Soy Milk and Tofu Okara:
Baking Industry Demonstration

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Performed for:
Minnesota Soybean Research & Promotion Council

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EXECUTIVE SUMMARY

While tofu is held in high regard as a highly nutritious food source, its byproduct okara (also known as soy pulp) has proven to be a difficult coproduct stream from which to extract significant value despite its fiber, protein, and nutrient content. Since the time of the earliest production of tofu or soymilk, the great majority of all okara use is for livestock feed or fertilizer. Today, we recognize the value of this byproduct stream, but because of fresh okara's high water content, it is a perfect environment for microorganism growth, putrefying rapidly, and typically resulting in landfill disposal.

This project focused on unlocking the nutrient content of okara, for traditional and sourdough bread, via fermentation with the tempeh culture *Rhizopus oligosporus*, taking advantage of fresh okara's high moisture content by using the culture's metabolism of oils, protein, and carbohydrates into higher nutritional value compounds.

Results demonstrated that fresh okara can be reliably fermented, with a subsequent improvement in its nutrition profile. However, okara moisture content and the presence of competing spoilage microorganisms greatly impacts fermentation efficiency. As an ingredient in both traditional and sourdough bread, okara is not usable at sufficiently significant levels to

deliver a meaningful nutrition benefit, due to its negative impact on product taste and texture. Interestingly, fermented okara at a 5 percent level in traditional bread demonstrated a potential benefit as a clean label bread or dough improver, which is a potential future research area.

The final goal of this project focused on the development of non-human food value-added applications of okara on behalf of project partner MinnTofu. Unfortunately, the most promising opportunities for okara were in lower value applications such as waste to energy, specifically anaerobic digestion, and composting, as utilization can occur without necessarily needing to reduce the moisture content of wet okara.

BACKGROUND

First mentioned in a Chinese document called the *Qing yi lu* over a millennium ago, tofu is one of the most important domestic and international value-added food products derived from soybeans (Shurtleff and Aoyagi 2013). In the manufacture of tofu, soybeans are soaked, ground, pressed, and separated into two process streams: soymilk, which is coagulated into tofu, and a high moisture soy pulp byproduct known as okara. While many hold tofu in high regard as a nutritious food source, fresh okara has proven to be a difficult coproduct stream from which to extract significant value despite its fiber, protein, and nutrient content.

With sales in the United States of over $350 million in 2019, okara production spiked due to the significant increase in sales in 2020 as consumers demanded more plant-based foods in light of the COVID-19 induced meatpacking plant shutdowns (Bloomberg 2020). Tofu producers generate approximately 1.1 kilograms of fresh, wet okara from every kilogram of soybeans processed, with a dry matter content of approximately 26% protein, 12% oil, and 52% complex carbohydrates such as hemicellulose, pectin, and cellulose. Since the time of the earliest production of tofu or soymilk, the majority of all okara use is for livestock fodder, with a portion being used as an organic fertilizer.

Because of the high-water content of fresh okara, it is an ideal environment for microorganism growth and thus putrefies rapidly, typically resulting in landfill disposal if the manufacturer is located far from a livestock feedlot or rapid drying of the okara is not feasible. Fermentation of fresh okara, however, may be a way to take advantage of its high moisture content by using the metabolism of oils, protein, and carbohydrates by specific microorganisms into higher nutritional value compounds such as isoflavones. Previous Minnesota Soybean Research and Promotion Council (MSR&PC)-funded research from the Northern Crops Institute (NCI), outlined in the 2019 project report "*Improve Profitability for Soymilk Processors by Methods that Add Value to Okara Produced from Soymilk Processing,*" used this strategy to convert fresh okara into an ingredient to mitigate high fat and high-calorie human food products such as beef and baked cookies (Xu 2019).

GOALS AND OBJECTIVES

To further the prior research, AURI and NCI researchers sourced fresh okara from MinnTofu (a Twin Cities-local wholesale tofu manufacturer) for further evaluation and determination of the nutritional and sensorial benefits of okara fermentation in baking applications. Additionally, Back When Foods (an AURI collaborator with expertise in traditional sourdough applications) conducted a comparison demonstration with traditional sourdough fermentation of okara bakery products. Finally, AURI used its industry network to connect MinnTofu with regional opportunities for its core tofu business and identified opportunities to enhance profitability by supporting efforts to transform the okara waste stream into a value-added revenue generator.

Goal 1: Okara processing for value-added baking uses

Objective 1: MinnTofu okara processed by NCI using fermentation to flour process

Objective 2: MinnTofu okara processed by Back When Foods in sourdough fermentation

Goal 2: Baked product assessment

Objective 1: NCI prepared breads evaluated at NCI and in AURI's Food Product Evaluation and Sensory Laboratory

Objective 2: Back When Foods reported evaluation of okara sourdough opportunity

Goal 3: MinnTofu business development for advancing regional soy foods

Objective 1: Okara value-added opportunity assessment

Objective 2: Business development through connection to regional resources for scale-up and enhancement of product marketability

Objective 3: Business to business connections to MN soybean suppliers and product buyers

METHODS AND DATA

Goal 1, Objectives 1 and 2: Okara processing for value-added baking uses

MinnTofu provided fresh okara to NCI for fermentation by the microorganism *Rhizopus oligosporus*, a widely used starter culture for traditional tempeh. MinnTofu in Spring Lake Park, Minnesota produced the fresh okara according to the process outlined in Figure 1, and quickly transported it to NCI, located in Fargo, North Dakota.

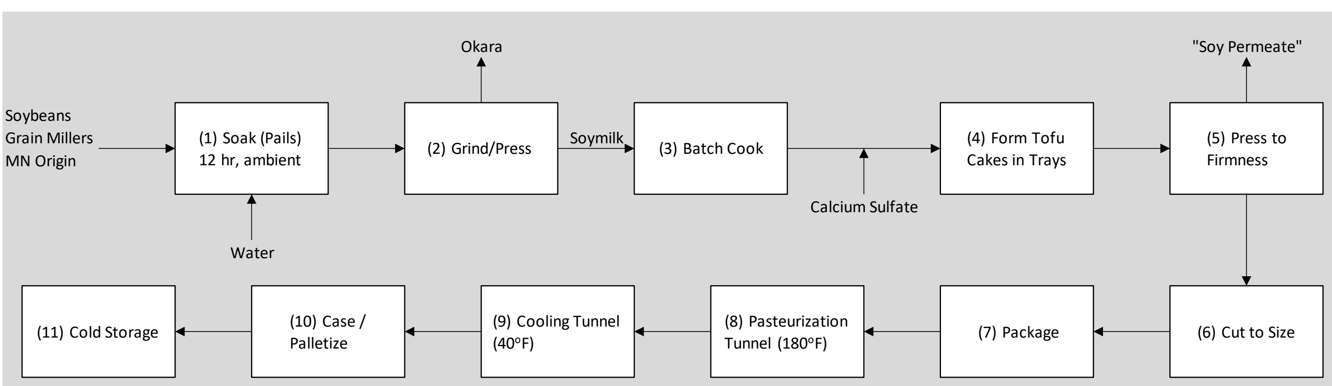


Figure 1. MinnTofu Process Flow Diagram. MinnTofu is a Spring Lake Park, MN-based wholesale tofu manufacturer. Note that okara is produced as a byproduct of grinding and pressing soaked soybeans in Step 2.

