Insights into Kernza a Potential Food Crop

The Food Science Group's Update

July 6th 2017

Team and Projects

PI	Students & post-docs	Topic
B. Pam Ismail	Catrin Tyl	Compositional analysis and chemical characterization
	Chathurada Gajadeera	Protein profiling and interactions with fiber
	Citra Rahardjo	Protein and starch functionality & baking quality
	Amy Mathiowetz	Storage stability
	Misen Luu	Impact of storage on flavor
Tonya Schoenfuss	Avi Goldstein Megan Parker Jaya Dhungana	Baking applications and functionality Product formulation Dough conditioners
Alessandra Marti	Xiaoxue Qiu	Protein interactions and rheology

Food Science Group Research

Composition, Functionality, Storage and Applications

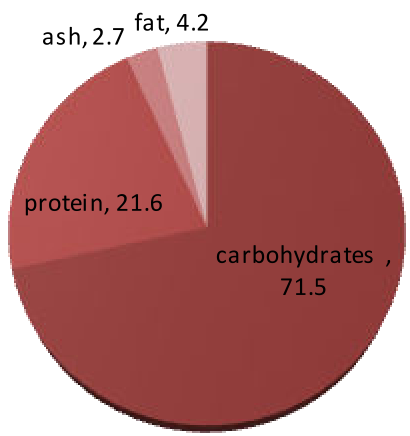
1. **What are the benefits and challenges?**
2. **What can we do to improve IWG's potential as an ingredient?**
   - Improve protein network formation for baking applications
   - Determine optimum bran content
   - Study effect of dough conditioners on functionality
3. **What are the key factors determining storage stability?**
   - Assess processing operations that can help to prolong shelf life
4. **How is the flavor profile of IWG impacted by level of refinement and storage?**

1

Benefits and Challenges of IWG - Composition of IWG Compared to Wheat

Composition IWG
(average of 13 breeding populations)

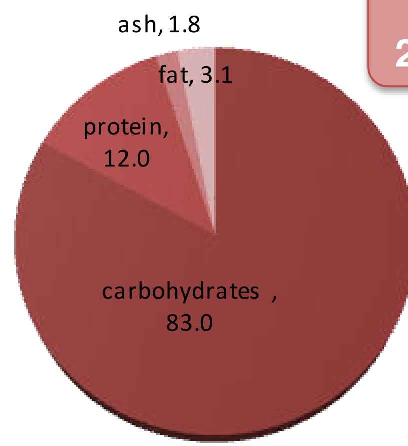
IWG (2004)
3.9 g/1000 seeds



More protein

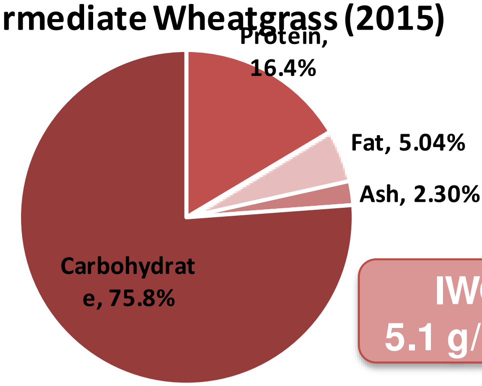
Composition Whole Wheat
(Hard red spring)

HRW
26 g/1000 seeds



More carbohydrates

Intermediate Wheatgrass (2015)



IWG (2015)
5.1 g/1000 seeds

The Chemical Composition Affects

Functionality

Baking Performance

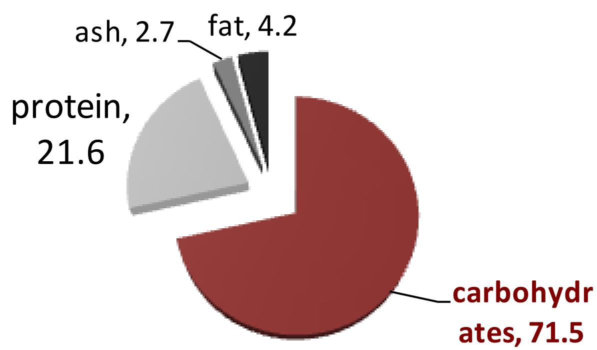
Stability over storage

Flavor

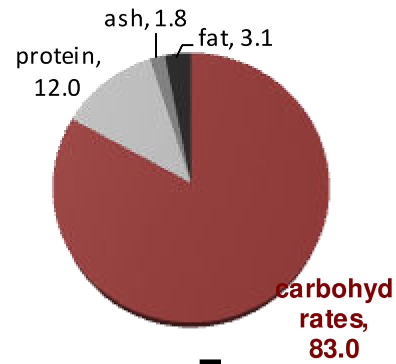
Physiological benefits

IWG's Carbohydrates

Composition IWG
(average of 13 breeding populations)



Composition Whole Wheat
(Hard red spring)

