PROJECT NO: New

TITLE: Developing Techniques to Quantify Wheat α-amylase and Measure its Activity and Investigate its Hydrolytic Mechanism

PERSONNEL:

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JUSTIFICATION/RATIONALE:

The falling number (FN) test is an indirect measurement of wheat α-amylase activity that measures the change in wholemeal flour visocisty during heating. Wholemeal flour viscosity is influenced by several factors: (1) the polymers in wholemeal wheat flour, (2) the interaction between polymers, and (3) the interaction between degrading enzymes (e.g., α-amylase) and their corresponding substrates (e.g., starch). The scientific literature simply attributes low FN in wheat to pre-harvest sprouting (PHS) or the synthesis of late mature α-amylase (LMA) because PHS and LMA are associated with changes in α-amylase quantity and activity. However, our research, sponsored by the Idaho Wheat Commission, has demonstrated other factors also affect FN. For example, the substrate (i.e., starch) has a significant influence on viscosity and consequently impacts FN. Our research also provides evidence that agronomic practice (e.g., irrigation) affects the substrate's composition (i.e., starch granule size distribution) and therefore impacts FN. In addition, accurately measuring FN can be challenging because the measurement itself is affected by many variables, such as elevation. Understanding and addressing the complexities of low FN in wheat necessitates the ability to accurately measure α-amylase quantity and activity. Unfortunately, the widely accepted direct measurement immunoassay (antibody-antigen binding-based analysis) is not available in the United States. Even if it were available, the immuoassay requires high levels of technical training and investment. An efficient and economic assay is still under development. With Idaho Wheat Commission support in FY18 (BKK403), we have proved the concept of using non-antigen-antibody binding to quantify α-amylase and measure its activity. The proposed study is to complete development of this methodology and further examine the hydrolytic mechanism of wheat α-amylase that will help us to understand the difference between PHS and LMA toward developing potential solutions to utilize low FN wheat.

HYPOTHESIS & OBJECTIVES:

We propose to test several hypotheses: (1) the quantity and activity of α-amylase can be measured by specific bindings with non-antibody molecules (Objective 1 &2), (2) starch granules have different susceptibility to various α-amylase isozymes (Objective 3), and (3) sprouting- and non-sprouting low FN wheat are impacted by α-amylase differently and non-sprouting low FN wheat, with intact starch granules, potentially has market value (Objective 4).

Our research will allow us to understand the real impacts of α-amylase on wheat quality (i.e., viscosity) and lead us to develop a solution to utilize non-sprouting low FN wheat. Our objectives are listed below.

Objective 1: Develop a methodology to quickly and economically **quantify** α-amylase in wholemeal flour.

- We generated preliminary data and submitted an invention disclosure to the UI Office of Technology Transfer in FY18. We will complete the technology development in FY19.
- *If we complete the development of the new technology to quantify α-amylase, then the next goal will be assisting other programs (e.g., wheat breeders) to use the new technology.*

Objective 2: Develop a methodology to quickly and economically determine α-amylase **activity** in wholemeal flour.

- We generated preliminary data and submitted an invention disclosure to the UI Office of Technology Transfer in FY18. We will complete the technology development in FY19.
- *If we complete the development of the new technology to determine α-amylase activity, then the next goal will be conducting an intra-laboratory evaluation for promoting the new method as an AACC standard method.*

Objective 3: Investigate the hydrolytic mechanism of wheat α-amylase and the susceptibility of wheat starch to α-amylase isozymes.

- We will generate antibodies of α-amylases for separating isozymes and investigate the hydrolytic mechanism of each isozyme on both A- and B-type wheat starch granules. We will also characterize each isozyme, including its optimal hydrolytic conditions (e.g., temperature, pH, and stability).
- *If we complete the investigation of the hydrolytic mechanism of wheat α-amylase, then the next goal will be identifying the difference between PHS and LMA from α-amylase degradation perspective.*

Objective 4: Investigate α-amylase impacts in sprouting- (i.e., PHS) and non-sprouting (i.e., LMA) low falling number (FN) soft white wheat.

- We will study the difference of the interaction between α-amylase and starch in sprouting and non-sprouting low FN soft white wheat and identify the α-amylase-affected portion in non-sprouting low FN grains.
- *If we successfully distinguish the biochemical difference between PHS and non-sprouting low FN wheat, then the next goal will be developing technology to utilize undamaged wheat grains to avoid being discounted.*

DURATION: 1 year (FY19)

COOPERATION/COLLABORATION:

Lin will design and conduct chemical and biochemical assays, manage the data, present research findings at professional conferences, and communicate research progress and results to the Idaho Wheat Commission. **Schroeder** will provide wheat samples. The graduate student assigned to this project will submit weekly progress reports to **Lin**.

