The application of CEM technologies to improve the analysis and environmental footprint of Dairy and Non-Dairy processing

**Presenter** – Devin Darrell – Global Key Account Manager
Green Movement in Dairy

Sustainability

Current State

- Dairy industry responsible for 4% of global GHG emissions
  - 2.7% for milk production, processing, and transportation
- 11% reduction from 2005-2015, even with a 30% production increase
Steps and developments in the market

• Ireland GHG 51% reduction by 2023
• Agriculture currently accounts for 1/3 of total emissions
  • Plan outlines 22-30% cut from Agriculture
• Multi-organizational plan for the first carbon neutral farm
  • Shinagh Farm in West Cork
• Irish Dairy contributes 1.1 kg CO₂ per 1 kg of fat/protein corrected milk
  • One of lowest global values
• Feed additives, biorefining waste, carbon sequestering, etc
• Gene-editing in beef cows
• Research already addressing antibiotics resistance, emission reduction, production increase
• Methane reduction through Algae as a feed additive
• Peer-review shows potential, but obstacles scaling technology
• Narrow focus on feedlot use (small % of total emissions)
Impact of lab testing

- In-house Lab testing USED to be an oversight
  - Requires skilled chemists
  - Demands Accuracy
  - Must have confidence in data
  - Speed is critical to making adjustments

- Fewer batch rejections
- Reduce value-added waste
- Optimize production efficiency
- Confirm ingredient CoA’s
- Improve in-line testing
GAME CHANGER

• Raw and processed milk testing by FT-IR
• Faster/better alternative to existing extraction methods
  • Gerber, Mojo, RG
• Strong global calibration library
Total FAT tests globally/regionally

• 906 million tonnes of annual milk production (FAO market review 2021)
  • 1/3 used for further dairy processing - ~300 million tonnes
    • 1/3….and growing
  • 1/2 of that performed in mid-large size processing - ~150 million tonnes

• Average QA test rate is about 1 test / 10,000 Liters
  • Total of ~40,000,000 tests each year globally

• Fat Test solvents for 1 test

<table>
<thead>
<tr>
<th></th>
<th>RG/Mojo</th>
<th>Gerber</th>
<th>Babcock</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5mL NH4OH</td>
<td>10mL HCl</td>
<td>17.5mL H2SO4</td>
<td></td>
</tr>
<tr>
<td>55mL Ether</td>
<td>25mL Ether</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55mL Pet Ether</td>
<td>25mL Pet Ether</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Aim to bring to ZERO

Visualize a world without solvents…not just environmental benefits

High Cost
Chemicals, disposal, labor, consumables, and more

Time per Test
Modified methods can take 15-20 minutes, full methods can take up to 16 hours

Safety Issues
Uses various hazardous solvents and exposed hot surfaces such as air ovens and hot plates

Difficulty of SOP
Multiple opportunities for human error leading to poor repeatability and reproducibility
Reference materials to evaluate measurement systems for the nutrient composition of foods: results from USDA’s National Food and Nutrient Analysis Program (NFNAP)

Table 1 Summary of $Z'$-scores for reported nutrient concentrations analyzed in certified reference materials (CRMs)

<table>
<thead>
<tr>
<th>Class</th>
<th>Nutrient</th>
<th>Total CRMs</th>
<th>Total labs</th>
<th>Total values</th>
<th>Count of 0 to</th>
<th>Count of 1 to</th>
<th>Count of 2 to</th>
<th>Count of &gt;3</th>
<th>Percent &gt;2</th>
<th>Percent &gt;3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximates</td>
<td>Moisture</td>
<td>11</td>
<td>7</td>
<td>118</td>
<td>82</td>
<td>22</td>
<td>9</td>
<td>5</td>
<td>11.9</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>9</td>
<td>5</td>
<td>106</td>
<td>60</td>
<td>24</td>
<td>12</td>
<td>10</td>
<td>20.8</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td>Ash</td>
<td>11</td>
<td>5</td>
<td>107</td>
<td>55</td>
<td>26</td>
<td>11</td>
<td>15</td>
<td>24.3</td>
<td>14.0</td>
</tr>
<tr>
<td></td>
<td>Total Fat</td>
<td>11</td>
<td>6</td>
<td>129</td>
<td>52</td>
<td>39</td>
<td>15</td>
<td>23</td>
<td>29.5</td>
<td>17.8</td>
</tr>
</tbody>
</table>
3 Innovations from CEM
ORACLE Fat Analyzer

Universal NMR Fat Analysis of any sample without calibration
CEM’s ORACLE Fat Analyzer

- “Crude” Fat by Low-Res 1H NMR analysis
- Universal Calibration validated for 2,000+ unique food matrices
- No solvents or calibration maintenance
- 30-90 second analysis time
- AOAC Accredited, ISO accreditation by end of 2021
- Over 500 installed globally
• 36 CRM’s initially validated on the ORACLE
  • Samples extensively tested in collaborative studies

• CEM outsourced 1000’s of samples to Eurofins and other contract labs
  • Submitted samples in “blind” and “non-blind” fashion to capture true sample variability
Actalia Cecalait Validation