Following receipt of the fresh okara, NCI separated it into two product streams:

1. Okara flour was prepared by drying at 60 degrees Celsius and 40% relative humidity for 15 hours, followed by exposure at 110 – 120 degrees Celsius in a Lincoln Impingement

oven for 3 hours. The dried okara was then ground into fine flour for further use in baking applications.

2. Fermented okara flour was prepared by first fermenting with *Rhizopus oligosporus* according to the process outlined by Xu in the previous research project "*Improve Profitability for Soymilk Processors by Methods that Add Value to Okara Produced from Soymilk Processing*" (2019). Following fermentation, NCI dried the okara at 110 – 120 degrees Celsius in a Lincoln Impingement oven for 4 hours. In the final step, NCI ground the dried, fermented okara into fine flour for further use in baking applications.

<u>Goal 2, Objective 1: NCI prepared breads evaluated at NCI and in AURI's Food Product Evaluation and Sensory Laboratory</u>

NCI prepared bread samples in January 2021, following a 2x3 experimental design with a control, using the variables and formulation outlined in Tables 1 and 2.

Table 1. Experimental Design for NCI Baking Applications Study.

Bread Type	Whole Wheat %	Okara %	Fermented Okara %
Control	100	0	0
Okara	95, 90, 85	5, 10, 15	0
Fermented Okara	95, 90, 85	0	5, 10, 15

Table 2. NCI Bread formulation. This formulation is representative of basic whole wheat bread, with the flour composition varying according to Table 1.

Ingredients	Baker's Percent	Weight Percent
Whole wheat flour Okara Flour Fermented Okara Flour	100	47
Vital wheat gluten	5	2.3
Yeast	3	1.4
Salt	2	0.9
Light brown sugar	10	4.7
Granulated sugar	5	2.3
Dependox	0.4	0.2
Veg oil	3	1.4
Honey	4	1.9
Water	80.5	37.8

Appendix 1 contains the complete procedure.

NCI conducted quantitative bread quality analysis, measuring the following attributes:

- Baked Weight
- Loaf Volume
- Crumb Firmness
- Crumb Color
- Bread cellular structure

AURI and NCI individually conducted sensory analyses of the bread variables with trained sensory evaluators. Appendix 1, *Okara Baking Report* compiles NCI sensory analyses. Appendix 2, *Okara Breads Sensory Evaluations* includes AURI sensory analyses.

Goal 2, Objective 2: Back When Foods reported evaluation of okara sourdough opportunity

The sourdough process of making bread products is one of the oldest biotechnological processes in cereal food production. Generally, sourdough bread is prepared from a combination of flour and water and fermented using lactic acid bacteria as a means of leavening. The result is typically a pleasant and sour-tasting end product, which has gained popularity as a means to improve the quality and flavor of wheat breads (Chavan and Chavan 2011).

In this project, Back When Foods, Inc. added previously unfermented okara flour to its Type 1 Sourdough French Bread process, whereby fermentation begins by using a "starter" from a previous fermentation. This method requires a 6-hour fermentation cycle before baking at 450 degrees Fahrenheit and uses only the flour blends outlined in Table 3 with distilled water, Himalayan salt, and Type 1 culture as ingredients.

Table 3. Experimental Design for Back When Foods Type 1 Sourdough French Bread Baking Study.

Bread Type	Bread Flour %	Okara %
Control	100	0
Okara	95, 90, 85	5, 10, 15

A full description of the Back When Foods, Inc. test protocol and results are in Appendix 3, *Okara Flour as an Inclusion to Type 1 Sourdough French Bread.*

Goal 3: MinnTofu business development for advancing regional soy foods

Using AURI's scientific and business development expertise, coupled with extensive regional networking capabilities, AURI assessed tofu production efficiency improvements and value-added okara opportunities for MinnTofu. Results of these assessments are included in the next section of this project report.

RESULTS & DISCUSSION

Goal 1, Objectives 1 and 2: Okara processing for value-added baking uses

Unfermented okara: Approximately 170 pounds were dried and ground into flour for use in a baked whole grain bread experimental design.

Key learning: Okara must dry quickly to avoid the onset of spoilage.

Fermented okara: Approximately 30 pounds were produced, dried, and ground into flour for use in a baked whole grain bread experimental design.

Key learning: Upon receipt at NCI, wet okara (approximately 80% moisture) showed signs of spoilage. As a result, the team saw a significant reduction in fermentation efficiency. While project partners obtained enough fermented okara flour to complete the baked whole grain bread experimental design, they undertook additional studies to better understand the root cause and impact on fermentation efficiency:

1. Qualitative Shelf-Life Study: NCI conducted a qualitative shelf-life study on raw, wet okara from MinnTofu. Results indicated that raw, wet, uncooked okara from MinnTofu exhibits noticeable spoilage microorganism growth within the first 24-48 hours of ambient storage. Refrigeration will delay this growth curve, but product spoilage will be obvious within 72 hours of production if one does not take mitigation steps.

2. Fermentation Efficiency Study: With the decrease in fermentation efficiency observed by NCI (which was noticeably different from the fermentation efficiency observed when using okara generated from the NCI process outlined in the previous research project "*Improve Profitability for Soymilk Processors by Methods that Add Value to Okara Produced from Soymilk Processing*" (2019)), NCI conducted a trial to mimic MinnTofu's process conditions in the NCI lab. The purpose of this study was to evaluate the fermentation efficiency of MinnTofu-processed okara versus NCI-processed okara without the transport lag time from the MinnTofu facility in Spring Lake Park, MN, to the NCI lab in Fargo, ND, that allowed for the growth of competing spoilage microorganisms.

   Note that the primary processing differences involve (1) a cook of the bean mash before okara and soymilk separation (effectively pasteurizing the okara), which is present in the NCI process but absent in the MinnTofu process; and (2) a soymilk/okara separation press at NCI (resulting in 70% moisture okara) versus a centrifugation process at MinnTofu (resulting in 80% moisture okara). See Figure 1 process schematic repeated here.

