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2 on lipid oxidation products and consumer acceptability

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Abstract

The antioxidant capacity of dried *Agaricus bisporus* mushrooms (DAB) in beef has previously been assessed. However, interactions between lipid oxidation products, mushroom polyphenols, and bovine proteins present in beef to explain the mushroom's antioxidative effect, has not been determined. Oven-dried or lyophilized DAB with and without 15 g NaCl/kg beef (1.5%) or 20 g NaCl/kg beef (2%) were added to sarcoplasmic protein homogenates from top round beef. Malondialdehyde and volatile aldehyde binding to sarcoplasmic protein (SP) were monitored. Oven dried had 64% higher total phenolic compared to lyophilized DAB, leading to ~50% lower malondialdehyde content in beef with oven dried DAB compared to lyophilized DAB. The addition of 20 g NaCl/kg beef (2%) acted as a pro-oxidant, while addition of 15 g NaCl/kg beef (1.5%) increased binding of lipid oxidation (LOX) products to SP. The results suggest that addition of mushrooms to beef can enhance the binding of sarcoplasmic protein to lipid oxidation products, thereby decreasing lipid oxidation compounds.

Keywords: *Agaricus bisporus*; aldehyde binding; sarcoplasmic proteins; sodium reduction

35 1. Introduction

36 Lipid oxidation begins immediately after beef slaughter and compromises the quality of
37 beef over time by producing volatile aldehydes that contribute to the development of rancid off-
38 flavors and odors, thereby limiting the overall consumer acceptability (Sammet, Duehlmeier,
39 Sallmann, Von Canstein, Von Mueffling, & Nowak, 2006). In the USA, the estimated
40 supermarket shrinkage (food loss) resulting in uneaten meat from 2011-2012 was about 13
41 percent which was three times higher than the 4.5 percent shrinkage from 2005-2006 (Buzby,
42 Bentley, Padera, Campuzano, & Ammon, 2016). This shrinkage may partially be due to changes
43 in color and production of off aromas, which are indicators of freshness in beef products for
44 consumers (Font-I-Furnols & Guerrero, 2014).

45 Secondary products of lipid oxidation, which include malondialdehyde (MDA) and
46 volatile aldehydes such as propanal formed by oxidation of linolenic acid, hexanal formed by
47 oxidation of linoleic acid, and octanal formed from oxidation of oleic acid (Pavan & Duckett,
48 2013) are responsible for the development of rancid off-flavors and aromas that consumers often
49 associate with spoilage and the increasing shrinkage. To inhibit lipid oxidation in ground beef,
50 natural plant-based extracts rich in antioxidants, such as rosemary, have been used. *Agaricus*
51 *bisporus* mushrooms (DAB) contain antioxidant phenolic and ergothioneine compounds
52 (Dubost, Ou, & Beelman, 2007), making these *Agaricus bisporus* mushrooms a readily available
53 source of antioxidants to inhibit lipid oxidation and prolong shelf life stability of foods.

54 Alnoumani, Ataman, & Were (2017) found that ground cooked beef with DAB had 66–
55 96% lower free MDA when compared to the control. The antioxidant capacity of DAB compared
56 to rosemary also increased over time, indicating that mushrooms can be a good alternative to
57 rosemary (Alnoumani et al, 2017). *Agaricus bisporus* mushrooms have been added to beef to

58 inhibit oxidation through radical scavenging from phenolic compounds, however, the degree to
59 which these mushrooms affect the interaction of specific bovine proteins in beef, such as
60 sarcoplasmic protein, to influence lipid oxidation products has not been studied. Thus, the
61 experimental objective was to investigate the antioxidant capacity of DAB as a function of
62 interaction with beef homogenates containing sarcoplasmic bovine proteins.

63 **2. Materials and Methods**

64 **2.1. Ground Beef Preparation and Protein Extraction**

65 Bovine top round beef was purchased twice (10 lbs each time) from American Beef
66 Packers Incorporation (Chino, CA, USA) from a female Holstein carcass, slaughtered less than
67 21 hours before transportation in coolers covered with bags of ice to the Chapman University
68 laboratory (26 miles). Meat was then ground through a 3-mm grinding plate attached to a
69 KitchenAid food processor (St. Joseph, MI, USA) and patties were formed.

70 Sarcoplasmic extraction was done as described by Stapornkul, Prytkova, & Were (2016).
71 Sarcoplasmic protein was then lyophilized and stored in a -80°C freezer for the duration of the
72 research.

73 **2.2. Blanching and Dehydration of Mushrooms**

74 *Agaricus bisporus* mushrooms grown in Pennsylvania (Country Fresh Mushroom Co.,
75 289 Chambers Road, Toughkenamon, PA, 19374) were obtained from B & C Fresh Sales
76 (Orange, CA) twice (two 5 lbs boxes of mushrooms, totaling to 10 lbs of mushrooms each time).
77 Mushrooms were washed under a narrow stream of tap water for 5-10 sec. All mushrooms were
78 blanched by placing one layer of mushrooms in a steam basket located 76.2 mm above boiling
79 water or in a 1 g ascorbic acid/100 mL water (1%) solution for 6 min to inactivate polyphenol
80 oxidase that could contribute to browning (Lespinard, Goni, Salgado, & Mascheroni, 2009).

81 After 6 min of blanching, mushrooms were then placed in either cold water or ascorbic acid
82 solution (1 g ascorbic acid/100 ml water) for an additional 6 min. Half of the blanched
83 mushrooms were oven dried at 60°C for 20 hr, and the other half were lyophilized with a Harvest
84 Right Scientific Freeze-Dryer (North Salt Lake, UT, USA) as per manufacturer's instructions
85 using the Food Profile parameters. The dried mushrooms were ground into fine powder using a
86 KitchenAid Blade Coffee Grinder (BCG111OB Onyx Black) and sieved through #40 mesh
87 (0.841 mm). A composite mix of the powdered mushrooms were stored in amber bottles in a -
88 80°C freezer for the duration of the study.

