

**PROJECT NO: BKK311**

**TITLE:** Investigate the influence of Pre-Harvest Sprouting (PHS) on Wheat Starch Properties Associated with Falling Number Measurement and Flour End-Use Quality

**PERSONNEL:**

**Principal Investigator:** (Amy) Hui-Mei Lin, Associate Professor, School of Food Science, University of Idaho (Moscow, ID), 208-885-4661, amylin@uidaho.edu

**Co-Investigators:** Yeuguang Wang, Research Associate, Department of Plant, Soil and Entomological Sciences, University of Idaho (Moscow, ID), 208-885-9110, ywang@uidaho.edu

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**JUSTIFICATION:**

Unpredictable low falling number (FN) issues have generated a lot of frustration for Idaho wheat growers. It is still unclear to growers and researchers what triggered the decrease in FN and caused economic disasters in 2014 and 2016 in Idaho. Falling number (FN) measures the pasting property of hot flour pastes. Starch is the major component of flours. During heating (e.g. FN measurement) or cooking, starch granules swell and are gelatinized, which makes flour paste viscous. Such a viscosity determines FN and affects wheat end-use quality. Starch pasting properties are mainly determined by its structural characteristics. However, it is not clear *which starch structure characteristics are sensitive to environmental changes (e.g., temperature and rainfall) and responsible for decreasing FN*. Based on an on-going study, we found the increase of the population of B-type wheat starch (sphere-shaped small granules) in 2014 [a pre-harvesting sprouting (PHS) year]. In the same study, we also suspect the decrease of amylopectin chain-length that is associated with a low viscosity. Literature also shows these two structural features are sensitive to other environmental stresses, such as drought and high temperature. However, we hesitate to make such a novel conclusion that the development of B-type granules and the change in chain length are sensitive to environmental stresses and responsible for a low FN. The major concern was that the materials used in our research were grown for another research activity. They were not grown in a well-controlled environment. Additionally, we lacked detailed agronomy data.

In addition to starch structural characteristics, our on-going project has been extended to consider the influence of extrinsic factors on FN. Flour paste is a complex system that consists of several macromolecules, such as protein (e.g.,  $\alpha$ -amylase and storage protein), lipid, and non-starch polysaccharides (e.g., arabinoxylan,  $\beta$ -glucan, and cell walls). The interaction between  $\alpha$ -amylase and starch has been discussed in another proposal. Some macromolecules, such as arabinoxylan, can compete with starch for water, which is a critical component for starch to fully swell and gelatinize. Lipid and cell walls are another two macromolecules to restrict starch granule to swell and negatively influence the FN. Some discussion in the literature has occurred about individual macromolecule's influence in starch pasting, but we have not found any reports investigating FN issues from a macromolecular interaction perspective with a mechanism-driven approach.

In fiscal year 2018, we propose to validate our findings of starch structure features using research materials developed in a controlled environment. We also propose adjusting an objective to examine macromolecular interactions and their influence in FN. The success of this project will advance the knowledge about FN measurement from an integrated interaction, instead of an individual component, and open a new era when designing a solution to the low FN issue will be possible.

#### **HYPOTHESIS & OBJECTIVES:**

We **hypothesize that** (1) some starch characteristics (e.g., granule type and amylopectin chain length) are sensitive to environmental stresses PHS and late mature  $\alpha$ -amylase (LMA), which results in low FN in these two circumstances; and (2) the interaction between starch and other macromolecules can influence starch pasting properties and influence FN. Our **objectives** are to (1) examine starch molecular characteristics and identify structural features responsible for the low FN of LMA and PHS wheat, and to (2) develop techniques for examining the macromolecular interactions among starch, protein, lipid, and non-starch polysaccharides and (3) identify the changes in macromolecules caused by the environmental stresses (i.e., PHS and LMA).

