Using Perennial Grain Crops in Wellhead Protection Areas to Protect Groundwater: *Objective 3 – Determining Optimal End-Uses for IWG*

UNIVERSITY OF MINNESOTA

Agricultural Utilization Research Institute

Submitted to the LCCMR

Project Title:	Using Perennial Grain Crops in Wellhead Protection Areas to Protect Groundwater – *Activity 3: Grain & Biomass testing to determine optimal end uses for IWG/ Kernza®*
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EXECUTIVE SUMMARY

AURI, as a partner with the University of Minnesota, spent time under this LCCMR grant working towards developing Activity 3- focused on grain and biomass testing to determine optimal end uses for IWG/ Kernza®. Work centered on assessing food quality metrics for the newly released Kernza variety from the University of Minnesota, analyzing biomass for utilization potential, as well as initial process development investigation on processing, cleaning, and dehulling. AURI's business development team also began initial work of starting discussions with potential end users in the state of Minnesota, in order to facilitate supply chain development. There are several areas within this report that can be pulled out as "one-pager" informational sheets or application bulletins. This allows the work done by the technical team under the grant to be easily distributed by the business development team to potential end-users or processors. AURI is currently partnering on a grant led by the Stearns County Soil and Water Conservation District focused on technical and Central MN supply chain development of Kernza. The information and knowledge gained through this project will allow AURI to make an informed transition into this second grant, and to build upon accomplishments of this first project.

ACKNOWLEDGEMENTS

Funding provided by the Minnesota Environment and Natural Resources Trust Fund.

PROCESS DEVELOPMENT at AURI—CLEANING AND DEHULLING

AURI researched and tested various cleaning, dehulling and separation techniques for Kernza grain in this project. AURI was able to source small samples of both MN-1502 and Clearwater (experimental name MN-1504) grain from project partners at the University of Minnesota. MN-1502 was an unreleased variety candidate, but has traits similar to the released variety, MN-Clearwater. This grain procurement allowed AURI to test different methods of cleaning and dehulling, and also provided grain and hulls for other analytical testing carried out under this project. Three lots of grain were received in total, two lots of MN-Clearwater and one lot of MN-1502. The MN-1502 lot and one of the MN-Clearwater lots were sourced from Roseau, MN, while the other batch of MN-Clearwater was a composite of four different fields in different regions of Minnesota. The batch of MN-Clearwater from Roseau was cleaned prior to AURI receiving it. The other two lots were received directly from the combine and were found to have between 20-30% dockage. The makeup of the dockage, or waste material, varied by where and how the grain was grown, but generally it consisted of hulls, weed seeds, other grain seeds from previous growing seasons and pieces of straw and other biomass that may have come through the combine. After removal of dockage, about 20-30% of the grain weight in the MN-Clearwater lots appeared to be free-threshed or dehulled in the combine and/or post-harvest handling of the grain. The MN-1502 sample had a majority (90+%) of the grain free threshed, believed to be due to significant post-harvest handling of the grain and not considered representative of other samples. After removal of dockage and hulls the final weight of dehulled grain was around 40% of the original lots. There was some ergot affected grain which remained in the finished lots and was not removed. Roughly 1-5% of Kernza kernels received were affected by ergot.

AURI's lab experience, as well as discussions with project contacts, indicated a significant spread regarding dockage, free threshed grain, and ergot-affected grain due to many different factors. Factors included, but were not limited to, harvesting practices, number of times the lots were handled, and dead heads/naked grains (closed hulls with no grain inside). Additionally, ergot levels created differences between lots. There can be a small percentage of good grain sacrificed during processing due to grain size inconsistencies and requirements to satisfy an extremely pure (99.9%), food grade end-product. Therefore, one cannot assume the numbers measured on the few lots which came through the AURI lab to be a completely accurate depiction or representative of all cases with Kernza grown throughout the state.

AURI summarized the breakdowns into ranges, but it is important to note the ranges are broad, and will change over time, as genetics improve, and handling and growing practices evolve.

Figure 2 shows the estimated range of product loss as the grain progresses through a food grade cleaning and dehulling process. Using the MN 2019 average yield of approximately 500lb/acre, anywhere from 50-150 lb/acre (10-30% of original lot) is estimated to be removed as dockage and free-threshed hulls, an estimated 250-400 lb/acre (70-90% of *post dockage removal*) would be grain sent to the dehuller, 35-135 lb/acre (10-30% of lot post dockage removal) will have been either free-threshed in the combine or separated from handling and shipping.

The final weight of clean, dehulled grain with ergot affected grain and any dead heads removed, is estimated to be anywhere from 150-250 lb/acre. For a 30-acre plot this would equate to between 4,500-7,500 lbs of clean grain. This breakdown is displayed visually in the Cleaning and Dehulling one-page handout.

*** Several possible implementable setups exist that could lead to a cleaned grain. AURI recommends working closely with your equipment supplier to optimize the process setup and select proper screens and equipment sizing. Please reach out to AURI with questions about utilizing your existing equipment to process Kernza. The identified process flow pictured below, was discovered by working with multiple equipment manufacturers and distributors, many of whom have experience working with Kernza. Conversations with current cleaners and processors of Kernza grain also provided supplemental Information.*

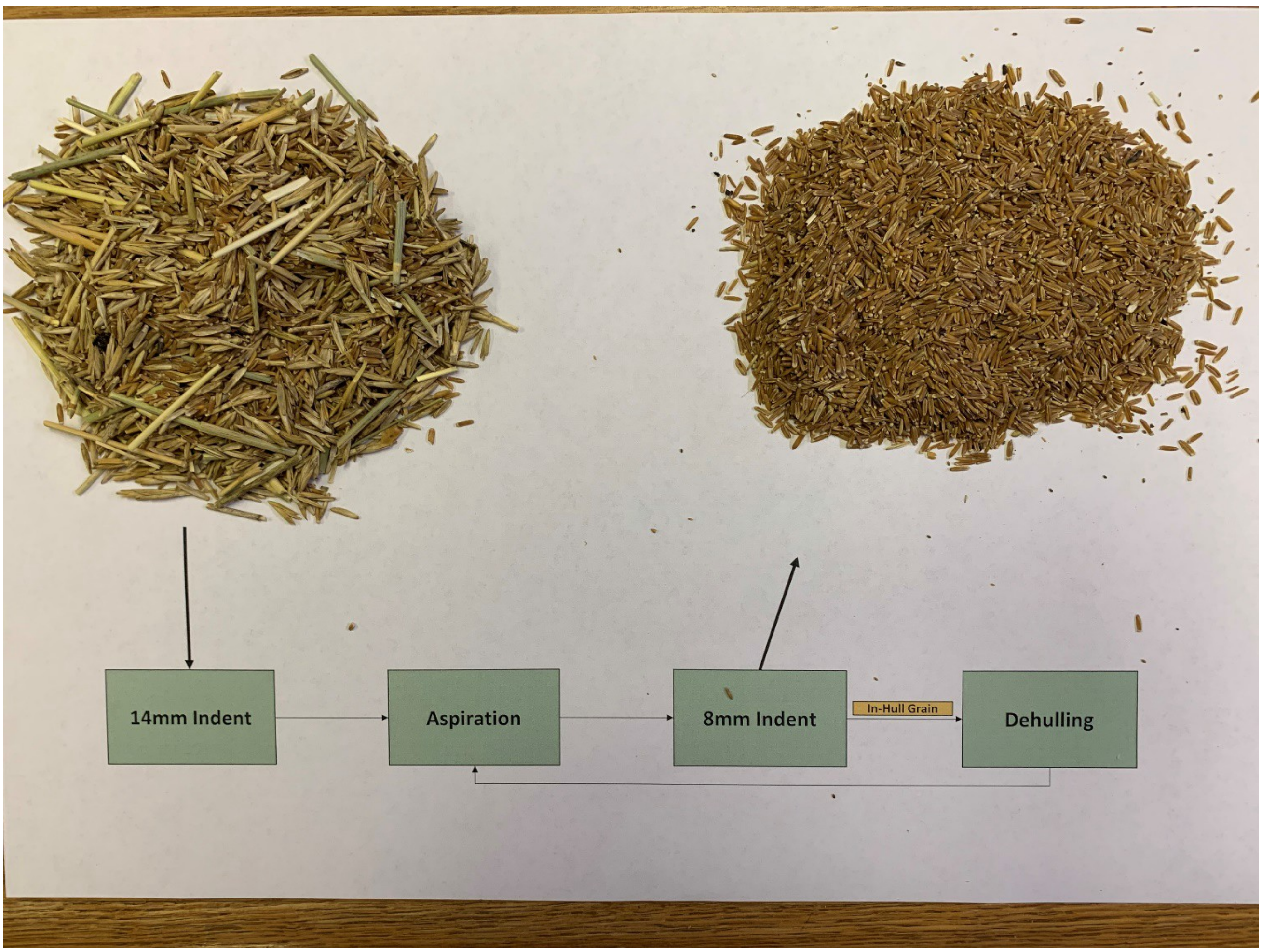

Figure 1: Kernza® Grain fresh from Combine vs. after Identified Cleaning and Dehulling Process

Kernza® Perennial Grain: Cleaning & Dehulling Process

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There are several processing steps which need to take place to clean and prepare Kernza for end users. AURI worked with equipment manufacturers and current cleaners/processors to identify the steps and equipment necessary to clean Kernza and remove/separate hulls to achieve a product for milling or to be supplied directly to end users. The identified processing steps are outlined in the diagram.

Inconsistencies in grain size and characteristics between various grain lots is currently a significant factor when processing Kernza. As a result, AURI recommends building flexibility into cleaning and processing equipment setups. The University of Minnesota and others are working to improve seed genetics, which will likely impact grain characteristics such as percentage of free threshed grain in the combine and size of individual grain kernels. Therefore, post-harvest dehulling and cleaning of Kernza grain are processes which are likely to change with time.

- As indicated on page 2, current expectations are for 30-50% of the harvested material to be dehulled usable grain

Kernza from Combine

These steps can be done in conjunction with an air screener or fanning mill type setup

Test Grain for: Moisture, Molds and Mycotoxins

Vomitoxin and Aflatoxin among the common issues

Removal of Dockage via Shaker Table/ Screens or Indent

3/32” x 1/2” slotted screen; 14mm indent

Removal of Hulls (Aspiration)

Removes hulls and additional dockage

Hulls to be used as feed, fuel or bedding

Grain in-hull and hulled grain

Grain Separation: Indent Separation or Slotted Screen

Separation of hulled grain from in-hull grain

8mm indent (length grader) or 3/64” x 3/8” slotted screen

Grain in-hull

Ddehulled Kernza Grain

Option 1: Dehuller discharge recycled to aspiration and separation until all grain is dehulled

Dehulling (Impact Huller)

1,000—1,500 RPM

Stone impact surface works best

Option 2: Dehuller discharge sent to separate aspirator and gravity table for increased process efficiency

Removal of Hulls (Aspiration)

Grain in-hull and hulled grain

Gravity Table Separation

Grain in-hull

Dehulled Grain

Color Sorting

If ergot is an issue, grain will need to be run through a color sorter

Cleaned, Dehulled Kernza Grain

Kernza® Perennial Grain: Cleaning & Dehulling Process

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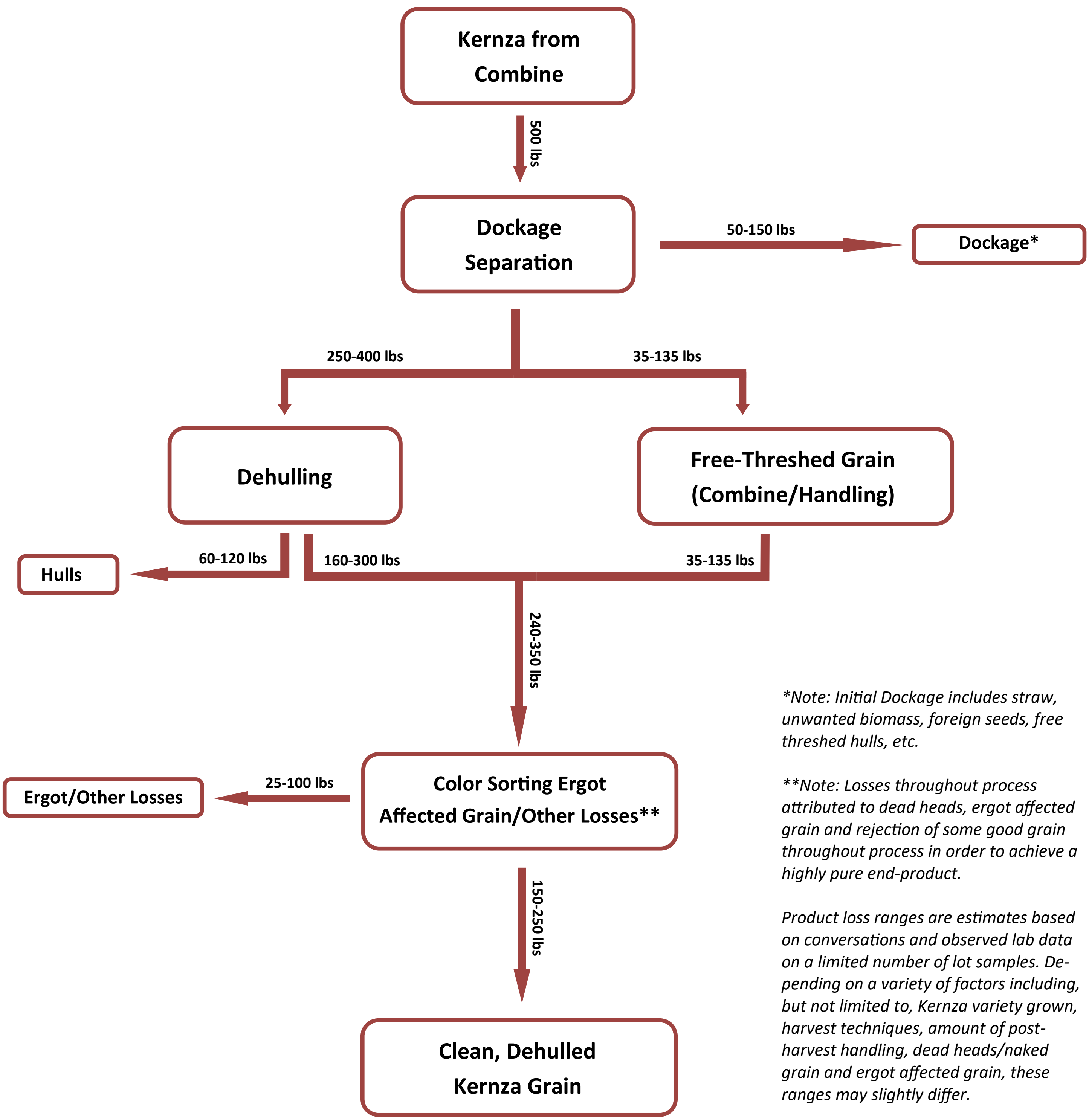
Identified Ranges of Product Weights and Losses Throughout Cleaning and Processing Steps – One Acre Example



**Note: Initial Dockage includes straw, unwanted biomass, foreign seeds, free threshed hulls, etc.*

***Note: Losses throughout process attributed to dead heads, ergot affected grain and rejection of some good grain throughout process in order to achieve a highly pure end-product.*

Product loss ranges are estimates based on conversations and observed lab data on a limited number of lot samples. Depending on a variety of factors including, but not limited to, Kernza variety grown, harvest techniques, amount of post-harvest handling, dead heads/naked grain and ergot affected grain, these ranges may slightly differ.

