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20

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"Our team approach in responding to this pandemic has been key in communicating and managing through this."

14

28

ON THE COVER
Mike Steinert, Ardent Mills, wears a mask while on the mill floor at one of the company's flour milling facilities. Photo courtesy of Ardent Mills.

Commercial Scale Decontamination of Wheat Flour using Neo-Temper: Efficacy and Flour Functionality Analysis

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Wheat flour has been implicated in several outbreaks caused by Shiga-toxin producing *Escherichia coli* (STEC) (Thomas-Popo et *al.* 2019). This is because wheat can become contaminated at many steps throughout processing into flour, including harvesting, milling, storage and transport (Rose et *al.*, 2012). Additionally, the steps involved in milling, including cleaning, tempering and grinding, can redistribute and concentrate microbial contaminants, if present (Sabillon and Bianchini 2016). Therefore, additional measures are necessary when processing wheat to control for pathogen contamination.

In this study, Neo-Temper, a patented, organic, antimicrobial solution developed by Agri-Neo Inc., was evaluated for efficacy against STEC with the final objective of validation in a commercial mill located in Quebec, Canada (Soulanges Mill, Saint-Polycarpe). There were three objectives of this study:

1. To identify a suitable surrogate for STEC to validate Neo-Temper at Soulanges Mill.
2. To achieve an efficacy of > 2 log cfu/g, identified as the target log reduction by Soulanges Mill, against the identified surrogate when

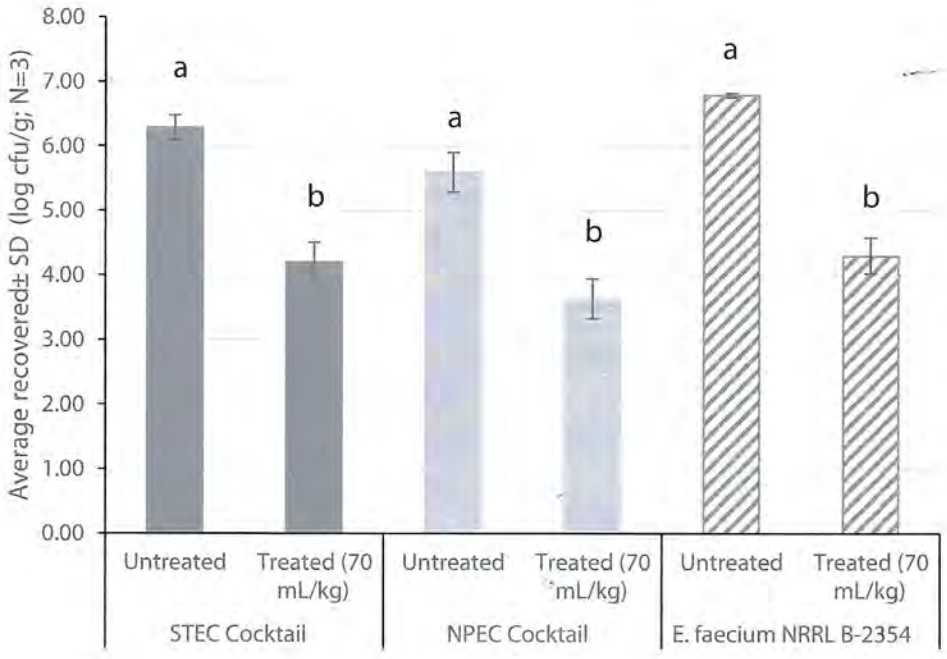

Figure 1. Recovery (detection limit = 0.30 log cfu/g, n =3) of a 7-strain STEC cocktail (serotypes O26:H11, O103:H2, O11:NM, O121:H19, O145:NM, O45:H2 and O157:H7), a 5-strain NPEC cocktail (ATTC strains BAA-1427, BAA-1428, BAA-1429, BAA-1430 and BBA-1431) or *E. faecium* NRRL B-2354 from irradiated and inoculated wheat berries before and after treatment of 100 g with a total volume of 7 milliliters, consisting of 0.3 milliliters of Neo-Temper combined with 6.7 milliliters of de-ionized water, followed by an 8-hour hold. Columns labeled with different letters are significantly different ($P < 0.05$). Columns labeled with the same letter are not significantly different ($P \geq 0.05$).