#### **PROCEDURES:**

**Objective 1: Examine starch molecular characteristics and identify structural features responsible for the low FN of LMA and PHS wheat**

We will grow research material UI Stone wheat – a soft white wheat, on the field following treatments to mimics PHS and LMA in the greenhouse and chambers. We will mill flours, isolate starch, and examine flour characteristics, including  $\alpha$ -amylase activities of flours, viscosity profile of starch and flour, particle size distribution of starch granules, starch granule morphology, amylose content, and the chain-length distribution of amylopectin and internal amylopectin.

**Objective 2: Develop techniques for examining the macromolecular interactions among starch, protein, lipid, and non-starch polysaccharides**

We will continue using UI Stone wheat grown in 2015 for methodology development. We are able to distinguish protein and starch pastes at this point. We will continue testing various dyes (e.g. fluorescent dyes) and chemical reactions to examine lipid and non-starch polysaccharides. We will apply enzymatic treatments to hydrolyze the individual macromolecules to identify their role in determining hot flour pasting properties.

**Objective 3: Identify the changes in macromolecules caused by the environmental stresses-PHS and LMA**

We will use UI Stone with PHS and LMA treatment as described in Objective 1 and the techniques developed in Objective 2 to examine the change of macromolecular interaction triggered by the environmental stresses. The quantity of individual macromolecules will be also measured.

The proposed project will be carried out at the University of Idaho (Moscow, ID). All chemical and biochemical analyses will be performed in triplicate. One-way ANOVA will be applied to

evaluate the significance of differences. Multivariate analysis will also be applied to identify the connection between various starch characteristics and the FN. Lin will also consult with the statistics support staff in the University of Idaho College of Agriculture and Life Science (CALS) to analyze the data. A master graduate student who is working on the first year of this project will continue work on the second year of the study. Lin has been using active-learning and problem-solving teaching styles to mentor the student.

#### **DURATION:**

BKK311 was a two-year project (FY 2017 & 2018). We would like to request an extension to a three-year project (FY 2017-2019) for having sufficient time to grow research materials, apply environmental stresses, and expand the research scope to include both starch characteristics and macromolecular interactions. The Objective 1 and 2 fall into year two, and the Objective 3 falls into year three of a three-year project.

**COOPERATION:** 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. Wang will grow and manage plants for research material. Lin and Wang will apply PHS and LMA treatments to the research materials. Fu will support this project by sharing part of greenhouse space.

#### **ANTICIPATED IMPACT AND EXPECTED OUTCOMES:**

This project will

- 1) Advance knowledge of starch structural features causing low FN to provide guidance to breeders for developing wheat varieties with consistent FN and end-use quality.
- 2) Advance understanding of the effect of macromolecular interaction on FN to assist the development of research plans for providing solutions to unpredictable low FN issues.

We plan to publish our findings in research journals with a focus on agriculture chemistry, such as the *Journal of Agriculture and Food Chemistry*. We will also present our research to the wheat and cereal communities at conferences such as at the American Association of Cereal Chemists International annual meeting and through relevant publications (e.g., *Grain* magazine).

#### **LITERATURE REVIEW –*The influence of macromolecules in flour pasting properties***

Wheat flour has a complex system that contains several macromolecules, such as starch, non-starch polysaccharides (e.g., arabinoxylan and  $\beta$ -glucan), protein, and lipid. Several researchers reported that increasing grain protein content had a positive influence in rising FN<sup>1-4</sup>, and the increase in the usage of nitrogen fertilization after anthesis can increase protein content<sup>4</sup>.