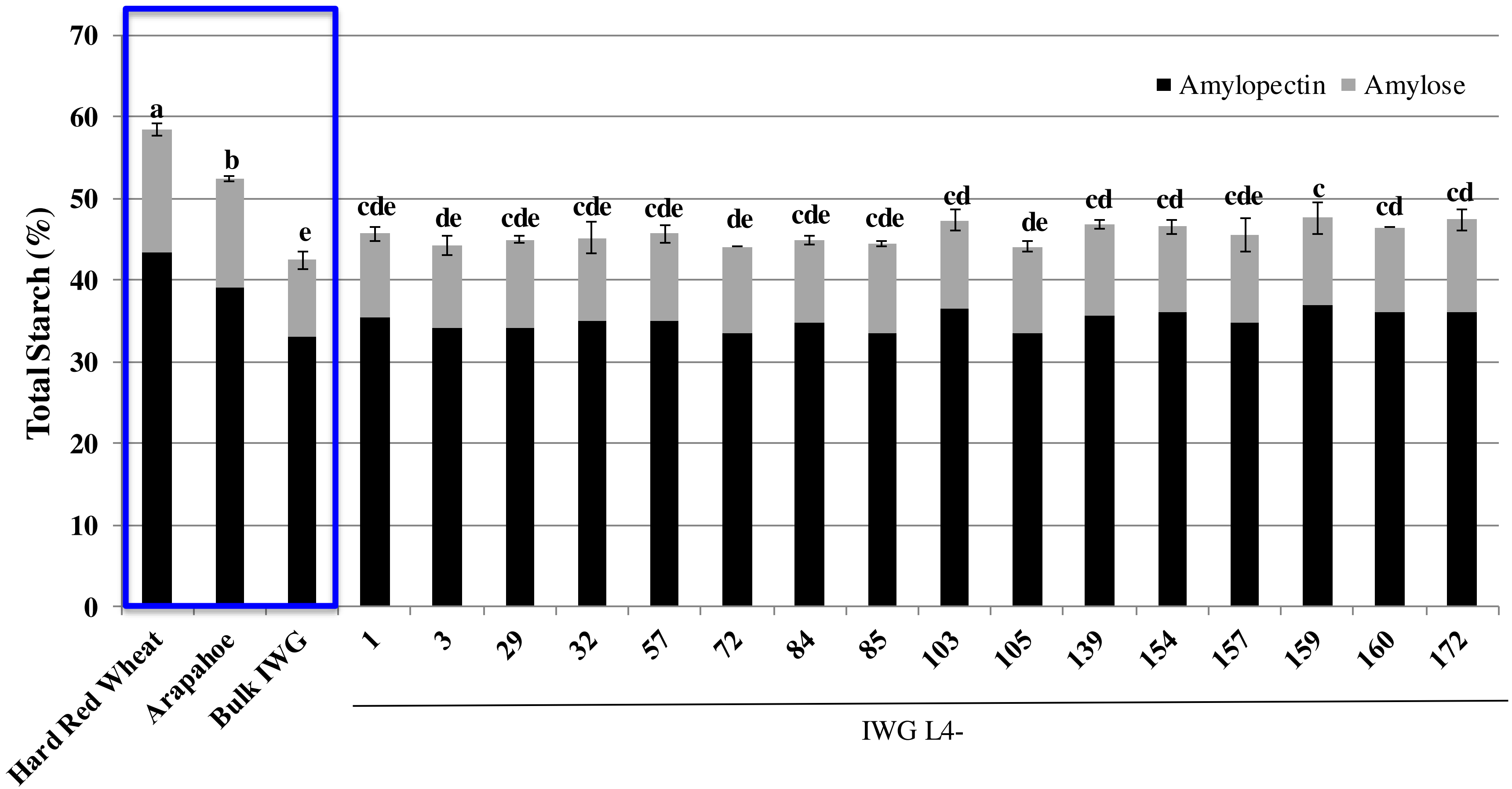


	IWG		Whole Wheat
more bran!	21.8	**% Fiber**	15
	47.7	**% Starch**	67.4

IWG	Milling yield	Whole Wheat
59	% bran	40
41	% refined flour	60

- Low amounts of starch may impair functionality of IWG – some starch characteristics are similar to wheat (amylose/amylopectin ratios)
- Higher dietary fiber content is nutritionally beneficial but may impede gluten net work formation

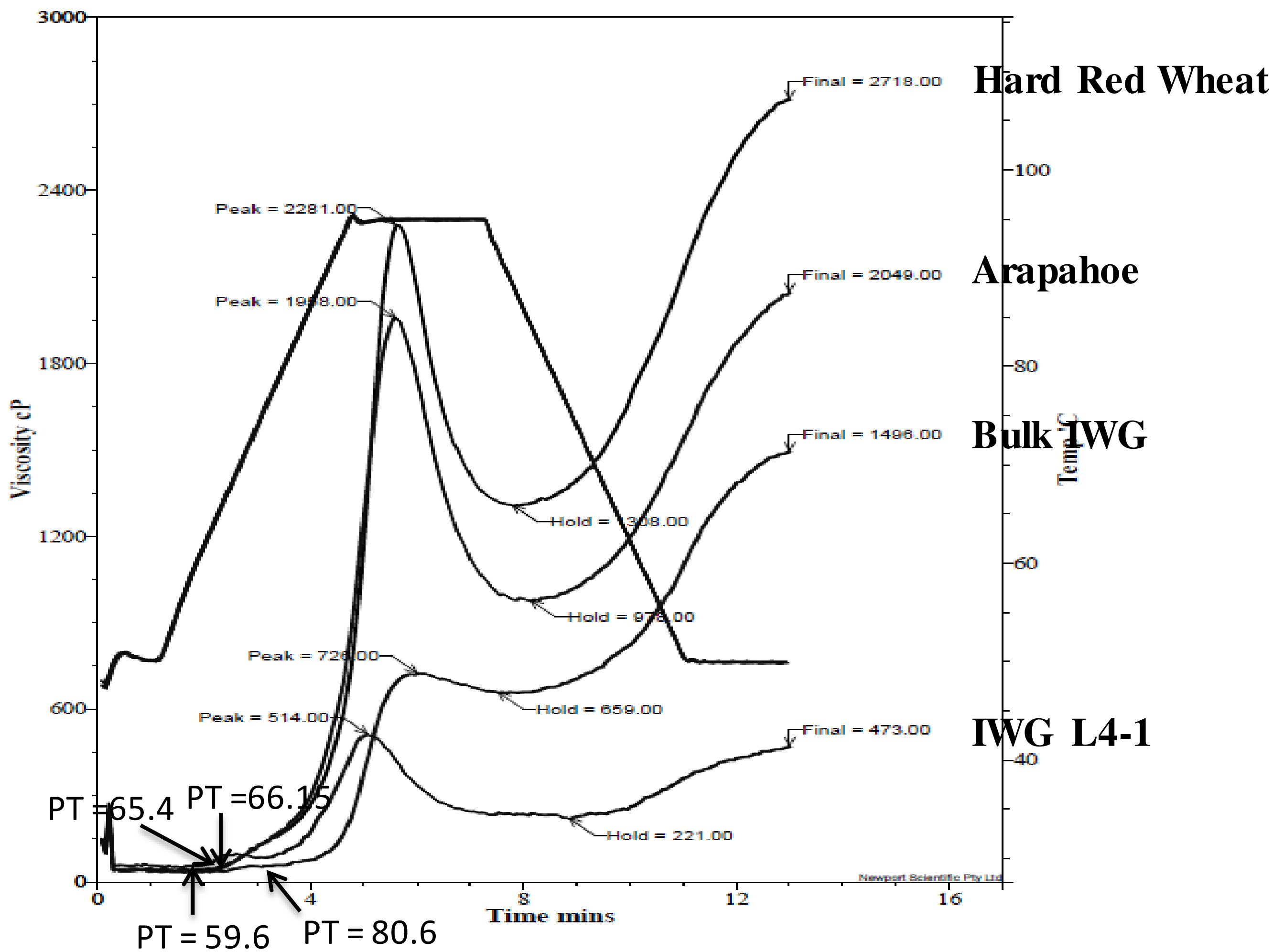
IWG's Carbohydrates



Error bars represent standard errors (n=3). Lowercase letters indicate significant differences among samples according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$).