Treatment Condition	Formula	Component #1 Volume (mL)	Component #2 Volume (mL)	Water Volume (mL)	Hold Time (h)
1	1	2	3	65	16
2	1	2	3	15	6
3	2	3	1	66	16
4	2	3	1	15	6

Table 1. Treatment conditions for lab scale surrogate efficacy studies with 100-gram wheat berries.

applying Neo-Temper under representative tempering conditions.

3. To evaluate functionality of flour treated under the validation conditions compared to untreated.

IDENTIFICATION OF A SUITABLE SURROGATE

Wheat berries used for surrogate compatibility testing were irradiated and confirmed to be free of background microbiota, allowing recovery to be done on non-selective agar (Tryptic Soy Agar; TSA) for direct comparison of two potential surrogates: 1) *Enterococcus faecium* ATCC 8459 (NRRL B-2354), and 2) a 5-strain cocktail of non-pathogenic *Escherichia coli* (NPEC), with a 7-strain cocktail of STEC. Recovery levels were compared before and after treatment with Neo-Temper (100 g of inoculated wheat berries were treated with a total volume of 7 milliliters, consisting of 0.3 milliliters of Neo-Temper combined with 6.7 milliliters of de-ionized water) and a holding step of 8 hours at ambient temperature (□22 °C) to simulate wheat tempering conditions (Fig. 1).

There was no significant difference ($P \geq 0.05$) in log reduction for either *E. faecium* (2.49 cfu/g) or the NPEC cocktail (1.96 cfu/g) compared to the STEC cocktail (2.09 cfu/g) (Fig. 1). However, *E. faecium* survival (6.80 ± 0.03 log cfu/g) was closer to the STEC cocktail (6.29 ± 0.19) compared to the NPEC cocktail (5.60 ± 0.31).

Additionally, previous studies have established the safe use of *E. faecium* for industrial validations (Villa-Rojas et *al.* 2017), and culturing a single bacteria

