Ammonium Hydroxide and Orange Juice Synergistic Effects on *Escherichia coli*

John Lynch
Pittsburgh Central Catholic High School
Grade 9
pH

**pH Scale**
- Measures how acidic or basic a substance is
- Scale from 0-14
- pH<7 acidic, pH>7 basic, pH=7 neutral

**Neutralization**
- The mixture of an acid and a base.
- Mixture of acid and base commonly yields water
Navel Orange Juice

• Very commonly sold orange

• Measured pH of 3 (raw)

• Contains large amounts of Vitamin A and C
  • May help animals form connective tissue
  • Also contains many other Vitamins
Components of Orange Juice

- Sugars
- Proteins and amino acids
- Vitamins A, C, D, E, K, B12, B6
- Thiamin
- Pantotheic Acid
- Sodium
- Potassium

- Water
- Mono/Polysaturated fats
- Omega-3 fatty acids
- Calcium
- Iron
- Magnesium
- Phosphorus
Ammonium Hydroxide

- Basic liquid
  - Measured pH of 10 (2 Molar)

- Very corrosive and antibacterial
  - Used at very small molarities in cleaners

- Releases Ammonia vapors when mixed with an acid or water.
  - Toxic to humans at high concentrations
Escherichia coli (E. coli)

- Large and diverse group of gram (-) bacteria
- Free living, symbionts, or pathogens
- Most strains are not pathogenic
- Serve as a common prokaryotic cell model
- Common mammalian intestinal symbiont
- Environmental prokaryotic model
Rationale

• Individual Orange Juice:
  • Orange falling from tree
  • Disposal of peels

• Individual Ammonium Hydroxide
  • Disposal of Ammonium-based cleaners

• Mixture of both:
  • Possible synergistic effects
  • Possible remedy for Ammonium-based cleaner spills

Purpose

• To determine the individual and synergistic effects of Navel orange juice and Ammonium Hydroxide on *E. Coli* survivorship.
Problem

• Will there be a significant effect when Ammonium Hydroxide, Navel orange juice, or a mixture of both is added to *Escherichia coli*?
Hypothesis

**Alternative Hypothesis:**

Ammonium Hydroxide and Orange juice will significantly reduce *E. coli* survivorship.

In combination, a synergistic effect will improve survivorship compared to individual exposures.
Materials

- Borosilicate sterile test tubes
- Latex Gloves
- Laboratory Goggles
- Micropipettes
- Sidearm Flask
- Sterile Filters
- Incubator (37° C)
- Vortex Machine
- E. coli (DH5 Alpha)
- LB Agar Plates (LB Media) (1% Tryptone, 0.5% Yeast extract, 1% NaCl)
- Spread bars

- Ethanol
- pH strips
- Matches
- Sterile Dilution Fluid (SDF) (100mM KH2PO4, 100mM K2HPO4, 10mM MgSO4, 1mM NaCl)
- Navel Orange Juice
- Juicer
- Knife
- 2 Molar Ammonium Hydroxide
- Sterile Dilution Fluid
- Bunsen Burner
Procedure

1. *E. coli* was grown until a density of 50 klett spectrophotometer density was reached. This was approximately $10^8$ cells/mL

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

3. Stocks of the variables were created;
   1. 10mL of orange juice was sterile filtered
   2. A $10^{-2}$ stock of Ammonium Hydroxide was made

4. The variables were added to the tubes as follows:
### Experimental Exposures

<table>
<thead>
<tr>
<th></th>
<th>Zero</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Orange Juice</strong></td>
<td>0 mL</td>
<td>0 mL</td>
<td>0 mL</td>
</tr>
<tr>
<td></td>
<td>0 mL</td>
<td>10 uL</td>
<td>1 mL</td>
</tr>
<tr>
<td></td>
<td>100 uL</td>
<td>100 uL</td>
<td>100 uL</td>
</tr>
<tr>
<td></td>
<td>9.9 mL</td>
<td>9.89 mL</td>
<td>8.8 mL</td>
</tr>
<tr>
<td><strong>Ammonium Hydroxide (10-2 stock)</strong></td>
<td>0 mL</td>
<td>0 mL</td>
<td>0 mL</td>
</tr>
<tr>
<td></td>
<td>100 uL</td>
<td>100 uL</td>
<td>100 uL</td>
</tr>
<tr>
<td></td>
<td>9.9 mL</td>
<td>9.89 mL</td>
<td>8.8 mL</td>
</tr>
</tbody>
</table>