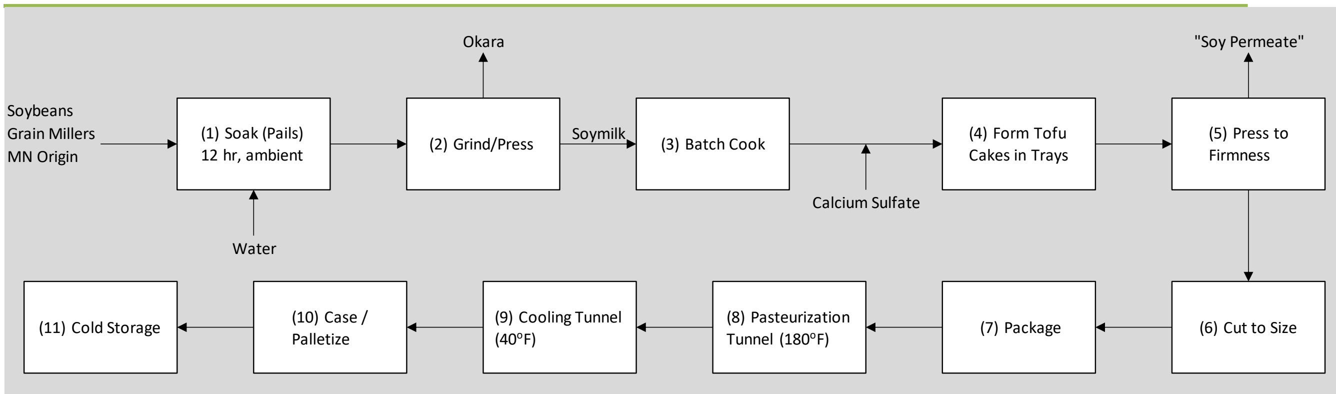
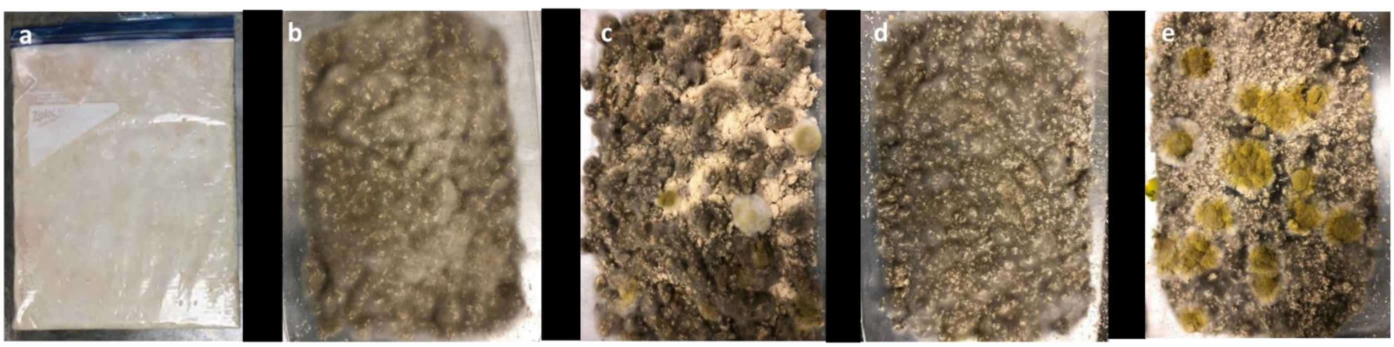


Figure 1. MinnTofu Process Flow Diagram. In the NCI tofu process, the batch cook (step 3) comes before the grind/press separation of soymilk and okara (step 2). Additionally, separation of the soymilk and okara (step 2) is done with a press at NCI, versus a centrifugation process at MinnTofu. This results in 70% moisture okara from NCI versus 80% moisture okara from MinnTofu.

Results of the fermentation efficiency study indicated a significant correlation to the okara pre-fermentation moisture content and microorganism load. Figure 2 summarizes these results.



70% Mois Pasteurized (2 day fermentation) | 70% Mois Unpasteurized (4 day fermentation) | 80% Mois Unpasteurized (7 day fermentation) | 24 hr Inoculation Delay 80% Mois Unpasteurized (5 day fermentation) | 48 hr Inoculation Delay 80% Mois Unpasteurized (5 day fermentation)

Figure 2. Okara Fermentation Efficiency Dependence on Moisture Content and Initial Microorganism Load. Note the source of (a) was from previous NCI research (Xu 2019), while samples (b) – (e) were produced in the current study at NCI. Okara samples (a), (b), and (c) were inoculated with *Rhizopus oligosporus* immediately upon creation, while okara samples (d) and (e) were held in refrigerated storage for 24 and 48 hours, respectively, before inoculation.

Okara fermentation efficiency is dependent on moisture content. The high moisture content of MinnTofu okara (approximately 80%, Figures 2c, 2d, and 2e) relative to NCI-produced okara (at 70% moisture, Figures 2a and 2b) reduces fermentation efficiency substantially. In Figures 2a and 2b, the culture has completely matured within 4 days when the starting okara moisture was at 70%, regardless of reduction of the okara microorganism load through a pasteurizing treatment. In contrast, Figure 2c demonstrates incomplete growth after 7 days with a starting moisture of 80%.

Okara fermentation efficiency is dependent on the starting microorganism load. Microorganisms present in unpasteurized okara compete with the tempeh culture *Rhizopus oligosporus* after inoculation. Figure 2 illustrates this through:

- *Color of fermentation products*. Figure 2a represents previous research completed by NCI (Xu 2019). Contrast the white fermentation products created when pasteurized okara (low initial microorganism load) was inoculated with *Rhizopus oligosporus* to the gray and multicolor fermentation products created when unpasteurized okara (high initial microorganism load) was the substrate inoculated (Figures 2a – 2e).
- *Speed of fermentation*. Fermentation time to reach culture maturity doubled when using unpasteurized okara (Figure 2b, 4 days) versus pasteurized okara (Figure 2a, 2 days).
- *Unknown microorganism growth if delayed inoculation occurs*. When inoculation of unpasteurized okara is delayed, unknown microorganisms propagate and compete with *Rhizopus oligosporus*. Note the multicolor fermentation products in Figures 2d (24-hour inoculation delay) and 2e (48-hour inoculation delay) versus the monotone fermentation products in Figures 2b and 2c (with no inoculation delay).

Nutritional impact of fermentation. Chemical analysis of both fermented and unfermented okara determined the nutritional impact and value add of okara fermentation. A literature review of published research prioritized the selection of appropriate chemical analyses. The results of these analyses are in Table 4.

Table 4. Chemical analysis results comparing key nutritional attributes of unfermented versus fermented okara flour.