89 **2.3. Preparation of Beef, NaCl, and Mushroom Treatments**

90 The treatments consisted of two concentrations of NaCl [15 g NaCl/kg beef (1.5%), 20 g
91 NaCl/kg beef 92%], two concentrations of mushrooms [10 g mushroom/kg beef (1%), 20 g
92 mushroom/kg beef (2%)], two different dehydration methods of mushrooms (oven dried or
93 lyophilized), and two blanching methods of mushrooms (ascorbic acid or water). Duplicate
94 samples for each treatment were prepared for each of the 5 time-points (Days 1, 3, 6, 9, and 12),
95 resulting in 160 patties for the entire experiment. These different combinations of mushrooms
96 and NaCl added to ground beef and formed into patties were then used for the different assays.

97 **2.4. Preparation of Mushroom and NaCl Solutions**

98 The concentration of *Agaricus bisporus* mushroom added to bovine proteins were
99 prepared based on concentrations used in initial ground beef from Alnoumani, Ataman, & Were
100 (2017)'s study. Since ground beef is comprised of ~220 g protein/kg beef and SP accounts for
101 ~300 g SP/kg total muscle protein, addition of 10 g mushroom/kg beef (1%) and 20 g DAB/kg
102 beef (2%) to ground beef equated to adding 3 g DAB/kg beef (0.3%) and 6 g DAB/kg beef
103 (0.6%) in SP homogenates. Addition of 15 g NaCl/kg beef (1.5%) and 20 g NaCl/kg beef (2%) to

104 ground beef equated to addition of 4.5 g NaCl/kg beef (0.45%) and 6 g NaCl/kg beef (0.6%) in
105 SP homogenates.

106 **2.5. Phenolic Quantification in Dehydrated Mushroom Powder**

107 Gallic acid (0-1.30 mg/L) dissolved in 70 mL methanol in 100ml was used for
108 quantification. Dehydrated mushroom powders were dissolved in 70% methanol and incubated at
109 room temperature for 10 min based on Su, Zhang, Hou, Zhang, Guo, Huang, et al., (2014) study.
110 All samples were filtered using 0.45 µm nylon membrane filters and analyzed using a Luna
111 reverse phase C8 column (150 x 4.6 mm, Phenomenex, Torrance, CA) and an Agilent HPLC
112 1100 series (Agilent Technologies, Waldbronn, Germany) at 30°C. The solvents used were:
113 0.1mL formic acid/100 mL HPLC water (A) and 0.1mL formic acid/100 mL (B). The gradient
114 employed was 100% A for 2 min, 99.8% A for 6.0 min, 10% A for 8 min, and 95% A for 2 min
115 at a flow rate of 0.2 mL/min at 280 nm.

116 **2.6. Lipid Oxidation Analysis of Bovine Proteins with Mushroom Infusions and Salt**

117 **2.6.1. Interaction of Mushrooms, Salt, and Bovine Proteins on Malondialdehyde**

118 Thiobarbituric acid reactive substances (TBARS) in raw ground beef with added oven
119 dried or lyophilized mushroom powder blanched in either 1 g ascorbic acid/100 mL water (1%)
120 ascorbic acid (AA) or HPLC water at NaCl concentrations of 15 g NaCl/kg beef (1.5%) or 20 g
121 NaCl/kg beef (2%) were measured after refrigerated storage in Ziploc® bags at 4°C after days 1,
122 3, 6, 9, and 12. TBARS was also performed for 0.066 g/mL SP samples with mushroom [0 g
123 DAB/kg beef (0%), 3 g DAB/kg beef (0.3), 6 g DAB/kg beef (0.6%)] and NaCl [(0 g NaCl/kg
124 beef (0%), 4.5 g NaCl/kg beef (0.45), 6 g NaCl/kg beef (0.60%)]. The binding of MDA to
125 protein was assessed as outlined by Stapornkul, Prytkova, & Were (2016) with modifications as
126 follows. The 164 µL of 1,1,3,3 –tetramethoxypropane (TMP) was hydrolyzed in 10 g

127 trichloroacetic acid (TCA)/100 mL DI water at 70°C for 15 min to obtain 0.1 mol/L MDA. Serial
128 dilutions were performed to obtain a final concentration of 0.15 mmol/L MDA. Each sample
129 contained 0.25 mL of NaCl (0 g NaCl/kg beef (0%), 15 g NaCl/kg beef (1.5%) or 20 g NaCl/kg
130 beef (2%)), 0.25 mL of mushroom powder [0, 10 or 20 g DAB/kg beef (0,1 or 2% DAB)], 0.25
131 mL of 0.15 mmol/L MDA, and 0.25 mL of SP bovine protein. The controls for each variable (0
132 g NaCl/kg beef (0%), 0 g DAB/kg beef (0%)) contained 0.25 mL of either NaCl [0 g NaCl/kg
133 beef (0%), 15 g NaCl/kg beef (1.5%), or 20 g NaCl/kg beef (2%)], 0.25 mL of mushroom [0 g
134 DAB/kg beef (0%), 10 g DAB/kg beef (1%), or 20 g DAB/kg beef (2%)], or 0.25 mL of SP and
135 0.75 mL of DI water. All samples were incubated at 4°C for 1 hr before day 0 baseline
136 measurements. Each 0.6 mL of sample was mixed with 0.75 mL of 10 g TCA/100 mL in micro-
137 centrifuge tubes, and then centrifuged using an accuSpin™ micro-centrifuge (Pittsburg, PA,
138 USA) at 8000 g⁻¹ for 5 min. The 0.02 mol/L TBA: supernatant (1:1) samples were incubated at
139 60°C for 90 min. Absorbance readings at 532 were recorded using a FLUOstar Omega
140 Microplate Reader (BMG Labtech, Cary, NC, USA).

141 A standard solution of MDA in 10 g TCA/100 mL was prepared from TMP and standard
142 curves ranging from 0 to 10 mM were used to quantify MDA in homogenates. The bound MDA
143 was expressed as mg bound MDA/ kg SP (Stapornkul, Prytkova, & Were, 2016).

