Effects of Caffeine on Yeast Survivorship

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First Year in PJAS
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Introduction

- Caffeine is regularly consumed by humans
- Monster Energy Drink is caffeinated
- Cellular Model - Yeast
- Variable - Monster Energy Drink
- Yeast will be used as a model for human cells
Caffeine

- Molecular Formula: $C_8H_{10}N_4O_2$
- Average mass: 194.191 Da
- Monoisotopic mass: 194.080383 Da
- Systematic name: 1,3,7-Trimethyl-3,7-dihydro-1H-purine-2,6-dione
Human Consumption of Caffeine

- In North America 90% of adults consume caffeine daily
- Most consumed psychoactive drug
- Coffea arabica coffee plant
- Effects of caffeine consumption on humans
Human Consumption of Caffeine (continued)

• Used to treat bronchopulmonary dysplasia of prematurity
• Known to help fight Parkinson’s disease, types of cancer, etc.
• Negative effects of caffeine
• Caffeine dependence
Monster Energy

• Ingredients: carbonated water, sucrose, glucose, citric acid, natural flavors, taurine, sodium citrate, color added, panax ginseng root extract, L-carnitine, caffeine, sorbic acid, benzoic acid, niacinamide, sodium chloride, glucuronolactone, inositol, guarana seed extract, pyridoxine hydrochloride, sucralose, riboflavin, naltodextrin, and cyanocobalamin.
Yeast/Experimental Cell Model

- Eukaryotic, unicellular fungi
- Used in genetics and cell biology
- Saccharomyces cerevisiae - common yeast
- Similar genetic makeup to human cells
- Most studied cell in the world
Purpose

• Does caffeine affect the survivorship of yeast?
• Optimal amount of caffeine that causes the most significant change?
• Is caffeine safe for human consumption?
• How much caffeine is too much?
Hypotheses

• Null Hypothesis: The Monster Energy will have no significant effect on yeast survivorship.
• Alternate Hypothesis: The Monster will increase yeast survivorship.
• Alternate Hypothesis: The Monster will decrease the yeast survivorship.
### Materials

- YEPD media and agar plates (1% yeast extract, 2% glucose, 2% peptone)
- Sterile Dilution Fluid (100mM KH$_2$PO$_4$, 100mM K$_2$HPO$_4$, 10mM MgSO$_4$, 1mM NaCl)
- Sterile micropipettes and tips (1000 mL and 200 mL)
- Sharpie
- Spreader bar
- Stop watch
- Incubator
- Klett spectrophotometer
- Monster Energy Drink
- Saccharomyces cerevisiae
- Pencil
- Paper
- Vortex
Procedures

1. Saccharomyces cerevisiae was grown overnight in sterile YEPD media.

2. The culture was placed in an incubator (30° C) until a density of 50 Klett spectrophotometer units were reached. This represents a density of approximately $10^7$ cells/mL.

3. The culture was diluted in sterile dilution fluid to a concentration of $10^5$ cells/mL.

4. 8.99 mL of was poured into the test tube

5. .01 mL of Monster Energy was pipetted into the test tube

6. 1 mL of yeast was poured into the test tube
7. The test tube was vortexed for 3 seconds
8. After exactly 5 minutes 1mL of solutions was poured from the test tube into 10 YEPD plates
9. The solution was spread with a sterilized spreader
10. The spreader was resterilized
11. Steps 1-7. were repeated 4 times
12. Steps 1-7. were repeated 5 times each with the 4 other according varying concentrations
13. Recorded the number of yeast colonies in each plate after days 2 of incubation
<table>
<thead>
<tr>
<th>% of Monster</th>
<th>0%</th>
<th>0.10%</th>
<th>1%</th>
<th>2.50%</th>
<th>5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile Fluid</td>
<td>9.9 ml</td>
<td>9.89 ml</td>
<td>9.8 ml</td>
<td>9.65 ml</td>
<td>9.4 ml</td>
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<tr>
<td>Yeast</td>
<td>0.1 ml</td>
<td>0.1 ml</td>
<td>0.1 ml</td>
<td>0.1 ml</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Monster</td>
<td>0 ml</td>
<td>0.01 ml</td>
<td>0.1 ml</td>
<td>0.25 ml</td>
<td>0.5 ml</td>
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</table>
Effects of Monster Energy on Yeast Survivalship

P-value: 0.024816
<table>
<thead>
<tr>
<th>Amount of Monster</th>
<th>T Value</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>.1%</td>
<td>0.021</td>
<td>Accept Null</td>
</tr>
<tr>
<td>1%</td>
<td>1.176</td>
<td>Accept Null</td>
</tr>
<tr>
<td>2.5%</td>
<td>3.38</td>
<td>Reject Null</td>
</tr>
<tr>
<td>5%</td>
<td>0.882</td>
<td>Accept Null</td>
</tr>
</tbody>
</table>

T Crit = 3.056
Null hypothesis was rejected for 2.5% Monster. Null hypothesis was accepted for .1%, 1%, and 5% Monster Energy.

It appears Monster Energy has a significant positive effect on yeast survivorship at around 2.5%, but increasing or decreasing that amount causes less and less significant change.
### Limitations and Extensions

<table>
<thead>
<tr>
<th>Limitations</th>
<th>Extensions</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Number of concentrations only survivorship analyzed (growth)</td>
<td>- Effects of pure caffeine could be tested</td>
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<tr>
<td>- More replicants could help</td>
<td>- Yeast could act as a model for micro flora rather than a model for human cells</td>
</tr>
</tbody>
</table>
Sources

http://www.chemspider.com/Chemical-Structure.2424.html
http://www.caffeineinformer.com/energy-drink-ingredients


Anova: Single Factor

<table>
<thead>
<tr>
<th>Groups Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
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<tbody>
<tr>
<td>0</td>
<td>4</td>
<td>704</td>
<td>176</td>
</tr>
<tr>
<td>0.001</td>
<td>4</td>
<td>705</td>
<td>176.25</td>
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<tr>
<td>0.01</td>
<td>4</td>
<td>760</td>
<td>190</td>
</tr>
<tr>
<td>0.025</td>
<td>4</td>
<td>865</td>
<td>216.25</td>
</tr>
<tr>
<td>0.05</td>
<td>4</td>
<td>746</td>
<td>186.5</td>
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ANOVA

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
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<tbody>
<tr>
<td>Between Groups</td>
<td>4325.5</td>
<td>4</td>
<td>1081.375</td>
<td>3.812581</td>
<td>0.024816</td>
<td>3.055568</td>
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<tr>
<td>Within Groups</td>
<td>4254.5</td>
<td>15</td>
<td>283.6333</td>
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<tr>
<td>Total</td>
<td>8580</td>
<td>19</td>
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