IWG's Carbohydrates

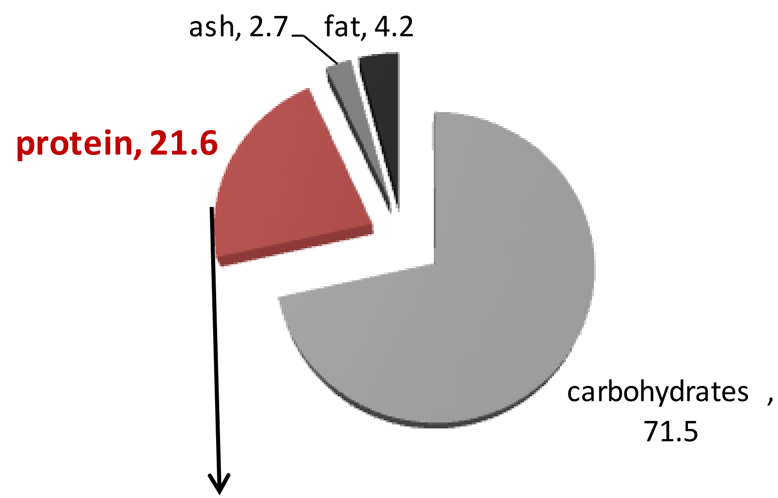
Starch Pasting Properties

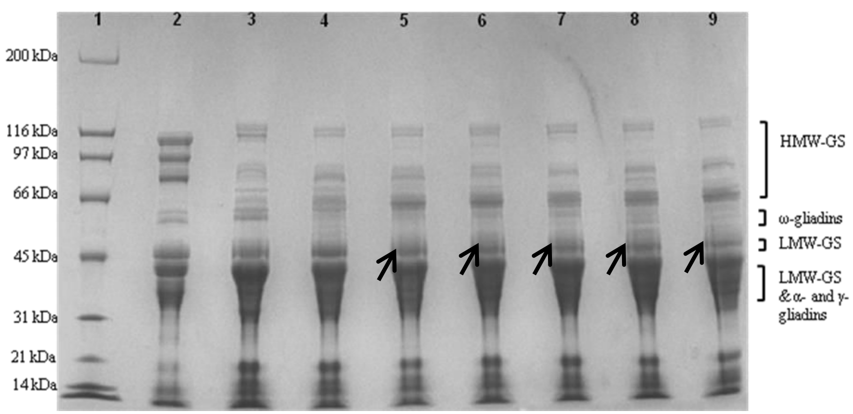


PT stands for pasting temperature (°C). Viscosities are expressed in centipoise (cP).

IWG's Proteins

Composition IWG
(average of 13 breeding populations)





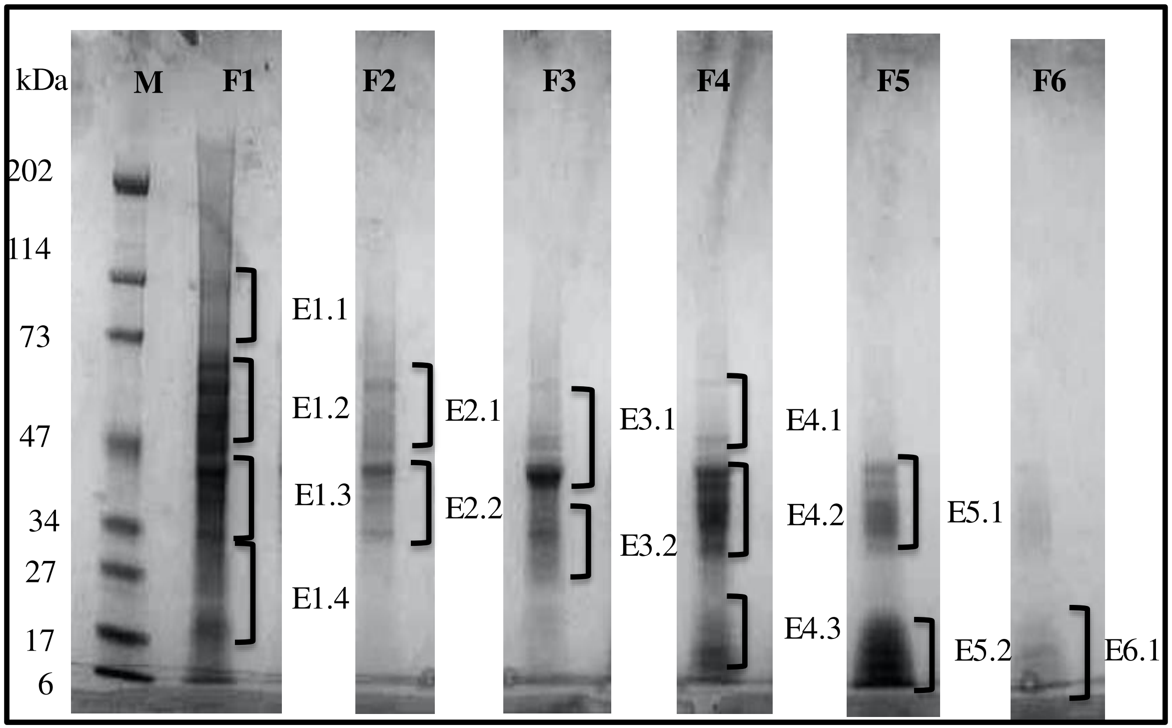
Lane 1: Molecular weight marker
Lane 2: Whole wheat flour gluten
Lane 3: Bulk IWG
Lanes 4-9: Different IWG populations

IWG's Proteins

Protein Molecular Weight Distribution by SE-HPLC

	% Total Area of Extractable Protein					% Total Area of Unextractable Protein				
Sample ID	**HMWPP***	**LMWPP^**	**ω-gliadins**	**α,β and γ-gliadins**	**Albumins and Globulins**	**HMWPP**	**LMWPP**	**ω-gliadins**	**α,β and γ-gliadins**	**Albumins and Globulins**
Hard Red Wheat	13.67$^{h\#}$	4.44^{f}	5.00^{i}	31.71^{e}	18.16^{efg}	12.83^{a}	3.90^{a}	2.13^{a}	4.85^{abcd}	3.33^{abcde}
Arapahoe	14.44^{gh}	4.67^{f}	5.10^{hi}	32.82^{de}	18.95^{efg}	10.92^{b}	3.59^{a}	2.01^{ab}	4.43^{bcd}	3.07^{abcde}
Bulk IWG	16.18^{abcde}	7.42^{bcd}	5.71^{bcdef}	33.80^{bcde}	21.17^{bc}	7.73^{c}	1.78^{bcd}	1.05^{def}	2.80^{d}	2.34^{e}
IWG L4-1	15.75^{cdefg}	8.20^{a}	5.89^{abc}	37.52^{a}	21.49^{abc}	3.77^{fg}	1.24^{de}	0.83^{ef}	2.90^{d}	2.40^{de}
IWG L4-3	14.99^{defgh}	7.79^{abc}	5.67^{bcdefg}	34.47^{abcde}	19.52^{de}	5.45^{de}	1.93^{bcd}	1.34^{cde}	5.11^{abcd}	3.72^{abc}
IWG L4-29	15.08^{defgh}	6.66^{e}	5.08^{hi}	35.46^{abcd}	18.93^{efg}	5.01^{def}	1.92^{bcd}	1.36^{cde}	6.49^{ab}	4.00^{ab}
IWG L4-32	17.45^{ab}	7.88^{ab}	5.80^{abcde}	35.60^{abc}	22.57^{a}	2.94^{g}	1.04^{e}	0.77^{f}	2.94^{d}	3.00^{bcde}
IWG L4-57	17.90^{a}	8.21^{a}	6.28^{a}	37.58^{a}	19.01^{efg}	3.36^{fg}	1.18^{de}	0.86^{ef}	3.17^{cd}	2.46^{cde}
IWG L4-72	17.15^{abc}	7.94^{ab}	5.39^{defghi}	33.41^{cde}	20.55^{cd}	5.31^{de}	1.73^{bcd}	1.13^{def}	4.13^{bcd}	3.26^{abcde}
IWG L4-84	14.57^{fgh}	6.77^{e}	5.24^{ghi}	36.34^{ab}	17.61^{g}	6.40^{cd}	2.17^{b}	1.45^{cd}	5.85^{abc}	3.60^{abc}
IWG L4-85	14.98^{efgh}	6.75^{e}	5.08^{hi}	35.95^{abc}	18.92^{efg}	5.36^{de}	1.95^{bc}	1.34^{cde}	5.86^{abc}	3.81^{ab}
IWG L4-103	16.71^{abcd}	7.87^{ab}	5.82^{abcd}	33.48^{cde}	21.91^{ab}	3.48^{fg}	1.43^{cde}	1.09^{def}	4.49^{bcd}	3.71^{abc}
IWG L4-105	16.07^{bcdef}	7.22^{cde}	5.46^{cdefgh}	36.63^{a}	21.67^{abc}	4.27^{efg}	1.36^{cde}	0.92^{ef}	3.49^{cd}	2.93^{bcde}
IWG L4-139	15.88^{cdefg}	7.53^{abcd}	5.31^{fghi}	35.55^{abc}	19.20^{ef}	4.87^{ef}	1.91^{bcd}	1.28^{cde}	5.01^{abcd}	3.46^{abcd}
IWG L4-154	15.85^{cdefg}	7.98^{ab}	5.99^{ab}	37.19^{a}	21.45^{abc}	4.33^{ef}	1.32^{cde}	0.87^{ef}	2.72^{d}	2.29^{e}
IWG L4-157	14.88^{efgh}	7.03^{de}	5.57^{bcdefg}	36.32^{ab}	21.42^{abc}	4.16^{efg}	1.54^{bcde}	1.12^{def}	4.66^{abcd}	3.30^{abcde}
IWG L4-159	15.56^{defg}	7.22^{cde}	5.42^{defghi}	33.46^{cde}	17.98^{fg}	5.22^{de}	2.20^{b}	1.62^{bc}	7.19^{a}	4.13^{a}
IWG L4-160	16.63^{abcd}	7.63^{abcd}	5.36^{efghi}	36.04^{abc}	21.77^{abc}	4.23^{efg}	1.41^{cde}	0.93^{ef}	3.2^{cd}	2.78^{bcde}
IWG L4-172	15.29^{defg}	7.95^{ab}	5.76^{bcde}	35.32^{abcd}	21.72^{abc}	4.28^{efg}	1.49^{cde}	1.07^{def}	3.77^{bcd}	3.36^{abcde}