144 **2.6.2. Interaction of Mushrooms, Salt, and Bovine Proteins on Volatile Aldehydes**

145 Volatile aldehyde binding was determined in sarcoplasmic protein homogenates with 10
146 g DAB/kg beef (1%) and 20 g NaCl/kg beef (2%). These concentrations of mushroom and NaCl
147 were determined based on results from **Section 2.6.1**, which showed that 10 g DAB/kg beef (1%)
148 and 20 g NaCl/kg beef (2%) resulted in higher free MDA production compared to 20 g DAB/kg
149 beef (2%) and 15 g NaCl/kg beef (1.5%). The SP homogenates with added mushroom and NaCl

150 were spiked with volatile aldehydes (pentanal, hexanal, and octanal). Volatile aldehydes bound
151 to protein were then measured using gas chromatography, and binding was expressed as mg/g SP
152 (Stapornkul, Prytkova, & Were, 2016).

153 **2.7. Consumer acceptability of salt reduced patties with added mushroom powder**

154 Each 454g of beef and mushroom mixture contained 9.08 g DAB. To control ground beef
155 with 1.5% salt, 6.81g NaCl was added, while to the beef and 2% DAB samples, 4.54g NaCl (1%)
156 was added. The ingredients were mixed for two minutes (Hobart, HL 120, Troy, OH, U.S.A),
157 divided into 100g portions, formed as 11.5cm diameter patties and cooked for 3 min on each side
158 to an inner temperature of 71°C (Rojas & Brewer, 2007). Each beef patty was divided into eight
159 pieces. Each piece was placed in a small plastic cup coded with a random three digits number.
160 The samples were served around 54°C. Two sensory evaluations were conducted: in the first one,
161 samples with 2% DAB added had 33% less NaCl compared to control sample while in the
162 second trial, NaCl reduction was 50% in samples with 2% DAB.

163 The effect of incubation time on DAB's impact on saltiness, aroma, and acceptability was
164 conducted over 3 days, given that microbial testing was not part of the current study and 3 days
165 was considered microbially safe. Consumers rated the patties using a nine-point liking scale with
166 0 being dislike extremely and 9 being like extremely (da Silva, da Silva, Ferreira, Minim, da
167 Costa, & Perez, 2013). Panelists were provided with crackers and water to cleanse their palates
168 in between samples.

169 **2.8. Statistical Analysis**

170 Differences in MDA and volatile aldehyde production and binding to sarcoplasmic
171 protein of three mushroom treatments [0 g DAB/kg beef (0%), 10 g DAB/kg beef (1%), and 20 g
172 DAB/kg beef (2%)], three NaCl levels [0 g NaCl/kg beef (0%), 15 g NaCl/kg beef (1.5%), and

173 20 g NaCl/kg beef (2%)] and two drying methods of mushrooms (oven dry or lyophilized)
174 combinations were assessed. Differences between treatments were determined by Analysis of
175 Variance (ANOVA) and Tukey's Honest Significant Difference (HSD) test using Statistical
176 Analysis Software (SAS 9.3, Cary, NC, USA). A level of significance of $\alpha = 0.05$ was used
177 throughout the study.

178 **3. Results and Discussions:**

179 **3.1. Effect of Mushroom Dehydration Method on Phenolic Acid Composition**

180 After washing, blanching, and drying, the yields of oven dried and lyophilized mushrooms
181 were 8.35% and 6.59%, respectively, resulting in a 1.79% higher yield in oven dried mushrooms
182 compared to lyophilized mushrooms. Oven dried mushroom powder had 64.15% higher total
183 phenolics of 5.227 mg/g compared to lyophilized mushroom powder at 3.189 mg/g. Alnoumani,
184 Ataman, & Were (2017) quantified total phenolic content in dry roasted DAB to be 5.45 mg/g
185 using Folin-Ciocalteu reagent rather than the HPLC method used in this study. Although
186 lyophilization prevents undesirable shrinkage and produces products with high porosity,
187 unchanged nutritional quality, superior aroma and flavor, and color retention (Oikonomopoulou,
188 Krokida, & Karathanos, 2011), heating at 100°C can disrupt the plant cell wall, thereby
189 liberating polyphenolic compounds more easily compared to the raw material (Choi, Lee, Chun,
190 Lee, & Lee, 2006). Heating mushrooms can decompose polyphenols and decrease their
191 antioxidant activity at high temperatures. However, an increase in phenolic concentration may
192 occur at low heating temperatures due to enhanced extraction when the cell wall is disrupted to
193 release bound polyphenolic compounds (Ferreira, Barros, & Abreu, 2009). Oven drying is
194 considered a harsher dehydration method, however, the oven dried *Agaricus bisporus*
195 mushrooms in this study were oven dried at 60°C for 20 hours, whereas Giri & Prasad, (2007)

196 used temperatures set 10°C higher or used microwave-vacuuming, which lowered phenolic
197 content. When Mphahlele, Fawole, Makunga, & Opara, (2016) compared lyophilization and
198 oven drying of pomegranate peels, they found that lyophilization yielded a higher phenolic
199 content compared to oven dried, however, the highest temperature reached for lyophilization in
200 their study was 60°C for 16 hours, whereas this study reached a maximum temperature of 120°C,
201 which was twice the temperature of that of Mphahlele, Fawole, Makunga, & Opara (2016) study.
202 Higher temperature may explain the lower yield and phenolic content in lyophilized mushrooms
203 compared to oven dried mushrooms. Differences in total phenolics and type of phenolics with
204 the different dehydration methods could impact functionality. Gallic acid in oven dried and
205 lyophilized mushrooms was 1.353 and 0.9241 mg/g, respectively. Lyophilization and oven
206 drying at 60°C could impact other reducing bioactive compounds besides phenolic compounds.
207 For instance the different dehydrations can affect enzymatic versus non-enzymatic browning
208 which could further affect reducing capacity of the powders.