**Key:**
- Orange Juice
- Ammonium Hydroxide
- *Escherichia coli*
- Sterile Dilution Fluid

**Concentration in %:**
- Low
- High

<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ammonium Hydroxide</strong></td>
<td>0.00005%</td>
<td>0.005%</td>
</tr>
<tr>
<td><strong>Orange Juice</strong></td>
<td>1%</td>
<td>10%</td>
</tr>
<tr>
<td><strong>Sterile Dilution Fluid</strong></td>
<td>0.005%</td>
<td>0.00005%</td>
</tr>
</tbody>
</table>
5. 0.1mL of *E. coli* stock was added to each tube, and allowed to incubate at room temperature for 15 minutes

6. 0.1mL of the aliquots were spread onto the agar plates

7. The plates were then incubated for 24 hours and the colonies were counted
Individual Variable’s Effects

Number of Colonies

Concentration Levels

- [Zero]
- [Low]
- [High]

Orange Juice
- P-value: 0.024806

Ammonium Hydroxide
- P-value: 0.008053

Alpha: 0.05

Effects of Orange Juice and Ammonium Hydroxide on the Number of Colonies
Synergistic Effects on E. Coli

Averages of All Solutions

Number of Colonies

Zero Orange Juice | Low Orange Juice | High Orange Juice

Zero Ammonium Hydroxide | Low Ammonium Hydroxide | High Ammonium Hydroxide
Data Analysis: Two-factor ANOVA

- Two-Way ANOVA for both Variables:
  - Interaction P-value: Yes, the p value was at 0.000262

- Interpretation: Reject null; there was a significant effect

- Type of effect: The mixed variables appeared to reduce the negative effects
Further Interpretation

**AVERAGES OF COLONIES**

<table>
<thead>
<tr>
<th>Orange Juice</th>
<th>Ammonium Hydroxide</th>
<th>Zero</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero</td>
<td>179</td>
<td>138</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>153</td>
<td>151</td>
<td>151</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>136</td>
<td>145</td>
<td>179</td>
<td></td>
</tr>
</tbody>
</table>
### pH Chart and Interpretation

<table>
<thead>
<tr>
<th>pH Levels</th>
<th>Ammonium Hydroxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero</td>
<td>Low</td>
</tr>
</tbody>
</table>

**Orange**
- Zero: 7
- Low: 7
- High: 7

**Juice**
- Low: 7
- High: 6

• pH solely doesn’t seem to be the affecting factor
Key Questions

• Did the Navel Orange Juice alone have a significant effect on *E. coli*?
  • Yes, p-value was less than 0.05

• Did the Ammonium Hydroxide alone have a significant effect on *E. coli*?
  • Yes, p-value was less than 0.05

• Did the mixture of both have a significant effect on the *E. coli*?
  • Yes, p-value was less than 0.05
Overall Conclusions

• There were negative individual effects of the variables, but a positive synergistic effect.
Limitations and Extensions

**Limitations:**
- Only one exposure time
- Chance of contamination
- Uneven distribution of E. coli in solution
- Small exposure to light
- Narrow range of variable concentrations

**Extensions:**
- Vary exposures and concentrations
- More replicates
- Other cultures of bacteria
- Different types of orange juice
- Other synergistic effects
Sources

- us.sensodyne.com
  http://dl.clackamas.edu/ch105-04/molarity.htm
  http://www.aqion.de/site/191
  http://www.nutrition-and-you.com/orange-fruit.html
  https://ods.od.nih.gov/factsheets/VitaminA-Consumer/
  http://people.ku.edu/~igmdoc/ecoli.html
  http://www.foodinsight.org/Questions_and_Answers_about_Ammonium_Hydroxide_Use_in_Food_Production
# Data Chart

<table>
<thead>
<tr>
<th></th>
<th>Zero</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Zero</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ammonium Hydroxide</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>193</td>
<td>179</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>127</td>
<td>107</td>
<td>118</td>
</tr>
<tr>
<td></td>
<td>195</td>
<td>130</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>174</td>
<td>217</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td>148</td>
<td>134</td>
<td>116</td>
</tr>
<tr>
<td><strong>Orange Juice</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Zero</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Orange Juice</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Low</strong></td>
<td>140</td>
<td>145</td>
<td>237</td>
</tr>
<tr>
<td></td>
<td>240</td>
<td>163</td>
<td>178</td>
</tr>
<tr>
<td></td>
<td>139</td>
<td>158</td>
<td>132</td>
</tr>
<tr>
<td></td>
<td>169</td>
<td>136</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td>165</td>
<td>105</td>
<td>149</td>
</tr>
<tr>
<td><strong>High</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>High</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>119</td>
<td>55</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>157</td>
<td>171</td>
</tr>
<tr>
<td></td>
<td>147</td>
<td>167</td>
<td>196</td>
</tr>
<tr>
<td></td>
<td>165</td>
<td>151</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td>147</td>
<td>105</td>
<td>128</td>
</tr>
</tbody>
</table>
One-Way ANOVA: Ammonium Hydroxide