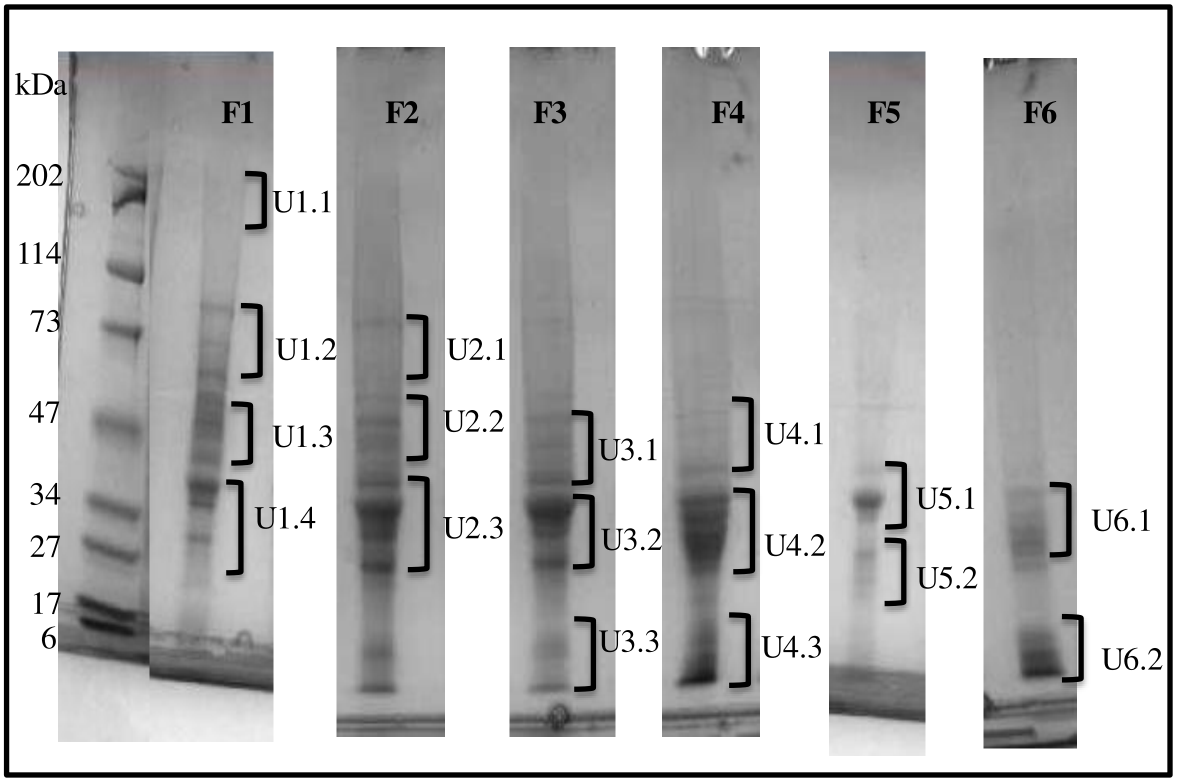
Proteomic Analysis of Extractable Proteins



Protein Band	Assigned Protein
E1.1	HMW glutenin*
E1.2	HMW glutenin*
E1.3	HMW glutenin#
E1.4	LMW glutenin#
E2.1	LMW glutenin#
E2.2	LMW glutenin*
E3.1	LMW glutenin*
E3.2	LMW glutenin*
E4.1	γ-gliadin#
E4.2	γ-gliadin#
E4.3	γ-gliadin#
E5.1	Avenin-like precursor#
E5.2	Alpha amylase inhibitor#
E6.1	Avenin-like precursor#

#*Triticum aestivum*
**Thinopyrum intermedium*

Proteomic Analysis of Unextractable Proteins



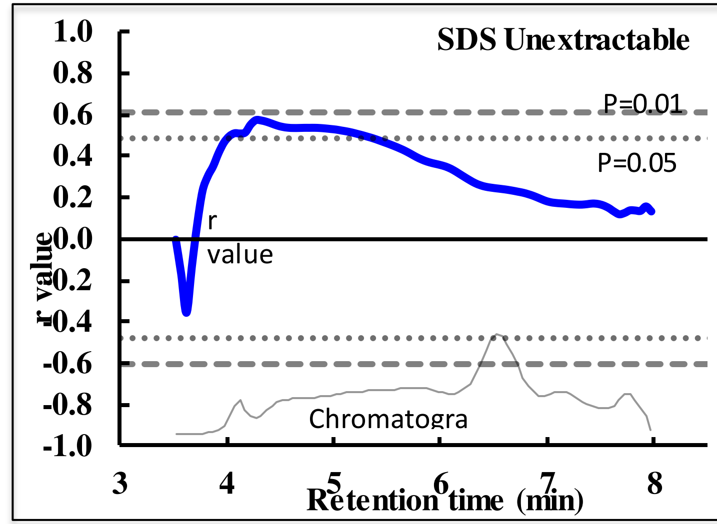
Protein Band	Assigned Protein
U1.1	HMW glutenin*
U1.2	HMW glutenin*
U1.3	HMW glutenin*
U1.4	HMW glutenin*
U2.1	LMW glutenin#
U2.2	LMW glutenin*
U2.3	LMW glutenin#
U3.1	LMW glutenin*
U3.2	LMW glutenin#
U3.3	LMW glutenin*
U4.1	α-gliadin*
U4.2	alpha/beta gliadin precursor#
U4.3	γ-gliadin#
U5.1	γ-gliadin#
U5.2	γ-gliadin#
U6.1	Puroindoline b#
U6.2	Endosperm transfer cell specific PR60 precursor#