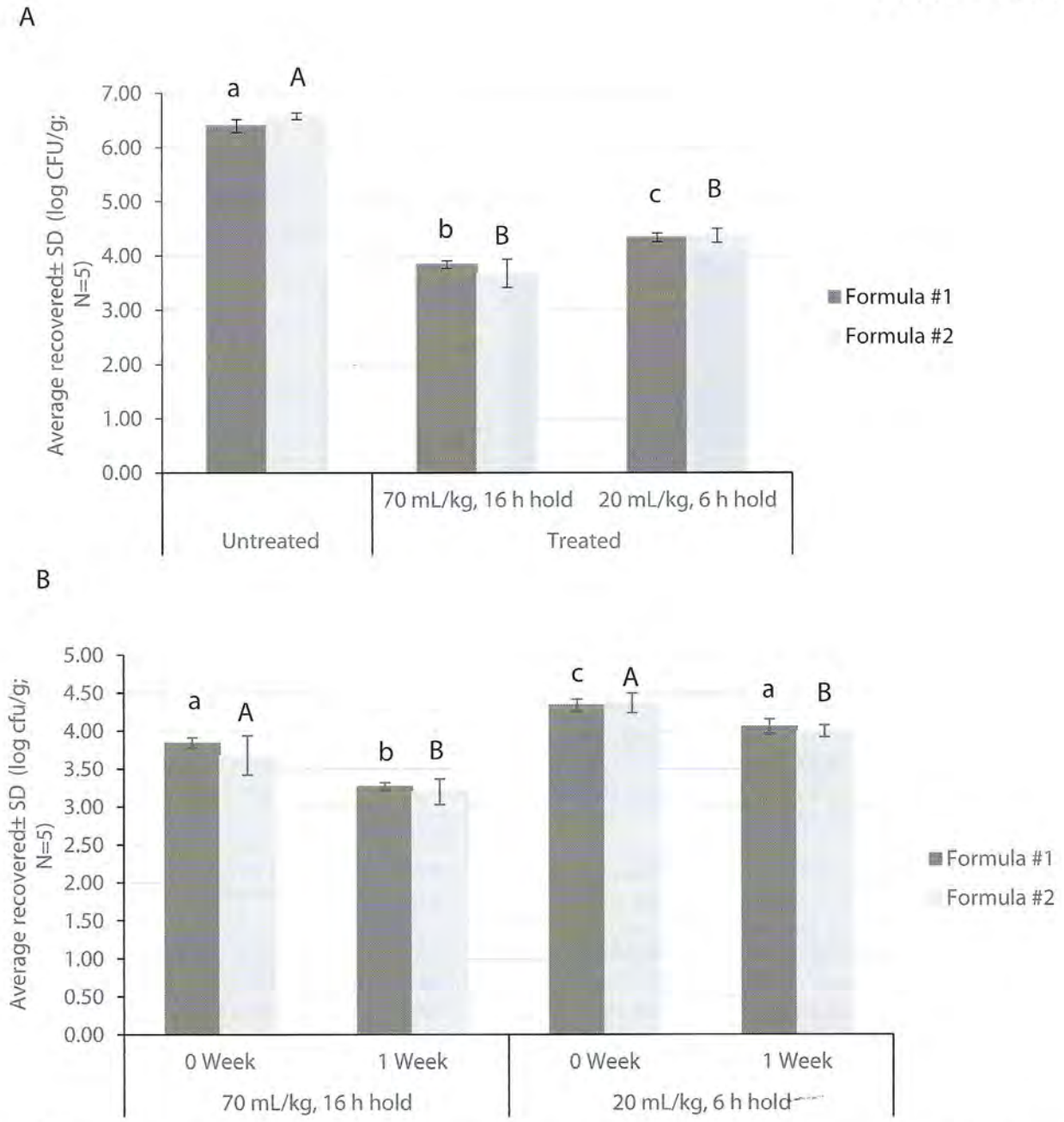

Figure 2. Recovery (detection limit = 0.30 log cfu/g, n =5) of E. faecium from inoculated wheat berries compared to that recovered from inoculated wheat berries treated under two different tempering conditions (Condition #1: 70 milliliters/kg water, 16 h hold, Condition #2: 20 milliliters/kg water, 6 h hold). Under these two different tempering conditions, a set volume of the tempering water was replaced with one of two different Neo-Temper formulations (Formula #1: 5 milliliters, Formula #2: 4 milliliters). Columns labeled with different letters (uppercase: formula 2, lowercase: formula 1) are significantly different ($P < 0.05$). Columns labeled with the same letter (uppercase: formula 2, lowercase: formula 1) are not significantly different ($P \geq 0.05$). Enumeration was conducted immediately after milling and one week after milling.

species was more feasible than five different strains for a cocktail. For these reasons, *E. faecium* was chosen over the NPEC cocktail as a surrogate for STEC for further testing. For further testing, plating on agar growth medium selective for the identified surrogate (Slanetz and Bartley Enterococcus selective agar) meant that wheat berries did not have to be irradiated. Preliminary testing confirmed the same recovery was achieved for both media.

EFFICACY OF NEO-TEMPER AGAINST THE SURROGATE

At a lab scale, to identify a treatment with Neo-Temper that would achieve a > 2 log cfu/g reduction in E. faecium, un-irradiated wheat berries inoculated with E. faecium were subjected to two different conditions to account for tempering variations used by Soulanges Mill (Condition #1: 70 milliliters/kg water, 16 hour hold; Condition #2: 20 milliliters/kg water, 6

