Zemea® USP-FCC Propanediol for Food and Flavor Applications

Performance is in our nature.

July 14, 2016
Agenda

- Company Overview
- Zemea® USP-FCC Propanediol Overview
- Sensory and Flavor Modification
- Antimicrobial Testing
- Life Cycle Analysis
Company Overview
Who is DuPont Tate & Lyle?

DTL is a joint venture formed in 2004 between DuPont and Tate & Lyle to produce bio-based propanediol from fermentation of glucose.

DuPont is a world leader in science and innovation across a range of disciplines, including agriculture and industrial biotechnology, chemistry, biology, materials science and manufacturing. CY2015 net sales were $25 billion.

Tate and Lyle is a global provider of renewable ingredients, solutions and services to the food, beverage and industrial customers. Revenues were $4.3 billion for Fiscal Year ending March 31, 2015.
Process Technology
Renewably sourced feedstocks are harvested, fermented, and refined to manufacture Zemea® USP-FCC propanediol.

**Harvest**
Renewably sourced feedstocks are harvested, dried and then wet-milled to create a range of carbohydrate rich feedstocks such as glucose.

**Fermentation**
Glucose is converted into 1,3 propanediol using a patented microorganism under exact temperatures and conditions.

**Refining**
The 1,3 propanediol is refined to a final purity of 99.9% by deactivating and removing the microorganism, water, and other byproducts.
Production
Biotechnology enables our global headquarters and production in Loudon, Tennessee to produce a stable supply of renewably sourced 1,3 propanediol

Awards
• 2003 EPA Presidential Green Chemistry Award
• 2007 ACS Heroes of Chemistry Award
• 2009 ACS-BIOT Industrial Biotechnology Award
• 2010 State of Tennessee Governor’s Award for Trade Excellence

Production
• Started November 2006
• Capacity expanded 35% in 2010
• Current Capacity = 140 million lb.
• Purity = 99.9%
• 100% sustainable and renewably sourced
Zemea® USP-FCC Propanediol
Overview
Zemea® USP-FCC Propanediol

What is it?
• A pure, naturally derived food and beverage ingredient
• Carrier for flavor and extracts
• Offers formulators the potential for reduced bitterness and overall improved sweetness perception in their final products
• 100% sustainably and renewably sourced

Approvals/Certifications:
• Food Chemicals Codex (FCC)
• United States Pharmacopeia (USP)
• Generally Recognized As Safe (GRAS)
• 100% Bio-based – USDA BioPreferred® Program
• Halal
• Kosher
• Gluten-free

Registrations:
• INCI Name: Propanediol
• CAS Number: 504-63-2
• FDA Registration Number: 12130619810
• FEMA: 4753
• EINECS Number: 207-997-3
• REACH Registration Number: 01-2119489383-28-0000
## Regulatory Status

<table>
<thead>
<tr>
<th>Country</th>
<th>Direct Food Additive</th>
<th>Flavor Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States of America</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Mexico, Brazil, Argentina, Chile, Peru, Australia, New Zealand</td>
<td>X</td>
<td>✓</td>
</tr>
</tbody>
</table>

- ADME studies are underway to support additional approvals globally with short-term target of Canada and the European Union.
# Zemea® USP-FCC Propanediol Attributes

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduces bitterness</td>
<td>✓</td>
</tr>
<tr>
<td>Enhances sweetness</td>
<td>✓</td>
</tr>
<tr>
<td>Non-chemical taste (low, sweet)</td>
<td>✓</td>
</tr>
<tr>
<td>Improved humectancy</td>
<td>✓</td>
</tr>
<tr>
<td>Lowers water activity</td>
<td>✓</td>
</tr>
<tr>
<td>Antibacterial and antifungal properties</td>
<td>✓</td>
</tr>
<tr>
<td>Naturally-derived (from glucose)</td>
<td>✓</td>
</tr>
<tr>
<td>Easily substituted/blended for/with PG or Glycerin</td>
<td>✓</td>
</tr>
<tr>
<td>High freeze/thaw, heat stability</td>
<td>✓</td>
</tr>
<tr>
<td>Environmentally sustainable</td>
<td>✓</td>
</tr>
<tr>
<td>High purity, colorless (&gt;99.9%)</td>
<td>✓</td>
</tr>
</tbody>
</table>
# Functionality and Applications