AURI visited Forsberg in Thief River Falls, MN and Bratney Companies in Des Moines, IA to test dehulling and cleaning equipment. AURI also toured the Healthy Food Ingredients (HFI) facility in Valley City, ND. HFI partnered with a major customer to build a Kernza processing line at the Valley City plant. At the time of AURI's visit, it was the only processing line available in the Midwest dedicated solely to cleaning and dehulling Kernza grain.

As a note, AURI has leveraged LCCMR funding to build capacity to clean and dehull small amounts of Kernza. AURI will also have the capability to mill small amounts of flour using a recently purchased Burr Mill located at AURI's Waseca site. The AURI Waseca site is currently in the process of developing a small food grade space, to operate this equipment.

Forsberg Dehulling

In September 2019, Al Doering and Riley Gordon travelled to the Forsberg testing lab in Thief River Falls, MN to process Kernza (MN-Clearwater) through two different hulling machines. The two technologies tested included the Model 7-F Impact Huller and the Model 2 Huller Scarifier (or friction huller). While both pieces of equipment successfully removed the hull from some of the grain, the impact huller was able to remove a larger percentage of the hulls. This machine removed approximately 55% of the hulls through one pass, compared to the friction huller which removed around 42%. See table 2 for trial results, rough costing and throughputs of various hullers. Dehulling efficiency was calculated by sending 100% in-hull grain through the various dehullers at various speeds, aspirating the hulls and separating the in-hull grain from the kernels, and then comparing the ratio of hulls and kernels to in-hull grain. In addition, it appeared that the friction huller resulted in more broken kernels when compared with the impact huller. For these reasons, AURI has moved forward with the purchase of a Forsberg Model 7-F Impact Huller with Stainless Steel contact parts utilizing funding under the 2019 Kernza LCCMR grant with Stearns County. This pilot scale huller will process several hundred pounds per hour and serve as a valuable resource to begin supplying businesses and end users with small amounts of Kernza for new product testing.

Figure 2: Forsberg Model 2 Huller Processing Kernza® Grain

Figure 3: Forsberg Model 15D Huller Processing Kernza® Grain

Figure 4: Separated Kernza® Grain and Hulls post dehulling on Model 15D Forsberg Huller

Bratney Company Cleaning Testing

In December of 2019, AURI had the opportunity to take a batch of the Kernza grain to the Des Moines, IA based headquarters and testing lab of Bratney Companies. Bratney Companies is highly regarded in the Midwest as a leader in quality seed cleaning equipment and process design. AURI has worked with Bratney Companies on other projects and was confident they would offer valuable guidance on process development in the cleaning of Kernza.

The MN-Clearwater which was dehulled at Forsberg and then subsequently aspirated at AURI (to lift off the light hulls) was taken to the Bratney lab in order to research a best method to separate the hulled grain from the in-hull grain in the dehulling process. The first test was to try and screen off grain still in the hull from the dehulled grain using an oblong screen. It was found prior to the visit at AURI, that using standard mesh screens would not be effective for this type of separation. The screen size yielding the closest fit at Bratney was a 1/18'' (W) X 3/4'' (L). However, as demonstrated in the photo in **Figure 5**, the inconsistencies in the width of the grain still caused some grain with the hull remaining to pass through the screen, and some dehulled grain to not pass through.

The Northern Crops Institute's (NCI) milling trial later confirmed that a 3/64" x 3/8" oblong screen worked quite well to make the separation. Upon testing at AURI it was found that this screen worked well for one of the MN-Clearwater samples, however, did not perform as consistently on the sample of MN-1502 grain. Further tests with a 4/64" x 1/2" screen were performed by AURI and confirmed to work better for the latter sample. A critical process consideration can be highlighted as a result of this work: It is important to plan for inconsistencies in grain size and allow flexibility in your setup by keeping alternative screens on site during processing. The best way to determine screen sizing will be to work with your equipment supplier to run a series of tests. Utilizing sieve balls underneath screens will also be beneficial to reduce the amount of grain that gets stuck due to inconsistencies in grain size.

The second method tested at Bratney was successful. This method was able to separate the grain based on length, rather than width. Bratney achieved this using a CIMBRIA lab indent separator. The indent drum size found to work most effectively was a 8mm pocket size, s7.9 as shown in **Figure 6**.

Figure 5: 1/18'' x ¾'' oblong screen used in attempt to separate Kernza® hulled grain from dehulled grain.

Figure 6: Lab scale indent machine used to separate Kernza® hulled grain from dehulled grain

The other equipment tested at Bratney was the CIMBRIA Delta Super 101 Air Screener/ Fanning Mill machine (**Figure 7**). Researchers fed the MN-1502 grain from the combine into this machine to see how it would perform in removing dockage. It was successful in removing about 20% of the weight of the grain as dockage. The overs and pass-through cuts on the machine contained both dehulled grain and grain still in the hull, which then needed to go through the indent separator. The Bratney team advised it would be possible to tweak this machine to get a cleaner separation of dehulled grain and grain in-hull, effectively utilizing one machine to clean out dockage and separate in-hull and hulled grain. However, the time was not available on the day of the visit to conduct further tests, and the indent separator delivered excellent results in the separation of in-hull and dehulled Kernza grain.

Figure 7: CIMBRIA Delta Super Cleaner 101 setup to run Kernza® Grain

Bratney Company offers other equipment of interest to the processing of Kernza including hullers, color sorters, gravity tables and BoMills. The Schule impact huller that Bratney markets would work as an excellent huller for Kernza grain, as demonstrated through testing. AURI decided to move forward with purchase of a Forsberg dehuller based on cost and scale. However, the Schule huller achieved a much greater efficiency, or greater percentage of grain dehulled, on the first pass (up to 90% after optimization), versus 65% on the Forsberg Model 15D huller. The CIMBRIA color sorters which Bratney represents, would work very well for removing any ergot or sclerotia-affected Kernza. Samples of the MN-1502 and MN-Clearwater received at AURI appeared to have a considerable amount of ergot affected grain (although this was not verified by a lab scale color sorter). Color sorting would appear to be a necessary processing step on a Kernza cleaning line. The BoMill is a unique piece of equipment which can selectively remove individual grains from a lot based on a range of qualities and/or defects.

The most critical quality factors for Kernza include protein content and vomitoxin levels. There were many lots of Kernza grown in Minnesota in 2019 which contained a high level of vomitoxin in the finished product. Vomitoxin levels above 2 parts per million (PPM) is typically a guideline for utilizing grains in food use to meet a 1 ppm advisory level in finished food products. . It is worth noting this is not a Kernza specific problem, as 2019 was a historically wet year, most small grains were affected by high vomitoxin levels in the upper Midwest. If vomitoxin proves to be a recurring issue for Kernza producers, it will be a major hurdle to overcome. The BoMill could be utilized to bring vomitoxin levels in a lot of Kernza grain to the recommended food grade limit. A BoMill works by optically inspecting individual grains and using air to reject grain that doesn't meet set parameters. The speed of the larger Bo-mill (Tri-Q model) is fully compatible with the needs of industrial grain handling, delivering a sorting capacity of 3 metric tons per hour[1]. Multiple units can be combined into higher capacity production systems.The BoMill can be useful when segregating for a variety of mycotoxins, as well as other grain parameters such as protein.

Bratney Company has generously offered to play a part in AURI efforts to process small amounts of Kernza grain for individual businesses or end users to utilize. Bratney has offered to clean small amounts of grain with their lab scale color sorter and BoMill to meet the research and development needs for food grade quality grain. This is a key step in building small scale capacity for producing this grain and getting it in the hands of end users to grow awareness about the ecosystem services and market opportunities of Kernza.

Figure 8: Schule Huller

Figure 9: CIMBRIA Optical Color Sorter

Figure 10: BoMill

Bratney provided some rough cost estimations for the equipment required to clean and dehull Kernza, as outlined in Table 1 below.

It is important to note that these prices are rough estimates and vary based on options, conveyance, automation and so on, but provide a rough idea of the required capital cost to install. The equipment included in Bratney's estimates are a Cimbria Delta cleaner (fanning mill), Schule dehuller, Schule aspirator, Cimbria Heid gravity table and Cimbria SEA color sorter. As capacity increases, the size of the machines and/or the quantity of machines increases as well.

Cleaning Equipment	Fanning Mill, Dehuller, Aspirator, *Gravity Table*** and Color Sorter			Fanning Mill, Dehuller, Aspirator and Color Sorter		
Throughput (ton/hr)	1	3	5	1	3	5
Price	$325-$375k	$690-$790k	$950-$1200k	$280-$330k	$605-$705k	$780-$1030k

Table 1. Capital Cost Estimates of Cleaning and Dehulling Equipment Options for Kernza® (Provided by Bratney Company)

*** Included in this table are setups with and without a gravity table. One could consider the gravity table optional, but Bratney highly recommends one for separation. The 5 ton/hr capacity option includes two of the Cimbria Heid gravity tables used in the 3 ton/hr setup, in parallel.*

Bratney invites anyone seeking more detailed information regarding its equipment in the context of cleaning Kernza to contact them directly.

Table 2 shows various estimated costs, throughputs and efficiencies pertaining to Kernza, of various dehullers which AURI researched as part of the project scope of work.

Dehuller Model	Calculated Efficiency (on first pass)	Estimated Cost	Estimated Throughput
Forsberg Model 2	42% (1190 RPM)	$10k	200-300lb/hr
Forsberg Model 7F	31%-55% (1800-2400 RPM)	$7k	200-300lb/hr
Forsberg Model 7F (Stainless Steel Contact Parts)	31% (1800 RPM) 50% (2100 RPM) 55% (2400 RPM)	$10k	200-300lb/hr
Forsberg Model 15D (Stainless Steel Contact Parts)	40% (1800 RPM) 60% (2100 RPM) 65% (2400 RPM)	$18k	1000lb/hr
Forsberg Model 15D	40-65% (1800-2400 RPM)	$12k	1000lb/hr
Schule FKS 500	90%+	$30k	3500lb/hr
Buhler	Not Tested at time of Reporting	$49k	3500lb/hr*
Codema VSH - 2096	Not Tested at time of Reporting	$22K	3000lb/hr*

Table 2. Estimated Costs, Throughputs and First Pass Efficiencies of various dehullers which AURI evaluated

**Estimated throughputs which require testing to confirm. Overall throughputs will depend on first pass efficiency as recycling through aspiration and separation will slow down the overall process.*

Healthy Food Ingredients (HFI) – Valley City, ND Facility

In November 2019, AURI toured the HFI Kernza Cleaning and Dehulling facility in Valley City, ND. Riley Gordon and Michael Sparby from AURI travelled to the facility and met with Chris Wiegert, who has taken a lead role for HFI in the area of Kernza. Chris is the Chief Soil Health and Sustainability Officer for HFI and has been extremely involved in efforts to bring the Kernza cleaning and dehulling facility online in Valley City.

HFI partnered with one of their close customers and early Kernza adopters to develop the cleaning and dehulling line for Kernza grain, which is the first of its kind in the Midwest, and likely the only cleaning and dehulling line dedicated to Kernza in the U.S. at the time of this report (June, 2020). The facility has been in operation since December 2018. The facility offers toll processing in specialty cases but mainly operates to clean and process grain grown for HFI and its primary customers for use in product development activities.

HFI and its partners have learned much about the current challenges and hurdles with processing Kernza. AURI interviewed Mr. Wiegert about his experience processing Kernza. One of the bigger challenges identified was an inconsistency of grain sizes and weights in different lots, and even among the same lot. This makes cleaning more challenging and requires constant adjustments to equipment settings. Chris confirmed similar numbers to those found by AURI regarding dockage. He reported that 20-30% of any given lot would likely be dockage. He also confirmed the final weight of clean, hulled grain at 99.9% purity could be anywhere from 30-50% of the original lot weight. The efficiency of operations is a challenge due to inconsistencies in lots and a fairly large amount of ergot affected grain. HFI has found that it must run ergot affected grain through an optical sorter several times for removal.

HFI is optimistic about the interest in Kernza and is supportive of efforts to identify and develop end markets for the grain. Chris mentioned that HFI would be open to cleaning and processing grain for future research purposes. This processing option is critical to providing larger quantities of food grade quality grain for various product development efforts planned as part of these grants.

HANDLING

AURI explored the requirements for a facility to be compliant with food grade handling regulations for cleaning, dehulling and milling of cereal grains, such as Kernza.