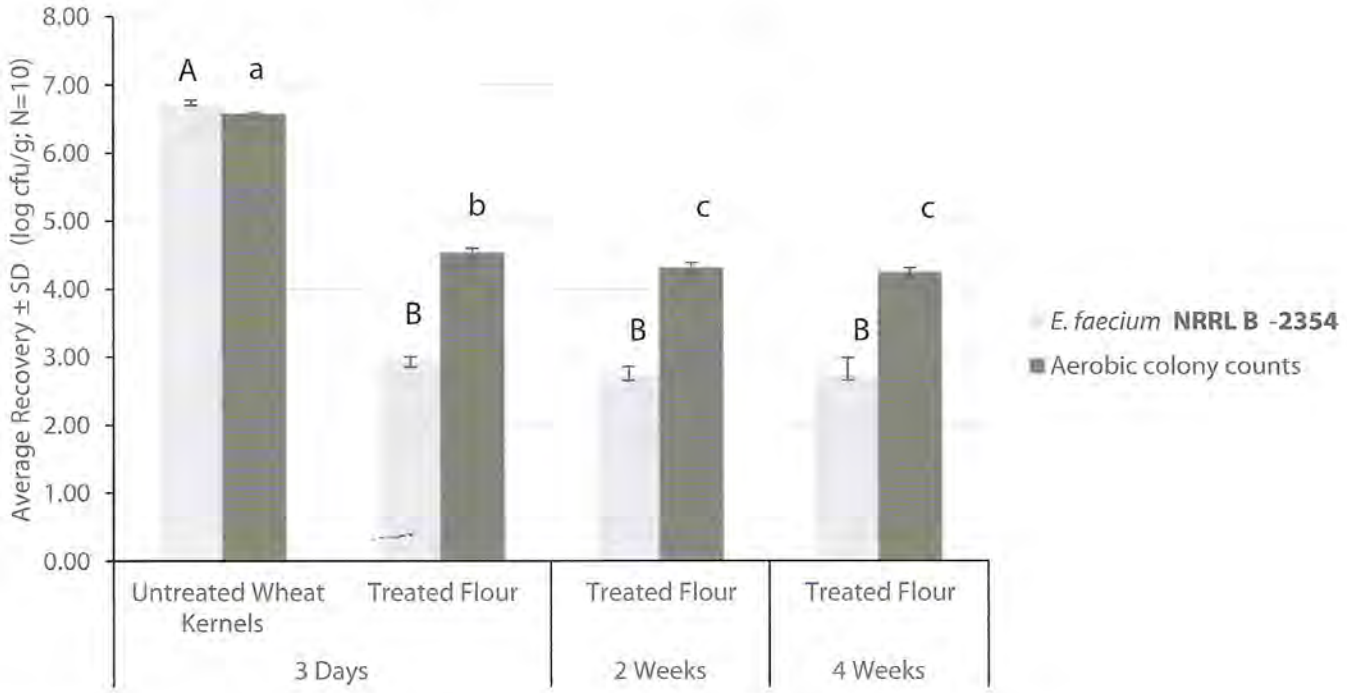

Figure 3. Plate counts (detection limit = 0.30 log cfu/g, n =10) of E. faecium NRRL B-2354 (light grey) and aerobic colonies (dark grey) recovered from inoculated wheat berries (control) and flour (treated). At the validation site, wheat was tempered in two stages. Neo-Temper was added during the first stage (5 milliliters/kg Formula #1 and 10 milliliters/kg water) followed by a hold of 12 hours before milling. In the second stage of tempering, 23 millimeters/kg of water was added, followed by a hold of 4 hours. Columns labeled with different letters (uppercase: E. faecium, lowercase: aerobic colonies) are significantly different ($P < 0.05$). Columns labeled with the same letter (uppercase: E. faecium, lowercase: aerobic colonies) are not significantly different ($P \geq 0.05$).

hour hold). Under these two different conditions, a set volume of water was replaced with one of two different Neo-Temper formulations. One formulation (Formula #1), consisted of 2 milliliters of one antimicrobial (Component #1) and 3 milliliters of a second antimicrobial (Component #2). The second formulation (Formula #2) consisted of 3 milliliters of Component #1 and 1 milliliter of Component #2. Therefore, a 5 milliliters volume of water was replaced for both conditions with Formula #1, whereas a 4 milliliters volume of water was replaced with Formula #2. In total, four different treatment conditions were tested (Table 1). Recovery was compared before and after treatment for all four tests post-treatment and again after one week of storage at ambient temperature (approx. 22 °C) to evaluate for re-growth.

Although slightly greater log reductions were observed for Formula #2 compared to Formula #1 under both conditions tested (Fig. 3), Formula #1 was chosen for further study because: 1) both formulas were able to achieve > 2 log cfu/g reductions, and 2) no re-growth in *E. faecium* was observed with either formulas.

COMMERCIAL MILL VALIDATION FOR EFFICACY

Prior to the on-site validation, the inoculation method was scaled up using Agri-Neo's patent-pending process and 500 kg of wheat was inoculated with *E. faecium*.