Analysis		Units	Unfermented Okara	Fermented Okara
Isoflavones	Daidzein	Parts per million	54.3	428
	Daidzin	Parts per million	61.5	17.3
	Genistein	Parts per million	81	682
	Genistin	Parts per million	95.6	34.9
	Glycitein	Parts per million	10.8	67.6
	Glycitin	Parts per million	14.9	< 10
	Total as Glucosides	Parts per million	407	1950
Vitamin B_{12}		mcg/100g	< 0.1000	1.066
Antioxidant Activity		micromoles TE/100 g	2800	3300
Dietary Fiber	Total	%	46.4%	52.8%
	Soluble	%	13.0%	7.8%
	Insoluble	%	33.4%	45.0%
GABA		micrograms/g	< 5.00 (ND)	< 5.00 (ND)

Soy Isoflavones and dietary fiber. Previous research completed by NCI (Xu 2019) indicated an increase in soy isoflavones and conversion of fiber from insoluble to soluble upon fermentation with *Rhizopus oligosporus*. Past reports indicate soy isoflavones have estrogenic, anticarcinogenic, antiosteoporotic, anti-hypertensive, and other positive biological effects (Kuligowski et al. 2017). Dietary fiber intake is associated with reduced body mass index, body weight, and body fat. There are indications soluble fiber helps reduce low-density lipoprotein (LDL cholesterol), while insoluble fiber aids in digestive health and prevents constipation. The Food and Drug Administration's Recommended Daily Value for total dietary fiber is 14 grams per 1,000 calories consumed but does not make a recommendation as to the appropriate ratio of soluble to insoluble fiber.

Key Learning (Isoflavones): Fermentation increased the total isoflavone content, while also converting glucoside isoflavones (daidzin, genistin, and glycitin) into aglycone isoflavones (daidzein, genistein, and glycitein). Figure 3 summarizes these data.

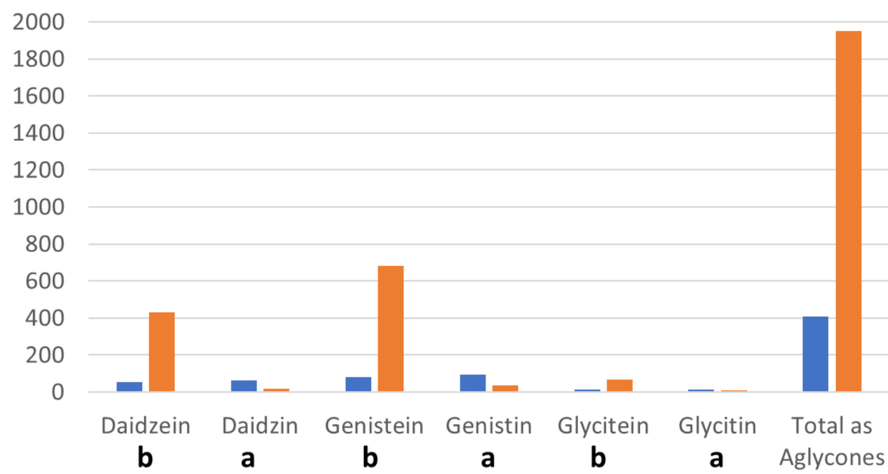


Figure 3. Fermentation Impact on Okara Isoflavones. Isoflavones are characterized as glucosides (a) and aglycones (b).

Humans absorb isoflavone aglycones faster than their glucosides, thus indicating an improvement in the amount and speed of absorption (Izumi et al., 2000).

Key Learning (Dietary Fiber): Interestingly, total dietary fiber increased upon fermentation, likely due to the presence of the *Rhizopus* organism itself, reported in research to contain 5% crude fiber (Che Omar & Suan Li, 1993). The conversion of insoluble fiber to soluble fiber upon fermentation, as reported in the MSR&PC study titled *Improve Profitability for Soymilk Processors by Methods that Add Value to Okara Produced from Soymilk Processing* (2020), was not consistent with this research. This fiber conversion is an interesting phenomenon, the mechanism of which requires additional research beyond the scope of this project.

Vitamin B_{12} and Gamma aminobutyric acid (GABA). Vitamin B_{12} and GABA levels significantly increase in fermented soy products (Jayachandran & Xu 2019). GABA is an amino acid that acts as a neurotransmitter and has hypotensive effects, while Vitamin B_{12} is vital for DNA synthesis and healthy blood cells. According to the National Institutes of Health, Office of Dietary Supplements, Vitamin B_{12} is only found naturally in animal products, thus a non-animal-derived source is especially important for individuals following vegetarian and vegan diets.

Key Learning (Vitamin B_{12}): Fermentation increased (by at least a factor of 10) the content of Vitamin B_{12} in okara. According to the National Institutes of Health, Office of Dietary Supplements, the Recommended Daily Intake of Vitamin B_{12} for an adult is 2.4 micrograms – meaning that fermented soy okara could contribute a meaningful amount of Vitamin B_{12} to the human diet.

Key Learning (GABA): Despite research indicating the formation of GABA upon fermentation in soy foods ((Jayachandran & Xu, 2019), the presence of GABA in soy okara, whether fermented or not, was below the detectable limit. Thus, this study would conclude that the conditions of fermentation were unable to form detectable amounts of the amino acid GABA.

Antioxidant activity. Oxidative stress in the body occurs because of an imbalance between free radicals and the antioxidant mechanism, resulting in a variety of diseases including diabetes, cancer, etc. (Jayachandran & Xu, 2019) – thus a measure of the antioxidant activity of fermented okara versus unfermented okara is crucial to understanding the benefits of fermentation.

Key Learning (Antioxidant Activity): Fermentation increased the antioxidant capacity of okara by 18%. In comparison with published values also using the 2,2'-Diphenyl-1-Picrylhydrazyl (DPPH) method described by Plank et al (2012), the absolute values of antioxidant activity for both fermented and unfermented okara fall within the range of data reported for red wine. This indicates that the 18% increase in antioxidant capacity seen when fermenting okara may not be statistically significant, though more data points would be required to reach a statistically valid conclusion.

A ranking of the antioxidant activity of okara (whether fermented or unfermented) versus published common foods data demonstrates it is in line with ready-to-eat cereal, red wine, and pomegranate juice, significantly above blueberry juice, green tea, and carrot juice, and significantly below cocoa powder, pomegranate concentrate, pomegranate by-product/pulp, and pomegranate arils (Figure 4).