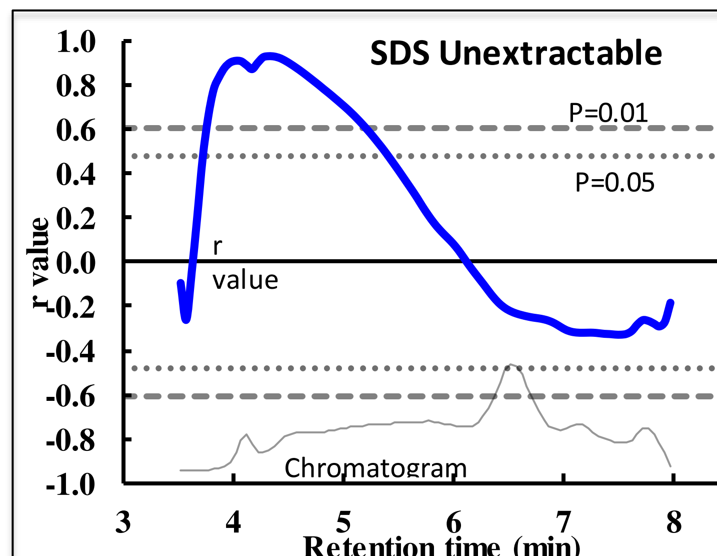
Triticum aestivum

* *Thinopyrum intermedium*

Correlation Coefficients Between Quality Parameters and Protein Fractions

	Farinograph		Kieffer	
Protein Fraction	Water Absorption	Stability	Extensibility	Protein %
SDS Extractable				
HMWPP	0.46 (ns)	0.04 (ns)	-0.38 (ns)	0.58*
LMWPP	0.27 (ns)	-0.07 (ns)	-0.40 (ns)	0.45 (ns)
ω-gliadins	0.28 (ns)	-0.03 (ns)	-0.40 (ns)	0.61**
α,β and γ-gliadins	0.51*	-0.43 (ns)	-0.42 (ns)	0.69**
Albumins and Globulins	0.20 (ns)	0.02 (ns)	-0.33 (ns)	0.130 (ns)
SDS Unextractable				
HMWPP	0.41 (ns)	0.81***	0.69**	0.52*
LMWPP	0.52*	0.27 (ns)	0.62**	0.69**
ω-gliadins	0.44 (ns)	0.05 (ns)	0.51*	0.66**
α,β and γ-gliadins	0.40 (ns)	-0.21(ns)	0.32 (ns)	0.61**
Albumins and Globulins	0.34 (ns)	-0.32 (ns)	0.26 (ns)	0.32 (ns)





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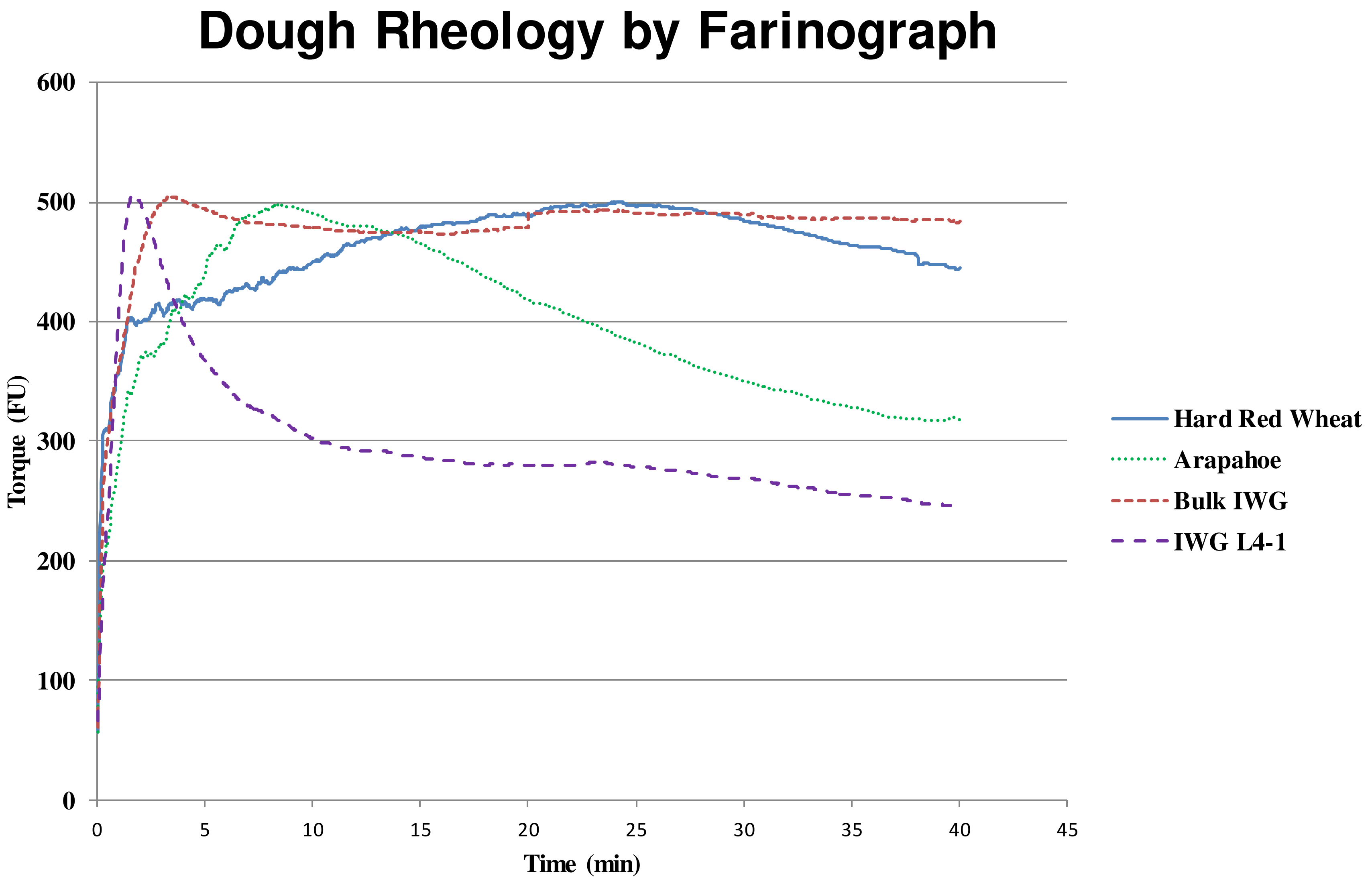
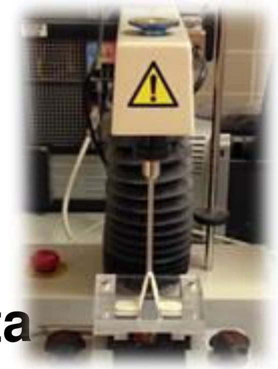
IWG's Protein Composition Affects Functionality: Elasticity & Extensibility