At Soulanges Mill, inoculated wheat berries were tempered in two stages. Neo-Temper was applied in the first stage (5 milliliters/kg of Formula #1 and 10 milliliters/kg water) followed by a hold of 12 hours in a silo. In the second stage of tempering, 23 milliliters/kg of water was added, followed by a hold of four hours in a second silo. Ten 45-g samples of wheat and flour were taken for enumeration before and after treatment, respectively.

EA 3.79 cfu/g log reduction was achieved in *E. faecium* and a 2.05 cfu/g log reduction was achieved in aerobic colony counts (ACC) (Fig. 6). Aerobic colony counts were evaluated as an indicator of overall product quality. Samples replated after two weeks of storage at ambient temperature gave a 3.99 and 2.27 log cfu/g reduction, for *E. faecium* and ACC, respectively, and a 4.00 and 2.33 log cfu/g reduction, respectively, when plated after four weeks (Fig. 6). The observed reduction in ACC levels could potentially improve shelf-life and overall quality of the treated wheat (Ibanoglu 2001).

FLOUR FUNCTIONALITY AND ASSESSMENT OF BAKED BREAD

Flour, treated and processed under the same con-

Property	Treated	Untreated
% Protein	11.94	11.68
% Moisture	13.58	15.00
% Ash content	0.58	0.56
% Absorption	58.70	58.10
Development time (min)	2.50	2.50
Stability time (min)	6.50	10.50
Dough softening number (Nm)	0.12	0.09
Falling number	381.00	318.00

Table 2. Property of flour and resultant dough from wheat treated with Neo-Temper added during tempering compared to wheat without the antimicrobial solution added during tempering.

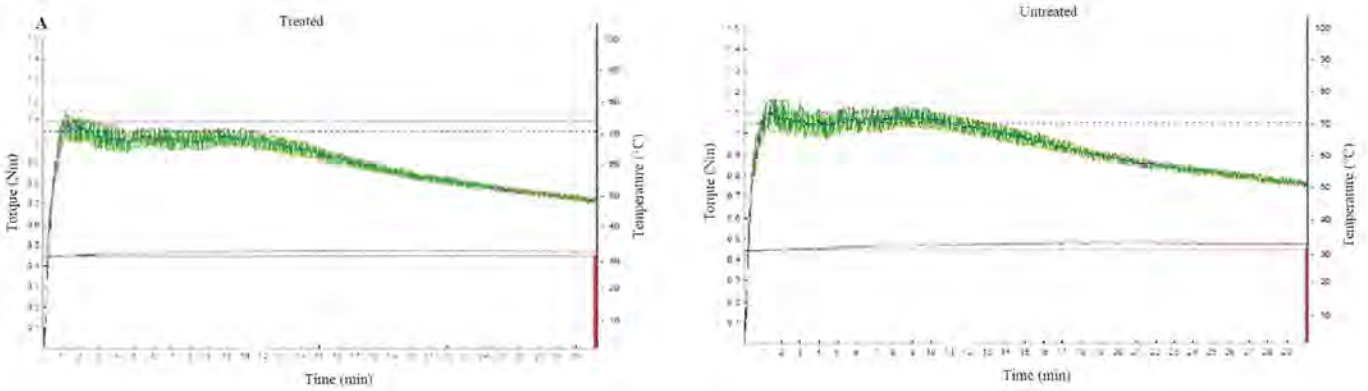

Figure 4. Torque-time graph generated from the Mixolab System (Chopin Technologies, Series #78) for wheat treated with the antimicrobial solution added during tempering (A) compared to wheat without the antimicrobial solution added during tempering (B).

ditions used for the validation, was evaluated for percentage of protein, moisture, ash and absorption content, as well as dough development time, dough stability, dough softening and dough falling number using a Mixolab System (Chopin Technologies, Series #78) (Table 2.). The measurements for each property were similar between the treated and untreated flour and the torque-time curve generated by the Mixolab System for the treated dough resembled that generated for untreated dough (Fig. 7). Upon baking of the dough, no difference in rising, appearance, texture, or taste, was observed compared to control.

