



Novel, All-Natural Mustard Extract Influences Beef Patty Shelf Stability

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Abstract: Non-allergenic, plant-based water binders could improve the shelf life of beef patties, thereby reducing food waste. The objective of the current study was to optimize mustard extract addition for improvement of shelf stability and physical appearance of fresh beef patties. Non-allergenic water binder treatments included mustard extract (0.25%, 1.0%, and 2.0%), potato extract (2%; positive control), rosemary extract (2%; positive control), and no binder (negative control). Six batches of each treatment were made with shoulder clod and chuck roll. Two patties from each batch were analyzed for subjective color, objective color, fluid loss, oxidation, and pH. There was a difference between treatments for subjective color ($P = 0.001$) and objective color a^* ($P = 0.002$). The 0.25% mustard extract-treated patties displayed less discoloration than patties treated with 2% mustard extract. The negative control patties had the highest amount of fluid loss, and rosemary-treated patties had the least ($P = 0.014$). The greatest amount of oxidation was seen in negative control patties ($P = 0.001$). Patties treated with mustard extract at all levels performed similarly to the positive controls at reducing oxidation.

Key words: beef patties, mustard extract, oxidation, shelf life, water binders

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Introduction

The US beef industry loses \$3.73 billion annually due to discoloration of meat products (Ramanathan et al., 2022). The total amount of beef discoloration per year in the US corresponds to 194.70 million kg, which is equivalent to wasting 780,000 head of cattle and the natural resources used to produce and harvest those animals (Ramanathan et al., 2022). Discoloration is a driving factor in consumer acceptance. Consumers routinely select or reject meat products based on visual appearance and color (King et al., 2023). If the US decreases discoloration in beef by 1.0%, it would reduce the environmental impact by reducing natural resources wasted by 23.95 billion L of water, along with associated energy consumed (Ramanathan et al., 2022).

Moreover, ground beef is widely consumed by Americans, and it is estimated that 45% of all beef consumed in the US is ground beef (Ishmeal, 2020). Likewise, Speer et al. (2015) reported ground beef accounts for 49% of all beef sold at the retail level. It is important to note the differences between ground beef and beef patties as defined in Title 9 chapter 3 a319 of the Code of Federal Regulations. Ground beef is not permitted to include any extenders or added water, whereas beef patties may include binder, extenders, and added water.

Meat is composed of protein, lipids, vitamins, and water (Martins et al., 2018). These components make meat an excellent nutrient source but also make it susceptible to degradation. Oxidation has significant impacts on flavor, shelf life, and color

(Domínguez et al., 2019). The addition of antioxidants is a strategy used in the meat industry to reduce oxidation (Trindade et al., 2010) and to improve color stability (Zahid et al., 2018). Antioxidants function by inhibiting the process of oxidation (Masuda et al., 2010). There are various types of antioxidants that can be sorted into 2 categories: synthetic and natural. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are widely used in the industry (Horbańczuk et al., 2019). Several plant products serve as natural antioxidants (Descalze and Sancho, 2008; Shan et al., 2009; Rababah et al., 2011; Tayel and El-Tras, 2011; Kim et al., 2013). Fruits, vegetables, and herbs like olives, pomegranate, and rosemary are high in bioactive compounds, making them excellent natural antioxidants (Loussouarn et al., 2017; Horbańczuk et al., 2019).

Oils derived from *Sinapis alba* and *Brassica juncea* mustard have been identified as viable raw materials for biofuel and biodiesel production (Aslan, 2023). The residual mustard meal resulting from the biofuel production process is considered a low-value by-product (Usman et al., 2023). Mustard is rich in phenolic compounds known to inhibit lipid oxidation, thus demonstrating potential as a natural antioxidant (Cui, 1997). Incorporating mustard as an antioxidant in beef patties presents a sustainable solution utilizing a low-value by-product, offering a natural alternative to synthetic antioxidants. However, there is little to no published research regarding the effects of mustard extract on fresh beef patty shelf life. Therefore, the objective of the current study was to optimize mustard extract addition for the improvement of shelf stability and physical appearance of fresh beef patties.