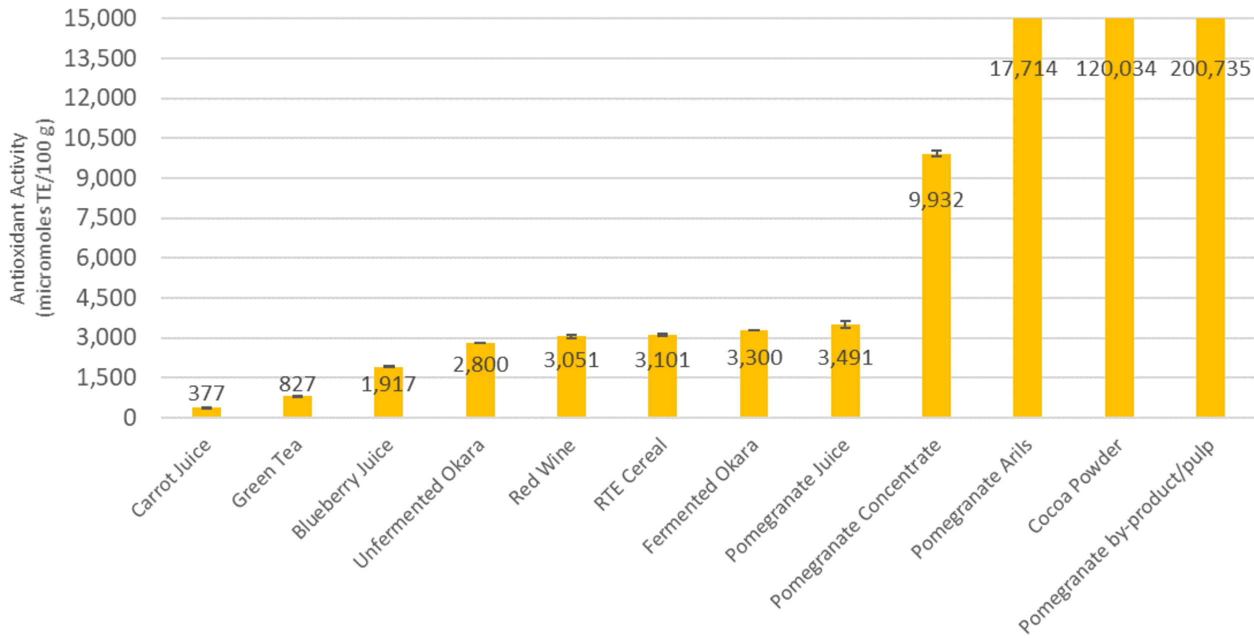


Figure 4. Antioxidant Activities of Common Foods as Compared to Okara. Common food antioxidant activities reported by Plank et al., 2012 using the 2,2'-Diphenyl-1-Picrylhydrazyl (DPPH) method. Error bars represent a 95% confidence interval around the dataset means.

Goal 2, Objective 1: NCI prepared breads evaluated at NCI and in AURI's Food Product Evaluation and Sensory Laboratory

Bread quality analysis and sensory results indicate a noticeable impact on the performance of breads formulated with okara and fermented okara, respectively, relative to the control. See bread variable comparisons in Figures 5 and 6. Full results are available in Appendix 1, *Okara Baking Report* (NCI), and Appendix 2, *Okara Breads Sensory Evaluations* (AURI). Key learnings are outlined below.

Figure 5. Baked Bread Loaf Comparison at Varying Levels of Okara Flour Replacement.

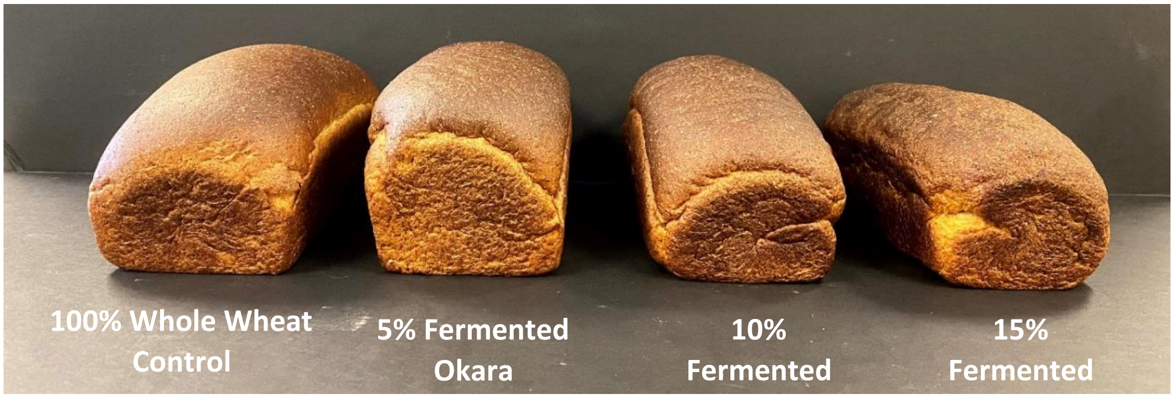


Figure 6. Baked Bread Loaf Comparison at Varying Levels of Fermented Okara Flour Replacement.

Bread Quality Analysis. Loaf volume decreased (up to 41%) and crumb firmness/bread hardness increased (up to 120%) with increasing unfermented okara in the formulation. Loaf volume increased slightly, by 4.6%, at the 5% inclusion rate of fermented okara, then decreased with increasing amounts of fermented okara. Crumb firmness/bread hardness dropped significantly (up to 48%) at low levels of fermented okara, with this softening effect disappearing at the highest inclusion rate of fermented okara.

Sensory Evaluation of Bread. Sensory evaluation results align with the baked product performance attributes outlined above, relative to the whole grain control.

The inclusion of okara flour resulted in tougher, denser, tighter crumb structure bread that wicks moisture from the mouth, with a notably bitter flavor and aftertaste concentrated in the darker baked crust. These effects increased linearly with the okara inclusion rate, producing an unpleasant experience at rates higher than 5%.

The inclusion of fermented okara flour demonstrated similar appearance effects, though the texture was notably softer and more cohesive upon chewing. These effects seemed to decrease with an increase in the fermented okara inclusion rate. Bifurcation of bread flavor between slightly sweet on the interior and unpleasantly bitter in the crust occurred.

Overall, these results suggest that replacement of wheat by okara is tolerable up to 5%, whether fermented or not. Further, fermented okara improved the performance and sensory characteristics of bread at the 5% level, indicating the potential for fermented okara to act as a "clean label bread texture improver," though further research is necessary to validate these findings and identify the mechanism of this improvement activity.

<u>Goal 2, Objective 2: Back When Foods reported evaluation of okara sourdough opportunity</u>

Back When Foods, Inc. prepared and evaluated Type 1 Sourdough French Bread using unfermented okara flour produced at NCI according to the method previously described. Full results are available in Appendix 3, *Okara Flour as an Inclusion to Type 1 Sourdough French Bread* (Back When Foods, Inc.).

In summary, the baked performance of okara sourdough bread produced at Back When Foods, Inc. was very similar to that seen in whole wheat bread baked at NCI.