Dr. Charles Hurburgh, a professor of Agricultural and Biosystems Engineering at Iowa State University and Professor in charge of the Iowa Grain Quality Initiative, informed AURI that steps include registering as a food facility under the Food Safety Modernization Act (FSMA). This requires following the FSMA process to document that an operation is a qualified facility with less than $500,000 in food product sales annually and documenting that a hazard analysis was conducted with no hazards found, or if hazards were identified, preventative controls were enacted to eliminate hazards. Dr. Hurburgh also mentioned it would be necessary for at least one individual at the facility to have taken the FSMA preventive controls training course. He recommended reviewing the new FSMA rule for current good manufacturing practice, hazard analysis and risk based preventative controls for human food.

AURI also consulted with the Minnesota Department of Agriculture (MDA) on necessary requirements to be in compliance with state regulations. MDA provided several helpful documents with guidance on required steps to ensure compliance with FSMA's rules, depending on the processor's situation. Requirements vary depending on if operations would solely clean and dehull a personal supply of Kernza, conduct toll cleaning and dehulling for several farms, or milling the grain. If the intention is to mill, one must classify the facility as a manufacturing/processing step under FSMA, and attestation as a food facility or qualified facility. The size of a business or operation is also another key factor in determining if a facility qualifies for the exemption of the preventative control's requirement under the FSMA food safety plan.

The following documents can be found online or provided by AURI upon request:

1. ***"Classification of Activities as Harvesting, Packing, Holding, or Manufacturing/Processing for Farms and Facilities: Guidance for Industry" – Document released by the FDA Center for Food Safety and Applied Nutrition***
2. ***"Key Facts about Preventive Controls for Human Food" – Document Released by the FDA***
3. ***"Understanding the Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food – 21CFR-117" – Document released by the Minnesota Department of Agriculture***
4. ***"IF YOUR FACILITY IS A VERY SMALL BUSINESS OR A SMALL (OR VERY SMALL) FARM MIXED-TYPE FACILITY, WHAT PC HUMAN FOOD EXEMPTIONS/MODIFIED REQUIREMENTS APPLY TO YOU?" – Document released by the FDA***

The first distinction is when the grain goes from a Raw Agricultural Product (RAC) to consideration as a food product. These documents supply guidance to businesses that only want to clean and dehull a grain of being exempt from having to register with the FDA as a food facility. In order to qualify, the operation would either have to be located at the farm site of grain harvest and/or be a majority owned facility by the farmers who grew the grain. If an entity plans to operate as a food grade cleaning and dehulling facility as a separate business (not farmer owned), which is processing lots of grain from various farm sites, it would need to go through the FSMA steps to be approved as either a food facility or a qualified facility.

The second distinction is whether a food grade facility that is cleaning, dehulling and/or milling Kernza must follow all the rules under the "*Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food"* document. Generally, any food facility has to register with the FDA, with one exception being the above example of a farm or mixed farm facilities either at the site of grain harvest or majority owned by the farmer(s) growing the grain. The other permissible exemption is if a business meets the size requirements to attest as a "qualified facility" or a "very small business". Table 3 below shows the exemption conditions of a qualified facility/very small business. This exemption still requires the facility to register with the FDA, but the facility would be exempt from the requirement to submit a full food safety plan. Instead, the business would only focus on risk analysis and preventive controls to eliminate risks or follow state food safety requirements. These exemptions would apply to any cleaning, dehulling and milling facility for Kernza that meets size requirements. Kernza cleaning and processing steps should be fairly low risk level operations, with foreign materials or metal pieces from equipment being the main potential concerns. It is important to keep in mind that a majority of the end uses of Kernza (baking, brewing, etc.) must involve a kill step of some form. See subparts A, B, D and F of FDA document 21CFR-117 for the considerations pertaining to a qualified facility.

Businesses not included in Table 3 will be subject to registering with the FDA as a food facility and be required to submit a full food safety plan shown in Figure 13 (preventive controls, hazard analysis, risk-based supply chain program, PCQI Training, recall plan, etc.).

Steps for Achieving a License to Operate as a Qualified Facility in Minnesota

1. Contact a local Minnesota Department of Agriculture Advisor and schedule an initial consultation visit to the facility under consideration for conversion to a food grade operation. During this initial site visit, an agricultural advisor will advise on the requirements needed to convert a chosen space into a qualified facility.
2. Work towards addressing and satisfying the comments from an agricultural advisor, identifying any potential hazards, and how to control them.
3. Reconnect with an MDA agricultural advisor to schedule a final licensing inspection of the facility. Upon approval, permission to operate as a qualified facility will be granted and a license application and corresponding application fee (fee amount dependent on gross annual food sales) submitted to the MDA to obtain a physical license.
4. Submit an attestation form (either online or by mail) to the FDA. The attestation essentially requires that an operation state 1) how the requirements of a qualified facility are being met and 2) what, if any, hazards an inspection finds, and how they will be controlled

Note: Food grade considerations under a food safety plan begins at the onloading of the grain lots or grain receiving and carries through until the final product leaves the facility.

Exemption or Modified Requirement	Conditions
Qualified Facilities – businesses (when including the sales by any subsidiaries, affiliates, and any entity of which the facility is a subsidiary or affiliate) with average annual sales of less than $500,000 with at least half the sales to consumers or to local retailers or restaurants or Indian reservation (within the same state or within 275 miles) or very small businesses as defined below. *Very small businesses* (including any subsidiaries or affiliates) averaging less than $1,000,000 (adjusted for inflation) -- in both sales of human food *plus* the market value of human food that is manufactured, processed, packed, or held without sale (e.g. held for a fee), per year during the 3-year period preceding the current calendar year. (21 CFR 117.5(a))	To be eligible for modified requirements, a qualified facility is required to notify FDA about its status; and attest that it is either: 1. Addressing identified hazards through preventive controls and monitoring the preventive controls; or 2. Complying with applicable *non-Federal* food safety regulations, and notifying consumers of the name and complete business address of the facility where the food was manufactured or processed. A qualified facility must submit these notifications to FDA during the same two year timeframe that the facility is required to update its facility registration. An otherwise Qualified Facility that does NOT notify FDA is subject to the requirements for Hazard Analysis and Preventive Controls.
Low-risk, on-farm activities performed by small businesses (less than 500 full-time equivalent employees) or very small businesses as defined above. (21 CFR 117.5(g) and (h))	For specific information on which activities are covered, see page 20.

Table 3. Exemptions to Preventative Controls Requirements and Conditions of Modifications

Excerpted from "IF YOUR FACILITY IS A VERY SMALL BUSINESS OR A SMALL (OR VERY SMALL) FARM MIXED-TYPE FACILITY, WHAT PREVENTATIVE CONTROLS (PC) HUMAN FOOD EXEMPTIONS/MODIFIED REQUIREMENTS APPLY TO YOU?"

Figure 11. FSMA Food Safety Plan Requirements

It is highly recommended that any incoming grain be tested for molds and mycotoxins as a standard practice. As mentioned previously, vomitoxin was a prominent issue during the 2019 grow season. Although not a Kernza specific problem, anyone considering setting up a qualified facility to clean and dehull Kernza for a food grade end use, should make mycotoxins a part of their Preventive Control Plan to test for these types of issues and detect them before entering the facility to avoid contamination. The mycotoxins affecting Kernza primarily reside in the hulls, and not the kernels themselves. Testing the hull of the incoming grain could be important to prevent equipment contamination, but testing the final, dehulled product on the back end of processing will also be important to ensure the product did not become contaminated through processing, to still meets required specifications. AURI had vomitoxin level testing run at Montana State University on cleaned and dehulled samples of MN-1502 and

MN-Clearwater. The 1502 came back at 0 ppm and the MN-Clearwater tested at 0 ppm, indicating no issues on these particular samples.

INSURANCE

AURI recommends obtaining general liability and recall insurance for anyone wishing to operate a food grade facility of any kind[6], including cleaning, dehulling and/or milling of Kernza. There are several agencies which offer general food manufacturing insurance policies satisfying this need. The researchers also recommended obtaining several quotes before moving forward. Looking at annual production and revenue will help make decisions on insurance coverage.

STORAGE

Storage of Kernza should be in a cool, dry area in either individual super sacks or feed bags. While keeping the grain below 14% moisture should ensure Kernza maintaining its condition, drying the grain to 8-12% moisture is preferable. Estimates show grain between 8-12% moisture should remain stable for two or more years. Once milled, the flour will likely have a shelf life of somewhere between six months to a year, depending on the environment and moisture content. *It's important to note the timeline guidance is currently only an **estimate**, based on similar grains, and further testing and research will need to be conducted to truly understand the storage of Kernza grain under ideal conditions.* Based on storage conditions of similar grains, such as wheat and barley, a storage temperature of under 60 degrees Fahrenheit (F), and relative humidity under 70%, should be safe for Kernza grain at between 8-12% moisture. Storage under 40F could eliminate the possibility of mold formation and insect activity. A good rule of thumb, if storing grain in the bin, comes from a University of Minnesota Extension paper on storing wheat and barley[7]. Extension advises maintaining the grain temperature to within 20F of the outside temperature, to avoid any moisture migration or relative humidity issues. This paper also states that winter bin storage temperatures for wheat and barley in Minnesota are ideally about 25F. *Again, further testing and research is necessary to validate this for Kernza.* Additional research on off-flavoring and oxidation effects of Kernza also have merit after hearing concerns from several industry contacts. Further testing to identify if there are advantages to storing grain after cleaning and dehulling, compared with dried, raw grain would be interesting to explore as well.

The University of Minnesota looked at the stability of Kernza through storage and after steam treatment[9]. The work was done by UMN grad student Amy Mathiowetz, under the guidance of her advisor Dr. Pam Ismail and is summarized in the thesis paper entitled "Evaluation of the Chemical and Functional Stability of Intermediate Wheatgrass (Thinopyrum Intermedium) Over Storage and in Response to Steam Treatment". The study concluded that Kernza or Intermediate Wheat Grass (IWG) had competitive storage stability when compared with hard red wheat. Due to IWG's lower lipoxygenase activity, lower hydroperoxide content and overall higher antioxidant content, it was found to hold its functionality over storage significantly better than hard red wheat. Secondly, this study concluded and provided information that, by using a steam treatment on the grain prior to storage, the storage stability could increase. The steam treatment also displayed evidence of improving certain grain qualities in the IWG over storage, such as dough strength, dough development time and starch pasting properties.

Northern Crops Institute Kernza Milling and Flour Analysis Information

AURI's Kernza utilization research included milling tests and subsequent refined and whole grain flour analysis at the Northern Crops Institute (NCI) in Fargo, ND. NCI's laboratories offer technical, testing, and analytical services aimed at supporting the promotion and market development of crops grown in Minnesota, North Dakota, South Dakota, and Montana. The results from these milling and flour tests will be used to help guide further pilot-scale milling and product development activities as part of AURI's LCCMR-funded Kernza projects.

Milling Information

Summary

Kernza Whole Grain Flour and Kernza Refined Flour are relatively high in protein and ash content. Kernza Whole Grain Flour and Kernza Refined Flour did not form gluten matrixes that are necessary for the production of most yeast raised bakery products, however, chemically leavened products, such as pancakes, could work well with Kernza flour.

Kernza flour could be used in combination with traditional or ancient grain wheat flour types to produce a wide variety of baked goods. Kernza flour could be a good candidate for extruded food products such as snack foods and cereals due to its similarity in composition to other cereal grain commodities.

When larger quantities of Kernza are available, NCI recommends the employment of a wider array of roll stands, sifters, and purifiers using its pilot milling system to increase the extraction rate for Kernza Refined Flour. NCI recommends milling Kernza Whole Grain Flour to produce a range of batches in specific particle sizes to determine functionality of the flours.

AURI sent approximately 75 pounds of raw, Clearwater variety Kernza grain to NCI.

NCI removed dockage and hulls from the Kernza sample using a Carter Day® Dockage Tester XT3.

Figure 12. Carter Day® Dockage Tester XT#

Hulled and dehulled Kernza kernels were collected. Hulled Kernza kernels were dehulled using a Strand® Sizer Shaker Model P, Seedburo Equip. Co.

Figure 13. Strand Sizer Shaker Model P

Note – Kernza has a tightly bound hull and a soft kernel. A triple stacked Seedburo Equip Co. barley pearler was tried, however the test was unsuccessful as it was unable to remove the hull due to Kernza's soft kernel characteristic. Using an Impact Huller, as discussed in previous section of the report, is a more applicable method to achieve efficient dehulling of Kernza.

Figure 14. Seedburo Barley Pearler Stack

Kernza Refined Flour Pilot Production

Researchers passed Kernza kernels through a Creason® 3rd Break Dual Fine Corrugated Roller Mill. The rotation ratio between the two rollers was 2:1. The gap between the rollers was set approximately 0.005 of an inch less than the average width of Kernza kernels. NCI selected the 3rd break roll stand to release as much endosperm flour as possible.

Figure 15. Creason® 3rd Break Corrugated Rollers

Approximately 5% by weight of refined Kernza® flour was obtained through a 150-micron screen (100 mesh) after the first pass of kernels through the 3rd break roll stand.

The Kernza coarse material (+150-micron) then passed through a 3rd break roller mill two additional times with a 0.005 inch gap reduction before each pass.

The extraction rate was very low (less than 15%) after three passes through the 3rd break corrugated rollers. NCI was not able to increase and optimize the extraction rate due to having a limited sample size available to work with.

Typical commercial extraction rates for producing refined flour from wheat range from 72% to 75%. A main reason for the low extraction was a low supply of Kernza to experiment in new settings to improve milling extraction rates. Kernza's bran and endosperm are softer than traditional classes of wheat In order to obtain a higher extraction rate from Kernza, the bran covering of Kernza should be analyzed with different degrees of tempering to improve Kernza's resistance to grinding.   After improving tempering conditions, Kernza milling would require re-configuring a soft wheat roller mill layout so that users can segregate more Kernza endosperm from the outer portion of Kernza kernels.