Methods and Materials

Mustard extract

The mustard extract used in this study was derived from a pilot plant process outlined in detail by Popova et al. (2023). Initially, yellow mustard (*Sinapis alba*, IdaGold variety) seed meal was procured from Farm Fuel Inc. (Watsonville, CA). The mustard seed meal, produced through cold pressing to extract the oil, was free from any additives or preservatives and contained less than 15% oil on a dry weight basis. In the pilot plant setup, the mustard seed meal underwent extraction with heated water, followed by centrifugation and filtration through a 10- μ m filter. The resulting liquid extract was then transferred to trays and placed in

a freeze dryer for further processing. This unique process creates the novel, all-natural mustard extract used in this study.

Pattie preparation

Shoulder clods (Institutional Meat Purchasing Specifications (IMPS) 114), as well as chuck rolls (IMPS 116A), were obtained from USDA-inspected steers (<30 mo) harvested at the University of Idaho meat lab. The meat blocks were coarse ground (10 mm) and then fine ground (3 mm) (Thompson Meat Machinery Model 3000 meat mixer-grinder, Ixonia, WI, USA).

Six batches of each treatment were made with approximately 85:15 lean:fat ratio (Univex FA73 Fat Analyzer, Salem, NH, USA). Each batch consisted of 4.5 kg of ground beef along with 1% salt, 15% water, 0.2% onion granules, and the designated treatment. Batch size was determined by the size of the mixer and not based on the number of patties needed for analysis. Antioxidant treatments included mustard extract (0.25%, 1.0%, and 2.0%), potato extract (2.0%; X-TRATOS, Item 207085, Basic American Foods [BAF], Blackfoot, ID, USA; positive control), rosemary extract (2.0%; Fortium R-WS 20, Kemin Industries, Des Moines, IA, USA; positive control), and no binder (negative control). The percent of antioxidant treatments and other ingredients were percentages of the meat block. Due to time and equipment constraints, 3 batches of each treatment were made on 2 separate days. Each treatment batch was mixed for 2.5 min at 29 rpm (revolutions per minute) (Daniels Foods Equipment DMX 50 mixer, Parkers Prairie, MN, USA) and then formed (Patty-O-Matic 330A, Farmingdale, NJ, USA) into 16-mm-thick, circular (11.4-cm diameter) patties weighing 151 g each. Twelve beef patties per treatment were placed, one patty per tray, in 21 cm by 14.6 cm commercially available white Styrofoam trays (WebstaurantStore, Lancaster, PA, USA) overwrapped with oxygen-permeable polyvinyl chloride film (oxygen transmission rate: 1,450 cc/645 cm² per 24 h; water vapor transmission rate: 17.0 g/645 cm² per 24 h; Koch Industries, Inc. #7500-3815; Wichita, KS, USA). Soaker pads were not utilized. Patties were displayed in a glass-fronted retail display case (Model GDM-69, True Manufacturing Co., O'Fallon, MO, USA) equipped with natural white Hg 40W lights with an average light intensity of 409 lux (Sylvania Lighting, Erlangen, Germany) at 38°C for 4 d. The position of the patties in the display case was systematically rotated a row forward and to the left

each day to minimize location effects in the display case. Two patties per batch were randomly selected and assessed for each parameter of interest to optimize the analysis process. In total, 10 patties per treatment were analyzed for each of the 5 parameters.

Batch pH and retail fluid loss

Immediately after mixing and prior to patty formation, the pH of each batch was taken by inserting the probe of the portable pH meter (Apera PH8500-MT, Model SevenGo, Mettler Toledo, Woburn, MA, USA) into the grinds. The pH meter was calibrated with pH 4.0, 7.0, and 10.0 buffers.