ANTICIPATED BENEFITS, EXPECTED OUTCOMES AND IMPACTS AND TRANSFER OF INFORMATION:

This project will

1) benefit wheat researchers and the industry by providing two economical, efficient, and accurate methods to quantify α-amylase and measure its activity;
2) provide a new direction to potentially utilize low FN wheat caused by LMA by increasing understanding of the hydrolytic mechanism of α-amylase needed to distinguish the biochemical difference between PHS and LMA; and
3) continue providing high-quality wheat research education in Idaho and promoting leadership at the international level.

We will present our results to the Idaho Wheat Commission and submit manuscripts to research journals with a strong impact in agriculture, such as the *Journal of Agriculture and Food Chemistry*. We will deliver our research findings through social media (e.g., YouTube, LinkedIn, or other professional websites) using short messages, cartoons, or videos to stakeholders. We will also present our research to wheat and cereal professional communities (e.g., the Wheat Quality Council meeting, the American Association of Cereal Chemists International annual meeting) and in relevant publications, such as *Grain* magazine.

LITERATURE REVIEW: - *The immunoassay for detecting α-amylase in wheat*

FN measurement is an indirect measurement of α-amylase activity that has low precision and high inconsistency. The technique "monoclonal antibodies to α-amylase," invented by John H. Skerritt and patented in 2010, provides a precise and consistent direct measurement to detect wheat α-amylase isozymes using the specific binding between antibodies and the antigen.[1] The immunoassay consists of two specific antibodies to qualify (capturing the function of α-amylase) and quantify α-amylase in weather-damaged wheat (i.e., LMA and PHS). LMA is the synthesis of high pI α-amylase that can be caught by monoclonal antibodies, which were produced by a single B cell and bound to one unique location of the antigen (α-amylase). PHS wheat has both high and low pI α-amylase isozymes that can be detected by the polyclonal antibodies that were generated from different B cells and can recognize and bind to multiple locations on the antigen.[2-3] This immunoassay can be performed in different formats. The sandwich enzyme-linked immunosorbent assay (ELISA) was one of the formats recommended and protected from use by the patent.[1] Skerritt and Bayer CropScience AG launched the commercialized kit, WheatRite®, that has been widely adopted by researchers in Australia.[2] However, WheatRite® is inaccessible in the United States because Bayer CropScience AG didn't succeed in North American markets (Personal communication at LMA Forum, Spokane, WA, November 27, 2017). It is not feasible to generate wheat α-amylase antibodies for commercial usage without violating the patent because the patent has restricted a broad range of the amino acid sequences of wheat α-amylase isozymes. In the patent, the longest sequence contains 425 amino acids in the five sequences.[1] Though the patent will expire soon, the immunoassay is expensive and requires trained personnel to perform the analysis. To benefit growers and wheat researchers across multiple disciplines, economical and specific assays to qualify and quantify wheat α-

amylase are still a critical need.

REFERENCES:

1. Skerritt, J. H. Monoclonal antibodies to alpha-amylase. Jul 20, 2010.
2. Skerritt, J. H.; Heywood, R. H., *Crop Science* **2000,** *40* (3), 742-756.
3. Verity, J. C. K.; Hac, L.; Skerritt, J. H., *Cereal Chemistry* **1999,** *76* (5), 673-681.

IDAHO WHEAT COMMISSION - BUDGET FORM

Principal Investigator: Amy Lin

Allocated by	**Idaho Wheat Commission**	**during FY 2017**	**$ -**
Allocated by	**Idaho Wheat Commission**	**during FY 2018**	**$ 42,098**

REQUESTED FY2019 SUPPORT:

	Salary (*staff, post-docs, etc.*)	Temporary Help	Fringe	Travel	OE	Graduate Tuition/Fees	TOTALS
Idaho Wheat Commission	**$ 18,720**	**$ -**	**$ 449**	**$ 3,500**	**$ 10,000**	**$ 10,006**	**$ 42,675**

TOTAL BUDGET REQUEST FOR FY 2019: **$ 42,675**

BREAKDOWN FOR MULTIPLE SUB-BUDGETS:

	(Insert CO-PI Name)	*(Insert CO-PI Name)*	*(Insert CO-PI Name)*	*(Insert CO-PI Name)*
Salary	$ -	$ -	$ -	$ -
Temporary Help	$ -	$ -	$ -	$ -
Fringe Benefits	$ -	$ -	$ -	$ -
Travel	$ -	$ -	$ -	$ -
Operating Expenses	$ -	$ -	$ -	$ -
Graduate Student Tuition/Fees	$ -	$ -	$ -	$ -
TOTALS	$ -	$ -	$ -	$ -

Total Sub-budgets $ -

Explanatory Comments: (see FY2019 RFP for definition)

Fall 2017 Version