Bread Quality Analysis. Loaf volume correlated inversely to okara content: as the percentage of okara in the bread increased, the baked volume of the bread decreased, to approximately 20% of the control at the highest level of okara replacement (see Figure 7).

Figure 7. Baked Sourdough Bread Loaf Comparison at Varying Levels of Okara Flour Replacement. Note that the 0% okara flour control sample is not shown.

Sensory Evaluation of Bread. Sensory evaluation indicated that increasing levels of okara in the sourdough bread resulted in a moister, "gummy," undesirable texture, with a strong acetic acid/vinegar flavor profile. Additionally, crust color darkened noticeably as the level of okara in the bread increased.

In the words of owner and chief baker of Back When Foods, Inc., Brian LaPlante, "We would not choose to add a bread containing okara flour to our product line. The flavor and quality of the final product are not beneficial. The okara flour creates a flavor that is not complementary to our flavor profiles."

Goal 3: MinnTofu business development for advancing regional soy foods

The U.S. Department of Agriculture (USDA) estimates that 30 to 40 percent of food in the United States goes to waste (Lemke 2020). According to ReFED, a national nonprofit dedicated to ending food loss and waste across the U.S. food system, further analysis reveals that, in Minnesota, 28% of this total waste is attributable to manufacturing, highlighted in Figure 8 (ReFED 2021).

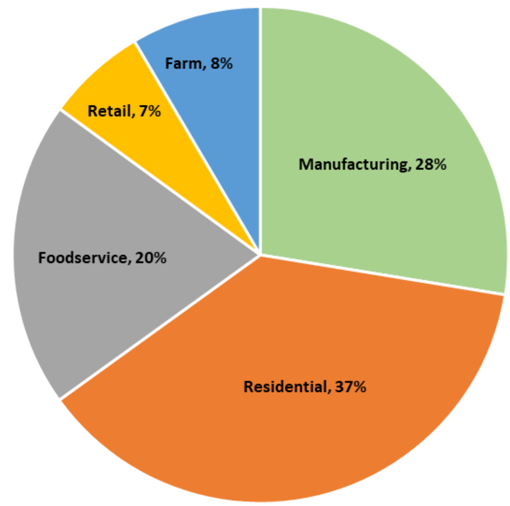


Figure 8. Food Loss and Waste by Sector in Minnesota – 2019. Data from the ReFED Food waste monitor (2021). Total estimated Minnesota food waste in 2019: 997,000 tons.

In response, the U.S. Environmental Protection Agency developed a Food Recovery Hierarchy (Figure 9), which prioritizes actions organizations can take to prevent and divert wasted food. Each tier of the hierarchy focuses on different management strategies for dealing with wasted food. The top levels of the hierarchy are the most impactful ways to prevent and divert wasted food because they create the most benefits for the environment, society, and the economy. The hierarchy places a higher value on preventing food waste and loss or on repurposing those products for food use than on diverting the waste materials to animal feed or energy generation (Lemke 2020).

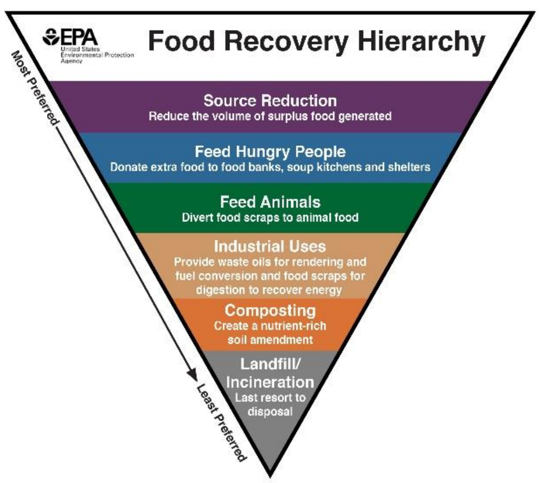


Figure 9. Food Recovery Hierarchy from the U.S. Environmental Protection Agency. https://www.epa.gov/sustainable-management-food/food-recovery-hierarchy

In conjunction with the Food Recovery Hierarchy, AURI assessed multiple scenarios to manage the okara byproduct. Currently, MinnTofu disposes of up to one ton per week of raw, wet okara in the local landfill.

Scenario 1: Source Reduction of Okara

MinnTofu's process currently produces 80% moisture okara, at a rate of up to one ton per week. By comparison, the NCI pilot-scale process produces okara at 70% moisture. This indicated that MinnTofu could potentially improve the efficiency of their process by extracting more soymilk from their soybeans, thereby increasing their tofu output and reducing their okara output (by weight). AURI conducted a process test with the AURI Waseca, MN facility to determine the value of a dewatering screw press after generating the okara, to extract this additional soymilk, on April 6, 2021.

Key Learning: In summary, the texture of wet okara (similar to mashed potatoes) did not allow for successful testing on the Vincent Dewatering Screw Press. This technology operates on the principle of "squeezing" liquid out of organic material by pressing the organic material against a screen, operating in a continuous fashion, whereby the liquid would be expressed through the screen and the organic material would remain on the interior side of the screen, conveyed away by the screw (see Figures 10 and 11). In this case, wet okara was pushed directly through the screen, and the screw press was unable to achieve sufficient pressure to extract additional liquid from the solid soy material.

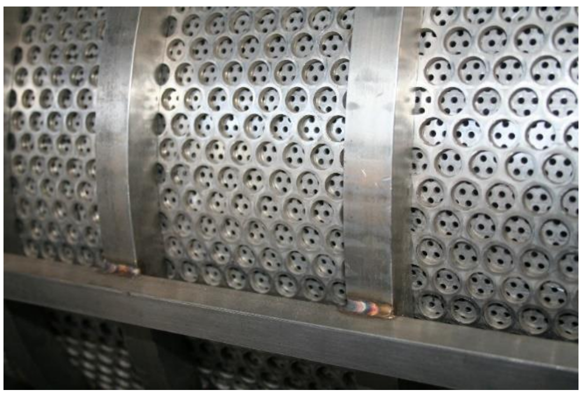


Figure 10. Screen Assembly for Screw Dewatering Press. A screw mechanism operating inside this screen assembly pushes organic material against the screen to dewater. Photo courtesy of https://vincentcorp.com/content/operating-hints-cp-6-thru-cp-12-presses

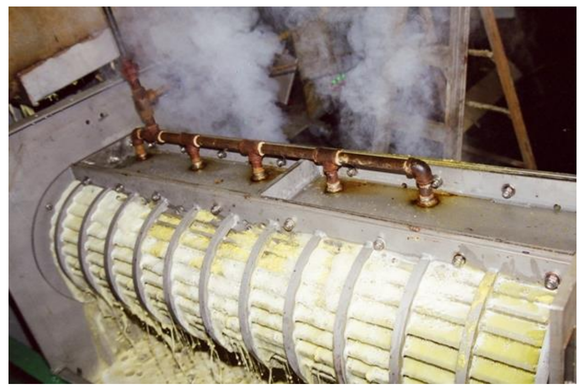


Figure 11. Dewatering Press in Operation – Pineapple Juice. Note the liquid flowing through the screen. Photo courtesy of https://vincentcorp.com/content/operating-hints-cp-6-thru-cp-12-presses

As a result of this unsuccessful trial, AURI determined that the current MinnTofu centrifuge process cannot extract more soymilk from soybeans and that a source reduction of okara by-product was not achievable.