Two patties from each batch were weighed on day 0 and day 4 of retail display to determine retail fluid loss. The fluid loss from both patties was averaged to get a retail fluid loss value for each batch. Percent retail fluid loss was calculated using the following equation:

$$\% \text{Fluid Loss} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100\%$$

Lipid oxidation analysis

Oxidation was evaluated using the rapid, wet method following the procedure in Colle et al. (2016). Briefly, 2 patties per batch were evaluated for oxidation on day 0 and day 4 of retail display. A section of approximately 0.5 g of each patty designated for oxidation analysis was cut on day 0 and day 4 and evaluated using the thiobarbituric acid reactive substances (TBARS) assay protocol provided in Appendix D, Protocol Q of the AMSA Meat Color Measurements Guidelines (King et al., 2023).

Retail color analysis

Two patties per batch were evaluated for objective color using the Nix Pro 2 Sensor (Nix Sensor Ltd., Hamilton, Ontario, Canada). The Nix Pro 2 Sensor is equipped with a 14-mm-diameter measuring area. Illuminant A₁₀ and Commission Internationale de l'Éclairage (CIE) *a** (redness) values were recorded daily throughout the entire retail display period. Objective color readings were taken through the packaging film to simulate consumers' points of view. Two readings were randomly taken in 2 different places from each patty and averaged to get an objective color score. Patty color was evaluated each day of retail display.

Two patties per batch were evaluated for subjective color by 3 trained University of Idaho research

assistants each day of retail display starting on day 0, no sooner than 2 h post-processing, similar to the methods described in Colle et al. (2016). Patties were scored following ground product display scoring from the Meat Color Measurement Guidelines (King et al., 2023). Each research assistant assigned a score to each patty scored on a 1 (very bright red to reddish pink) to 8 (tan to brown) scale for a total of 3 scores per patty per day. The 3 scores were then averaged to assign one value per patty per day.

Statistical analysis

The experiment was a factorial design with 2 factors, antioxidant treatment and day of retail display. Data were analyzed using a mixed model analysis, with batches as the experimental unit. There were 5 antioxidant treatments and 1 negative control. Each treatment was replicated 6 times using the same meat and ingredients, totaling 36 batches. Batches within a treatment were averaged to represent the treatment as a whole. Antioxidant treatment, retail display time, and their interactions were assumed as fixed effects. Day of retail display was considered a repeated measure. When comparing the 2 groups of patties made on different days, there was no significant difference ($P < 0.05$); therefore, they were combined and evaluated as one. The values of both patties per batch were averaged to obtain a single value per batch for each parameter on each day. Treatment least-squares mean differences were assessed through pair-wise comparisons for significant effects. Significance was determined at $P < 0.05$. All statistical analyses were done using the Statistical Analysis System V 9.4 (SAS Institute, Inc., Cary, NC, USA).

Results and Discussion

pH and retail fluid loss

The antioxidant treatments did not have an effect on the pH of the beef patties ($P = 0.277$; Table 1). The pH measurements were within normal range (5.64 to 5.77) (Page et al., 2001; Holdstock et al., 2014). Conversely, when the pH of meat reaches the isoelectric point ($pI = 5.4$), it has no net charge. This affects the ability of the meat to bind to water, resulting in greater fluid loss (Huff-Lonergan and Lonergan, 2005). The consistent pH values between the treatments suggest that any observed differences are not attributable to pH variations. If the retail display had

Table 1. Estimated mean effect of antioxidant treatments on fresh beef patties pH and retail fluid loss ($n = 36$)

Trait	Antioxidant Treatment ¹						SEM	P Value
	Control	Mustard 0.25%	Mustard 1.0%	Mustard 2.0%	Potato 2.0%	Rosemary 2.0%		
pH	5.70	5.64	5.73	5.77	5.74	5.75	0.04	0.277
Retail Fluid Loss ² , %	1.45 ^a	1.40 ^{ab}	1.36 ^{bc}	1.31 ^{bc}	1.28 ^c	1.29 ^c	0.36	0.014

^{a-c}Within a row, means without a common superscript differ ($P < 0.05$).

¹Treatments include no binder (negative control), mustard (0.25%, 1.0%, 2.0%), potato extract at 2.0% and rosemary extract at 2.0% of the meat block.