Dough Functionality



Dough Rheology – Farinograph and Kieffer Measurable Data

Samples	Farinograph Data: Water Absorption Corrected for Default Moisture Content (14%)	Farinograph Data: Stability (sec)	Kieffer Data: Resistance to Extension (mN)	Kieffer Data: Extensibility (mm)
Hard Red Wheat	74.5^{a*}	598.5^{a}	340.33^{b}	17.68^{b}
Arapahoe	74.6^{a}	247.0^{b}	425.82^{a}	21.61^{a}
Bulk IWG	68.7^{hi}	121.5^{bc}	179.14^{c}	13.51^{c}
IWG L4-1	73.2^{b}	44.0^{c}	66.69^{ijk}	6.04^{hi}
IWG L4-3	71.8^{cd}	88.0^{c}	67.47^{hijk}	7.72^{fgh}
IWG L4-29	69.0^{hi}	42.0^{c}	74.5g^{ghi}	6.69^{fgh}
IWG L4-32	67.2^{j}	42.0^{c}	65.53^{jk}	5.69^{hi}
IWG L4-57	68.5^{i}	30.0^{c}	44.99^{l}	4.41^{i}
IWG L4-72	69.6^{gh}	52.0^{c}	75.79^{fgh}	7.22^{fgh}
IWG L4-84	72.1^{c}	53.0^{c}	66.02^{ijk}	8.68^{ef}
IWG L4-85	70.3^{efg}	52.0^{c}	72.72^{ghij}	10.02^{de}
IWG L4-103	70.2^{fg}	29.0^{c}	66.86^{ijk}	6.76^{fgh}
IWG L4-105	70.8^{def}	47.5^{c}	68.73^{hijk}	6.81^{fgh}
IWG L4-139	71.2^{cde}	56.0^{c}	91.98^{e}	11.66^{cd}
IWG L4-154	71.4^{cd}	48.0^{c}	61.70^{k}	6.05^{hi}
IWG L4-157	70.3^{efg}	38.0^{c}	84.10^{ef}	8.40^{efg}
IWG L4-159	69.5^{ghi}	64.5^{c}	143.92^{d}	13.46^{c}
IWG L4-160	70.3^{efg}	42.0^{c}	71.57^{ghij}	6.34^{ghi}
IWG L4-172	68.9^{hi}	49.5^{c}	79.00^{fg}	8.42^{efg}

* Lowercase letters in each column indicate significant differences among samples according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$), where n=2 for farinograph data and n=5 for Kieffer data.

Bread Quality

Sample	Specific Volume (mL/g)	Bread Height (cm)	Bread Firmness (g)
Hard Red Wheat	2.00^{b}	3.81^{b}	5.71^{c}
Arapahoe	2.34^{a}	4.13^{a}	5.74^{bc}
Bulk IWG	1.58^{gh}	3.26^{d}	6.05^{a}
IWG L4-1	1.97^{bc}	3.54^{c}	6.01^{ab}
IWG L4-3	1.96^{bcd}	3.50^{cd}	6.12^{a}
IWG L4-29	1.61^{gh}	3.31^{cd}	5.88^{abc}
IWG L4-32	1.91^{bcd}	3.31^{cd}	5.98^{abc}
IWG L4-57	2.02^{b}	3.32^{cd}	5.95^{abc}
IWG L4-72	1.86^{bcde}	3.35^{cd}	5.92^{abc}
IWG L4-84	1.80^{cdef}	3.39^{cd}	5.98^{abc}
IWG L4-85	1.40^{i}	3.33^{cd}	5.95^{abc}
IWG L4-103	1.63^{fgh}	3.23^{d}	5.95^{abc}
IWG L4-105	1.72^{efgh}	3.34^{cd}	5.89^{abc}
IWG L4-139	1.87^{bcde}	3.44^{cd}	5.91^{abc}
IWG L4-154	1.67^{fgh}	3.40^{cd}	5.92^{abc}
IWG L4-157	1.55^{hi}	3.35^{cd}	5.89^{abc}
IWG L4-159	1.75^{defg}	3.45^{cd}	5.95^{abc}
IWG L4-160	1.63^{fgh}	3.31^{cd}	5.93^{abc}
IWG L4-172	1.76^{defg}	3.25^{d}	6.07^{a}

* Lowercase letters in each column indicate significant differences among samples according to the Tukey-Kramer HSD means comparison test ($P \leq 0.05$), n=2.

Composition and Functionality: Summary

- ✓ Seed size and bran/endosperm ratio have direct bearing on the grain's chemical compositions
- ✓ IWG samples had higher protein and total dietary fiber contents but lower starch content than HRW
- ✓ IWG samples were deficient in high molecular weight glutenin (HMWG) commonly found in wheat
- ✓ IWG dough system is generally weaker than that of HRW
- ✓ IWG is not as strong of a viscosity builder as HRW

Capitalizing on IWG's Potential as an Ingredient

- ✓ **Blend IWG flour with wheat flour**
- ✓ **Refine IWG to reduce bran content**
- ✓ **Use of dough conditioners**

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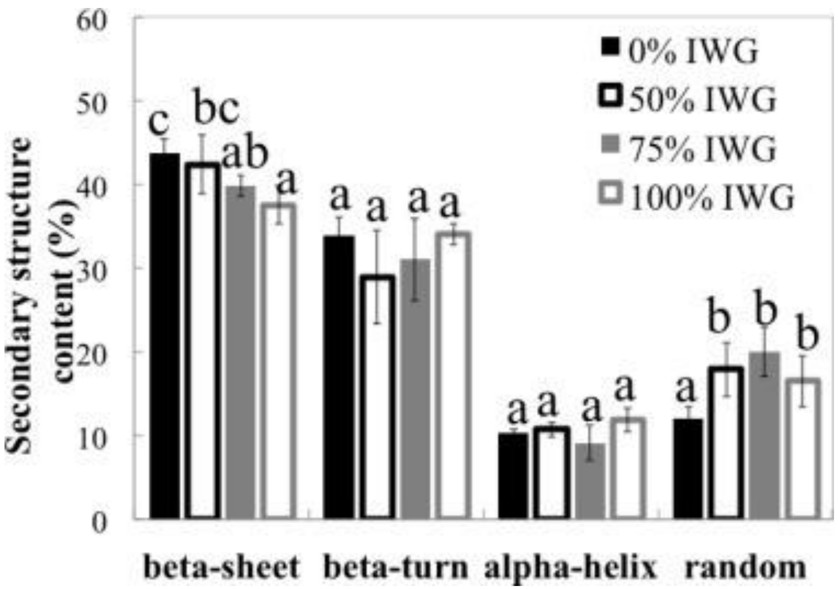
2