The process of re-configuring a roller mill would require trial and error experimentation in order to successfully increase the yield of Kernza endosperm into a refined flour. A much larger quantity of Kernza grain would increase the likelihood of increasing milling extraction rates. A goal would be an extraction rate of 50% or higher through the use of a larger set of roll stands, sifters, and purifiers within a milling system.

Once a larger quantity of Kernza is available (more than 600 pounds of grain in kernel form), NCI's Creason® Pilot Mill is configurable to increase the extraction rate to obtain more Kernza flour with a reasonably low ash content.

Kernza Whole Grain Flour Pilot Production

Kernza Whole Grain Flour was produced from Kernza Grain using a Fitzpatrick® Hammermill.


Figure 16. Fitzpatrick® Hammermill

After this milling, the Kernza Whole Grain Flour passed through an 841-micron screen (20 mesh).

Kernza Whole Grain Flour and Refined Flour Analytical Results

NCI conducted a number of analytical tests on Kernza Whole Grain Flour and Kernza Refined Flour as follows:

1. Moisture Content:
    a. Kernza Whole Grain Flour – 7.94%
    b. Kernza Refined Flour – 8.16%
2. Ash Content (dry-weight basis):
    a. Kernza Whole Grain Flour – 2.67%
    b. Kernza Refined Flour – 0.72%
    c. Hard Red Spring Wheat Grain* – 1.76%
    d. Hard Red Spring Wheat Refined Flour* – 0.59%
    e. Ash content in Kernza Refined Flour reduced to nearly 25% of the ash content of Kernza Whole Grain Flour.
3. Protein Content (dry moisture content basis):
    a. Kernza Whole Grain Flour – 20.78%
    b. Kernza Refined Flour –19.12%
    c. Hard Red Spring Wheat Whole Grain Flour* – 16.1%
    d. Hard Red Spring Wheat Refined Flour* – 15.3%

    The protein content in the Kernza refined flour was 1.4% lower than the protein content of the Kernza whole grain flour. This percentage reduction in protein content for Kernza is greater than for hard red spring wheat, when going from a whole grain to a refined flour.

4. Falling Number (indication of the level of alpha-amylase enzyme activity – dry moisture basis):
    a. Kernza Whole Grain Flour – 193 seconds
    b. Kernza Refined Flour – 283 seconds
    c. Hard Red Spring Wheat Whole Grain (Avg.) – 397 seconds**
    d. Hard Red Spring Wheat Refined Flour* - 393 seconds

** Wheat Quality Council - Hard Spring Wheat Technical Committee 2018 Crop report

In testing hard red spring wheat for alpha-amylase enzyme activity, a falling number time of > 300 seconds indicates minimal sprout damage. It is interesting to see the wide disparity in falling number seconds between whole grain flour and refined flour. Hard wheat kernel and resultant flour falling number tests are normally fairly close to each other.

5. Color (Minolta CR-310):

Sample	L (lightness 0-100)	A (green (-) to red (+)	b (blue (-) to yellow (+)
Kernza® WG Flour	81.24	-0.22	15.61
Kernza® Refined Flour	86.59	-2.69	15.77
HRSW Refined Flour	90.5	-1.1	9.5

Table 4. Kernza® vs. HRSW color

A higher lightness score means a lighter colored flour. Kernza Refined Flour is lighter in color compared to Kernza Whole Grain Flour, while hard red spring wheat flour is lighter in color than the refined Kernza flour

6. Wet Gluten/Gluten Index
    a. Kernza Whole Grain Flour – No Result
    b. Kernza Refined Flour – No Result

Gluten balls could not be formed to conduct the test. The protein in Kernza Whole Grain Flour and Kernza Refined Flour is different than traditional wheat due to Kernza's inability to form a gluten structure.

7. Farinograph

Farinograph Result	Kernza®WG Flour	Kernza® Ref Flour	HRSW Flour Avg.*
Absorption % (as is moisture)	79.2%	61.5	62.9
Peak Time (minutes)	2:09	1:31	7:24
Stability (minutes)	0:53	1:02	10:48
Mixing Tolerance Index (bu)	151	216	~30

Table 5. Kernza® Farinograph results

Farinograph data shown above and compared to the 5-year average for hard red spring wheat flour* from 2015 through 2019 as compiled by North Dakota State University. Results show Kernza flours mix quickly to produce weak dough. Both Kernza Whole Grain Flour and refined flour have high water absorption rates, and Kernza Refined Flour is similar to hard red spring wheat flour. Both Kernza Whole Grain Flour and Refined Flour shows low mixing resistance and mixing extensibility.

8. Total Starch and Amylose

	Kernza® Whole Grain Flour	Kernza® Refined Flour	Hard Red Spring Wheat
Amylose %	24.0	30.0	25.0
Total Starch%	46.0	66.0	73.0

Table 6. Kernza® Amylose and Starch results

Amylose content in hard red spring wheat flour averages 25.0%. Hard red spring wheat flour contains on average 73% total starch. (can you add this column to the table?)

*Note: U.S. Wheat Associates 2019 Crop Quality Report – 5 Year Average from 2015 to 2019

COPRODUCT EVALUATION

The main Coproducts associated with Kernza are the straw and the hulls. AURI tested these materials for various properties to evaluate where these coproducts will have the highest value. It will be important to find a home for the lower value coproducts associated with Kernza to increase the viability of the crop.

	Lignin	Hemi-Cellulose	Cellulose
Kernza Straw	5.98%	23.03%	31.86%

Table 7. Kernza® Straw Lignin, Hemi-Cellulose and Cellulose Content (Results of testing at Dairyland Labs)

Initial conversations with potential end users of the straw for bio composite and pressed board applications have indicated that Kernza straw would be worthwhile to test in these areas. AURI plans to conduct some initial prototype development of biobased materials utilizing the straw under the Kernza focused LCCMR grant led by the Stearns County Soil and Water Conservation District.

	Oat	Wheat	Kernza	Rye
Total Phenolic Content (mg/g)	4.06	3.26	6.82	3.04
Protein (%)	6.5	9.33	5.13	4.83

Table 8. Total Phenolic Content of Kernza® Straw with Comparisons (Results from AURI Marshall, MN Analytical Lab)

Elevated levels of Phenolic compounds in Kernza straw compared to other common straws could be an indicator of potential extractable value. Polyphenols are often full of antioxidant capabilities and other potential attributes providing health benefits[8]. Further research into classifying the polyphenols contained within the Kernza straw is necessary to understand if there is value in extracting these compounds from the straw. What is known for now, is that the total level of Phenolic compounds in the straw is more than double the content of wheat or rye straw.

KERNZA STRAW and HULLS as FEED

	Crude Protein	TDN	ADF	aNDF	Lignin	Fat	Ash
Kernza Straw	6.28%	55.27%	43.17%	66.20%	5.98%	2.21%	5.33%
Kernza Hulls	10.98%	55.97%	37.52%	54.01%	6.41%	2.39%	11.71%

Table 9. Observed Feed Value of Kernza® Straw and Hulls

Feed analysis shows that crude protein in Kernza straw is better than average for a straw (6.28% for Kernza vs. 3.5-6% on average for most barley, oat, or wheat straw). Energy is greater than most straws with a Total Digestible Nutrients (TDN) for Kernza at 55.2% compared to 39% for most straws. If feeding dry cows, one would need to watch the potassium level. The sharpness of

hulls could damage the throats of animals. This issue could be resolved through additional processing (milling). An added milling step would increase the cost and reduce the competitiveness of the material as compared to alternatives. The protein level observed in the hulls is also substantially higher than other grain hulls. However, it is important to note that the numbers in the above table are based only on one sample. AURI plans to conduct additional tests on the protein level of Kernza hulls to identify a reliable protein level. If the number provided in Table 9 is accurate, Kernza hulls would make for a viable feed product, outperforming products like oat or rice hulls, due to a higher protein level.

Sugar Extraction from Kernza Straw - Sasya

AURI contracted with Sasya LLC to explore further value-added opportunities for Kernza straw. The initial report from Sasya suggests the presence of valuable extractable sugars in Kernza straw, which may prove worthwhile for further examination and utilization research. Budget permitting, AURI and Sasya may partner for continued work in this regard to explore additional opportunities for the use of these sugars and Kernza biomass. Any noteworthy findings from this work will be included in the final report for the other Kernza LCCMR project, which will be completed in 2021.

The following information summarizes the results of the research performed by Sasya under the Service Provider Agreement for the Agricultural Utilization Research Institute (AURI). The experimental methods used are generic methods implemented in the industry and no effort was made to optimize the methods or processes to improve the efficiency for better results. Therefore, AURI must interpret the results as a "proof-of-concept" only. Sasya made reasonable effort to ensure the validity and accuracy of the methods, results and procedures.

Kernza

1. Background

Kernza is a domesticated variety of intermediate wheat grass. In addition to the potential use as grain, the plant biomass can also be used to extract fermentable sugars. Given the consistency of the dry straw, this section of the report presents the results from a study that evaluated the applicability of established extraction methods from dried straw. Due to the lack of information on the biomass composition or fermentable sugar extraction from Kernza, a sample of corn stover was included as a control. Cellulose, hemicellulose and lignin make up a major portion of biomass samples. The nature of these three components is quite different. Hemicellulose is composed of both pentose and hexose molecules, such as xylose, arabinose, mannose, and galactose, while cellulose is a crystalline structure that is only composed of glucose. These constituents were measured to understand the general make-up of the biomass.

1.1. Sample preparation

Kernza straw (provided by AURI) and Corn Stover were oven dried at 60°C overnight and milled to Mesh 35 and used as the starting material for biomass extraction. As shown in Figure 1, the samples milled very cleanly leaving little residue.

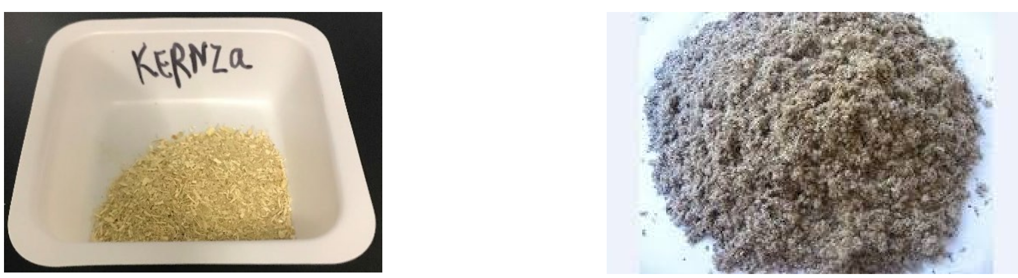


Figure 1. Ground dry samples of Kernza® straw (left) and corn stover (right) that were used as the starting material for quantification and extraction of fermentable sugars.

In comparison to milled corn stover, Kernza was less dense and flaky. Interestingly, milled Kernza was also more hydrophobic at alkaline pH. For carbohydrate quantification, the extraction solvent was acidified to 5.

1.2. Biomass pretreatment

Analysis procedure involved a two-step acid hydrolysis to fractionate the biomass into forms that are more easily quantified. The lignin fractionates into acid insoluble material and acid soluble material. The acid insoluble material may also include ash and protein, which must be accounted for during gravimetric analysis. The underlying principle involves hydrolyzing the polymeric carbohydrates into their monomeric forms which are readily soluble in water and can be measured using an HPLC. Acetic acid is released from the hemicellulose with xylan backbone, but not from mannan and can also be measured using an HPLC.

1.3. Biomass composition assessment

1.3.1. Solids and Ash quantification

Four aluminum boats were pre-weighed and approximately 500 mg of Kernza or corn stover was transferred into the boats. The samples were incubated at 105°C in a convection oven for five hours (when constant weight was achieved) and re-weighed. The difference in the weight corresponded to the moisture content, allowing calculating the accurate solids content.

Four crucibles (two for Kernza and two for corn stover) were heated to 575°C for four hours and cooled in a desiccator before recording their weight. A pre-weighed sample of Kernza or corn stover (~500 mg) was transferred into the crucible and dried to ash over an ashing burner and transferred to 575°C for 3 h. The weight of ash was measured in the duplicate samples. Using this procedure, the ash content in corn stover was ~1.5% but that in Kernza was twice that at ~3.1%.

1.3.1. Cellulose quantification

To the ~ 4 g of bone-dry plant biomass (from Section 1.3.1), 4 mL of acetic/nitric/water (8:1:1, by volume) was added in a glass sample vial with PTFE (spell out) seals and placed in a water bath at 98°C for 1 h. After cooling, the volume was brought up to 10 mL with deionized (?) (DI) water. The liquid was freeze-dried overnight, and the solids resuspended in 2 mL acetone, which evaporated during incubation at 45°C. The fibers were depolymerized by adding 1 mL 50% H_2SO_4 and shaking at room temperature for 1 hour followed by the addition of 1 mL of 3% anthrone and thoroughly mixed. The samples were incubated at 121°C for 1 hour to release glucose and absorbance at 620 nm was measured. Glucose was quantified by a standard curve using known amount of glucose which was also subjected to the exact process. Avicel was used as a positive control. Cellulose content is calculated as the $\frac{G}{W} \times D \times 100$, where G is the weight of glucose (mg) as calculated from the standard curve for each sample, W is the weight of the dried biomass in mg (shown in Appendix 2.2) and D is the dilution factor. The cellulose values were calculated in duplicate and corrected for ash (Section 2.1).

1.3.2. Hemicellulose quantification

Hemicellulose was isolated from 4 g of dried plant biomass (from Section 1.3.1) by alkaline hydrolysis of the ester bonds. Unlike previous procedures that described using concentrated alkali which can mostly hydrolyze xylans and arabinans, a gradient elution was performed using NaOH gradient from 0.1 M to 5 M (spell out M – not clear to me). The lower concentration alkali can hydrolyze glucans and mannans and the analysis is expected to facilitate an accurate estimation of hemicellulose.

