BME 174: Module 3 Lab Report
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Abstract
Meat oxidizes over time, changing its appearance, texture and flavor. An important aspect of cultivated meat is long-term storage and freshness. Antioxidants combat oxidation of lipids and other cellular material. Here, we treat bovine satellite muscle cells with different concentrations of two types of antioxidant treatments. Higher cell viability and lower oxidation is demonstrated under food-grade antioxidant treatment.

Introduction

Oxidation of meat is a challenge to its long-term storage. Oxidative stress leads to the creation of free radical oxygen and reactive nitrogen species, which cause discoloration, change in flavor and formation of toxic compounds. Lipid and protein oxidation are the mechanistic reasons for colorectal cancer's link to red meat. Overall, it is desired for cultivated meat to be resistant to oxidative stress.

Antioxidants scavenge free radical species and slow down oxidation. Endogenous production of carotenoids phytoene, lycopene and β-carotene in mammalian cells through metabolic engineering has been demonstrated [1]. This was done to combat lipid oxidation's role in decreasing shelf-life, and impairing color stability and flavor.

Here, we added an exogenous antioxidant to growth medium to study its effect on viability and lipid oxidation of bovine satellite muscle cells. We compare food-grade and lab-grade antioxidant treatments at different concentrations and select one of each kind based on highest cell viability. We add optimal lab-grade and food-grade antioxidant concentrations to growth medium with cells. Lipid oxidation levels of cells were tested with a thiobarbituric acid reactive substances (TBARS) assay using malondialdehyde (MDA), a byproduct of lipid oxidation.

Methods

Cell thawing, seeding and viability testing
Bovine satellite muscle cells were thawed from cryopreservation at -80 ^{0}C and incubated (37 ^{0}C, 5% CO_2). 10mL of food-grade antioxidant treatment stock solution was prepared by mixing commercially available turmeric powder and water at a concentration of 10,100 ng/mL. 10mL of lab-grade antioxidant treatment stock solution was prepared by mixing commercially available curcumin (carotenoid) powder and dimethyl sulfoxide (DMSO) at a concentration of 10,000 ng/mL. 2 control growth medium formulations and 10 growth medium formulation with differing concentrations of food-grade and lab-grade antioxidant treatments were made as described in Table 1. DF 10, DF100 and DF1000 growth medium formulations were prepared by serial dilution. Standard growth medium (Gibco DMEM 1X + GlutaMAX) for bovine satellite cells used. Cells were seeded at 910 cells/cm^2. Each condition was triplicated and 200uL of respective media formulation was exchanged every 3 days for 1 week. The well-plate was incubated (37 ^{0}C, 5% CO_2). After 1 week, cell viability was measured for each of the 12 media formulations by adding 20uL of Prestoblue dye to each well and performing fluorescence plate reading assay at 560/590nm. 1X growth medium with 10% Prestoblue dye was used as control for cell viability assay.

Name	Formulation	Antioxidant concentration	Grade
Control GM	1X standard growth medium	None	Control
Control DMSO	1X growth medium and 0.1% DMSO	None	Control
DF1 L	1X growth medium, 0.1% DMSO and 0.1% lab-grade stock solution	10 ng/mL curcumin	Lab
DF10 L	1X growth medium, 0.1% DMSO and 10% DF 1 L solution	1 ng /mL curcumin	Lab
DF100 L	1X growth medium, 0.1% DMSO and 10% DF 10 L solution	0.1 ng/mL curcumin	Lab
DF1000 L	1X growth medium, 0.1% DMSO and 10% DF 100 L solution	0.01 ng/mL curcumin	Lab
DF10000 L	1X growth medium, 0.1% DMSO and 10% DF 1000 L solution	0.001 ng/mL curcumin	Lab
DF1 F	1X growth medium and 5% food-grade stock solution	500 ng/mL turmeric	Food
DF10 F	1X growth medium and 10% DF 1 F solution	50 ng/mL turmeric	Food
DF100 F	1X growth medium and 10% DF 10 F solution	5 ng/mL turmeric	Food
DF1000 F	1X growth medium and 10% DF 100 F solution	0.5 ng/mL turmeric	Food
DF10000 F	1X growth medium and 10% DF 1000 F solution	0.05 ng/mL turmeric	Food

Table 1: Growth medium formulations varying in antioxidant concentration and type

Cell seeding for oxidation test

Three T-175 flasks were seeded at 17,000 cells/cm^2 each, with 25 mL of standard growth medium in the first, 25mL of DF 1 F food-grade medium in the second and 25mL DF 10000 L lab-grade medium in the third. Flasks were incubated (37 ^{0}C, 5% CO_2) for 1 week with media exchanged every 3 days. Cells were counted using a hemocytometer after scraping. Cells in all flasks were normalized to have the same count by removing 214uL of cell suspension solution from lab-grade and 610uL from

food-grade. Cell suspensions were then centrifuged (300g, 5 minutes) and resuspended in 2mL PBS each. 900uL of each cell suspension solution was stored in 6 Eppendorf tubes, 2 tubes for each condition (control, food-grade, lab-grade). 1 tube of each condition was stored for 1 week at room temperature (22 ^{0}C) and another of each was refrigerated for 1 week (4 °C) to introduce multiple oxidative challenges.