²Retail fluid loss = (day 0 weight – day 4 weight)/day 0 weight *100%.

SEM = standard error of the mean.

been observed over an extended period, it is plausible that a reduction in pH could have been noted, leading to a higher incidence of retail fluid loss (Huff-Lonergan and Lonergan, 2005).

In assessing the retail fluid loss, a main effect was observed with respect to the antioxidant treatments ($P = 0.014$; Table 1). Patties treated with rosemary or potato exhibited less fluid loss than the negative control and 0.25% mustard extract, consistent with the findings of Colle et al. (2019), where beef patties made with potato binders improved water-holding capacity. Conversely, patties treated with mustard extract at 1.0% and 2.0% displayed similar levels of fluid loss as the positive controls. Retail fluid loss of less than 2% is considered normal, while fluid loss greater than 4% is considered excessive (Johnson, 1974). Treatments had an average fluid loss of less than 2% and therefore would be acceptable. Notably, the negative control group exhibited the greatest retail fluid loss, followed by the patties treated with mustard extract at 0.25%. Being that the patties treated with 0.25% mustard extract had such a small percentage of the antioxidant treatment added to

them, the lower amount of inclusion added could contribute to the higher fluid loss similar to the negative control. By minimizing retail fluid loss, the patties are expected to be juicier and result in improved yields (Kim et al., 2016).

Lipid oxidation

An interaction between the day of retail display and antioxidant treatment was noted for oxidation ($P = 0.001$; Figure 1). All antioxidant treatments demonstrated a reduction in oxidation compared to the negative control on day 0 and day 4, aligning with previous studies (Lee et al., 2010; Trindade et al., 2010). Negative control patties' TBARS values increased from day 0 to day 4 while the antioxidant-treated patties did not have significant increases from day 0 to day 4. These results are in agreement with Lee et al. (2010), where fresh ground pork treated with mustard extract at 0.2% and 0.05% did not increase in TBARS values from day 0 to day 4 of retail display. Notably, TBARS values of less than 1 mg malondialdehyde/kg meat are

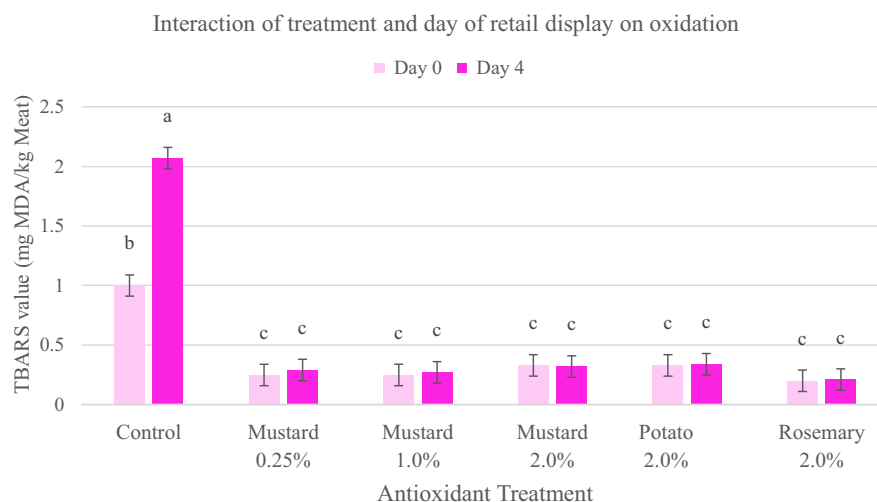


Figure 1. Oxidation measured by thiobarbituric acid reactive substances (TBARS) for antioxidant treatments by day of retail display for fresh beef patties. Each batch ($n = 36$) was assigned to a treatment which was included as a percentage of the meat block. Treatments included 0.25% mustard, 1.0% mustard, 2.0% mustard, 2.0% potato, 2.0% rosemary, and no binder. TBARS were measured on day 0 and day 4 of retail display. Values are shown as least-squares means \pm standard error (0.02). ^{a-c}Means without a common superscript differ ($P < 0.05$).