Hot DI water (80 mL), glacial acetic acid (0.5 mL) and 1 g of $NaClO_2$ was added to the plant biomass and heated at 70°C for 1 h. After 2 h, fresh bolus of acetic acid and $NaClO_2$ were added and the delignification continued for 5 h. The delignified plant biomass (called holocellulose) was filtered and weighed. The holocellulose was packed in a 20 mL column and 10 mL of NaOH at different concentrations (low to high) was passed through the column and the fractions pooled. Glacial acetic acid was added (15% by volume) to precipitate the polymers. The process mixture (~100 mL) was filtered through a filter paper to collect the precipitate and dried at 105°C. The weight of the resulting precipitate was determined and hemicellulose content calculated as $\frac{P}{W} x\ 100$ where P is the weight of the precipitate (mg) and W is the weight of the dried biomass in mg. The values were calculated in duplicate, corrected for ash and the average value reported.

1.3.3. Biomass deconstruction

To release the carbohydrates from the biomass, 1 g of the biomass was weighed and 10 mL of 72% H_2SO_4 was added to it and thoroughly mixed for 1h at 30°C. The acid was diluted to 4% with DI water. Authentic samples of glucose, cellobiose, xylose, galactose, arabinose and mannose were also individually subjected to the same treatment to account for any sample losses during the deconstruction process. The samples were treated at 121°C for 1 h and cooled to room temperature. A 50 mL aliquot of the sample was stored at 4°C for subsequent analysis of lignin (see section 1.3.4).

1.3.4. Lignin analysis

The autoclaved solution from Section 1.3.3 was filtered to quantify acid-soluble lignin. The absorbance of the clarified filtrate was measured at 320 nm (for minimal interference any carbohydrates). The amount of acid-soluble lignin was quantified as $\frac{A_{320}\ x\, V_f\ x\, D}{\varepsilon\ x\, W\ x\, p}$, where A_{320} is the recorded absorbance at 320 nm, V_f is the volume of the filtrate, D is the dilution factor, ε is the extinction coefficient, which for corn stover is 30 L/g/cm, W is the dry weight of the biomass and p is the pathlength of the cuvette (1 cm). For the lack of better information, the same value of ε was also used for Kernza biomass.

Acid-insoluble lignin was calculated from the solids in the filter paper, which were rinsed with DI water and dried to uniform weight at 105°C. The dry solids are transferred into a muffle furnace and heated to 105°C for 10 minutes, ramping up the temperature to 250°C for 30 min and 575°C for 3 h before cooling down to room temperature in a desiccator. The weight of the dried solids was recorded. The amount of acid insoluble lignin was calculated as:

$$\frac{w_{res} - w_{ash} - w_{prot}}{w_{dry}}$$

Where w_{res} is the tared weight of all acid insolubles in the biomass, w_{ash} is the weight of ash left over and w_{prot} is the weight of protein. The exact amount of protein in Kernza straw was provided by AURI and was used in the equation to calculate insoluble lignin. Using this method, the total lignin in Kernza was 21% and in corn stover was 15.5%. The most interesting difference was that almost all the lignin in corn stover was acid insoluble. Kernza straw comprised of a substantial amount of acid soluble lignin.

1.3.5. Quantification of structural carbohydrates

The hydrolysis liquor from Section 1.3.3 was used to quantify glucose, cellobiose, xylose, galactose, arabinose and mannose. The sugars were measured using an enzymatic kit using colorimetric determination against a standard curve. The hydrolysis liquor (25 mL) was neutralized with $Ca(CO)_3$ to bring the pH to ~5.5 until the effervescence slowed down. The solids were removed by filtration and the clarified liquor was analyzed for sugars. Glucose was also measured by monitoring the absorption at 620 nm after anthrone treatment.

Based on the protocol and the calculations described, the composition of biomass in corn stover and Kernza straw was calculated and summarized in Table 1.

Table 1. Composition of biomass from Corn stover (used as control) and Kernza® straw, on a dry matter basis. The data are the average of duplicate analysis.

	Corn Stover	**Kernza®**
Cellulose	38.4%	42.1%
Hemicellulose	29.5%	32.8%
Lignin	15.5%	20.6%
Ash	1.5%	3.1%

Encouragingly, Kernza straw appears to comprise higher concentration of sugars as indicated by higher cellulose and hemicellulose content. The results conclusively demonstrate that Kernza straw contains significant amount of carbohydrates that can be extracted for use as fermentable sugars.

1.4. Extraction of sugars from Kernza straw

This section explores the conventional dilute acid method to extract cellulose and hemicellulose followed by their hydrolysis into fermentable sugars from Kernza. Dried, milled straw was pre-treated at different pH conditions - 3.0, 4.0, 5.0, 6.0 and 7.0 and three temperatures - 60°C, 75°C and 90°C. These conditions are relatively milder than the conventionally used conditions (pH < 2, 121°C). As a trade-off, the reaction time was extended to 6 hours. The workflow of extracting sugars is shown in the schematic below (Figure 2).

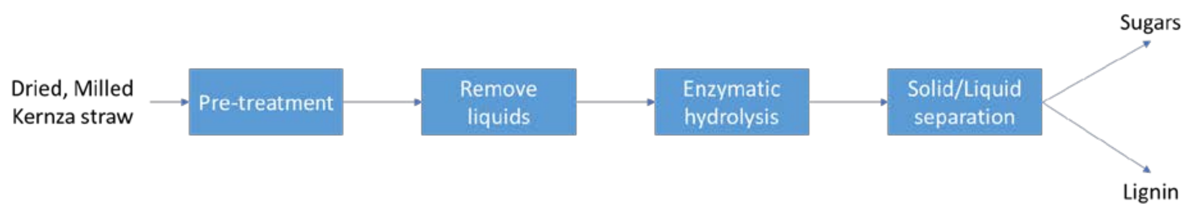


Figure 2. Schematic of the workflow for extracting sugars from Kernza® straw

The pre-treated biomass samples were collected after removing the acid and subjected to enzymatic hydrolysis. The enzyme used was a cocktail of carbohydrases, comprising of arabinase, cellulase, β-glucanase, hemicellulase, and xylanase (proprietary product from Novozymes). The enzyme was previously evaluated to efficiently depolymerize cellulose and hemicellulose and found to be resistant to inhibitors from biomass treatment (such as furfurals, etc.), high temperature and low pH conditions. In a previous study, the optimal hydrolysis condition was determined to be at a pH of 5.2 and a temperature of 50°C. These conditions were employed to hydrolyze the pre-treated biomass.

After 6 hours of incubation, the hydrolysis reaction was stopped and solids separated from the syrup by centrifugation. The solid portion, which predominantly comprises of acid-insoluble lignin and other residue, was weighed and was used as a proxy for lignin. The syrup was filtered and sent to AURI for quantification of glucose, galactose, xylose and arabinose. The data obtained was normalized to biomass weight (dry matter) and the amount of fermentable sugar released was expressed as a percentage.

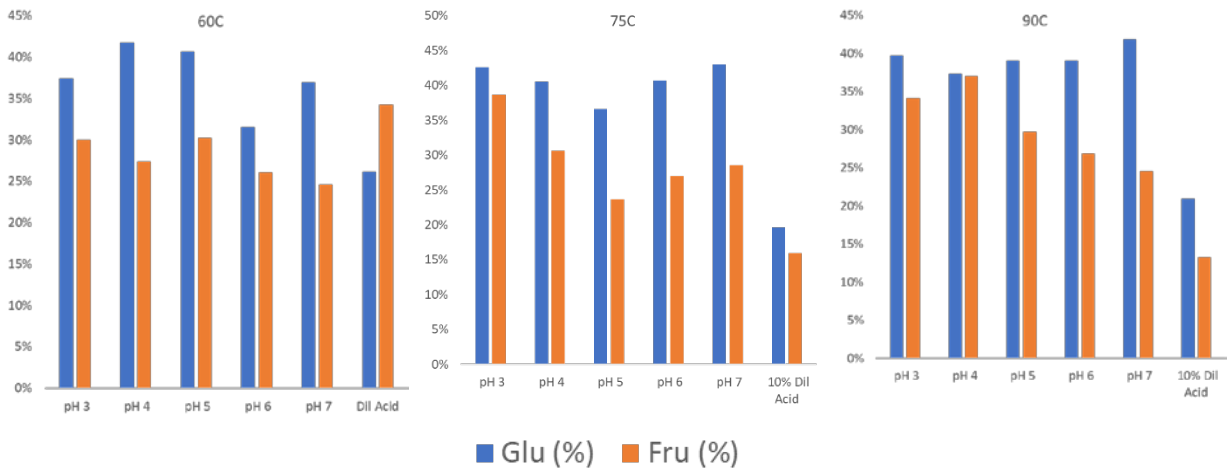


Figure 3. Extraction of hexose sugars from Kernza® straw at different pH values and temperatures. Shown in the figure are Glucose (blue) and Fructose (orange) as percentage of the biomass on a dry matter basis.

Acid pretreatment appeared to have a greater impact on the deconstruction of biomass into cellulose and hemicellulose. In general, at 60°C more acidic conditions were conducive to the release of hexoses (Figure 3). Interestingly, 10% H_2SO_4 which was also used to pre-treat Kernza straw, resulted in less hexose release than at a pH of 3. The result is strongly suggestive of an optimal pH for the release of hexoses. Interestingly, the amount of hexose released was the highest at pH 4. As the pretreatment temperature increased to 75°C and 90°C, the trend appeared to reverse, with higher pH treatment releasing more sugars. As indicated in the center panel of Figure 3, more glucose and fructose were released at pH 6 and pH 7 than at pH 3 or with dilute acid. If true, the trend has a valuable implication on process design that allows departure from the conventional conditions used in biomass pretreatment (dilute acid at a pH of 1.5 and 121°C). Overall, the dilute acid treatment followed by enzymatic hydrolysis was able to release hexoses that corresponded to ~65% of the biomass on a dry matter basis. This result alone conveys the strong promise of the applicability of Kernza straw for biofuel production.

The pretreatment condition and subsequent enzymatic hydrolysis had very different impact on the release of pentoses. Xylose and Arabinose were the major pentoses. Consistently across all pH values (3.0 to 7.0) for pretreatment, there was only a negligible amount of xylose and arabinose released (~1%) at the three temperature conditions studies. On the contrary, when 10% dilute acid was used as the pretreatment agent the amount of xylose released increased substantially to 20% at 75°C pretreatment (Figure 4). However, arabinose remained at 2%. Indeed, the large increase in xylose release was reproducible at all temperatures, with a maximum at 75°C. An important message from this result is that higher temperature is not necessarily required to depolymerize hemicellulose and xylan. As demonstrated with Kernza straw, pretreatment at an intermediate temperature of 75°C yielded higher xylose titer than pretreatment at 60°C or 90°C. The result agrees with the higher acetate concentration detected in the samples from dilute acid pretreatment. Acetals are produced as a result of hydrolysis of hemicellulose and not cellulose, higher xylose yields should be accompanied by higher acetate titers.

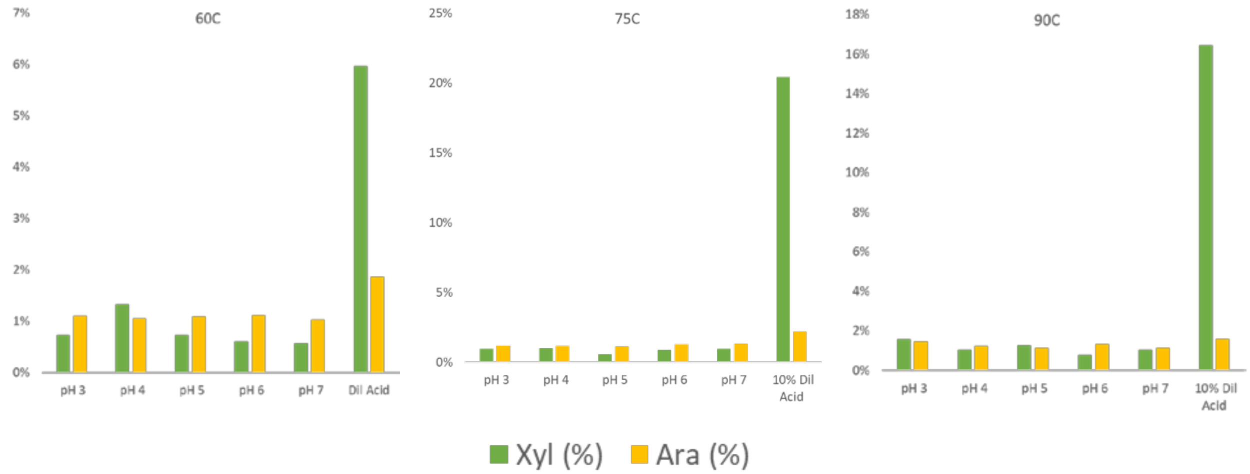


Figure 4. Extraction of pentose sugars from Kernza® straw at different pH values and temperatures. Shown in the figure are Xylose (green) and Arabinose (yellow) as percentage of the biomass on a dry matter basis.

The result is clearly indicative of a requirement of stringent pretreatment conditions to deconstruct hemicellulose from Kernza straw. The recalcitrant nature of hemicellulose in corn stover and switchgrass is widely reported and appears to hold true for Kernza straw. Given that the amount of arabinose content in biomass is typically very low, there is very little effort into optimizing the release of this sugar.

1.5. Conclusions

The main conclusion from the study is that Kernza is very amenable to extracting cellulosic sugars to provide feedstock for the biofuel industry. Given the significantly different pretreatment conditions required for the release of hexoses and pentoses from the straw, a one-pot, two-stage pretreatment process to deconstruct Kernza straw into monomeric sugars might be suitable. The first stage of the proposed process would comprise of moderate reaction conditions to deconstruct biomass into cellulose followed by a second stage harsh acidic conditions for hemicellulose.

