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Natalie Tom

Hatouf Ahmed Alnoumani

Lilian Were

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Interactions between mushroom powder, sodium chloride, and bovine proteins and their effects on lipid oxidation products and consumer acceptability

Natalie Tom, Hatouf Ahmed Alnoumani, Lilian Were*

Food Science Program, Schmid College of Science and Technology, Chapman University, 1 University Drive, Orange, CA 92866

*Contact information for corresponding author:

E-mail: were@chapman.edu

Address: Food Science Program, Schmid College of Science and Technology, Chapman University, 1 University Drive, Orange, CA 92866

Telephone: (714) 744-7895

Fax Number: 714-532-6048

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

1. Introduction

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

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

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

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

2. Materials and Methods

2.1. Ground Beef Preparation and Protein Extraction

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

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

2.2. Blanching and Dehydration of Mushrooms

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

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

2.3. Preparation of Beef, NaCl, and Mushroom Treatments

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

2.4. Preparation of Mushroom and NaCl Solutions

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

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

2.5. Phenolic Quantification in Dehydrated Mushroom Powder

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

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

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

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

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

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

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

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

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

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

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

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

2.8. Statistical Analysis

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

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

3. Results and Discussions:

3.1. Effect of Mushroom Dehydration Method on Phenolic Acid Composition

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

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

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

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

Addition of 20 g DAB/kg beef (2%) of oven dried and lyophilized DAB decreased free 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 beef (2%) salted ground beef, respectively, compared to the control with no DAB on day 12 of refrigerated storage (**Fig. 1**). For the 15 g NaCl/kg beef (1.5%) and 20 g NaCl/kg beef (2%) salted ground beef samples, added oven dried mushroom powder to raw beef lowered MDA compared to lyophilized mushroom powder (**Fig. 1**) attributed to the 64.15% higher phenolic content in oven dried mushrooms (**section 3.1 and Fig. S2)**. These mushroom phenolic 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

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

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

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

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

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

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

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

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

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

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

The addition of DAB to ground beef has been shown to inhibit LOX and decrease production of LOX products, such as MDA and volatile aldehydes. Although DAB's antioxidative capacity in meat has been demonstrated, the addition of DAB to ground beef could affect sensory qualities. **Table S1** indicated that panelists could differentiate between the sample 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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433

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			

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

[a]Days included 1, 3, 6, 9, and 12 of refrigerated storage,

[b]salt included 15 g NaCl/kg beef (1.5%) and 20 g NaCl/kg beef (2%)

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

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

[a]Days included 1, 3, 6, and 9 of 4°C refrigerated storage
[b]Salt included 0 g NaCl/kg beef (0%), 15 g NaCl/kg beef (1.5%), and 20 g NaCl/kg beef (2%)
[a]Treatments included sarcoplasmic protein with ± 0 g DAB/kg beef (0%), 10 g DAB/kg beef (1%) and 20 g DAB/kg beef (2%) and ± 0 g NaCl/kg beef (0%), 15 g NaCl/kg beef (1.5%) or 20 g NaCl/kg beef (2%).

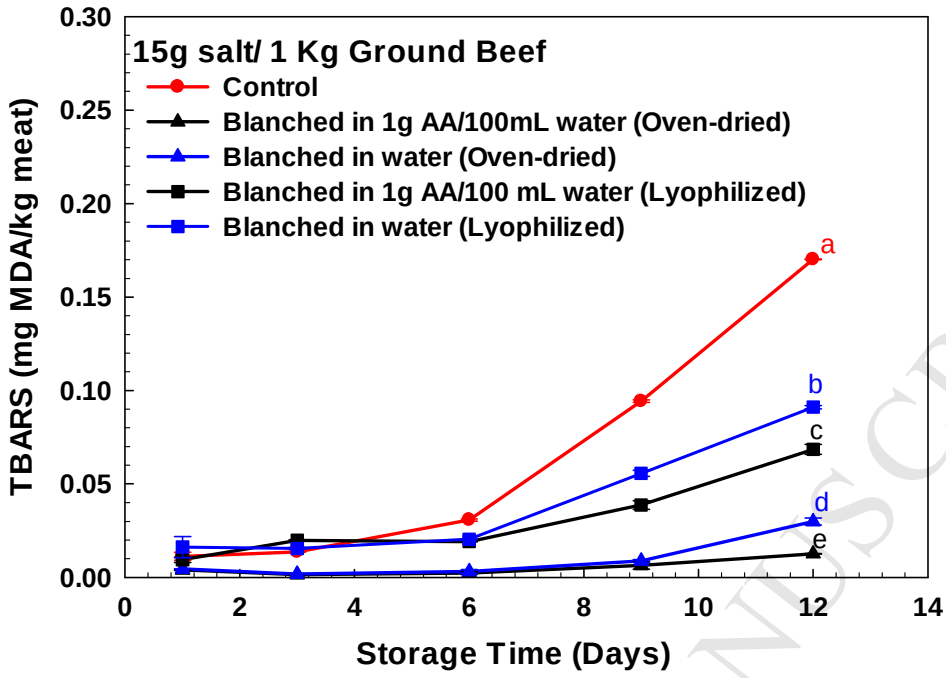
Table 4 Hedonic test results (Mean ± SD*)* of adding 20 g DAB/kg beef (2%) to beef patties with 33% salt reduction compared to the control.