undetectable to consumers, while a TBARS value above 1 mg malondialdehyde/kg meat is indicative that off-flavors could be detected by consumers (Greene and Cumuze, 1982). Patties treated with antioxidants had an average TBARS value of less than 1 mg malondialdehyde/kg meat, while negative control patties had a value of 1 mg malondialdehyde/kg meat on day 0 and a value exceeding that on day 4. Mustard, like most oil seeds, is rich in phenolic compounds (Cui, 1997). These phenolic compounds include sinapic acid isomers, which have antioxidant properties that retard oxidation by combating the free radicals (Cui, 1997). Saleemi et al. (1993) found that 1.5% and 2.0% of low-pungency ground mustard seed (LPGMS) was as effective at reducing oxidation as 200 ppm of BHT or 30 ppm of Tertiary butylhydroquinone. Al-Rubeii et al. (2009) found rosemary at 1.0% reduced TBARS levels in ground beef. Furthermore, Colle et al. (2019) displayed reduced oxidation on day 4 of retail display in beef patties treated with potato extract. Moreover, when mustard extract at all levels was added to beef patties, there was a delay in oxidation compared to the negative control.

Retail color

An interaction between the day of retail display and objective color for a^* values was observed ($P = 0.001$; Table 2). Likewise, an interaction between the day of retail display and antioxidant treatment was observed for ground product display subjective color ($P = 0.001$; Table 2). When comparing a^* values within a day, patties treated with 2.0% mustard extract displayed the lowest a^* values on day 2 of the retail display

compared to other treatments. Additionally, patties treated with 2.0% mustard extract had similar a^* values to the negative control on days 0, 1, 3, and 4 of retail display. Caglar et al. (2018) found meatballs treated with 2.0% yellow mustard had lower a^* values than the negative control on days 0 to 7; however, they displayed a higher a^* than the negative control on days 8 to 15. In the current study, discoloration was only observed over a 4-d retail display, and perhaps similar results to Caglar et al. (2018) could be seen if a longer retail display period was observed. Patties treated with 2.0% mustard extract displayed the greatest amount of ground product display subjective discoloration on days 1 to 4. Conversely, patties treated with rosemary exhibited the best ground product display subjective performance and the greatest a^* value. Rosemary-treated patties had the most favorable ground product display subjective scores on days 2 to 4 of retail display and had the highest a^* values on day 4 of retail display. This is consistent with the findings of Yin et al. (2016). In their study, rosemary extract was added to raw ground pork. The authors state that patties treated with rosemary exhibited greater a^* values than the negative control on days 4 and 7 of retail display. Of the mustard extract-treated patties, patties treated with mustard extract at 0.25% presented the highest a^* values on days 2 and 3 of retail display. Patties treated with mustard extract at 2.0% performed the worst for both a^* and ground product display subjective scoring. Patties treated with lower concentrations of mustard extract displayed less discoloration.

While there was no treatment-by-day interaction for b^* values ($P = 0.714$) or L^* values ($P = 0.255$), treatment differences were observed for b^* ($P = 0.001$;

Table 2. Effect of antioxidant treatment and day of retail display on retail color of fresh beef patties ($n = 36$)