However, the mechanism is still unclear, and research findings are not consistent<sup>5-6</sup>. In addition, a high protein content is not a desired trait in soft white wheat, especially for the Asian market. Protein does influence the starch paste profile, but the influence did not exist when the protein was hydrolyzed by protease<sup>7-8</sup>. Our microscopy data also demonstrate the integration between starch and protein pastes. Lipid is another macromolecule highly associated with starch, and it can restrict starch granule swell. Lipid also interacts with starch after gelatinization and forms a complex with starch molecules. Lipid also protects starch from mechanical damage during milling and decreases the amount of damaged starch. Arabinoxylan, also known as pentosan, is the major non-starch polysaccharide in wheat. Arabinoxylan can compete with starch for water

and has a negative influence on starch gelatinization. On the other hand, water soluble type arabinoxylan can increase viscosity after absorbing water<sup>9-10</sup>. In addition, our data indicates the quantity of arabinoxylan is associated with environmental stresses. Taken together, it is critical to think of the FN issue within a big picture of the complex macromolecular system. The adjusted objective aims to advance the knowledge in this area with a broad spectrum.

## BIBLIOGRAPHY

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# IDAHO WHEAT COMMISSION - BUDGET FORM

Allocated by Idaho Wheat Commission during FY 2016

Allocated by Idaho Wheat Commission during FY 2017 \$ 36,700

## REQUESTED FY2018 SUPPORT:

|                        | Salary    | Temporary Help | Fringe | Travel   | OE        | Graduate Tuition/Fees | TOTALS    |
|------------------------|-----------|----------------|--------|----------|-----------|-----------------------|-----------|
| Idaho Wheat Commission | \$ 15,600 | \$ 5,760       | \$ 738 | \$ 5,000 | \$ 15,000 | \$ 9,004              | \$ 51,102 |

TOTAL BUDGET REQUEST FOR FY 2018: \$ 51,102

## BREAKDOWN FOR MULTIPLE SUB-BUDGETS:

|                               | (PI name) | (PI name) | (PI name) | (PI name) |
|-------------------------------|-----------|-----------|-----------|-----------|
| Salary                        | \$ -      | \$ -      | \$ -      | \$ -      |
| Temporary Help                | \$ -      | \$ -      | \$ -      | \$ -      |
| Fringe Benefits               | \$ -      | \$ -      | \$ -      | \$ -      |
| Travel                        | \$ -      | \$ -      | \$ -      | \$ -      |
| Operating Expenses            | \$ -      | \$ -      | \$ -      | \$ -      |
| Graduate Student Tuition/Fees | \$ -      | \$ -      | \$ -      | \$ -      |
| TOTALS                        | \$ -      | \$ -      | \$ -      | \$ -      |
| Total Sub-budgets             |           |           | \$ -      | \$ -      |

Explanatory Comments: (see FY2018 Guidelines for definition)

11.21.2016 - Version

## ANNUAL REPORT

**PROJECT NO:** BKK311

**TITLE:** Investigate the influence of pre-harvest sprouting (PHS) on wheat starch properties associated with falling number measurement and flour end-use quality

**PERSONNEL:**

**Principle Investigator:** (Amy) Hui-Mei Lin, Ph.D.

**Graduate Students:** Yijing Shao and Yuezhen He

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**ACCOMPLISHMENTS:**

In this project, we have been investigating the influence of the extreme weather in soft white wheat on their starch characteristics. Our research materials are UI Stone, Alturas, and their inbred line SA043 provided by Dr. Jianli Chen. Wheat was grown in 2013 (normal year) and 2014 [pre-harvest sprouting (PHS) year]. All the lines grown in 2014 had a decrease of falling number (FN) compared with wheat grown in 2013. Our major findings are discussed below:

**1. Starch amount did not have a significant change in the PHS year (2014)**

It was a conventional view that PHS-affected wheat starch was damaged by  $\alpha$ -amylase and had a decrease of starch content. Our data showed the starch content did not decrease significantly in the PHS year. All flour samples contained 63~64% of starch. UI Stone even had a higher starch content, 66%, in 2014 compared with its starch content in 2013.