2. Appendix

2.1. Solids and Ash Quantification for Kernza

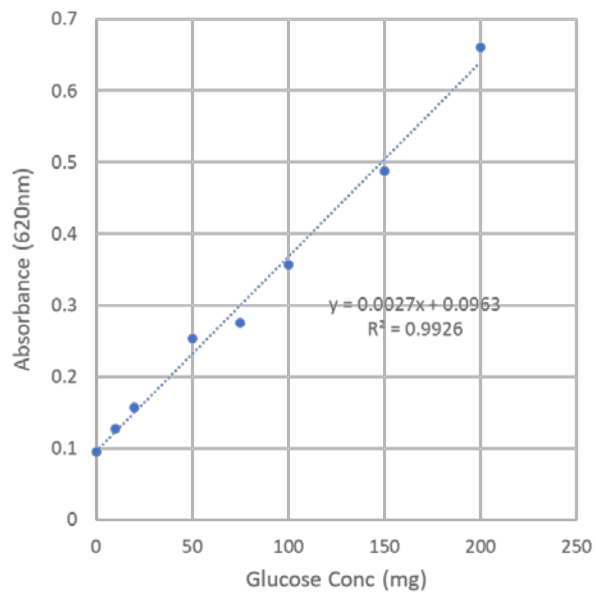
Quantification of solids in Kernza and Corn Stover samples

Boat	Init Wt (g)	Final Wt (g)	Final Wt (g)	Moisture
K1	4.35	4.05	4.06	6.90%
K2	4.62	4.28	4.3	7.36%
C1	4.89	4.41	4.4	9.82%
C2	4.12	3.7	3.68	10.19%

Quantification of ash in Kernza and Corn Stover samples

Crucible	Weight (g)	Final Wt (g)	Final Wt (g)	Ash
K1	64.1569	67.1280	65.1623	3.10%
K2	65.8506	68.8375	66.8775	3.05%
C1	66.1334	67.6351	66.596	1.49%
C2	65.9796	67.4738	66.4945	1.52%

2.2. Glucose released and cellulose calculation



Boat	Solids (g)♦	Glu (mg)	Dilution	Cellulose
K1	4.055	86.71842	20	42.77%
K2	4.29	83.322	20	38.84%
C1	4.405	80.00761	20	36.33%
C2	3.69	72.54624	20	39.32%

♦ Calculated from data in Section 2.1

2.3. Hemicellulose determination

Boat	Dry Solids (g)	P (mg)	Hemicellulose
K1	3.975	14.5715	36.66%
K2	4.055	10.9192	26.93%
C1	4.157	12.0416	28.97%
C2	4.054	11.8161	29.15%

2.4. Lignin quantification

Acid-soluble lignin

Sample	A320	Vf (L)	W	Soluble lignin
K1	0.388	0.06	0.244	3.18%
K2	0.402	0.06	0.254	3.17%
C1	0.118	0.06	0.422	0.56%
C2	0.131	0.06	0.512	0.51%

Acid-insoluble lignin

Crucible	Wbio	Wdry	Wash (calc)	Wres	% prot (from AURI)	Wpro (calc)	Insoluble lignin
K1	1.0321	0.9609	0.0295	0.2770	7.98%	0.0767	17.77%
K2	1.1002	1.0192	0.0313	0.2860	7.87%	0.0802	17.12%
C1	1.0442	0.9417	0.0289	0.2185	5.12%	0.0482	15.01%
C2	1.0875	0.9766	0.0300	0.2272	5.25%	0.0513	14.94%

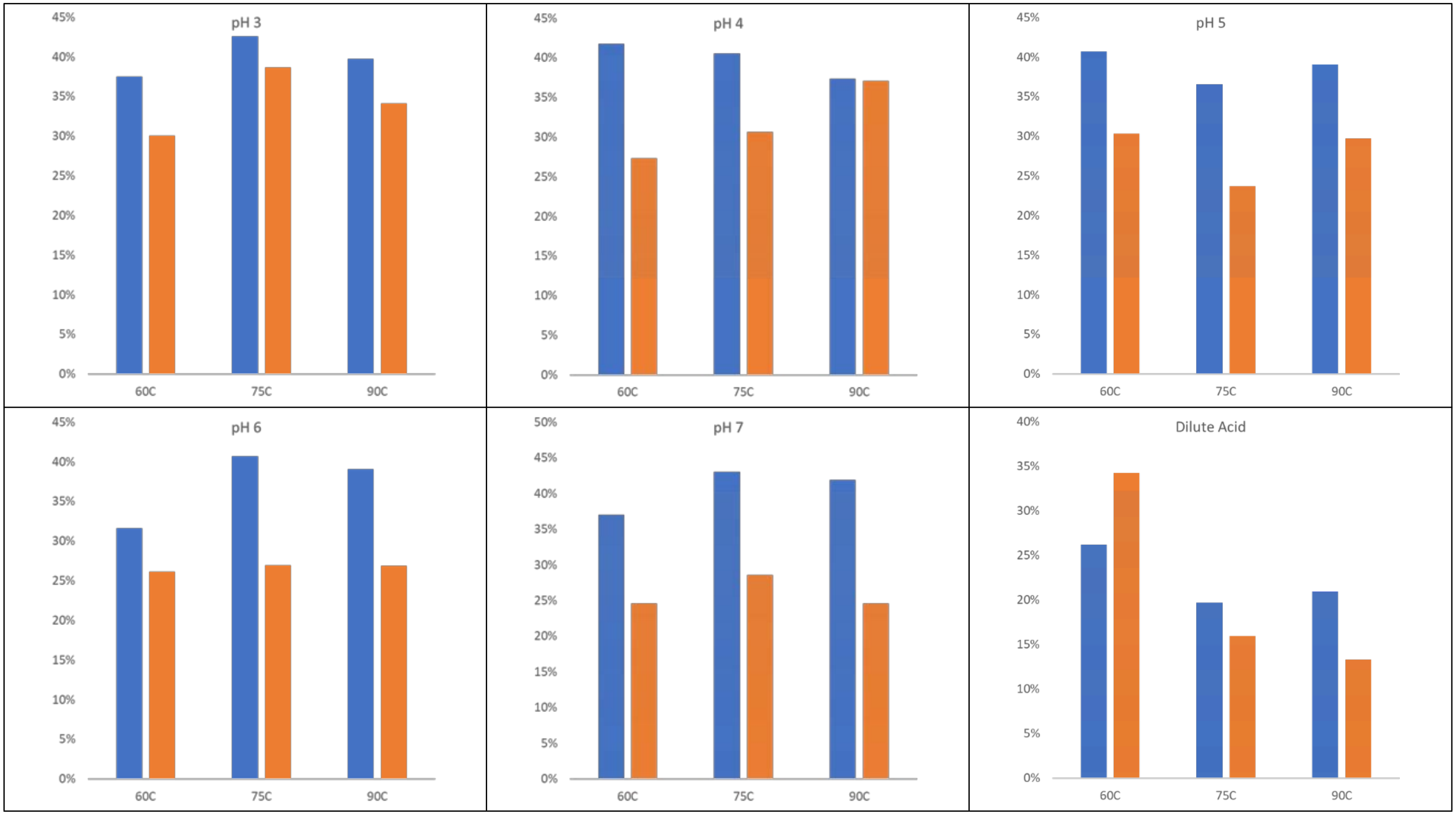
2.5. Sugar recovery

Sugar	Absorption	Measured Conc (g/L)	Actual Conc (g/L)	Recovery
Glucose	0.241	4.98	5.01	99.4%
Cellobiose	0.118	5.01	4.99	100.4%
Galactose	0.321	5.06	5.03	100.6%
Xylose	0.224	4.97	5.01	99.2%
Arabinose	0.13	5.01	4.99	100.4%
Mannose	0.0921	4.93	4.96	99.4%

2.6. Raw data from AURI

Pretreatment conditions							
pH	**Temp**	**Init biomass (g)**	**Glu (g/L)**	**Fru (g/L)**	**Xyl (g/L)**	**Ara (g/L)**	**Acetate (g/L)**
pH 3	60	5.99	11.33	9.08	0.22	0.33	0.08
pH 3	75	5.97	12.59	11.44	0.28	0.36	0.11
pH 3	90	6	12.00	10.30	0.47	0.44	0.20
pH 4	60	5.99	12.59	8.25	0.40	0.32	0.10
pH 4	75	6.01	12.09	9.13	0.29	0.34	0.10
pH 4	90	6.01	11.13	11.05	0.31	0.37	0.16
pH 5	60	5.96	12.03	8.96	0.22	0.32	0.07
pH 5	75	5.99	10.86	7.03	0.17	0.33	0.08
pH 5	90	5.99	11.60	8.83	0.37	0.34	0.19
pH 6	60	5.98	9.36	7.73	0.18	0.33	0.16
pH 6	75	5.99	12.24	8.11	0.27	0.39	0.12
pH 6	90	5.92	11.46	7.88	0.22	0.39	0.22
pH 7	60	6.02	11.06	7.35	0.17	0.31	0.27
pH 7	75	6.01	13.01	8.64	0.29	0.40	0.17
pH 7	90	5.97	12.38	7.26	0.31	0.34	0.27
10 % H_2SO_4	60	6	7.90	10.34	1.80	0.56	1.46
10 % H_2SO_4	75	6.01	5.95	4.81	6.17	0.66	2.09
10 % H_2SO_4	90	5.99	6.32	4.01	4.96	0.48	2.00

2.7. Hexoses released from Kernza® Straw



2.8. Pentoses released from Kernza® straw

3. References used to develop the methods

1. Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D (2008) Determination of structural carbohydrates and lignin in biomass, laboratory analytical procedure (LAP). Technical report NREL/TP-510-42618

2. Silvio Vas Jr. (2016) Analytical Techniques and Methods for Biomass. Springer International Publishing, Switzerland.

3. Handbook of Wood Chemistry and Wood Composites, edited by Roger M. Rowell, Taylor & Francis Group, 2012

FOOD APPLICATIONS OF KERNZA GRAIN

AURI obtained a sample of MN-Clearwater (1504) variety Kernza to assess for use in food applications. This sample consisted of grain grown at several locations throughout Minnesota. Working together with external partners, AURI generated data and analyzed grain attributes to identify potential Kernza food applications. The MN-Clearwater Kernza grain was sent to NCI in Fargo, ND for milling trials as well as several grain quality tests seen in the NCI section of this report. A number of nutritional analyses were completed on samples of both the dehulled, whole grain and milled flour by Minnesota Valley Testing Labs (MVTL) in New Ulm, MN.

Researchers compared proximate and nutritional data to values reported in the literature for other varieties of Kernza. The following table (Table 10) reports the data for several varieties. In comparison to the average values, the MN-Clearwater variety is in close agreement for all proximate and nutritional data reported.

Kernza Variant	Protein	Fat	Ash	Insoluble Fiber	Soluble Fiber	Starch	Amylose
Hard red wheat	12.01	1.8	1.84	11.72	3.23	67.38	24
C3-214	23.08	3.32	2.77	16.85	3.84	44.22	27.2
C3-2627	24.56	3.08	2.97	17.89	3.94	42.53	24.5
C3-2725	21.68	3.06	2.66	17.49	3.58	48.32	25.5
C3-3471	22.88	3.14	2.49	15.72	5.36	52.96	22.3
C3-3486	24.84	2.23	3.01	19.51	4.78	43.02	27.3
C3-448	19.76	3.78	2.44	20.11	4.96	49.79	27.1
C3-EB	20.46	3.86	2.56	18.35	4.66	46.84	25.9
TLI-C2	20.8	3.63	2.58	17.95	3.06	49.07	19.9
Beefmaker	21.32	3.22	2.55	17.62	4.72	48.79	19.5
Manifest	23.51	2.9	2.75	16.18	2.59	49.35	26.5
Manska	19.22	2.76	2.86	18.21	1.88	48.57	21.9
Oahe	18.18	4.02	2.65	17.19	4.67	47.59	26.3
Rush	20.87	3.91	2.67	17.04	4.05	48.52	21.7
Avg. IWG Varieties	21.63	3.3	2.69	17.7	4.01	47.66	24.28
Clearwater	21.03	3.64	2.61	15.76	3.94	46.97*	N/A

Table 10. Attribute comparison of Kernza® varieties. (Feels like TITLE SHOULD GO ON TOP VS BOTTOM)
Data reported on a Gram/100-gram dry basis

*Calculated value

A complete nutritional profile along with other technical aspects and considerations were also summarized in short Application Bulletins to guide adoption of and create demand for Kernza by new users. Additional analyses completed on MN-Clearwater include a flour Amino Acid Profile (Table 11) and Fatty Acid profiles on both the whole grain and the flour (Table 12).

Kernza® Perennial Grain as a Cereal Grain

Agricultural Utilization Research Institute

Introduction

Kernza® perennial grain (Kernza) is a new domesticated grain introduced by The Land Institute that is now being developed for commercial use in Minnesota. It originates from a forage grass called intermediate wheatgrass (*Thinopyrum intermedium*) and is a relative of wheat. In 2019, the University of Minnesota released its first named Kernza® variety: MN-Clearwater.

As a close relative of wheat, Kernza has application opportunities in the food industry. It contains a higher protein and dietary fiber content versus wheat but lacks in some gluten components that limit its functionality in some applications. Besides the potential for food applications, Kernza also provides environmental benefits. According to University of Minnesota Researchers, its deep roots can protect soil from erosion, improve soil health, and reduce nitrogen leaching, protecting water resources from

Nutritional Comparison to Wheat

Kernza contains more protein, dietary fiber and bioactive compounds such as carotenoids versus wheat but certain characteristics limit its use as a stand-alone flour. Although Kernza contains gluten, it is deficient in one of the gluten components (high molecular weight glutenin).