209 **3.2. Interactions of Mushroom Powder, NaCl, and Sarcoplasmic Proteins with Lipid** 210 **Oxidation Products**

211 **3.2.1. Binding of Malondialdehyde to Bovine Proteins in the Presence of Mushroom** 212 **Powder and NaCl**

213 Addition of 20 g DAB/kg beef (2%) of oven dried and lyophilized DAB decreased free
214 MDA by 46.51-92.60% and 56.73-92.77% in both 15 g NaCl/kg beef (1.5%) and 20 g NaCl/kg
215 beef (2%) salted ground beef, respectively, compared to the control with no DAB on day 12 of
216 refrigerated storage (**Fig. 1**). For the 15 g NaCl/kg beef (1.5%) and 20 g NaCl/kg beef (2%)
217 salted ground beef samples, added oven dried mushroom powder to raw beef lowered MDA
218 compared to lyophilized mushroom powder (**Fig. 1**) attributed to the 64.15% higher phenolic
219 content in oven dried mushrooms (**section 3.1 and Fig. S2**). These mushroom phenolic
220 compounds can donate hydrogens to free radicals and decrease production of lipid oxidation

221 products (Ghahremani-Majd & Dashti, 2015).

222 The free MDA in the 20 g NaCl/kg beef (2%) control with no added mushrooms was
223 34.50% higher compared to the 15 g NaCl/kg beef (1.5%) control with no added mushroom
224 powder on day 12 (**Fig. 1**), with the interaction between salt and treatments being significant
225 ($p<0.05$) (**Table 1**). Rhee & Ziprin, (2001) also found that NaCl at 25 g NaCl/kg beef promoted
226 lipid oxidation. The pro-oxidant action of NaCl at 20 g NaCl/kg beef (2%) was attributed to its
227 potential capacity to disrupt cell membrane integrity, facilitating access of free radicals to
228 unsaturated fatty acids, as well as liberating iron ions from heme protein, therefore leaving more
229 free iron ions to catalyze LOX and inhibit antioxidant enzymes (Mariutti & Bragagnolo, 2017).
230 Further investigations are suggested to pinpoint which of the aforementioned mechanisms
231 explain the pro-oxidant effect at higher NaCl concentrations.

232 Since ascorbic acid is an anti-browning agent that can prevent oxidation and discoloration
233 of beef during storage by reduction of metamyoglobin-Fe(III) to metamyoglobin-Fe(II), thereby
234 maintaining the red color in raw beef (Varvara, Bozzo, Celano, Disanto, Pagliarone, & Celano,
235 2016), blanching in 1 g ascorbic acid/100 mL water solution to inactivate the browning
236 polyphenol oxidase enzyme was compared to that in water. In beef with 15 g NaCl/kg beef, oven
237 dried and lyophilized mushroom powder from water blanching produced 24.79% and 58.67%
238 more free MDA, respectively, compared to samples with oven dried and lyophilized mushrooms
239 blanched in ascorbic acid (**Fig. 1**). Ascorbic acid has antioxidant properties (Ahn & Nam, 2004)
240 and can inhibit LOX, leading to lower ($p<0.05$) free MDA observed.

241 The 15 g NaCl/kg salted raw beef control with no added mushroom powder produced
242 82.42% and 46.60% more free MDA compared to raw beef with oven dried and lyophilized
243 mushrooms blanched in water, respectively. The 15 g NaCl/kg beef (1.5%) salted raw beef

244 control produced 92.74% and 59.84% more free MDA compared to raw beef with added oven
245 dried and lyophilized mushrooms blanched in ascorbic acid. Although mushrooms blanched in
246 ascorbic acid and water decreased free MDA by 10.31% and 13.23% on day 12, respectively, the
247 2.92% difference between the two blanching methods was relatively small when compared to the
248 92.74% increase in free MDA in the control compared to the blanched mushroom treatments.
249 Therefore, mushrooms blanched in 1 g ascorbic acid/100 mL water were not used in subsequent
250 assays.

251 Addition of lyophilized and oven dried mushrooms decreased free MDA content, where,
252 addition of oven dried mushrooms to raw ground beef lowered free MDA by 67.18% compared
253 to lyophilized mushrooms on day 12 (**Fig. 1**). Due to the higher phenolic content of oven dried
254 mushrooms compared to lyophilized mushrooms, oven dried mushrooms can inhibit lipid
255 oxidation to a greater extent, lowering amount of free MDA produced. Therefore, the binding of
256 MDA with bovine proteins that lowered free MDA was monitored using oven-dried mushrooms.
257 As seen in **Table 2**, adding 20 g oven dried DAB/kg beef (2%) to SP increased binding of MDA
258 by 52.56-71.19% compared to the control treatments with no added DAB on each day of
259 analysis. Similarly, Stapornkul, Prytkova, & Were (2016) found that adding green tea increased
260 MDA binding by 59% compared to the control with no added green tea. As seen in **Fig. 1**,
261 adding DAB decreased free MDA, because rather than having free MDA in the meat matrix, the
262 MDA was bound to the proteins in ground beef (Stapornkul, Prytkova, & Were, 2016). The *Bos*
263 *taurus* myoglobin in the sarcoplasmic protein is comprised of 13 histidine residues on its 154
264 amino acid chain, while *Agaricus bisporus* lectin protein, a major protein in *Agaricus bisporus*
265 mushrooms, contains 1 histidine residue and 9 tyrosine residues on its 142 amino acid chain
266 (Carrizo, Irazoqui, Lardone, Nores, Curtino, Capaldi, et al., 2004). Adding mushrooms may thus