SUMMARY

<table>
<thead>
<tr>
<th>Group</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>4</td>
<td>710</td>
<td>177.5</td>
<td>476.333</td>
</tr>
<tr>
<td>C2</td>
<td>4</td>
<td>550</td>
<td>137.5</td>
<td>907</td>
</tr>
<tr>
<td>C3</td>
<td>4</td>
<td>460</td>
<td>115</td>
<td>8.66667</td>
</tr>
</tbody>
</table>

ANOVA

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>Ftest</th>
<th>P-value</th>
<th>F-Critical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>8016.67</td>
<td>2</td>
<td>4008.33</td>
<td>8.63865</td>
<td>0.008053</td>
<td>4.2565</td>
</tr>
<tr>
<td>Within</td>
<td>4176</td>
<td>9</td>
<td>464</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>12192.7</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conclusions

Ho: \( \mu_1 = \mu_2 = \mu_3 = \ldots \)
Ha: At least 2 \( \mu \)'s differ

F-Test     8.63865
F-Critical 4.2565
Alpha       0.05
P-Value     0.008053
Decision:   Reject Ho
One-Way ANOVA: Orange Juice

**SUMMARY**

<table>
<thead>
<tr>
<th>Group</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6</td>
<td>4</td>
<td>710</td>
<td>177.5</td>
<td>476.333</td>
</tr>
<tr>
<td>C7</td>
<td>4</td>
<td>613</td>
<td>153.25</td>
<td>254.917</td>
</tr>
<tr>
<td>C8</td>
<td>4</td>
<td>543</td>
<td>135.75</td>
<td>188.917</td>
</tr>
</tbody>
</table>

**ANOVA**

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>Ftest</th>
<th>P-value</th>
<th>F-Critical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>3516.5</td>
<td>2</td>
<td>1758.25</td>
<td>5.73238</td>
<td>0.024806</td>
<td>4.2565</td>
</tr>
<tr>
<td>Within</td>
<td>2760.5</td>
<td>9</td>
<td>306.722</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6277</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions**

Ho: \( \mu_1 = \mu_2 = \mu_3 = \ldots \)  
Ha: At least 2 \( \mu \)'s differ  
F-Test: 5.73238  
F-Critical: 4.2565  
Alpha: 0.05  
P-Value: 0.024806  
Decision: Reject Ho
# Two Factor ANOVA With Replication

<table>
<thead>
<tr>
<th>Summary</th>
<th>O</th>
<th>L</th>
<th>H</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Sum</td>
<td>710</td>
<td>550</td>
<td>460</td>
<td>1720</td>
</tr>
<tr>
<td>Average</td>
<td>177.5</td>
<td>137.5</td>
<td>115</td>
<td>143.333</td>
</tr>
<tr>
<td>Variance</td>
<td>476.333</td>
<td>907</td>
<td>8.66667</td>
<td>1108.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Sum</td>
<td>613</td>
<td>602</td>
<td>603</td>
<td>1818</td>
</tr>
<tr>
<td>Average</td>
<td>153.25</td>
<td>150.5</td>
<td>150.75</td>
<td>151.5</td>
</tr>
<tr>
<td>Variance</td>
<td>254.917</td>
<td>151</td>
<td>380.917</td>
<td>216.273</td>
</tr>
<tr>
<td>Source</td>
<td>SS</td>
<td>df</td>
<td>MS</td>
<td>Ftest</td>
</tr>
<tr>
<td>------------------------</td>
<td>--------</td>
<td>----</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Samples (rows)</td>
<td>657.056</td>
<td>2</td>
<td>328.528</td>
<td>0.904735</td>
</tr>
<tr>
<td>Columns</td>
<td>775.056</td>
<td>2</td>
<td>387.528</td>
<td>1.06722</td>
</tr>
<tr>
<td>Interaction</td>
<td>11307.3</td>
<td>4</td>
<td>2826.82</td>
<td>7.7848</td>
</tr>
<tr>
<td>Within</td>
<td>9804.25</td>
<td>27</td>
<td>363.12</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>22543.6</td>
<td>35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Conclusions

Interaction
Ho: NO Interaction
Ha: Interaction Exists
F-Test: 7.7848
F-Critical: 2.72777
Alpha: 0.05
P-Value: 0.000262
Decision: Reject Ho

Samples (rows)
Ho: $\mu_1 = \mu_2 = \mu_3 = ...$
Ha: At least 2 $\mu$'s differ
F-Test: 0.904735
F-Critical: 3.35413
Alpha: 0.05
P-Value: 0.416567
Decision: Fail to reject Ho

Columns
Ho: $\mu_1 = \mu_2 = \mu_3 = ...$
Ha: At least 2 $\mu$'s differ
F-Test: 1.06722
F-Critical: 3.35413
Alpha: 0.05
P-Value: 0.358032
Decision: Fail to reject Ho