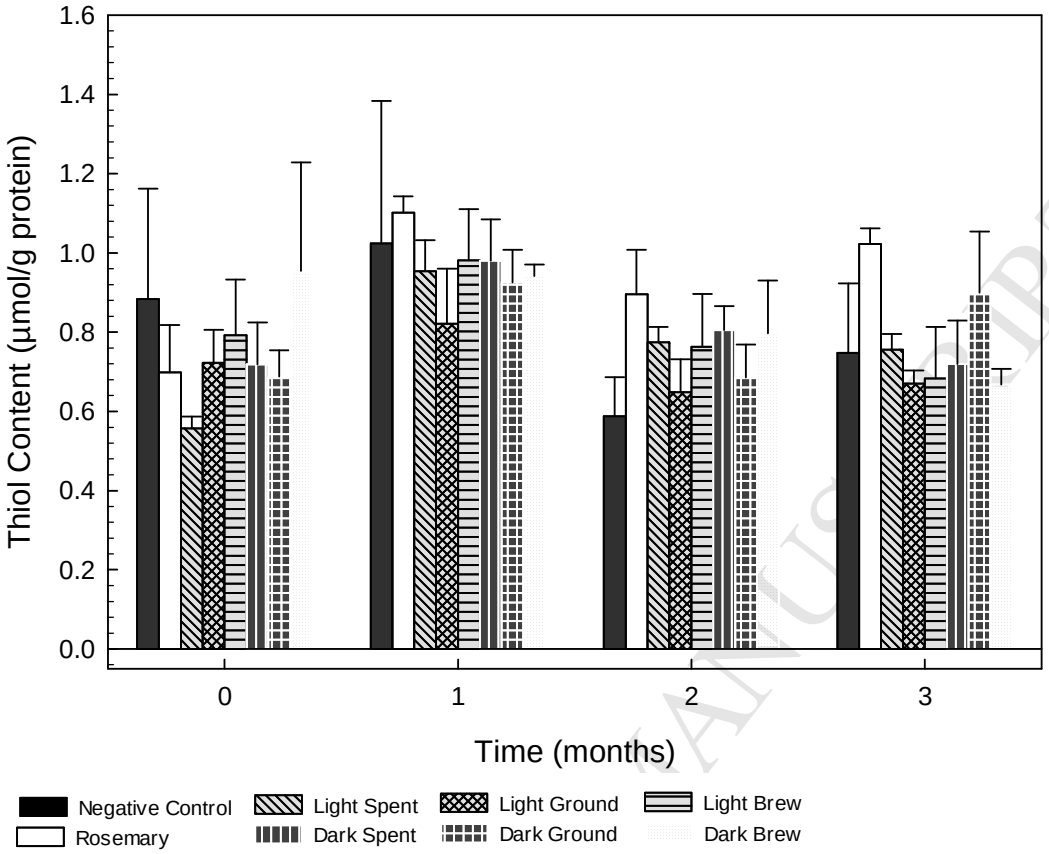


Fig. 3.

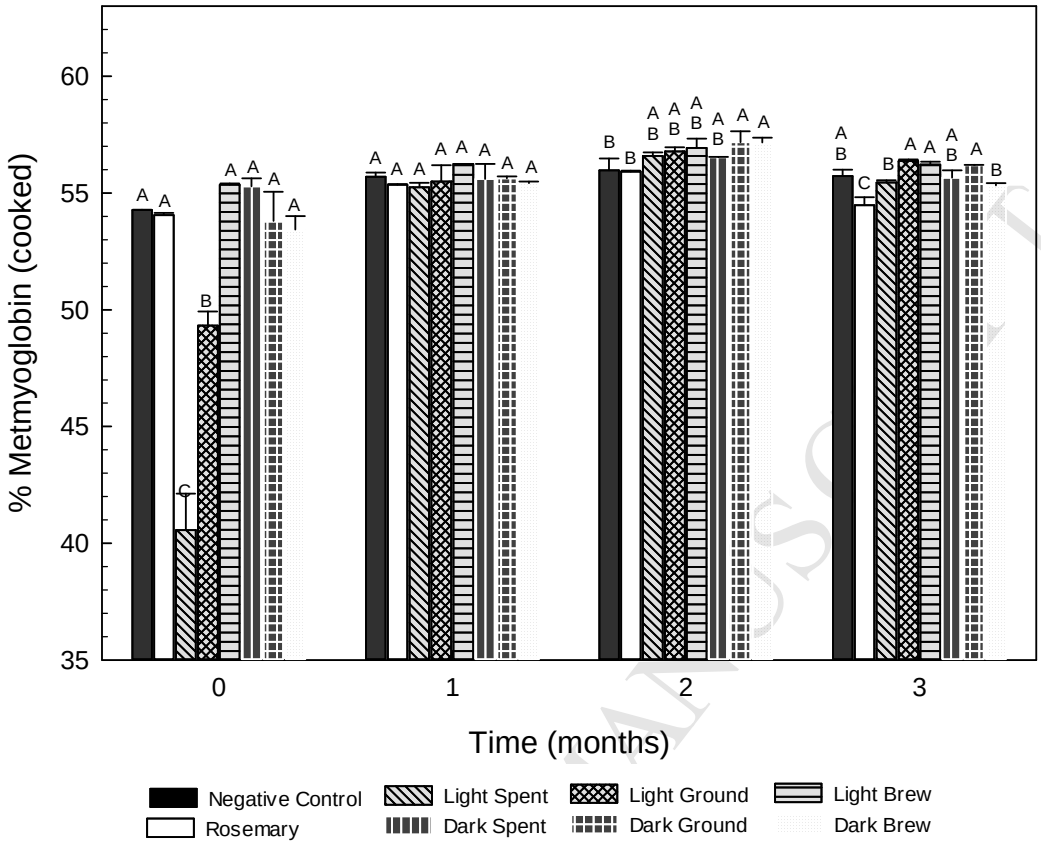


Fig. 4.

Highlights:

- Pork with coffee exhibited comparable TBARS values to pork with rosemary oleoresin.
- Aldehydes were lowest with added lyophilized brew compared to whole or spent coffee.
- Thiol content remained stable for all treatments under frozen conditions.
- Coffee inhibited lipid oxidation without adverse effects on protein oxidation.