Trait	Day of Retail Display	Antioxidant Treatments						SEM	P Value
		Control	Mustard 0.25%	Mustard 1.0%	Mustard 2.0%	Potato 2.0%	Rosemary 2.0%		
a^*	0	20.56 ^{b,w}	20.42 ^{b,w}	19.44 ^{b,w}	19.36 ^{b,w}	20.81 ^{b,w}	22.81 ^{a,v}	0.75	0.002
	1	16.58 ^{c,x}	19.55 ^{ab,w}	18.67 ^{b,w}	16.68 ^{c,x}	19.06 ^{ab,wx}	20.68 ^{a,w}		
	2	15.08 ^{cd,x}	16.61 ^{bc,x}	14.36 ^{d,x}	11.74 ^{e,y}	17.73 ^{ab,x}	18.65 ^{a,x}		
	3	12.10 ^{c,y}	14.61 ^{b,y}	11.92 ^{c,y}	10.48 ^{c,y}	15.73 ^{ab,y}	16.69 ^{a,y}		
	4	9.46 ^{bc,z}	9.93 ^{b,z}	9.33 ^{bc,z}	8.01 ^{c,z}	10.62 ^{ab,z}	12.00 ^{a,z}		
Sub. Color ¹	0	2.7 ^v	2.5 ^v	2.4 ^v	2.6 ^v	2.5 ^w	2.3 ^v	0.2	0.001
	1	3.4 ^{b,w}	3.2 ^{bc,w}	3.3 ^{bc,w}	4.0 ^{a,w}	3.3 ^{bc,x}	2.8 ^{c,w}		
	2	4.2 ^{b,x}	3.9 ^{cd,x}	4.7 ^{b,x}	5.9 ^{a,x}	4.2 ^{c,y}	3.4 ^{d,x}		
	3	5.4 ^{bc,y}	4.9 ^{cd,y}	5.8 ^{b,y}	6.6 ^{a,y}	4.6 ^{de,y}	4.4 ^{c,y}		
	4	6.6 ^{bc,z}	6.6 ^{bc,z}	6.9 ^{ab,z}	7.2 ^{a,z}	6.2 ^{cd,z}	6.1 ^{d,z}		

^{a-c}Within a trait and day, means without a common superscript differ ($P < 0.05$).

^{v-z}Within a trait and treatment, means without common superscripts differ ($P < 0.05$).

¹Sub. color = ground product display subjective colors scale from 1–8, 1 = very bright red, 8 = tan to brown.

SEM = standard error of the mean.

Table 3. Effect of antioxidant treatment on L^* and b^* of beef patties ($n = 36$)

Trait	Antioxidant Treatment ¹						SEM	P Value
	Control	Mustard 0.25%	Mustard 1.0%	Mustard 2.0%	Potato 2.0%	Rosemary 2.0%		
L^*	41.79 ^b	40.98 ^b	41.35 ^b	41.64 ^b	41.48 ^b	45.41 ^a	0.43	0.001
b^*	13.44 ^b	13.98 ^b	13.7 ^b	13.69 ^b	13.56 ^b	15.33 ^a	0.36	0.001

^{a,b}Within a row, means without a common superscript differ ($P < 0.05$).

¹Treatments include no binder (negative control), mustard extract (0.25%, 1.0%, 2.0%), potato extract at 2.0% and rosemary extract at 2.0% of the meat block.

SEM = standard error of the mean.

Table 3) and L^* ($P = 0.001$; Table 3). Rosemary displayed the highest b^* and L^* values, resulting in the lightest and most yellow color. This is contrary to Yoder et al. (2021), where rosemary-treated beef displayed lower L^* values compared to the negative control patties over a 7-d retail display. Rosemary and potato treated patties improved both ground product display subjective color and objective a^* values. Among the mustard extract-treated patties, those treated with 2.0% mustard extract performed the worst for retail color, while those treated with 0.25% mustard extract performed the best. Considering that the lower concentration of mustard extract produced a more acceptable retail display color, it could be postulated that the yellow color of the mustard extract is negatively affecting the color of the patties.

Conclusion

The novel, all-natural mustard extract is effective at reducing retail fluid loss, prolonging low oxidation levels, and improving the color of fresh beef patties during retail display. The addition of mustard extract could not only act as an antioxidant but also provide a potential water-binding effect, as it effectively reduced the percentage of fluid loss during retail display at all concentrations. All-natural mustard extract in fresh beef patties is an effective antioxidant displayed in this study by its ability to reduce fluid loss, delay oxidation, and improve retail color. However, mustard extract at higher concentrations had a negative effect on retail color. Future research would be needed to investigate the effects of mustard extract when combined with other antioxidants and the possibility of synergistic effects. Reducing oxidation and improving the color of fresh beef patties has the potential to prolong shelf life and reduce food waste in the beef industry.

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