Protein interactions in IWG/HRW blends

Bran-induced changes in model wheat dough studied by FT-IR



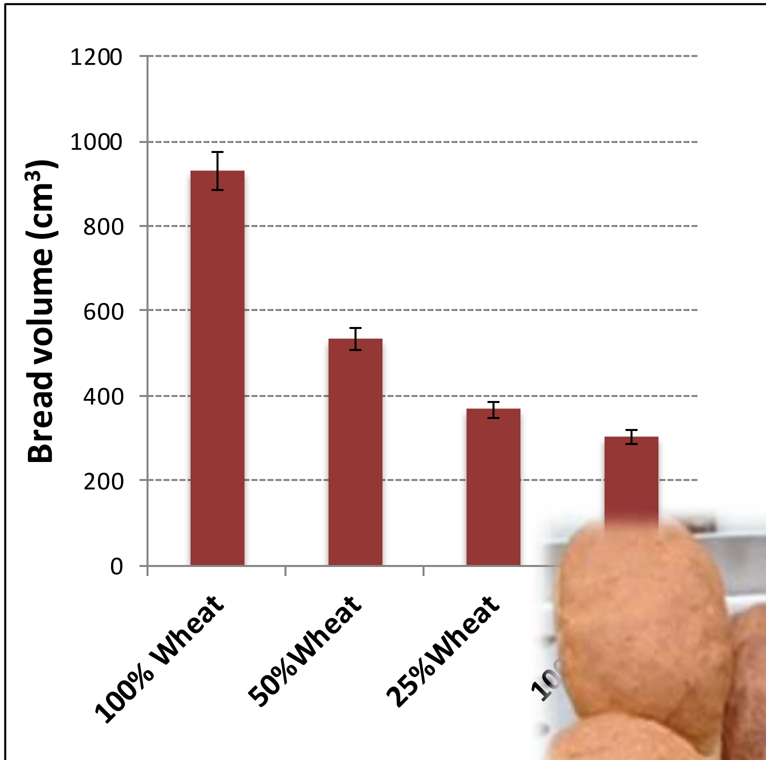
Marti *et al*. Food Chemistry 2016, 194, 994–1002

Bran causes partial gluten dehydration & gas cell disruption ⇒ bread volume ↓

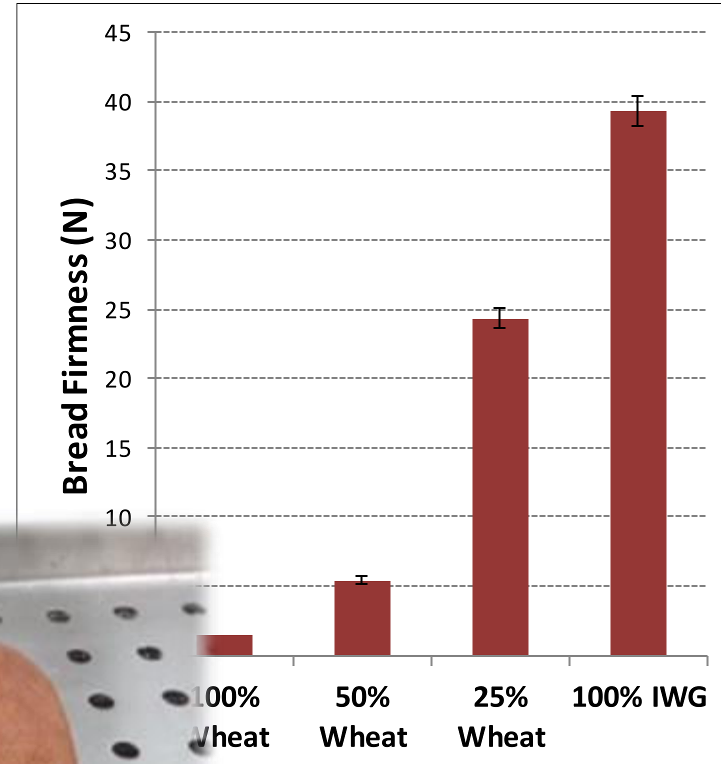
IWG/HRW blends

- **Adding ≥ 75% IWG to hard wheat flour resulted in protein secondary structure changes**
  - ✓ **fewer beta-sheets**
  - ✓ **more random coils**
- **Adding IWG affects dough handling and elasticity**
- **These differences are not only due to different protein profiles, but also IWG's composition (more bran!)**