Scenario 2: Repurpose Okara as Human Food

Given the remaining nutrient quality of okara post-tofu processing, this project intended to identify new human food applications, particularly in baking. As previously demonstrated, okara is readily fermentable using the standard tempeh starter culture *Rhizopus oligosporus*, resulting in an ingredient with an enhanced micronutrient profile from its starting point. However, this project also demonstrated limitations of okara use in human food, namely:

1. The moisture content of raw, wet okara limits its ingredient potential without significant additional processing (primarily, application of thermal energy to pasteurize and reduce its water content).
2. When prepared appropriately, the use of okara as an ingredient is limited by the sensorial attributes it imparts to the final product, demonstrated in both this and previous research (Xu 2019).

As such, the team suggests that, unless there is a willingness to invest in substantial drying capacity to stabilize okara, the producer should consider a soybean-forward product line such as tempeh, whereby the okara is the fermentable substrate. As a matter of fact, in a *FoodNavigator-USA* article from June 15, 2021, Greenleaf Foods president Dan Curtin stated, "there's tremendous growth in tempeh…it's probably the most versatile superfood product out there" (Watson 2021).

While defining and validating tempeh production from MinnTofu okara was outside the scope of this project, the previous research by Xu (2019) described a process whereby fresh okara is easily and efficiently fermented into tempeh using *Rhizopus oligosporus* inoculation following a soybean cook at 84^{o}C / 183^{o}F (effectively pasteurizing the resulting okara) and subsequent soymilk/byproduct separation, producing "clean" okara at approximately 70% moisture. To apply the described tempeh process, MinnTofu would need to invest in two unit operations:

1. A drying process that simultaneously (a) reduces okara moisture from 80% to a maximum of 70% and (b) effectively pasteurizes the raw okara to eliminate competitive microorganisms in preparation for inoculation of the tempeh culture *Rhizopus oligosporus.*
2. An incubation process that produces conditions to grow the tempeh culture effectively and efficiently.

Scenario 3: Repurpose Okara as Animal Food

While the protein content of raw, wet okara (26% by weight) indicates high value as an animal feed, particularly livestock feed, the economic analysis indicates considerations beyond simply the crude protein and digestible nutrients content as an indicator of value.

The feed market typically makes comparisons for new opportunities against the following commodities:

1. Corn: energy source for livestock and poultry diets
2. Soybean Meal (SBM): protein source for livestock and poultry diets
3. Alfalfa Hay: protein and energy source for ruminant animals (dairy, beef cattle)

With that background, AURI completed a feed value estimate for okara versus these primary commodities, outlined in Table 5.

Table 5. Feed value estimate for okara. Commodity prices listed are estimates only, thus this comparison is purely for directional purposes. Note that corn pricing is listed on a per bushel basis - using a standard weight of 56 pounds per bushel of corn, the commodity pricing of $4.50/bu is equivalent to $160.71/ton.

Product	$4.50/bu. Corn, $375/ton SBM & $175/ton Alfalfa hay
Okara "As-Is" with 81% moisture	$26.84 per ton
Okara if dried to 10% moisture	$217.43 per ton

According to this analysis, based solely on its feed value, the okara producer could expect to sell raw, wet okara at $26.84 per ton or dried okara at $217.43 per ton. However, the feed value of okara does not tell the entire economic story.

In the case of wet okara, freight adds a significant cost that reduces its feed value. Assuming a cost of $4.00 per loaded mile for a 24-ton truck, the freight cost to ship raw, wet okara just 50 miles reduces its feed value by approximately $8.00 per ton, to $18.84 per ton. In the specific case of MinnTofu, the rapid growth of spoilage microorganisms outlined previously limits animal feed usage opportunities for raw, wet okara to geographically adjacent feedlots for maximum value. Note that this analysis does not consider on-site storage costs for raw, wet okara, nor does it consider the potential cost discount for "old" okara suffering from extreme spoilage because of storage time to fill a 24-ton truck.

While dried okara value generates significantly more value for the okara producer (by concentrating the protein content and eliminating the potential for spoilage), it's important to consider the cost to dry okara from approximately 80% moisture to 10% moisture at $217.43 per dry ton of okara. This analysis assumes a natural gas fuel cost of $4.00 per million BTUs, with a resulting cost to dry okara of $31.50 per dry ton, reducing the feed value of dried okara to $185.93 per dry ton. This value is before adding in additional costs of labor, facility overhead, and capital to install the drying technology itself.

In conclusion, while there is value as a feed source, topline economic analysis indicates that the full okara feed value is likely in line with (and potentially lower than) existing commodity sources, presenting no significant benefit. Comprehensive economic analysis of all considerations is recommended before any capital investments are made to service the feed market.

Scenario 4: Repurpose Okara as feedstock for Anaerobic Digestion (Waste to Energy)

Anaerobic digestion is a process through which bacteria break down organic matter in the absence of oxygen, in a sealed vessel called a bioreactor. These reactors contain complex microbial communities that break down (or digest) the organic matter and produce resultant biogas, which is usable as an energy source like natural gas, and digestate (the solid and liquid material end-products), which can be used in many different applications as a coproduct revenue stream (EPA 2021).

As anaerobic digestion is a complex fermentation process regulated by a bacteria consortium, it is sensitive to the type and composition of organic matter, or substrate, used as the reactor input. Specific substrates are utilized by specific types of bacteria, thus the proportions of organic waste used in the reactor input determine the process rate and efficiency (Yulin et al. 2011). Reactor conditions also impact process rate and efficiency, as specific types of bacteria thrive under different temperature conditions – for instance, mesophilic bacteria at 11 – 45^{o}C (52 – 113^{o}F) and thermophilic bacteria at 46 – 75^{o}C (115 – 167^{o}F).

As a source of organic matter for anaerobic digestion, Yulin et al. found 93% of okara input into their theoretical bioreactor fermented to methane biogas, or biomethane, under mesophilic conditions (2011), indicating that the nutrient content of wet okara is an excellent substrate for anaerobic digestion. Additionally, AURI posits that one could consider the starch content of wet okara to be "rocket fuel" as it pertains to biomethane production under thermophilic conditions, with the appropriate bacteria consortium.