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

1. Figure Captions

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

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



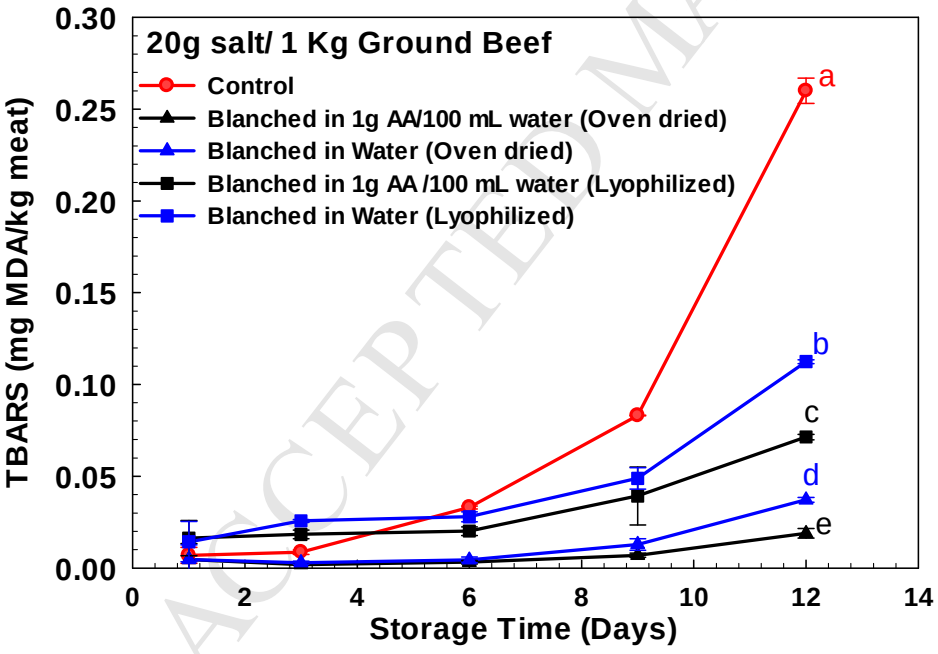


Fig. 1.

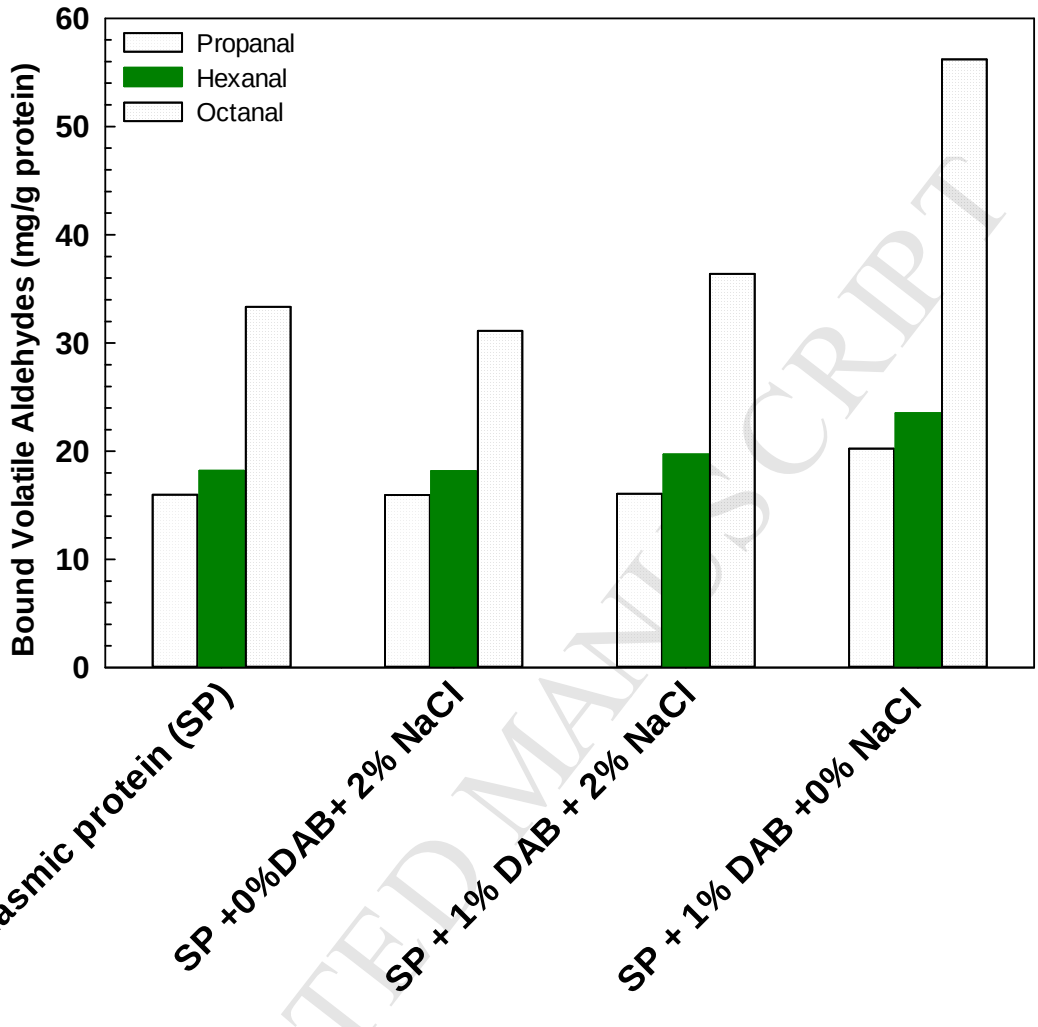


Fig. 2.

Highlights

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