Lipid oxidation test with TBARS assay

Cell pellet tubes were placed on a heat block (100 °C, 10 minutes) to simulate cooking. Cell pellets were mixed with 200uL water. Cells were lysed using the freeze-thaw method by placing tubes under refrigeration (-80 °C, 5 minutes) and thawing, repeating the freeze-thaw process for 3 cycles. 5 standard solutions were prepared as described in Table 2 to plot MDA standard curve. To conduct the TBARS assay, 100uL of 10% trichloroacetic acid reagent and 800uL of color reagent were added 100uL of standard solutions or 100uL of sample solutions from 6 Eppendorf tubes. The 11 vials were placed on the heat block (100 °C, 60 minutes). Vials were placed in the refrigerator (4 °C, 10 minutes). Vials were microcentrifuged (4 °C, 1,600g, 10 minutes). 200uL from each vial was triplicated on a 96-well plate. Absorbance was measured using a plate reader at 535nm. MDA standard equation was generated using Numpy, a Python library. MDA concentrations in each of the 6 conditions were extrapolated using the equation obtained for the MDA standard curve from absorbance data obtained for each vial. We use MDA absorbance measurements as a proxy for lipid oxidation in cells.

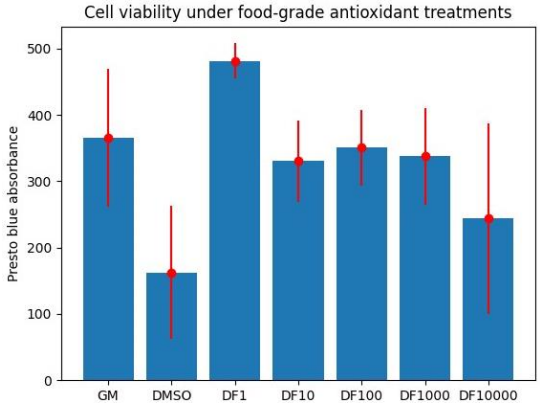
Tube	MDA (uL)	Water (uL)	MDA concentration (uM)
A	0	500	0
B	20	480	5
C	40	460	10
D	100	400	25
E	200	300	50

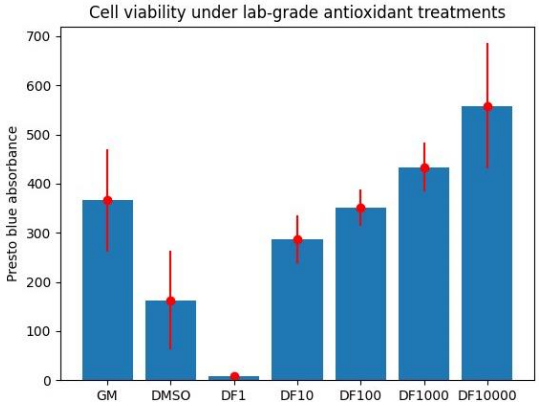
Table 2: MDA standard solutions

Results

500 ng/mL turmeric and 0.001 ng/mL curcumin treatments showed highest cell viability

As seen in Figure 1, cell viability is highest under DF1 for food-grade antioxidant treatments and DF10000 under lab-grade antioxidant treatments. 500 ng/mL turmeric in growth medium shows highest cell viability amongst all food-grade antioxidant treatments. 0.001 ng/mL curcumin in growth medium shows highest cell viability amongst all lab-grade antioxidant treatments. While viability decreases as concentration of turmeric goes down in growth medium, it increases with decrease in curcumin concentration. Surprisingly, high levels of curcumin in growth medium significantly impacted growth as seen in DF1 under lab-grade antioxidant treatments. Only DF1000 and DF10000 lab-grade treatments, and DF1 food-grade treatment show higher viability compared to growth medium control.





Figures 1-2: Cell viability measurements under food-grade and lab-grade antioxidant treatments. Prestoblue absorbance is a measure of cell viability. DF: dilution factor, GM: growth medium control, DMSO: dimethyl sulfoxide control.