<table>
<thead>
<tr>
<th>Functionality</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrier</td>
<td>Flavors, extracts, aroma chemicals</td>
</tr>
<tr>
<td>Direct food additive</td>
<td>Beverages</td>
</tr>
<tr>
<td>Taste modifier</td>
<td>Dairy (yogurt)</td>
</tr>
<tr>
<td>Humectant</td>
<td>Cereal and energy bars</td>
</tr>
<tr>
<td>Extraction Solvent</td>
<td>Bakery</td>
</tr>
<tr>
<td>Diluent</td>
<td>Snacks</td>
</tr>
<tr>
<td></td>
<td>Confectionery</td>
</tr>
</tbody>
</table>
Approved Use Rates (GRN000302)

Approved Applications:
• 97% for seasonings and flavorings
• 24% for confections and frostings
• 5% for nuts and nut products
• 5% for alcoholic beverages
• 2.5% for frozen dairy products
• 2% for all other food categories

Applications currently not Approved:
• Meat, Fish, Poultry (however meat flavorings are allowed)
• Infant Formula
• Pet food
• Tobacco and e-Cigarettes
Zemea® USP-FCC Propanediol Benefits vs. Propylene Glycol

<table>
<thead>
<tr>
<th>CommonIngredient</th>
<th>CAS #</th>
<th>Formula</th>
<th>Mol. Wt.</th>
<th>Bp,°C</th>
<th>Mp,°C</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG</td>
<td>1,2-Propanediol</td>
<td>57-55-6</td>
<td>C₃H₈O₂</td>
<td>76.1</td>
<td>187.3</td>
<td>-60</td>
</tr>
<tr>
<td>Zemea® USP-FCC Propanediol</td>
<td>1,3-Propanediol</td>
<td>504-63-2</td>
<td>C₃H₈O₂</td>
<td>76.1</td>
<td>214</td>
<td>-24</td>
</tr>
</tbody>
</table>

Sensorial:
- Better taste profile than PG for some flavoring compounds
- Non-chemical taste and odor

Solubility:
- Unique solubility properties

Preservative efficacy:
- Similar

Natural and Sustainable:
- Naturally derived and petroleum-free
- Life Cycle Analysis (LCA) demonstrates less greenhouse gas emission and less energy consumed in production

Certifications:
- 100% Bio-based – USDA BioPreferred® Program
Zemea® USP-FCC Propanediol Benefits vs. Glycerin

<table>
<thead>
<tr>
<th>Common Ingredient</th>
<th>CAS #</th>
<th>Formula</th>
<th>Structure</th>
<th>Mol. Wt.</th>
<th>Bp,°C</th>
<th>Mp,°C</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerin</td>
<td>1,2,3-Propanediol</td>
<td>56-81-5</td>
<td>C₃H₈O₃</td>
<td>92.1</td>
<td>290</td>
<td>18</td>
<td>1.261</td>
</tr>
<tr>
<td>Zemea® USP-FCC Propanediol</td>
<td>1,3-Propanediol</td>
<td>504-63-2</td>
<td>C₂H₈O₂</td>
<td>76.1</td>
<td>214</td>
<td>-24</td>
<td>1.053</td>
</tr>
</tbody>
</table>

**Sensorial:**
- Cleaner taste profile than glycerin

**Viscosity:**
- Lower than glycerin

**Antimicrobial properties:**
- Lower minimum inhibitory concentration (MIC) than glycerin
- Formulators use glycerin to lower water activity in order to reduce the incidence of bacterial growth in certain foods
- The MIC for Zemea® USP-FCC propanediol is 40-50% lower than glycerin for bacteria and fungi
- Zemea® USP-FCC propanediol is more efficient in inhibition of unwanted bacteria and fungi
- Formulations can achieve lower bacterial and fungal count at a higher water activity when compared to formulations with glycerin

**Certifications:**
- 100% Bio-based – USDA BioPreferred® Program
Sensory and Flavor Modification
Summary of Sensory & Flavor Modification Studies