***Values in table based on 100g sample**

Types of Grain		Kernza Whole Grain[a]	White Wheat Berries[b]	Kernza Refined Flour[a]	All Purpose White Flour[c]
Moisture	%	8.6	13.75	8.1	11.9
Ash	%	2.4	N/A	0.6	0.47
Calories	-	368	318	368	364
Protein	g	19.2	9.24	17.5	10.3
Carbohydrates	g	67.3	73.7	73.2	76.3
Dietary Fiber	g	18.0	10.3	4.3	2.7
Soluble Fiber	g	3.6	N/A	1.0	0.9
Sugar	g	1.7	1.1	N/A	0.3
Total Fat	g	2.9	2.3	1.2	1.0
Sat Fat	g	0.5	0.4	0.3	0.2
Mono Fat	g	0.5	0.3	0.1	0.1
Poly Fat	g	1.9	1.6	0.7	0.4
Trans Fat	g	0	0	0	0
Cholesterol	mg	0	0.10	0	0
Calcium	mg	120.0	25.0	50.0	15.0
Iron	mg	5.5	2.6	3.7	1.2
Potassium	mg	480.0	N/A	140.0	107.0
Sodium	mg	0	13.0	0	2.0

Characteristics

- Kernels are 80% smaller than Hard Red Wheat (breeding efforts are underway at the UMN and The Land Institute to increase kernel size)
- Hull/Kernel weight Ratio: 25-35% Hull to 65-75% Kernel
- Amber/Mahogany color

Bakers Field toasted the grain to assist in milling the product, which led to an enhanced flavor and a more consistent particle size for the flour.

—Steve Horton- Bakers Field Flour and Bread

***a:** Source: Results are directional only, data represents analysis of one sample of Clearwater Variety, MVTL, New Ulm, MN*
***b:** ESHA Database: Star of the West Milling Company*
***c:** ESHA Database: USDA Composition Data*

Kernza® Perennial Grain as a Cereal Grain

Agricultural Utilization Research Institute

Suggested Application Opportunities

Whole Grain
Granola or other cereal
Pilaf style side dish
Brewing (malted or unmalted)
Puffed or Sprouted

Flour	
Bread and Flatbreads	Pretzels
Biscuits	Pasta
Pancake/Waffle Mix	Crackers
Cupcakes	Brewing

Processing and Grain Stability

- On average 30-50% of the harvested material will be dehulled usable grain
- Inconsistent grain sizes could lead to inefficient dehulling and/or the need to regularly modify processing settings
- Mold/Mycotoxins: Kernza can be tested using existing methods for molds and mycotoxins
- Food Grade Storage and Handling: Kernza does not present any unique challenges for food grade storage and handling
- Higher fat content increases overall rancidity potential, but higher antioxidant content may offer protective effect
- Kernza showed reduced levels of peroxide formation during storage versus Hard Red Wheat which points to an increased resistance to oxidative rancidity
- Microbiological Spoilage: Kernza does not require special preventative measures
- Whole grain Kernza is stable when stored under typical grain storage conditions, though once hulled, the grain may benefit from refrigerated storage to help extend its shelf life

References

1. The Land Institute, 2020, https://landinstitute.org/our-work/perennial-crops/kernza/
2. *"Kernza in Southern Minnesota: Assessing Local Viability of Intermediate Wheatgrass"* Erik Muckey, January 2019, University of Minnesota Extension
3. Marti et al, *"Structural characterization of proteins in wheat flour doughs enriched with intermediate wheatgrass (Thinopyrum intermedium) flour"*, 2015, Journal of Food Chemistry
4. *"Evaluation of the Chemical and Functional Stability of Intermediate Wheatgrass (Thinopyrum intermedium) over Storage and in Response to Steam Treatment"* Amy Mathiowetz, December 2018, University of Minnesota

Kernza® Perennial Grain in Baking Applications

Agricultural Utilization Research Institute

Introduction

Kernza® perennial grain (Kernza) is a new domesticated grain introduced by The Land Institute that is now being developed for commercial use in Minnesota. It originates from a forage grass called intermediate wheatgrass (*Thinopyrum intermedium*) and is a close relative of wheat. In 2019, the University of Minnesota released its first named Kernza variety: MN-Clearwater. Besides the potential for food applications, Kernza also provides environmental benefits. According to University of Minnesota researchers, Its deep roots can protect soil from erosion, improve soil health, and reduce nitrogen leaching, protecting water resources from nitrate contamination.

As a close relative of wheat, Kernza has application opportunities in the food industry. It contains a higher protein and dietary fiber content versus wheat but lacks in some gluten components that limit its functionality in some applications. To overcome the gluten component deficiency, there are several additives or dough conditioners that can be utilized to help improve functional properties. Kernza can also be blended with wheat flour to improve baking (or baked good) quality.

Comparison with Traditional Wheat

*Values in table based on 100g sample

Grain Types		Kernza Refined Flour[a]	All Purpose White Flour[b]
Moisture	%	8.1	11.9
Ash	%	0.6	0.5
Calories	-	368	364
Protein	g	17.5	10.3
Carbohydrates	g	73.2	76.3
Dietary Fiber	g	4.3	2.7
Soluble Fiber	g	1.0	0.9
Sugar	g	N/A	0.3
Total Fat	g	1.2	1.0
Sat Fat	g	0.3	0.2
Mono Fat	g	0.1	0.1
Poly Fat	g	0.7	0.4
Trans Fat	g	0	0
Cholesterol	mg	0	0
Calcium	mg	50	15.0
Iron	mg	3.7	1.17
Potassium	mg	140	107.0
Sodium	mg	0	2.0

a *Source: Results are directional only, data represents analysis of one sample of Clearwater Variety, MVTL, New Ulm, MN*
b ESHA Database USDA Composition Data

Baking Properties

- Whole Grain Flour: Kernza berries have a higher bran-to-endosperm ratio which can lead to reduced loaf volume and increased crumb firmness when using whole grain flour

- Refined Flour: Removal of the bran through refining processes can lead to dough that has increased stickiness

Handling and Special Considerations

- With the proper storage conditions (maintaining low humidity and a temperature-controlled environment) flour from Kernza would be considered shelf stable

- Toasting the grain prior to milling may improve flour particle size consistency and help to highlight

Kernza® Perennial Grain in Baking Applications

Agricultural Utilization Research Institute

Challenges and Opportunities

- Glutenin: Kernza contains significantly less glutenin (a functional component of gluten) which limits the dough's ability to form viscoelastic networks required for certain baking applications
- Starch Content: Kernza dough contains less starch, leading to a reduced loaf volume and a weaker crumb structure when compared to conventional wheat dough
- Dough Conditioners: the addition of several dough conditioners, such as vital wheat gluten, ascorbic acid, transglutaminase, xylanase, and alpha amylase, can improve the overall quality of the food products made from Kernza dough
- Blending: Baked goods made from Kernza would benefit from the addition of wheat flour to make up for the lack of gluten proteins and starches
- Dietary Fiber: Kernza flour could be used as a fiber source in flour blends due to its higher dietary fiber content
- Sourdough fermentation was found to highlight the earthy notes in Kernza's flavor profile
- Kernza works well in flatbread applications combined with wheat flour, at a Kernza inclusion rate of about 10 to 15%

Baking Applications

- Bread & Sourdough
- Flatbreads such as Focaccia and Pizza Crust
- Pretzels
- Biscuits
- Muffins
- Cupcakes
- Cookies
- Scones
- Pancake/Waffle Mix
- Crackers
- Pasta

"I prefer the flour in non-yeasted applications, particularly products like pound cake, because the flavor of the grain resembles graham and works well with sweet applications. It reflects the flavor of the Earth quite well where conventional flour does not."

—Beth Dooley- Cookbook Author and Minneapolis Chef

References

1. *The Land Institute, 2020, https://landinstitute.org/our-work/perennial-crops/kernza/*
2. *Marti et al, Structural characterization of proteins in wheat flour doughs enriched with intermediate wheatgrass (Thinopyrum intermedium) flour, 2015, Journal of Food Chemistry*
3. *"Evaluation of the Chemical and Functional Stability of Intermediate Wheatgrass (Thinopyrum intermedium) over Storage and in Response to Steam Treatment" Amy Mathiowetz, December 2018, University of Minnesota*
4. *Interviews with Steve Horton from Bakers Field Flour and Bread 2020*
5. *"Effects of Dough Conditioners on Rheology and Bread Quality of Intermediate Wheatgrass" Jaya Dhungana Banjade, July 2018, University of Minnesota*
6. *"Chemical Characterization, Functionality, and Baking Quality of Intermediate Wheatgrass (Thinopyrum intermedium)" Citra Putri Rahardjo, May 2017, University of Minnesota*

Amino Acid profile of MN Clearwater Flour

Table 11. Kernza® Flour Amino Acid Profile. Data reported on both per 30 gram and per 100-gram basis.

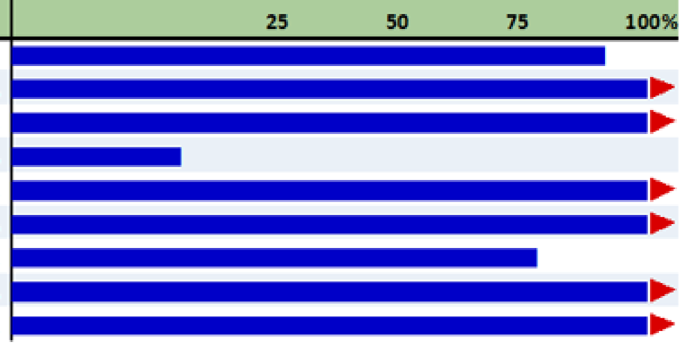
Nutrients	Per Serving	Per 100g	Nutrients	Per Serving	Per 100g
Basic Components			Isoleucine (g)	0.19	0.64
Gram Weight (g)	30.00	100.00	Leucine (g)	0.35	1.17
Calories (kcal)	110.49	368.31	Lysine (g)	0.08	0.27
Protein (g)	5.25	17.50	Methionine (g)	0.08	0.28
Amino Acids			Phenylalanine (g)	0.27	0.89
Alanine (g)	0.14	0.47	Proline (g)	0.04	0.14
Arginine (g)	0.18	0.60	Serine (g)	0.20	0.68
Aspartic Acid (g)	0.18	0.60	Threonine (g)	0.14	0.47
Cystine (g)	0.14	0.46	Tryptophan (g)	0.06	0.20
Glutamic Acid (g)	1.84	6.14	Tyrosine (g)	0.16	0.54
Glycine (g)	0.17	0.55	Valine (g)	0.22	0.74
Histidine (g)	0.09	0.31			

Based on the Amino Acid profile in Table 8 (11?), the Protein Quality report (Figure 19, below) shows actual versus ideal ratios of amino acids scored as percentages, as well as the limiting amino acid. The limiting amino acid is that with the lowest individual percent listed. Kernza is limiting in Lysine and low in Threonine and Histidine. In comparison, wheat flour is also limiting in Lysine and low in Threonine (but not Histidine). Because Kernza has amino acids that score below 100 %, it's not considered a complete protein. However, the overall protein content is fairly high, and pairable with other protein containing components to make a complete protein ingredient.

Figure 19 Protein Quality of MN-Clearwater

Weight: 100 g

Amino Acid	Actual Ratio	÷	Ideal Ratio	=	Score
Histidine	17.14	÷	19	=	90%
Isoleucine	36.19	÷	28	=	129%
Leucine	66.67	÷	66	=	101%
Lysine	15.24	÷	58	=	26%
Methionine + Cystine	41.9	÷	25	=	167%
Phenylalanine + Tyrosine	81.9	÷	63	=	130%
Threonine	26.67	÷	34	=	78%
Tryptophan	11.43	÷	11	=	103%
Valine	41.9	÷	35	=	119%



Fatty acid profile of MN Clearwater Flour

The amount of total fat, saturated fat and polyunsaturated fat are reported in the nutritional profiles contained in the Application Bulletins. The following table (Table 12) shows the detailed fatty acid profile for both the whole grain and the flour. The overall amount of fat in Kernza is slightly higher than in wheat but the largest proportion of the fat is polyunsaturated, similar to wheat.

Table 12 Fatty Acid Profile of MN Clearwater. Only non-zero fatty acids reported.

Saturated	Grain	Flour
4:0 Butyric	0.001	0.001
14:0 Myristic	0.003	0.002
15:0 Pentadecanoic	0.003	0.002
16:0 Palmitic	0.406	0.279
17:0 Margaric	0.002	0.002
18:0 Stearic	0.017	0.01
20:0 Arachidic	0.004	0.002
21:0 Heneicosanoic	0.001	N/A
22:0 Behenic	0.005	0.003
24:0 Liqnoceric	0.007	0.005
Totals (%)	**0.45**	**0.3**
% of Fatty Acid Components based on Total Fat	16.07	25.42
Monounsaturated		
16:1 Palmitoleic	0.005	0.002
18:1 Oleic	0.457	0.132
20: 1 Gadoleic	0.023	0.006
22:1 Erucic	0.005	0.003
24: 1 Nervonic	0.003	0.001
Totals (%)	**0.49**	**0.14**
% of Fatty Acid Components based on Total Fat	17.5	11.86
cis-cis Polyunsaturated		
18:2 Linoleic	1.678	0.674
18:3 Linolenic	0.178	0.06
20:2 Eicosadienoic	0.003	0.001
20:4 Arachidonic	0.002	0.001
22:2 Docosadienoic	0.001	N/A
22:4 Docosatetraenoic	0.001	0.001
Totals (%)	**1.86**	**0.74**
% of Fatty Acid Components based on Total Fat	66.43	62.71
Omega-3	0.178	0.06
Omega-6	1.685	0.272
Trans		
18:2 t-Octadecadienoic	0.002	0.001
Totals (%)	**0.00**	**0.00**
% of Fatty Acid Components based on Total Fat	0.000	0.000

Malting Testing

AURI partnered with Montana State University (MSU), Bozeman, MT, to conduct a malting analysis on Kernza grain. Both MN-1502 and MN-Clearwater (1504) Kernza varieties underwent a set of barley selection tests to inform malting methods and process, in order to understand the germination capacities and energies of the samples. MN-Clearwater (1504) was malted and compared to unmalted Kernza grain on several analytical measures. Also, researchers conducted tests on cleaned and dehulled grain. In addition to the malting studies, AURI summarized other technical aspects and considerations for the use of Kernza in brewing in an Application Bulletin.