**2. Starch granules were not degraded by  $\alpha$ -amylase in plants**

The decrease of FN is often associated with the increase of  $\alpha$ -amylase activities. We could not analyze the enzyme activity because wheat materials had been stored for 2-3 years when we received them. However, we examined starch granules with the scanning electronic microscopy (SEM). Our SEM images demonstrated that starch granules were only slightly hydrolyzed by  $\alpha$ -amylase. There was no significant difference between the wheat grown in 2013 and 2014 in all three lines.

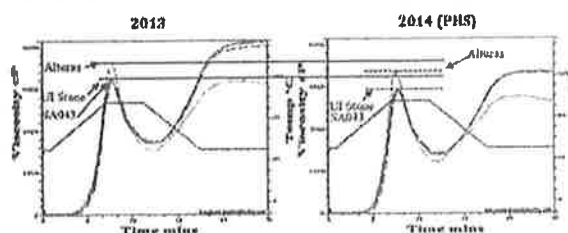
**3. Starch viscosity significantly decreased in the PHS year (2014)**

Wheat grown in 2014 had a significant decrease in FN; our data indicated the decrease of starch viscosity was a major cause of the decrease of FN. We isolated starch from flours and examined their pasting profile using a rapid viscosity analyzer (RVA). All the starches isolated from the wheat grown in 2014 had a lower peak viscosity than those in 2013. (Fig. 1)

Starch viscosity is determined by several structural characteristics, such as granule size, granular architecture, starch molecular composition (e.g., ratio of amylose and amylopectin content), and starch molecular structure (e.g., chain-length distribution). Our data showed:

**3.1. The ratio of amylose to amylopectin did not significantly change in the PHS year (2014)**

The ratio of amylose to amylopectin has an important role in affecting starch pasting properties. We measured amylose content using two methods, blue value (BV) and high-performance size exclusion chromatography equipped with a refractive index (HPSEC-RI). We used two methods because their measurement mechanisms are different and provided different information. According to the BV, UI



**Fig. 1 Pasting profiles of starch isolated from Alturas, UI Stone, and SA043 grown in 2013 and 2014 using RVA.**

Stone and SA043 did not have a significant change in the apparent amylose content in 2014 while Alturas had a slight increase in 2014. In contrast, the HPSEC-RI data showed that Alturas did not have a significant change in 2014 while UI Stone and SA043 had a slight decrease in 2014. The discrepancy between two measurements indicated three lines had different amylose and amylopectin structures. We later realized that Alturas is a partial waxy wheat with different starch synthesis enzyme activities from non-waxy wheat (e.g., UI Stone). Such a difference can affect starch molecular structure.

### ***3.2. The starch thermal properties changed slightly in 2014***

Starch thermal properties include the gelatinization temperature and enthalpy change. The gelatinization onset temperature ( $T_o$ ) of starch isolated from the wheat grown in 2013 was higher than it was in 2014. Our findings indicated (1) starches in PHS-affected wheat were not at the same gelatinization condition as the normal wheat while measuring FN, (2) the baking or cooking condition needs to be adjusted when using PHS-affected wheat.

### ***3.3. The population of B-type wheat granules increased in 2014***

Starch isolated from the wheat grown in 2014, especially UI Stone and SA043, contained a higher percentage of B-type starch granules compared to the normal year samples. The B-type granules display lower viscosity compared with A-type granules because B-type starch has a low amylose content and a high quantity of short amylopectin chains. Also, B-type wheat granules have a high susceptibility to  $\alpha$ -amylase due to the high surface areas. Currently, we are investigating the fine structure of starches to support our hypothesis that having a higher quantity of short amylopectin chains is another major cause of the decrease of FN. We are also separating A- and B-type granules to examine the changes in their fine structure between two years.

## **4. The protein content might decrease in 2014**

Besides starch content, protein and non-starch polysaccharides (mainly arabinoxylan) content also influence the flour viscosity. We quantified the crude protein of flours, and all the samples contained 13% protein except UI Stone in 2014, which decreased to 12%. We did not observe a significant correlation between protein content and FN. However, the interaction between starch and protein is likely to be more important than the quantity of protein.