Zemea® USP – FCC Propanediol Findings

- Potential for reduced bitterness and overall improved sweetness perception in final products
- Improved mouthfeel was observed in various beverage products

<table>
<thead>
<tr>
<th>Testing Completed</th>
<th>Confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensory Analysis – Electric Tongue</td>
<td>✓</td>
</tr>
<tr>
<td>Orange Juice Taste Panel Study</td>
<td>✓</td>
</tr>
<tr>
<td>Food and Beverage Taste Panel Study</td>
<td>✓</td>
</tr>
</tbody>
</table>
Sensory Analysis – Electronic Tongue

• Samples diluted in deionized water 1:1
  • Glycerol, USP (n=3)
  • Propylene Glycol, USP (n=3)
  • Zemea® USP-FCC Propanediol (n=3)

• 7 E-tongue sensors
  • Saltiness; Spiciness; Umami; Sweetness; Metallic; Bitterness; Sourness

• Discrimination of taste attributes resulting in distinct clustering of responses by principal component analysis (PCA)

PC1 represents correlated taste responses from SRS (sourness), GPS (metallic), SPS (spiciness), BRS (bitterness), SWS (sweetness)
PC2 represents correlated taste responses from UMS (umami) and STS (saltiness)
Individual E-Tongue Sensor Responses

• Glycerol is distinctly different than PG or Zemea® USP-FCC propanediol
  • High sourness and umami; low responses for other taste attributes

• Currently investigating alignment between human sensory panel data and E-tongue for more complex sensory phenomena
  • Bitterness suppression
  • Sweetness enhancement

- SRS: Sourness
- GPS: Metallic;
- STS: Saltiness;
- UMS: Umami;
- SPS: Spiciness;
- SWS: Sweetness;
- BRS: Bitterness;

Glycerol, USP Blue, n=3
Propylene Glycol, USP Red, n=3
Zemea® USP-FCC Propanediol Green, n=3
Bitterness Suppression by Zemea® USP-FCC Propanediol

Bitterness in foods and pharmaceuticals

- Bitterness in some foods like catechin, theophylline, theobromine, and caffeine in black tea and coffee.
- Bitterness in certain over-the-counter pharmaceuticals for example: dextromethorphan, acetaminophen, and pseudoephedrine.

Bitterness suppression has been investigated to improve palatability:

- Sodium (Na+) salts have been shown to suppress the bitterness of certain compounds.
- Zemea® USP-FCC propanediol has recently been shown to suppress bitterness of high-intensity sweeteners, tea, coffee, orange juice, and potassium chloride in foods (US2013/0236597A1).
- Bitterness suppression has not been reported for propylene glycol
The bitter taste cascade involves calcium efflux to generate cell membrane depolarization and/or ATP production.

1,3-propanediol has Ca2+ channel blocking or regulator activity.

Efflux (release) of Ca2+ appears to be inhibited resulting in lower bitter response.

Study design:
- Fresh pressed Valencia orange juice
- 8 sensory panel members
- USP glycols
- Values are the mean for 8 data points

- Up to a 60% bitterness suppression was observed for Zemea® USP-FCC propanediol formulations
- Zemea® USP-FCC propanediol formulations had enhanced mouthfeel
- PG formulations were statistically equivalent to the control
- Sour attributes were reported by some panelists for Zemea® USP-FCC propanediol containing juice above use rates of 0.4%
Sweetness Enhancement for Orange Juice

Study design:
- Fresh pressed Valencia orange juice
- 8 sensory panel members
- USP glycols
- Values are the mean for 8 data points

- Up to a 38% increase in sweetness was observed for Zemea® USP-FCC propanediol formulations
- PG formulations were statistically equivalent to the control
Food and Beverage Taste Panel

Introduction:

- The effect of Zemea® USP-FCC propanediol on the flavor of various food and beverage products was studied. The sensory testing was conducted by an experienced panel of Tate & Lyle employees.

Test Methods:

- Nine (9) Tate & Lyle employees experienced in the evaluation of food and beverages tasted three juices and two yogurts with and without the addition of Zemea® USP-FCC propanediol. Panelists indicated whether the test or control product in each pair was sweeter, more bitter, and had a stronger overall flavor.