Brewing with Kernza®
Perennial Grain

Agricultural Utilization Research Institute

Brewing Overview

Kernza® perennial grain (Kernza) is a new type of perennial intermediate wheatgrass that is under development in Minnesota for its environmental benefits. According to University of Minnesota researchers, Kernza has an extensive root system that helps protect soil from erosion, improves soil health, and reduces nitrogen leaching, protecting water resources from nitrate contamination. As a close relative of wheat, Kernza has many potential applications in the food and beverage industry.

Comparison of Brewing Characteristics

Type of Grain		2-row Barley Base Malt	Kernza Malted Hulled*	Unmalted Hulled Kernza*	Malted White Wheat	Unmalted White Wheat
Moisture	%	5.23	3.53	4.30	5.00	12.0
Total Protein	%	11.5	18.0	17.9	11.5	10.0
Alpha Amylase	D.U.	65.0	15	8	48	-
Germination Energy	%	>95**	NA	65	NA	>95**
Germination Capacity	%	>95**	NA	75	NA	>95**
Extract (FG Dry Basis	%	81.0	79.9	69.9	83.0	76.0
Color	°SRM	2.2	3.3	1.8	2.5	2
Turbidity	NTU	8.7	N/A	3.0	-	-
pH	-	5.8	6.0	6.3	-	-
Soluble Protein	%	4.7	8.9	4.6	4.7	-
S/T Ratio	-	41.0	49.4	25.7	41.0	-
ß-Glucan	mg/L	96	67	176	-	-
Free Amino Nitrogen (FAN)	mg/L	169	174	45	-	-
Diastatic Power	°L	129	104	108	160	-

*- Source: Data represents initial lab scale testing results at Montana State Malting Labs

** Montana State Lab does not recommend malting grain that does not have Germination Energy and Germination Capacity over 95%.

Malt test results based on one sample of MN Clearwater, numbers may vary slightly, sample to sample

Brewing with Kernza® Perennial Grain

Agricultural Utilization Research Institute

Comparison of Brewing Characteristics

- Compared to wheat, Kernza yields less extract on a fine ground basis
- S/T Ratio: Soluble Protein to Total Protein Ratio
    - Malted Kernza: Indicates thinner and lighter-bodied beer
    - Unmalted Kernza: indicates fuller-bodied beer with good head retention and foam stability
- FAN level, Free Amino Nitrogen, of malted Kernza suggests higher percentage usage will not negatively impact yeast growth or result in need for added yeast nutrients in the wort
- Low turbidity of unmalted Kernza suggests a clear, bright finished beer appearance
- Both the malted and unmalted Kernza made beers with low SRMs, Standard Reference Methods, suggest that Kernza usage in higher percentages (>50 percent) won't darken the final product

Challenges

- Grain size: Seed is approximately 80% smaller than conventional wheat, potentially leading to difficulties in milling and malting Kernza traditionally. Genetic modifications are currently under exploration at the University of MN and the Land Institute to increase Kernza grain size
- Processing: The addition of ß-glucans in unmalted Kernza and elevated protein levels in malted Kernza may lead to stuck sparges during brewing. Addition of rice hulls during the mash step could mitigate the frequency of these occurrences
- Supply: Low grain supply may impact availability of Kernza for brewing purposes

Typical Usage

- Suggested beer styles for Kernza use:
    - American Wheat Beer
    - German Hefeweizen
    - German Dunkelweizen
    - German Weizenbock
    - Belgian Witbier
- Typical usage levels- Small batch testing has suggested usage of 15-20% of Kernza to have no perceived negative effects. Specific brewing conditions and finished product sensory preferences may result in usage levels outside of this suggested range.
- For example, a 500-gallon batch of a traditional mild American wheat beer would use around 950 pounds of grain, 20% of which would be wheat. If Kernza were substituted in this recipe, the 500 gallon batch would require around 200lb of Kernza grain.

- Sensory Profile Impacts
    - Addition of Kernza at 15% added a slight sour-like acidity to the beer
    - Beer made with 15% Kernza had less lingering sweetness than a standard, malty beer
    - Inclusion of Kernza at 15% was shown to have a slight dampening effect on the perceived carbonation of the beer
    - Imminent Brewing out of Northfield, MN has used milled Kernza in a German Alt beer at 20% with success, noting a slightly lighter color and a pleasant nuttiness addition
    - Overall, the addition of Kernza at lower levels does not seem to negatively impact the sensory characteristic of beer and may add a unique flavor profile.

References

The Land Institute, 2020, https://landinstitute.org/our-work/perennial-crops/kernza/ • Interviews with Dr. George Annor from the University of Minnesota Food Science Department 2020 • Interview with Imminent Brewing 2020

Hannah Turner, Director of the Barley Malt and Quality Lab at MSU, provided analysis of the malting results through a series of email correspondences[8], summarized here:

- *Due to Kernza's seed size, traditional maltsters may have an issue with some seeds falling through the pores of malting cages. Similar issues are possible during grain de-culming.*
- *Malting Kernza had some positive effects including increases in Extract Yield and FAN, and a drop-in beta-glucan levels.*
- *Malting Kernza may also result in decelerating the lautering process in beers where Kernza makes up a majority of the grain bill, due to an increase in the protein content.*
- *Both malted and unmalted Kernza are low in the enzyme alpha amylase, thus requiring additional base malt in the grain bill to complete conversion. As such, researchers obtained this data using a mixture of 1:1 Kernza with a standard 2-row base malt.*
- *It's necessary to conduct more research to explore the effect of both intact hulls and a coarse grind on the quality of beer produced.*

Germination is of critical importance for any grain malted. We would not recommend malting a sample that has less than 95% germination capacity. If germination energy is lower than this there may be dormancy issues, in which case malting should wait until dormancy breaks and both values are greater than 95%. Malting is a germination process and if you don't have high germination there is no point to malting the grain. It is important to carefully manage aspects of agronomics and grain handling to ensure high germination of malting grain. The data here offers germination counts at 24, 48 and 72 hours – these numbers can be informative to a maltster to help them judge how vigorously the grain will germinate. The Germination Energy is a sum of these three counts.

Discussions with Montana State also resulted in malting in-hull Kernza to see how it may affect the results. Discussions are ongoing with multiple contacts in Minnesota about malting tests of in-hull Kernza, including the University of Minnesota and Sprowt Labs. This testing will occur in the near future. Through discussions, researchers believe malting the Kernza with the hull-on (similar to malting barley) will lead to a higher germination energy and capacity.

Next Steps on Kernza Application in Foods

As noted throughout this report, AURI is partnering with the Stearns County Soil and Water Conservation District on a separate LCCMR project to further explore the commercialization of Kernza. This project will continue exploring food applications, including with external partners. The initial research and analytical testing conducted as part of this work will help inform product development in the next project. Application Bulletins are planned for use of Kernza in pasta and crackers as well as treatment of the whole grain including puffing and sprouting.

Initial Supply Chain Identification – Potential Cleaners, Processors and End Users

Current MN Regional Cleaners and Processors

1. Healthy Food Ingredients (Valley City, ND) – Cleaning and Dehulling
2. Cal Spronk (Edgerton, MN) - Cleaning
3. MN Native Landscapes (Otsego, MN) – Cleaning
4. Richard Magnusson (Roseau, MN) – Cleaning
5. Swany White (Freeport, MN) – Milling
6. Northern Excellence (Williams, MN) – Cleaning
7. Sprowt Labs (Burnsville, MN) – Cleaning, Dehulling and Malting

Current/Interested End Users

2. Birchwood Café (Minneapolis, MN)
3. Bakers Field Flour & Bread
4. Bang Brewing (St. Paul, MN)
5. Imminent Brewing (Northfield, MN)
6. Beaver Island Brewing Co. (St. Cloud, MN)

Conclusions

AURI will continue working in the area of Kernza supply chain, product and process development through both individual client projects and the additional LCCMR-funded work with the Stearns County Soil and Water Conservation District. AURI has ordered a Forsberg 7F impact huller, several slotted screens and an indent separator to allow for a demonstration of the cleaning and dehulling process at its Waseca lab. AURI will explore the opportunity to have this space deemed a qualified facility through FDA's FSMA standards as well, in order to provide small amounts of cleaned, dehulled grain for food product development testing. Overall, the progress made under this research opportunity has provided excellent information on processing, product and supply chain development. The work will support future projects surrounding Kernza to further explore storage, processing, business and end-product development.

QUALIFICATIONS, RESOURCES AND FACILITIES

The Agricultural Utilization Research Institute (AURI) helps foster long-term economic benefit for Minnesota through value-added agricultural products. It accomplishes this mission by helping develop new uses for agricultural products through science and technology, while collaborating with businesses and entrepreneurs to bring ideas to reality. AURI provides a broad range of services including hands-on scientific technical assistance and technology transfer, a network of resources, and the applied research necessary to generate ideas for new ag-based products and processes and to help move them to market. With labs specific to analytical chemistry, coproducts, food, and microbiology, as well as staff experienced in science, technology and innovation processes, AURI is a unique resource, providing assistance to Minnesota businesses seeking to create more value for the state's agricultural products.

AURI staff included in this project are as follows:

- Principal Investigator: Riley Gordon, Engineer
- Project Manager: Matthew Leiphon, Innovation & Commercialization Project Manager
- Subject Matter Experts:
  - Ben Swanson, Scientist of Food and Nutrition
  - Lolly Occhino, Scientist of Food and Nutrition
  - Jason Robinson, Project and Business Development Director – Food
  - Dr. Michael Stutelberg, Analytical Chemist
  - Alan Doering, Senior Scientist, Coproducts
  - Abel Tekeste, Associate Scientist, Coproducts
  - Harold Stanislawski, Project Development Director
  - Michael Sparby, Senior Project Strategist

References

1. ***https://www.bratney.com/equipment/sorting--inspection/bomill-grain-quality-separator***
2. ***"Classification of Activities as Harvesting, Packing, Holding, or Manufacturing/Processing for Farms and Facilities: Guidance for Industry" – Document released by the FDA Center for Food Safety and Applied Nutrition***
3. ***"Key Facts about Preventive Controls for Human Food" – Document Released by the FDA***
4. ***"Understanding the Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food" – Document released by the Minnesota Department of Agriculture***
5. ***"IF YOUR FACILITY IS A VERY SMALL BUSINESS OR A SMALL (OR VERY SMALL) FARM MIXED-TYPE FACILITY, WHAT PC HUMAN FOOD EXEMPTIONS/MODIFIED REQUIREMENTS APPLY TO YOU?" – Document released by the FDA***
6. **Why Food Manufacturers Need the Best Insurance -** https://www.trustedchoice.com/n/31/food-manufacturing-insurance/
7. **Storing Wheat and Barley - William F. Wilcke, emeritus Extension engineer and Kenneth J. Hellevang, Extension engineer, North Dakota State University**
8. **What are Polyphenols? -** https://www.healthline.com/health/polyphenols-foods
9. **"Evaluation of the Chemical and Functional Stability of Intermediate Wheatgrass (Thinopyrum Intermedium) Over Storage and in Response to Steam Treatment". - Amy Mathiowetz Thesis Paper**
10. ***Full Email Correspondences on Malting with Hannah Turner of Montana State University***

APPENDIX A

The following Narrative is based on multiple email correspondences with Hannah Turner, the director of the Barley, Malt & Brewing Quality Lab at Montana State University.

"First *on malting -- we were able to make the process work in our system -- but I think it may be more difficult depending on the setup a craft maltster may have. We were worried about losing some of the grain through the pores of our malting cages - so purchased small organza bags to contain the grain in while malting - this worked great in this small scale - but a craft maltster may have to come up with larger creative ideas on the processing side. Also de-culming was a challenge as we typically use hand agitation and sieving over a 5/64" sieve - this works well for malted barley but the Kernza would have dropped through - we were able to use the natural static of the sample bag then doing several rounds of sample transfers to a second bag/cleaning culms from original bag to remove the culms. There was not a lot of material removed - so for a larger scale operation it may not make much difference removing culms or not. Quality analysis - the differences between un-malted and malted Kernza are interesting... I think overall there are pros and cons to both. Malting gives a bump to extract, and FAN, and drops the B-glucan - all items important to a brewer. Negatives to the malted Kernza are that the protein is quite high (this would be an issue if brewing with 100% Kernza - not so much when mixing with base malt) and surprisingly the filtration slowed way down (3-4 times slower than what we consider normal) -- I would have expected the un-malted sample to be slower -- so I'm not sure what is causing this. One hypothesis would be the reduced moisture of the malted sample is causing the grain to break into a finer flour at milling which could impact filtration. Both malted and un-malted samples are very low in alpha - again not an issue when brewing with a good base malt. One area that could make an argument one way or the other - but we have not evaluated here - is how malting impacts the flavor.*

Just looking at the data we have - its arguable that hulled Kernza can make an interesting addition to a brewers' grist bill - but malting it beforehand may be logistically more difficult than it is worth. It is also worthwhile to note that results here are based on a fine grind - where a brewer would be utilizing a coarser grist. Altering mill settings may be necessary - or at least experimenting to determine what will be most efficient for a brewer may be worthwhile as a recommendation.

Barley base malts make up a large proportion of a grist bill as they supply enzymatic power for conversion - particularly when utilizing adjuncts in the mash which will be lower enzymatically (as in the 8 and 15 DU reported here for the hulled Kernza). Also, it is important to note that the QC comparison malt is not something malted in our system

-- it is a homogeneous 50lb bag of 2-row that we purchase as a control and which provides consistency. Reasoning for including it is not really for direct comparison to the Kernza - but so that if a brewer were to look at the report it would inform them that the Kernza results were obtained when utilizing a base barley malt of x, x and x example quality at ratio of 1:1 to for conversion.

The filtration time we report would likely impact lautering for the brewer. 1:1 or 50% inclusion is quite high for most adjunct grains - but it is the standard method for comparison and helps to give a better idea of what the performance of the grain itself is. Impact on lautering time might be one of the main limitations on what % inclusion would really be functional for a brewer. This will likely also vary brewer to brewer as each will have different system set ups."