267 increase the number of amino acid potential binding sites for MDA, thereby increasing the
268 percent MDA binding compared to the control samples with no DAB added. Mushrooms added
269 to SP at 20 g DAB/kg beef had 27.57-63.95% higher binding to MDA compared to mushrooms
270 added to SP at 10 g DAB/kg beef on day 9, which may be due to the 20 g DAB/kg beef having
271 twice the amount of amino acid binding sites available for MDA to bind. The interaction of
272 treatment and day was significant ($p < 0.05$) for MDA binding (**Table 3**). The highest binding of
273 MDA to SP was on day 9 with 15 g NaCl/kg beef (1.5%) and 20 g DAB/kg beef (2%) at 21.69
274 mg MDA/kg beef, whereas the lowest binding of MDA to SP was on day 9 with no NaCl and
275 DAB added at 4.702 MDA mg/kg beef (**Table 2**). The higher MDA binding with 20 g DAB/kg
276 beef and 15 g NaCl/kg beef may explain the results found in **Fig. 1**, where added 20 g DAB/kg
277 beef (2%) and 15 g NaCl/kg beef (1.5%) lowered free MDA in raw beef. Jensen (2008) found
278 that at high salt concentrations of ~2%, the ionic strength of protein may be altered to increase
279 protein-protein interaction. This interaction could therefore decrease the percent MDA binding
280 seen in SP samples with added 20 g DAB/kg beef and 20 g NaCl/kg beef compared to SP
281 samples treated with 20 g DAB/kg beef (2%) and 15 g NaCl/kg beef (1.5%) (**Table 2**). As seen in
282 **Table 2**, the bound MDA to SP increased from day 0 (baseline) to day 1. However, the bound
283 MDA to SP began to decrease between day 1 to day 9. From day 0 to day 1, there may have been
284 more amino acid sites for MDA to bind to, thereby increasing amount of bound MDA to protein.
285 However, once MDA starts to bind to protein, the number of free amino acid sites available were
286 occupied, decreasing MDA bound to the protein after day 1 (**Table 2**).

287 **3.2.2. Binding of Volatile Aldehydes to Bovine Protein in the Presence of Mushroom** 288 **Powder and NaCl**

289 There was an increase in bound volatile aldehydes to protein from 20.24, 23.53, and 56.21
290 mg bound volatile/g SP as the carbon chain length of the aldehyde increased from propanal,

291 hexanal, and octanal, respectively (**Fig. 2**). Perez-Juan, Flores, & Toldra (2008) found that pork
292 actomyosin bound to octanal to a greater extent than did hexanal, which is similar to Stapornkul,
293 Prytkova, & Were (2016) findings where free percent volatile compounds were in the decreasing
294 order: pentanal > hexanal > heptanal > octanal > nonanal, suggesting that molecules with longer
295 carbon-chain length have higher binding with protein. The binding affinity of sarcoplasmic
296 protein to volatile aldehydes is dependent on hydrophobic interaction between the protein and
297 aldehydes.

298 The addition of 10 g oven dried DAB/kg beef (1%) to SP increased propanal, hexanal, and
299 octanal binding to protein by 20.90%, 22.65%, and 40.67%, respectively compared to SP control
300 (**Fig. 2**). Stapornkul, Prytkova, & Were, (2016) found that green tea phenolic compounds can
301 bind near the His 64 on the surface of myoglobin, blocking potential aldehyde binding to that
302 specific histidine. Although the phenolic compounds in green tea, such as catechin, can block
303 volatile aldehydes from binding, the volatile aldehydes are significantly lower in molecular
304 weight/MW compared to phenolic compounds in mushrooms such as gallic acid (MW 170.12
305 g/mol), therefore, propanal, hexanal, and octanal with MW of 58.08, 100.16, and 128.212 g/mol,
306 respectively, are more likely to bind to the histidine sites in myoglobin compared to the higher
307 MW phenolic compounds. In addition to the 12 histidine sites on the surface of myoglobin, the
308 *Agaricus bisporus* lectin protein, a major protein in *Agaricus bisporus* mushrooms' additional
309 histidine site (Carrizo, et al., 2004), could provide volatile aldehydes more histidine sites to bind
310 to, thereby decreasing the percentage of free aldehydes. Stapornkul, Prytkova, & Were (2016)
311 also found through molecular docking that at His 97, octanal bound to the greatest extent at 0.92
312 mg/g SP compared with hexanal at 0.63 mg/g SP (Stapornkul, Prytkova, & Were, 2016).

313 As seen in **Fig. 2**, there was a decrease in volatile aldehyde binding to SP with addition of 20

314 g NaCl/kg beef (2%) compared to the control SP for propanal, hexanal, and octanal. The binding
315 of propanal, hexanal, and octanal decreased by 0.31%, 0.22%, and 6.69%, respectively, with
316 added 20 g NaCl/kg beef (2%) in SP compared to SP control with no added NaCl. Adding salt
317 can preserve meat by inhibiting microbial growth in beef depending on the NaCl concentration,
318 however, Perez-Juan, Flores, & Toldra, (2008) found that addition of 0.05 M NaCl to protein
319 homogenates significantly increased free volatile aldehydes compared to unsalted homogenates.
320 The addition of 20 g NaCl/kg beef (2%) can inhibit the activity of antioxidant enzymes, such as
321 catalase, glutathione peroxidase, and superoxide dismutase, favoring oxidation (Pavan &
322 Duckett, 2013).

323 It was established that adding 10 g DAB/kg beef (1%) to SP increased the binding of volatile
324 aldehydes in SP by 20.90-40.67%, whereas adding 20 g NaCl/kg beef (2%) to SP decreased the
325 binding of aldehydes to protein by 0.31-6.69%. Although adding 20 g NaCl/kg beef (2%) to SP
326 decreased aldehyde binding to protein compared to SP control, adding a mixture of 10 g DAB/kg
327 beef (1%) and 20 g NaCl/kg beef (2%) to SP increased binding of volatiles to protein (**Fig. 2**)
328 indicating that 10 g DAB/kg beef (1%) is an effective ingredient for increasing binding of
329 volatile aldehydes to protein compared to the control with no added NaCl and mushroom.
330 Alnoumani, Ataman, & Were (2017) likewise found a 99% decrease in volatile aldehydes in
331 ground beef with added DAB compared to ground beef control with no added DAB.