<table>
<thead>
<tr>
<th>Product</th>
<th>Zemea® USP-FCC Propanediol Level, Wt. %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Plain Greek Yogurt</td>
<td>0</td>
</tr>
<tr>
<td>Vanilla Yogurt</td>
<td>0</td>
</tr>
<tr>
<td>Orange Juice (no pulp)</td>
<td>0</td>
</tr>
<tr>
<td>Cranberry Juice</td>
<td>0</td>
</tr>
<tr>
<td>Grapefruit Juice</td>
<td>0</td>
</tr>
</tbody>
</table>

*Zemea® USP-FCC propanediol was added directly to the final product.
Food and Beverage Taste Panel Results

Results:

• The plain Greek yogurt and grapefruit juice containing Zemea® USP-FCC propanediol were less bitter and clearly sweeter than the corresponding control products.

• The vanilla yogurt and orange juice containing Zemea® USP-FCC propanediol were less bitter and somewhat sweeter than the corresponding control products.

• Cranberry juice containing Zemea® USP-FCC propanediol was less bitter and less sweet than the corresponding control product.
Antimicrobial and Antifungal Testing
Summary of Bacterial & Fungal Studies

Zemea® USP – FCC
Propanediol Findings
• 1,3 propanediol is self-sterilizing providing a 2 year shelf life
• Inhibits bacterial, yeast, and mold growth as demonstrated throughout all testing
• Ease of mind as it performs similarly to propylene glycol

<table>
<thead>
<tr>
<th>Testing Completed</th>
<th>Confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum inhibitory concentration (MIC)</td>
<td>✓</td>
</tr>
<tr>
<td>Minimum lethal concentration (MLC)</td>
<td>✓</td>
</tr>
<tr>
<td>CTFA Antifungal/Mold Testing</td>
<td>✓</td>
</tr>
<tr>
<td>CTFA Antimicrobial Testing</td>
<td>✓</td>
</tr>
<tr>
<td>Preservation Efficacy in Beverages</td>
<td>✓</td>
</tr>
<tr>
<td>• Lemon Tea</td>
<td></td>
</tr>
<tr>
<td>• Peach Tea</td>
<td></td>
</tr>
<tr>
<td>• Pomegranate Cranberry Tea</td>
<td></td>
</tr>
<tr>
<td>• Apple Tea</td>
<td></td>
</tr>
</tbody>
</table>
Bacterial and Fungal Inhibition Testing

- Zemea® USP-FCC propanediol completely eliminates growth and viability of bacteria and fungi above an aqueous glycol concentration of approximately 15%.

- An inhibitory concentration (MIC50) is typically reached at a concentration of 7-10%.

- The manufactured product (>99.9% 1,3-propanediol) is self-sterilizing, which supports a maximum recommended shelf life of 2 years.
## MIC and MLC Testing for Glycols

<table>
<thead>
<tr>
<th>Organism</th>
<th>Zemea® USP-FCC Propanediol, % v:v</th>
<th>Propylene Glycol, % v:v</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC$_{50}$</td>
<td>MIC$_{90}$</td>
</tr>
<tr>
<td>Staphyloccoccus aureus sbsp. aureus</td>
<td>8.2%</td>
<td>9.0%</td>
</tr>
<tr>
<td>Bacillus megaterium</td>
<td>10.2%</td>
<td>11.0%</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>7.2%</td>
<td>9.0%</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>8.2%</td>
<td>9.0%</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>9.2%</td>
<td>10.0%</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>9.3%</td>
<td>10.0%</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>7.0%</td>
<td>8.0%</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>7.6%</td>
<td>13.2%</td>
</tr>
<tr>
<td>Mean for group, % v:v</td>
<td>8.36%</td>
<td>9.90%</td>
</tr>
<tr>
<td>Std. deviation for group, % v:v</td>
<td>1.12%</td>
<td>1.61%</td>
</tr>
</tbody>
</table>

- Minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) values for glycol testing.
- Zemea® USP-FCC propanediol and PG demonstrate similar results for these microbiological tests.
Zemea® USP-FCC Propanediol Neat Glycol Antifungal/Mold CTFA Challenge Tests

Testing Summary:
• Neat samples of Zemea® USP-FCC propanediol and PG evaluated using CTFA challenge test guidelines
• Neat glycol samples inoculated with approximately $6 \times 10^6$ mold and yeast colonies
• The number of colonies were counted periodically
• After 4 weeks the inoculation was repeated

Mold and yeast used in testing:
• Aspergillus niger
• Candida albicans
• Penicillium sp.
• Trichoderma sp.