Actalia Cecalait are COFRAC/ISO Dairy lab in France

- “Experts” in Dairy analysis and processing equipment
  - Validate and publish technologies for the dairy industry
- Manager currently chairs IDF/ISO TC34/SC5 committee

![Graph showing accuracy of ORACLE method for various dairy products](CEM_Celebra-CESM throwing.jpg)

\[ y = 0.999x + 0.009 \]

\[ R^2 = 1.000 \]
Stages of Approval

• March 2021 – Accepted as NWIP Method Guideline

• March 2022 – Final WD/CD submitted for voting

• May 2022 – Accepted as Draft International Standard (DIS)

• November 2022 – Accepted as Final DIS and published as ISO method

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100 fat test per day for 1 year will result in:

<table>
<thead>
<tr>
<th>Chemical Extraction</th>
<th>ORACLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,500 L Hazardous Solvents</td>
<td>Zero</td>
</tr>
<tr>
<td>17,500 hrs Labor Time</td>
<td>1,150 hrs</td>
</tr>
<tr>
<td>15 – 20 m Benchtop Space</td>
<td>2 m</td>
</tr>
<tr>
<td>$140K – 280K Consumable Cost</td>
<td>$30K</td>
</tr>
<tr>
<td>Up to 14,000¹ Out of Spec Results</td>
<td>Zero²</td>
</tr>
</tbody>
</table>

¹According to 2007 USDA study on CRM testing in certified labs. ²According to 2018 Ashley Ciesielski ISO Independent study
Sprint Rapid Protein Analyzer

Fully automated UDY dye-binding method
Typical Kjeldahl problems
Sprint Rapid Protein Analyzer

- AOAC approved Technology
  - 967.12, 2011.04
- Automates Udy Dye-Binding method from the 1970’s
- Under 4-minute analysis
- No hazardous chemicals
- Lower cost than Kjeldahl
The Three Basic Amino Acids

- Arginine
- Histidine
- Lysine

Common Amino Acid Structure
The Three Basic Amino Acids

These are the only amino acids with basic side chains

Arginine
Histidin
Lysine
iTAG™ Molecule

Acid side chain binds with Histidine, Lysine, and Arginine

Aromatic group which absorbs light, and appears orange
Direct Protein Binding

**Crude Protein**
- Simple conversion of N→%P in Kjeldahl testing
- Does not account for NPN variation
  - Natural or Synthetic

**True Protein**
- TKN-NPN method, requiring second Kjeldahl titration
- Usually lower than crude protein
- Global legislation trending toward True Protein reporting
  - US
  - Canada
  - France
  - Australia
MW-Based Hydrolysis

Novel method for FAMEs and AA preparation
Amino Acid Hydrolysis

- Current method requires 22–24 hour oven hydrolysis
- Separate techniques for Acid, Base, and SAA prep
- Pre- or Post-Oxidation depending on method and equipment

MARS 6
- High Throughput
- 40 similar type samples per 2 hours
- Acid and Base Hydrolysis capable, but only 1 option per run

Discover 2.0
- Sequential analysis with Automated option
- Mix and match Base and Acid tests
- Full temperature and pressure control per test
Traditional FAMEs Method

A lengthy traditional total fat determination must be performed using acid or alkaline hydrolysis and soxhlet type extraction.

1. Weigh out a new **DRY** sample
2. Dry sample at 100°C for 3 hours
3. Add toxic BF3 (esterification)
4. Add DCM/water and homogenise for 1 hour
5. Separate organic fat layer for 1/2 hour
6. Set up and perform reflux over steam bath for 40 minutes with stirring
7. Reflux over steam bath (45 minutes, 100°C)
8. Any evaporation and repeat whole process
9. Add pentane
10. Remove pentane extract
11. Dry pentane over sodium sulphate
12. Inject into GC
Traditional FAMEs Method

A lengthy traditional total fatty acid extraction must be performed using acid or alkaline hydrolysis and soxhlet type extraction.

Weight and dry sample
Add Methanol/KOH
Dry sample at 100°C for 2 hours
Add water and heat beaker
Set up and perform reflux
Remove extract
Dry pentane over sodium sulphate
Inject into GC
CEM Improved FAMEs Procedure

40 Samples prepared simultaneously (ORACLE + MARS 6)

- Weigh out up to 40 wet or dry whole samples into vessels
- Add Methanol KOH solution
- Microwave up to 40 samples simultaneously for 4 minutes
- Saponification
- Add Methanol HCl Solution
- Microwave for 5 minutes
- Esterification
- Add pentane and shake
- Dry pentane over sodium sulphate
- Inject into GC
1 Day TAT for FAMEs

- 1 day TAT for full lipid profile, SFA/PUFA values, etc
- Removal of carcinogenic BF$_3$
- Extraction and derivatization done in same vessel for easy cleanup
Future of Dairy Testing
Future of Dairy Testing

- PDCAA and DIAA Scores
- SFA, PUFA, and Trans Fats
- Vitamin and Mineral reporting and testing
Future of Dairy Testing

Plant-Based Meat and Dairy

“Cultivated” cell grown products
Future of Dairy Testing

SUSTAINABILITY
ADAPTATION
INNOVATION
In Conclusion