In summary, the potential of wet okara from MinnTofu as a substrate for anaerobic digestion suggests further research is necessary to determine both its biomethane potential and the risk that okara spoilage microorganisms present to the digester bacteria. To minimize transport costs, the team recommends exploring the economic potential of regional or Twin Cities-based anaerobic digester projects.

Scenario 5: Repurpose Okara as Compost or Fertilizer

While exploring the potential of raw, wet okara as compost or fertilizer, AURI interviewed former client [Mississippi Topsoil] in addition to conducting literature research.

In comparison to cow manure, poultry manure, and sewage sludge composts, okara compost–treated soil had the most readily decomposable carbon fraction as determined by carbon dioxide evolution. Additionally, the microbial biomass of okara–treated soil, used as an indicator of soil quality and nutrient availability to plants, contained a higher amount of accumulated biomass carbon than the other composts (Miyittah and Inubushi 2003). Taken together, these results suggest that an okara compost is suitable for stabilizing and supplying carbon and increasing the humus content of soils.

However, Mississippi Topsoil, a business dedicated to sustainably creating superior soil products based in Cold Spring, MN, highlighted a familiar economic consideration for the use of okara as a compost – its high moisture content would require transportation of a significant amount of water (bound in the okara) to its composting facility. Additionally, due to the consistency and "compressibility" of wet okara, it would likely present challenges related to poor ventilation of the compost matrix, requiring significant management to overcome. As such, Mississippi Topsoil would not be interested in such a composting material for their business, but this does not discount the potential of okara as a material for a MinnTofu-adjacent compost site.

Fertilizer represents an alternative use for okara as a soil amendment. All commercial fertilizer products are characterized by the proportion of nitrogen (N), phosphorus (P), and potassium (K), known as the N-P-K ratio (or simply, NPK). A fertilizer's NPK reflects the percentage by weight of each nutrient in the fertilizer, thus allowing for comparisons based on the basic nutrients each commercial fertilizer formulation provides. According to AURI, okara, on a dry basis (0% moisture), has an NPK ratio of approximately 3-1-2, with a value of $39.84 per dry ton. On a wet basis (80% moisture), the NPK ratio for okara is approximately 0.6-0.2-0.3, with a value of $7.19 per ton. In comparison to commercially available home garden fertilizers, which typically have an NPK ratio of 10-10-10 at a cost to the end consumer of approximately $700 per dry ton, okara may represent a value to the home gardener. Practically speaking, however, the lower NPK ratio of okara (and in the case of wet okara, significantly lower) requires a higher application rate than commercial fertilizer. This is likely to impact the ability of water and other nutrients to reach the root system of garden plants; therefore, the ideal okara fertilizer application (if not used as an ingredient in a commercial fertilizer) is for home garden soil amendment to till in during the fall following harvest.

Note that this analysis, once again, identifies the greatest value from okara in its dry form, indicating that the fertilizer value of okara must be reduced by the capital and operating cost of drying. Using the same drying cost analysis as outlined for okara's feed value, the fuel cost to dry okara from 80% moisture to 0% moisture is $54.50 per dry ton – which exceeds its fertilizer value. Wet okara, while eliminating the cost of drying, offers little value as a fertilizer; hence, the recommendation of usage as a till-in fall soil amendment added post-harvest.

SUMMARY & OUTREACH

While tofu is held in high regard as a nutritious food source, fresh, wet okara has proven to be a difficult coproduct stream from which to extract significant value despite its fiber, protein, and nutrient content. Since the time of the earliest production of tofu or soymilk, the majority of all okara use is as livestock feed, fertilizer, or as "food for the poorer classes" (Shurtleff and Aoyagi 2013). Today, we recognize the value of this byproduct stream, but because of fresh okara's high water content, it is a perfect environment for microorganism growth, putrefying rapidly, and typically resulting in landfill disposal.

This project focused on unlocking okara's nutrient content for human food through fermentation with the traditional tempeh culture *Rhizopus oligosporus*, taking advantage of wet okara's high moisture content by using the culture's metabolism of oils, protein, and carbohydrates into higher nutritional value compounds. The fermented okara was then dried, ground into flour, and compared in leavened bread baking applications with unfermented okara.

Results demonstrated that fresh okara can be reliably fermented, with a subsequent improvement in its nutrition profile. However, okara moisture content and the presence of competing spoilage microorganisms significantly impacts fermentation efficiency. Future work should focus on optimizing the fermentation process by reducing the moisture content and decreasing the spoilage microorganism load in the fresh okara substrate.

As an ingredient in both traditional and Type 1 sourdough bread, neither fermented nor unfermented okara is usable at sufficiently significant levels to deliver a meaningful nutrition benefit, due to the negative impact of okara on product taste and texture. Thus, this study concluded one should hold okara inclusion as a bread ingredient to 5 percent of the total flour or less. Interestingly, the inclusion of fermented okara at the 5 percent level in traditional bread demonstrated a potential benefit as a clean label bread or dough improver, that could be explored in future research.

The final goal of this project focused on the development of non-human food value-added applications of okara using AURI's scientific and business development expertise on behalf of project partner MinnTofu. Unfortunately, AURI found that the most promising opportunities for the okara byproduct stream were in lower value applications such as waste to energy, specifically anaerobic digestion, and composting as they are applicable without necessarily needing to reduce fresh okara's high moisture content. However, all applications would see an increase in value if a cost-effective drying approach could be defined.

Following completion of the research, AURI used its AURI Connects *Webinar Wednesday* platform to disseminate results on August 11, 2021, incorporating all project partners. Promotion of the webinar event, "Value-Added Opportunities for Tofu Okara," included social media and AURI's email list of over 5,000 subscribers and past webinar attendees including government, industry, entrepreneurs, researchers, businesses, Research & Promotion Council. The event attracted 59 registrants of which there were 29 engaged and passionate unique viewers during the live session. A lively panel question and answer session necessitated extending the webinar 20 minutes past the originally scheduled ending time. A recording of the webinar is available on AURI's website at https://auri.org/events/value-added-opportunities-for-tofu-okara-webinar/, and the slides presented are included as an attachment in Appendix 4.

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Appendix 1: Okara Baking Report (authored by NCI)

Please see PDF Attachments to view this file.

Appendix 2: Okara Breads Sensory Evaluations (authored by AURI)

Report - AURI
Sensory Eval of NCI B

Appendix 3: Okara Flour as an Inclusion to Type 1 Sourdough French Bread (authored by Back When Foods, Inc.)

Okara Flour as an
Inclusion to Back Whe

Appendix 4: AURI Connects Webinar Wednesday Slide Deck – August 11, 2021

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Webinar FINAL.pdf