## **5. The increase of arabinoxylan in 2014**

We quantified arabinoxylan of flour samples and found a higher content in 2014 compared with their arabinoxylan content in 2013. Arabinoxylan can compete with starch for water and affect starch pasting properties. On the other hand, water-soluble type arabinoxylan can increase viscosity. Due to the limited amount of flour samples, we cannot quantify various types of arabinoxylan. It is hard to determine its influence in FN at this point.

Another macromolecule that has a significant influence in starch pasting is lipids, especially of soft white wheat. We could not quantify the lipid content due to the limited flour quantity in this project.

## **6. Developing microscopy techniques to examine the macromolecular interaction**

Microscopy techniques have been widely used to examine the microstructure of the wheat flour and wheat dough, but we have not seen reports using them to examine flour paste. We have been using several techniques including light microscopy, SEM, and a confocal laser scanning microscopy to visualize the microstructure of flour paste during the FN test. Our micrographs showed swollen starch granules were partially gelatinized (purple color) and embedded in the protein (gluten) matrix (green color) (Fig.



*Figure 2. Light microscopy image of the flour paste. Gelatinized starch (in purple-blue color) embedded in the protein matrix (in green color).*

2). Starch paste viscosity predominately contributes to the overall performance of the hot flour paste. However, protein (gluten) formed a matrix among starch granules and restrained the space for starch swelling and gelatinizing. This observation agreed with the literature discussing the influence of protein on starch pasting properties. We plan to continue investigating the influence of such an interaction in FN.

### **PROJECTIONS:**

#### **1. Continue analyzing the chain-length distribution and its influence in FN.**

The findings from our on-going research activities suggested that the population of B-type granules and amylopectin chain length are two key structure features that were sensitive to the environmental stresses happened in 2014. We are analyzing the chain-length distribution and projected to complete the assay in the late spring. The major challenge of this analysis is the lack of reliable instrument. We are using a retired instrument, high-performance anion-exchange chromatography equipped with pulsed amperometric detection (HPAEC-PAD), which we have not been able to gain stable signals for a couple of months. It severely delayed our progress. We borrowed this instrument from another university, and it is the only HPAEC-PAD in Moscow. There is another similar instrument located at Washington State University, but it is not available for us to access it. We will continue looking for a solution to complete this analysis.

#### **2. Continue developing microscopy techniques to examine starch granule swelling and the macromolecular interaction**

Our data have led us to expand our research plan and examine starch granule swelling, which happens at the early stage of gelatinization and has an important role in determining the viscosity. In addition to starch structural characteristics, the interaction between starch and other macromolecules also influence starch pasting. We can visualize the interaction between starch and protein pastes as shown above. We will continue the methodology development to reveal the influence of macromolecular interaction in FN.

#### **3. Complete the literature review of falling number**

We have been reviewing the literature to understand what have been done in the research area related to FN and plan to publish it in the spring semester. In this review article, we will discuss the mechanism of FN measurement, the relationship between  $\alpha$ -amylase and FN, the influence of PHS & LMA in FN, the relationship between environment and agronomical practice with starch characteristics, the relationship between starch structure characteristics and FN, and the influence of non-starch macromolecules and FN. Our review article will benefit the wheat researchers to have a broad understanding about FN and help us to develop a strategy for solving the low FN issues.

### **PUBLICATIONS:**

Lin, A., 2016. How does starch affect falling number? Grain Magazine. [Extension article]

Shao, Y., 2016. Elucidating the influence of unexpected rainfalls on wheat starch synthesis. 2016 Innovation Showcase. University of Idaho (Moscow, ID). [Student's oral presentation]

Shao, Y., 2016. Investigate the weather influence on wheat starch in Idaho in 2014. Institute of Food Technologists (IFT) Intermountain Annual Meeting (Sun Valley, ID). [Student's poster presentation]