Results:
• Zemea® USP-FCC propanediol performed equal to PG
• After 6 hours the number of colonies was less than 10 for both Zemea® USP-FCC propanediol and PG
• After 4 weeks the second inoculations occurred with similar results
Zemea® USP-FCC Propanediol Neat Glycol Antimicrobial CTFA Challenge Tests

Testing Summary:
• Neat samples of Zemea® USP-FCC propanediol and PG evaluated using CTFA challenge test guidelines
• Neat glycol samples inoculated with approximately 6x10^6 mold and yeast colonies
• The number of colonies were counted periodically
• After 4 weeks the inoculation was repeated

Bacteria used in testing:
• Group I – S.aureus, E.coli, P.vulgaris, E.cloacae and E. gergoviae
• Group II – P. aeruginosa, P.fluorescens, P.cepacia and Flavobacterium sp.
• Group III – Environmental (bacterial) isolates

Results:
• Zemea® USP-FCC propanediol performed equal to PG
• After 24 hours the number of colonies was less than 10 for both Zemea® USP-FCC propanediol and PG
• After 4 weeks the second inoculations occurred with similar results
Zemea® USP-FCC Preservative Efficacy in Beverages

Study:
- The preservative efficacy of Zemea® USP-FCC propanediol in water additive flavoring systems was studied. The challenge testing was conducted by Silliker Laboratories using standardized microbiology guidelines.

Experimental design:
- Based on several different water additive flavoring systems.
- Zemea® USP-FCC propanediol used at a final concentration of 25% (v:v).
- Each formulation contained both ascorbic and citric that were adjusted to a pH of 2.0 and the water activity (aw) was 0.7.

The tested formulations included:
- Lemon tea inclusive of 20% tea botanical.
- Peach tea with 20% tea botanical plus peach juice/essence.
- Pomegranate Cranberry tea inclusive of a high saccharide natural vegetable colorant.
- Apple tea flavor inclusive of apple fruit juice and aqueous apple essence.
Zemea® USP-FCC Test Methods and Results for Preservative Efficacy in Beverages

Test Methods and Organisms:

• Formulation variants were injected with the microorganisms as follows, under ambient (73-77°F) storage conditions, without a further “kill step”.

• Initial samples were analyzed within 15 minutes after inoculation.

• In addition, uninnoculated yeast and mold test were run concurrently with testing hurdles at three and four weeks.

Results:

• In all variants the death of the microorganisms occurred within the first 24 hours. These products also remained stable for the first milestone of three weeks and a final milestone of four weeks.

• Uninoculated yeast and mold was run concurrently with the microorganism testing. Zemea® USP-FCC propanediol did not support any yeast or mold growth, a secondary benefit to Zemea® USP-FCC propanediol ability to deter bacterial and fungal growth.

• Despite the introduction of nutritive substances, fruit and vegetable juices and other nutritive botanical matter, the systems inclusive of Zemea® USP-FCC propanediol did not support microbiological growth.
Life Cycle Analysis
Life Cycle Analysis Approach

LCA is the only standardized method to evaluate the environmental footprint of a whole supply chain. Energy consumption and Green House Gas (CO$_2$) emissions are key factors in determining environmental footprint.
Zemea® USP-FCC Life Cycle Analysis

From “cradle to gate” (extraction and production prior to delivery to the consumer), Zemea® USP-FCC propanediol produces 56% less greenhouse gas emissions and consumes 42% less nonrenewable energy than petroleum-based 1,3-propanediol. Compared with propylene glycol, Zemea® USP-FCC propanediol produces 42% less greenhouse gas emissions and uses 38% less nonrenewable energy from cradle to gate.

**Greenhouse Gas Emissions**
- 56% less than Propanediol
- 42% less than Propylene Glycol

**Non-Renewable Energy Use**
- 42% less than Propanediol
- 38% less than Propylene Glycol
David Shaw

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