332 The addition of DAB to ground beef has been shown to inhibit LOX and decrease
333 production of LOX products, such as MDA and volatile aldehydes. Although DAB's
334 antioxidative capacity in meat has been demonstrated, the addition of DAB to ground beef could
335 affect sensory qualities. **Table S1** indicated that panelists could differentiate between the sample
336 with a 33% and 55% salt reduction that were compensated with DAB. However, no differences

337 ($p>0.5$) in the liking of saltiness, smell, juiciness, overall flavor, and overall liking were detected
338 between control patties and patties with added DAB and 33% less salt (**Table 4**) demonstrating
339 potential replacement of 33% salt with DAB.

340 **4. Summary and Conclusions**

341 Oven dried mushroom powder yielded a higher phenolic content compared to
342 lyophilization, which accounted for a decrease in free MDA and volatile aldehydes produced.
343 The addition of DAB increased the binding of LOX products to sarcoplasmic protein. The
344 addition of 20 g NaCl/kg beef (2%) to raw beef acted as a pro-oxidant, however, addition of 15 g
345 NaCl/kg beef (1.5%) to raw beef increased the binding of LOX products, an indication that salt
346 concentration can affect lipid oxidation depending on concentration. The sodium chloride
347 concentrations that promote or have no negative effect on oxidation warrants further
348 investigation. The molecular basis determining if release of ferrous iron from ground beef or
349 competition of salt with anti- or pro-oxidants to explain results obtained should be explored.
350 Improving the knowledge and understanding of naturally sourced antioxidants, such as *Agaricus*
351 *bisporus* mushrooms utilized in the meat industry is important, as use of mushroom powder can
352 improve the chemical shelf life of raw beef at the retail and consumer level and can compensate
353 for NaCl reduction without negatively affecting sensory acceptance. Possible mushroom powder
354 applications may include a seasoning salt blend for beef products to reduce sodium chloride
355 content.

356 **Conflict of Interest**

357 Authors do not have any conflict of interest.

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Source	DF	Type II SS	Mean Square	F	Pr > F
Day ^a	4	0.08092	0.02031	1239.77	<0.0001
Salt ^b	1	0.00015	0.00015	9.30000	0.0037
Treatment ^c	4	0.05211	0.01303	798.400	<0.0001
Day*Salt	4	0.00294	0.00294	45.1100	<0.0001
Treatment*Day	16	0.06021	0.00376	230.610	<0.0001
Treatment*Salt	4	0.00039	0.00009	6.06000	0.0005
Treatment*Day*Salt	16	0.00337	0.00337	12.9100	<0.0001
Error	50	0.00082			
Corrected Total	99	0.20093			

1 **Table 1** Analysis of Variance Model of Free Malondialdehyde in Ground Beef

2 ^aDays included 1, 3, 6, 9, and 12 of refrigerated storage,

3 ^bsalt included 15 g NaCl/kg beef (1.5%) and 20 g NaCl/kg beef (2%)

4 ^cTreatments included beef with oven dried and lyophilized mushrooms blanched in 1% ascorbic
5 acid and water with 15 g NaCl/kg beef (1.5%) and 20 g NaCl/g beef (2%).

Table 2 Bound MDA (mg/kg protein) in refrigerated (4°C) bovine sarcoplasmic protein (SP) with added oven dried Agaricus bisporus (DAB) mushrooms and salt (NaCl).

Samples	Mean \pm SD of Bound MDA/Protein (mg/kg)				
	Baseline (Day 0)	Day 1	Day 3	Day 6	Day 9
<i>Unsalted Sarcoplasmic Protein (SP)</i>					
Sarcoplasmic Protein (SP)	4.71 \pm 0.021 ^h	16.42 \pm 0.064 ^g	7.840 \pm 0.099 ⁱ	11.09 \pm 0.035 ^f	4.702 \pm 0.001 ^g
SP + 10 g DAB/kg beef	6.10 \pm 0.014 ^e	17.43 \pm 0.049 ^f	17.23 \pm 0.021 ^d	14.11 \pm 0.022 ^e	11.82 \pm 0.014 ^d
SP + 20 g DAB/kg beef	6.99 \pm 0.016 ^d	20.08 \pm 0.028 ^b	20.58 \pm 0.057 ^a	18.78 \pm 0.085 ^b	16.32 \pm 0.042 ^c
<i>15 g NaCl/kg beef Salted Sarcoplasmic Protein (SP)</i>					
Sarcoplasmic Protein (SP)	7.90 \pm 0.014 ^b	15.02 \pm 0.148 ^h	12.65 \pm 0.078 ^h	9.220 \pm 0.134 ^g	7.508 \pm 0.221 ^f
SP + 10 g DAB/kg beef	7.35 \pm 0.021 ^c	18.47 \pm 0.021 ^d	20.01 \pm 0.014 ^b	16.76 \pm 0.070 ^d	7.820 \pm 0.007 ^f
SP + 20 g DAB/kg beef	8.49 \pm 0.0007 ^a	19.75 \pm 0.0007 ^c	16.73 \pm 0.007 ^e	18.08 \pm 0.049 ^c	21.69 \pm 0.049 ^a
<i>20 g NaCl/kg beef Salted Sarcoplasmic Protein (SP)</i>					
Sarcoplasmic Protein (SP)	4.99 \pm 0.078 ^g	17.36 \pm 0.042 ^f	13.61 \pm 0.052 ^g	6.806 \pm 0.006 ^h	7.898 \pm 0.023 ^f
SP + 10 g DAB/kg beef	5.35 \pm 0.035 ^f	18.19 \pm 0.071 ^e	15.93 \pm 0.014 ^f	14.08 \pm 0.007 ^e	8.239 \pm 0.018 ^e
SP + 20 g DAB/kg beef	5.40 \pm 0.035 ^f	20.47 \pm 0.057 ^a	19.69 \pm 0.042 ^c	21.38 \pm 0.049 ^a	16.65 \pm 0.007 ^b

Means in the same column with the same letters (a-e) are not significantly different

Source	DF	Type II SS	Mean Square	F	Pr > F
Day ^a	4	1508.69	377.17	102697	<0.001
Salt ^b	2	13.9202	6.9901	1903.27	<0.001
Treatment ^c	2	709.784	354.89	96630.1	<0.001
Day*Salt	8	27.6858	3.4607	942.290	<0.001
Treatment*Day	8	298.711	37.339	10166.66	<0.001
Treatment*Salt	4	14.6587	3.6647	997.8200	<0.001
Treatment*Day*Salt	16	157.358	9.8349	2677.850	<0.001
Error	45				
Corrected Total	89				

1 **Table 3** Analysis of Variance Model of Bound Malondialdehyde in Sarcoplasmic Proteins.