CONCLUSIONS

This is the first commercial-scale study to evaluate the efficacy of an antimicrobial solution applied during the tempering stage of wheat milling and the resultant flour functionality. With Neo-Temper, milling companies, like Soulanges Mill, not only achieve food safety, but also maintain flour functionality, giving them a competitive industry advantage. IM

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Chafik graduated in food science engineering from the National School of Agronomy and Food Science in Nancy, France. He has been the R&D Director at the Soulanges Mills for almost 10 years. With his deep cereal chemistry knowledge, he specializes in developing customized clean label flours and actively contributes to improving the baking value of Eastern Canadian wheat varieties. He has worked on many projects, including one that considered the kinetics of gluten aggregation to integrating the extensograph into the evaluation of flour during baking. His current project is to understand the variability of starch and gluten quality in current commercial winter wheats to efficiently address the needs of artisanal style bread bakers.

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Emily serves the baking industry as a subject matter expert on talent and continuing professional education. Prior to joining the non-profit sector, she served in multiple training and development leadership roles. As a performance consultant, Emily specialized in project management, program facilitation, and train-the-trainer. She has managed the organizational development function for a global manufacturer of packaging solutions. Emily has earned several certifications, as well as a bachelor's degree in elementary and early childhood education from Northwest Missouri State University and a master's degree in curriculum development from Baker University.

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Amir leads the food safety science team at Agri-Neo. He specializes in designing and implementing food safety technologies for low-moisture foods. Amir and his team have successfully completed commercial-scale validations for equipment and processes developed at Agri-Neo. Amir holds a Ph.D. in food science and technology with a food microbiology focus from the University of Lincoln, UK; a master's degree in food productions management from the University of Nottingham, UK; and a bachelor's degree in food science and technology from the University of Tabriz, Iran. He has published more than 10 peer-reviewed papers and is an inventor of three patents.

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Ken has a Ph.D. in engineering, is a registered professional engineer, and has obtained the academic rank of tenured professor. As an extension engineer of Agricultural & Biosystems Engineering at NDSU, he has provided education and technical assistance in grain drying and storage, structures with a focus on energy efficiency, indoor environmental engineering primarily related to moisture and mold, and flood preparation and recovery to farmers, citizens, agribusiness, and professionals across the United States and internationally since 1980.

His educational program utilizes numerous methods to provide education and technical assistance to clientele. He has authored or co-authored more than 220 publications, in addition to numerous resources that are on the internet, that are distributed by private business, professional societies, and universities internationally. Ken has presented hundreds of seminars and has provided engineering assistance to thousands of people across the United States and internationally.

He has conducted research on numerous grain drying and storage topics. In addition, he developed a system for and has conducted numerous grain dryer energy audits. He has been an expert witness and consultant for several clientele.

He is a Fellow in the American Society of Agricultural and Biological Engineers (ASABE), has served in numerous ASABE leadership roles and in other professional organizations. He was recognized in 2018 as Professional Engineer of the Year, the highest award given to a licensed professional engineer by the Society. In 2019, he was the recipient of the ASABE Sukup Global Food Security Award.

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In her role, Rebecca leads laboratory testing of foods for processing in the Neo-Pure Continuous System. She is also involved in the development and publication of external papers and presenting research findings of various projects. Prior to joining Agri-Neo, Rebecca was involved in numerous research studies specific to the prevention of foodborne diseases through the University of Guelph, Canada, where she earned her master's degree in food safety and quality assurance. She earned an honors bachelor's degree with a double major in molecular genetics and microbiology, and cell and molecular biology from the University of Toronto.

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Célia is a French food science engineer. After taking part in research projects in China and Germany during her studies at the AgroSup University in Dijon, France, she worked as a senior R&D product technologist at Unilever in the UK before becoming an R&D project manager for a major French cereals company where she was responsible for the development of new products in partnership with customers, formulation and process engineering. She joined Revtech Process Systems Inc. in Chicago where she is responsible for developing business through science by accompanying potential customers interested in existing application, developing new applications for the unique Revtech heat treatment system, and by forging close partnerships with industrial partners for mutual benefits.

BY THE NUMBERS

2019 Professional Development

IAOM-KSU Resident Courses

- **72** students from
- **7** countries in
- **6** courses

IAOM Mill Maintenance and Electrical Training

- **65** students from
- **3** countries in
- **3** classes

Milling Technician Certificate Program

- **25** students completed (since program inception)
- **58%** completion rate
- **7** - average # of students per cohort

Correspondence Course

- **773** Units sold since August 2019
- **46%** international students
- **60%** of testing online
- **111** Diplomas awarded