Food-grade antioxidant treatment shows lower lipid oxidation levels

Compared to control, food-grade antioxidant treatment containing 500 ng/mL turmeric in growth medium shows much lower oxidation level under refrigeration and slightly lower oxidation level at room temperature. Lab-grade antioxidant treatment shows lower oxidation levels at room temperature. Surprisingly, lab-grade antioxidant treatment shows much higher oxidation under refrigeration.

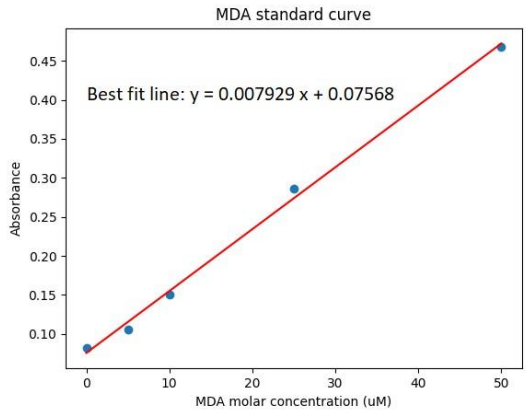
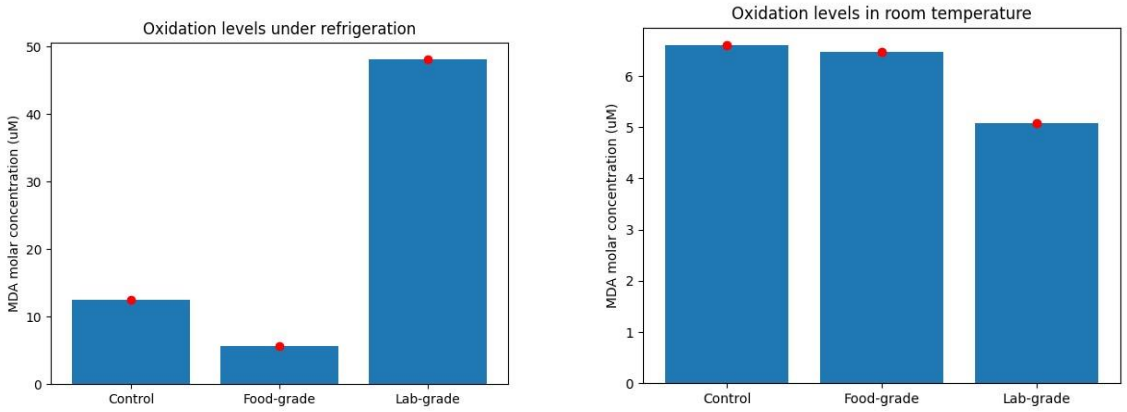


Figure 3: MDA standard curve obtained by plotting line of best fit to absorbance measurements of prepared MDA standards. MDA: malondialdehyde.



Figures 4-5: Oxidation level comparison between food-grade and lab-grade antioxidant treatments under room temperature and refrigeration. MDA molar concentrations are obtained from MDA standard curve using absorbance assay measurements to line of best fit in Figure 3. MDA concentrations to corresponding absorbance assay measurements from cells are noted.

Discussion

Increasing turmeric in growth medium leads to higher cell viability. Turmeric is known for its antioxidative properties. High concentrations of turmeric in growth medium showed high cell viability, with the highest concentration demonstrating higher cell viability than standard growth medium. This result highlights turmeric's potential in improving cell viability. In literature, turmeric has shown antioxidant properties by inhibiting lipid peroxidation [3]. Further research can be done to characterize turmeric's effects on the growth of bovine cells.

Curcumin, a component of turmeric, in the lab-grade antioxidant treatment is essentially purified turmeric. High concentrations of curcumin in our growth medium showed reduced cell viability. At low temperatures, curcumin has reduced bioavailability [7]. This seems to be a plausible reason for the much higher oxidation levels under lab-grade antioxidant treatment and refrigeration. Our lab-grade antioxidant treatment contains the lowest concentration of curcumin. The low concentration of curcumin coupled with reduced bioavailability of curcumin at low temperature may have resulted in increased oxidation. Further study should be done to confirm curcumin's lower bioavailability under refrigeration. Meat is generally refrigerated. Any detrimental effects of an antioxidant treatment to cultivated meat's shelf-life can significantly impact adoption and commercialization.

In conclusion, higher cell viability and lower lipid oxidation is shown in bovine satellite muscle cells under food-grade antioxidant treatment with commercially available turmeric. Lab-grade antioxidant treatment with curcumin demonstrates higher viability of cells under certain conditions, lower oxidation at room temperature but much higher oxidation under refrigeration.

References

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