2 ^aDays included 1, 3, 6, and 9 of 4°C refrigerated storage

3 ^bSalt included 0 g NaCl/kg beef (0%), 15 g NaCl/kg beef (1.5%), and 20 g NaCl/kg beef (2%)

4 ^cTreatments included sarcoplasmic protein with ± 0 g DAB/kg beef (0%), 10 g DAB/kg beef
5 (1%) and 20 g DAB/kg beef (2%) and ± 0 g NaCl/kg beef (0%), 15 g NaCl/kg beef (1.5%) or 20
6 g NaCl/kg beef (2%).

7

8 **Table 4** Hedonic test results (Mean \pm SD) of adding 20 g DAB/kg beef (2%) to beef patties with
 9 33% salt reduction compared to the control.

		Saltiness	Smell	Juiciness	Overall flavor
First Trial: NaCl was reduced by 33%					
Day 1	Control	5.49 \pm 1.56	5.23 \pm 1.26	5.34 \pm 1.85	5.23 \pm 1.78
	DAB	5.37 \pm 1.82	5.26 \pm 1.54	4.66 \pm 2.00	5.69 \pm 1.89
	p-value	0.768	0.93	0.148	0.302
Day 2	Control	5.03 \pm 1.58	5.31 \pm 1.67	4.82 \pm 1.78	5.20 \pm 1.90
	DAB	6.11 \pm 1.76	5.23 \pm 1.54	5.26 \pm 1.68	5.86 \pm 1.91
	p-value	0.0004	0.778	0.150	0.052
Second Trial: NaCl was reduced by 50%					
Day 1	Control	6.34 \pm 1.26	5.74 \pm 1.46	5.89 \pm 1.55	6.57 \pm 1.42
	DAB	5.77 \pm 1.68	5.51 \pm 1.63	4.26 \pm 1.92	5.31 \pm 1.69
	p-value	0.118	0.538	0.0004	0.0018
Day 3	Control	6.55 \pm 1.54	5.67 \pm 1.90	6.57 \pm 1.38	6.80 \pm 1.31
	DAB	5.80 \pm 1.54	5.27 \pm 1.75	4.29 \pm 1.71	5.39 \pm 1.71
	p-value	0.019	0.282	< 0.0001	< 0.0001

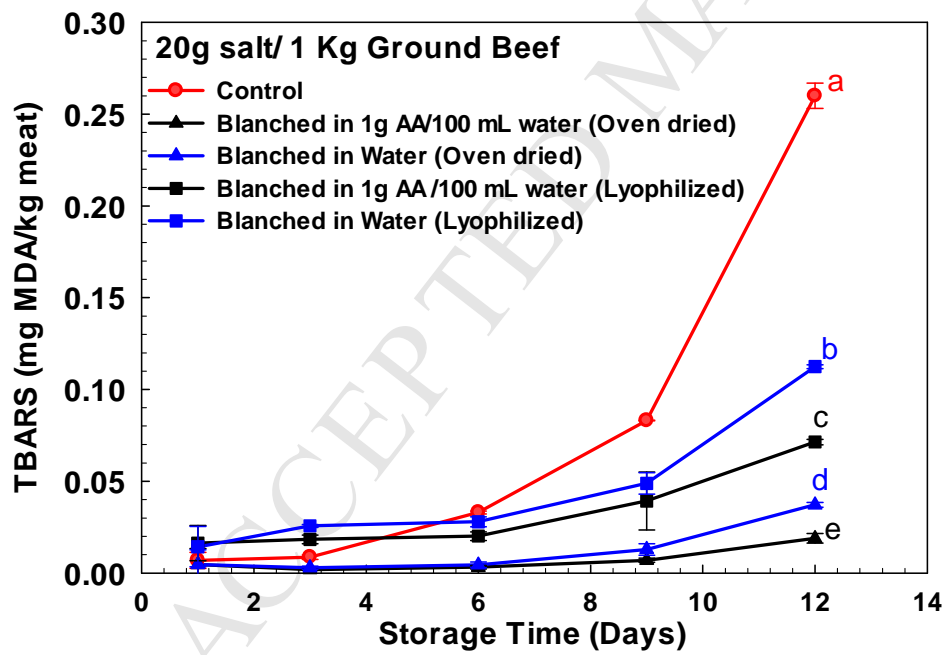
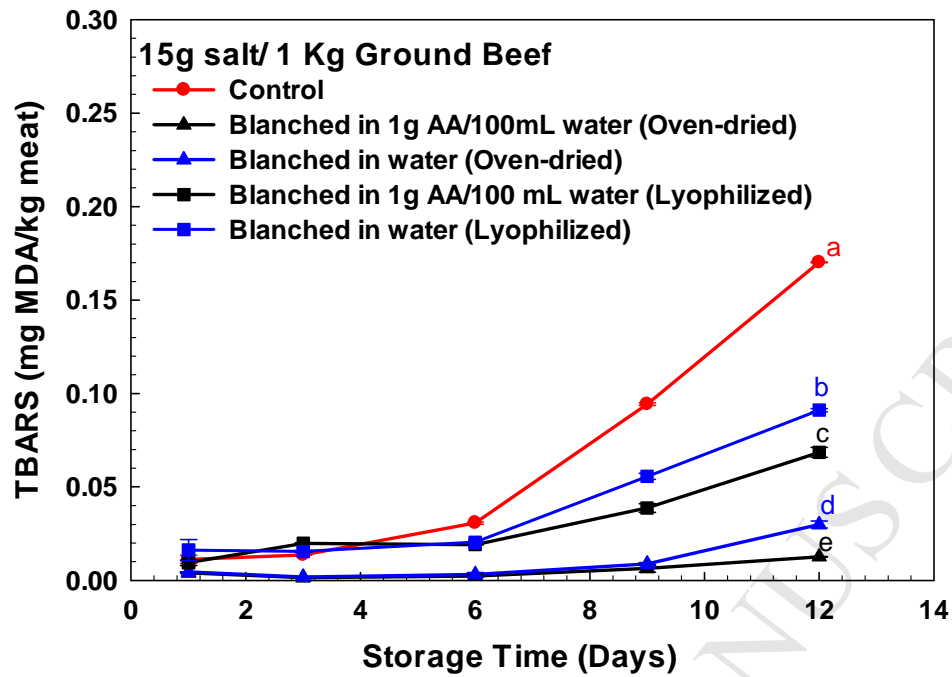
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1 **1. Figure Captions**

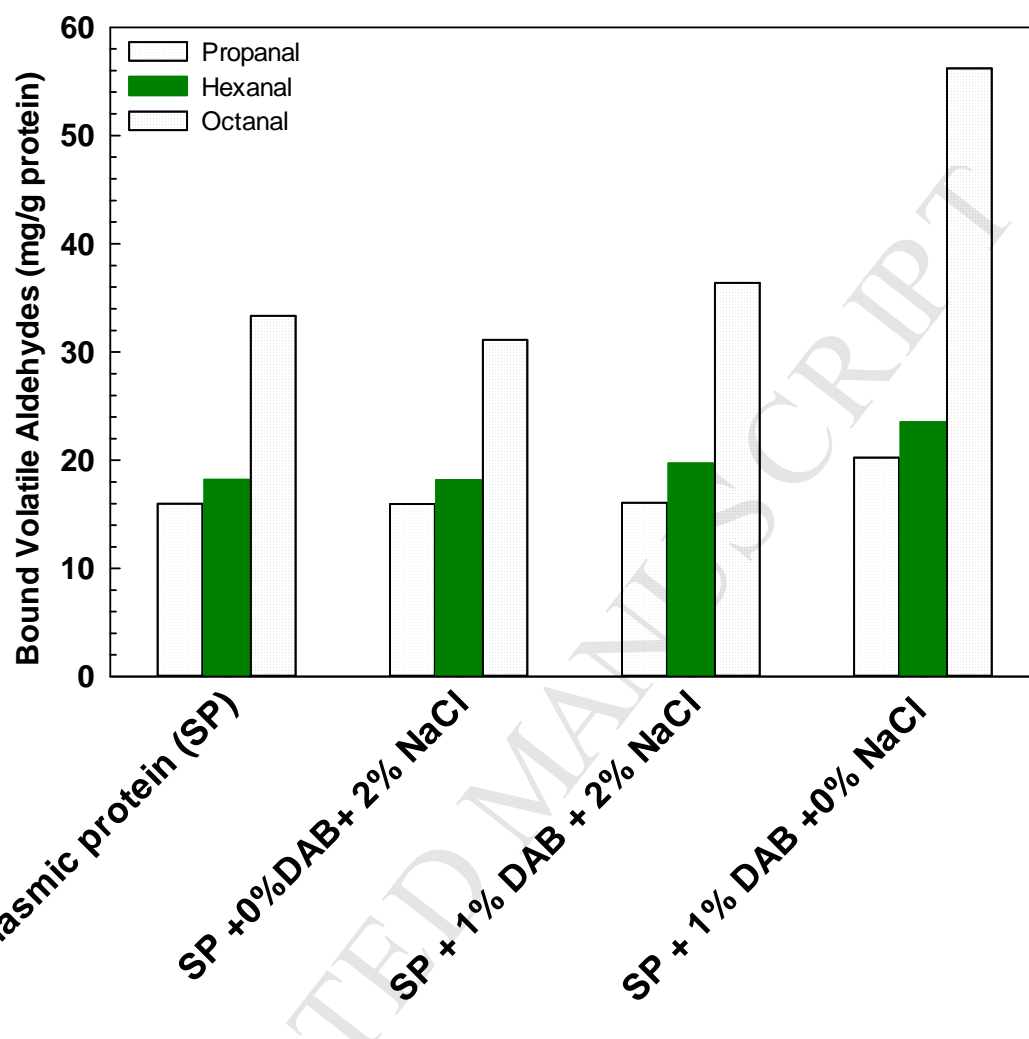
2 **Fig. 1.** Thiobarbituric Acid Reactive Substances (mg/kg) values of raw ground beef with added
3 oven dried and lyophilized mushroom powder blanched in 1 g ascorbic acid/100 mL water (1%)
4 ascorbic acid (AA) or water solutions with different salt concentrations (1.5% or 2%) stored at
5 4C for 12 days. *Means with the same letters are not significantly different on day 12.*

6
7 **Fig. 2.** Bound Volatiles (mg/g protein) in refrigerated (4°C) bovine sarcoplasmic protein (SP)
8 with and without 10 g oven dried *Agaricus bisporus* (DAB) mushroom powder/Kg beef (1%)
9 and with and without 20 g salt (NaCl)/Kg beef (2% NaCl).

10



11 Fig. 1.



12
13
14
15

Fig. 2.

1 Highlights

- 2 ▪ Free malondialdehyde increased with storage time
- 3 ▪ Oven dried mushroom powder had greater antioxidant capacity than lyophilized powder
- 4 ▪ Antioxidant capacity and lipid oxidation product binding was positively correlated
- 5 ▪ An increase in NaCl from 1.5% to 2% increased free lipid oxidation products
- 6 ▪ 2% mushroom powder compensated for 33% salt reduction in beef patties.