IWG/HRW blends



Standard AACC method: AACC 10-0

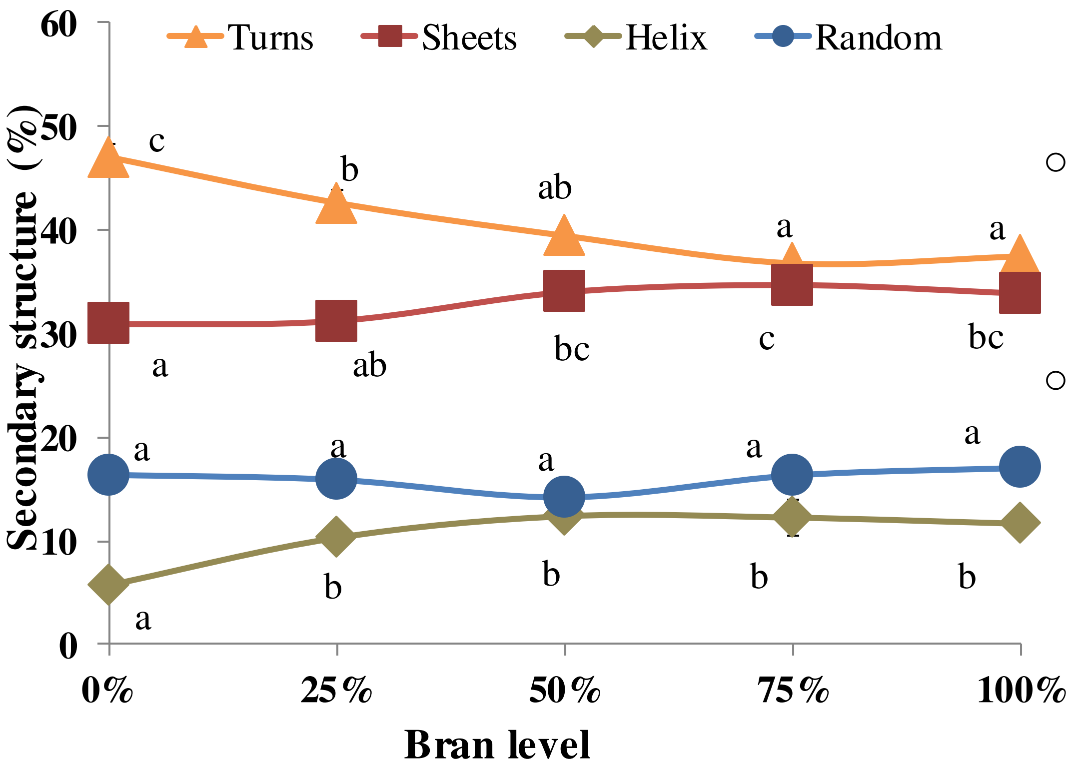


AACC method: AACC 74-09.01

100%W 50%W 25%W 100%IWG

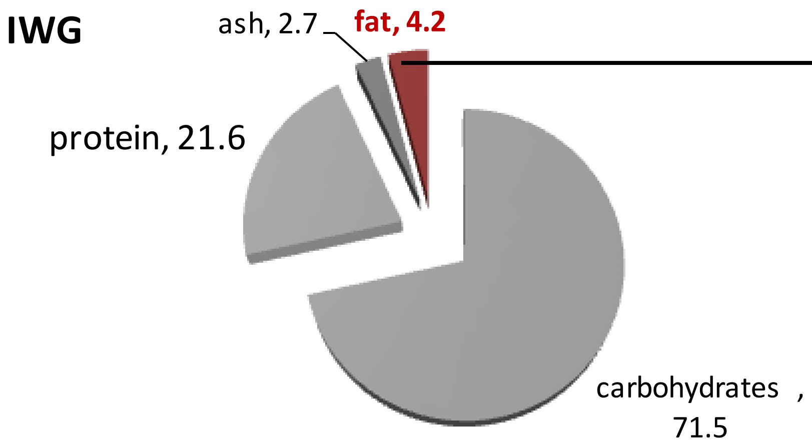
IWG Flour Refining

- ✓ Bran was added back to refined IWG flour at 100%, 75%, 50%, 25% and 0% of original bran content
- ✓ Dough rheology evaluated by farinograph (AACC 54-21)
- ✓ Changes in protein secondary structure determined by ATR-FTIR
- ✓ Effects of mixing temperature and time



- ○ Complete refinement does not lead to the optimum ratio of β-turns to β-sheets
- ○ 75%_Bran_IWG has the best sheets/turns ratio suggesting a good compromise between dough extensibility and elasticity

Storage Stability



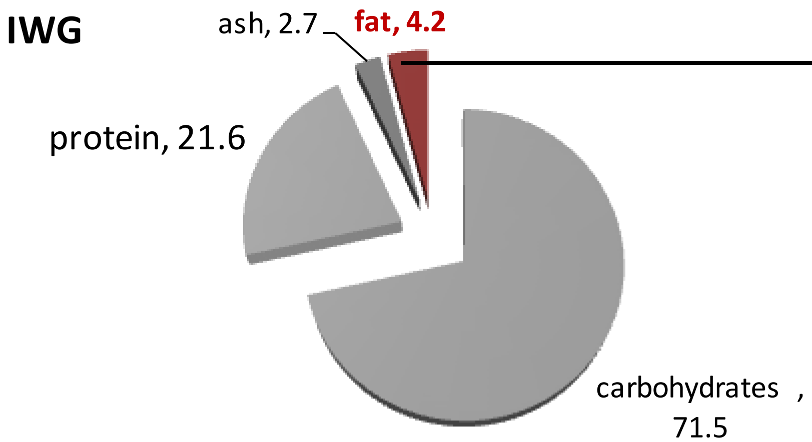
- ✓ Main fatty acids: linoleic, oleic and palmitic acid (similar to wheat)
- ✓ Higher in carotenoids than wheat

	HRW	IWG
Lipoxygenase Activity (U/g)	5.36*	5.00
Lipase Activity (U/g)	1.84	2.79*

Heat treatment of groats/flour during processing may be used to inactivate problematic enzymes

3

Storage Stability



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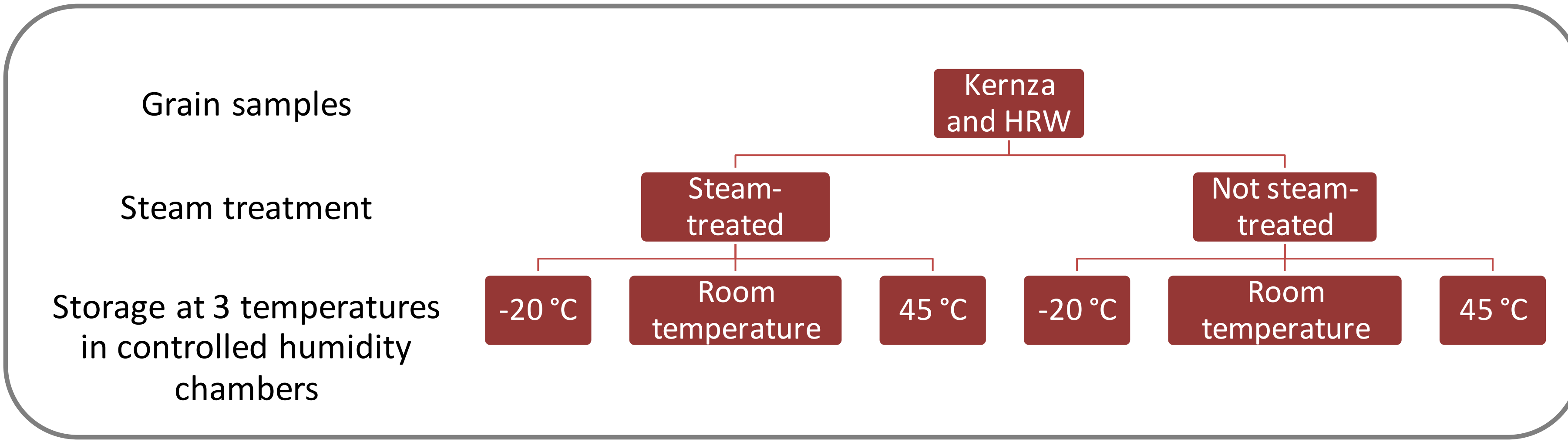

Samples removed from storage at regular intervals and analyzed for rancidity as well as functionality attributes

Storage Stability: Summary

- ✓ Steaming did not destroy the antioxidants hydroxycinnamic acid, nor disrupt their activity over accelerated storage; however carotenoid content decreased over storage
- ✓ IWG showed higher antioxidant activity and content over storage compared to HRW, as well as lower lipoxygenase values
- ✓ Oxidative rancidity in IWG did not increase over accelerated storage compared to HRW, while hydrolytic rancidity increased slightly
- ✓ Functionality testing is underway

Other Ongoing Work

- ✓ Monitor dough strength upon addition of dough conditioners
- ✓ Determine the overall safety of the grains by assessing presences of chemical residues, such as pesticides and mycotoxins, heavy metal, allergens, and anti-nutrient factors (phytate and trypsin inhibitor)
- ✓ Storage study of flour at different refinement levels (with and without heat treatment)
- ✓ Determine the impact of storage and refinement on IWG flavor
- ✓ Determine protein and starch digestibility
- ✓ Determine the physiological benefits of dietary fiber
- ✓ Research different product applications that do not require rising properties during baking.

Funding and Collaborators

- Initiative for Renewable Energy and the Environment (IREE)
- Forever Green Initiative (led by Dr. Donald Wyse)
- Minnesota Department of Agriculture
- The Land Institute

Collaborators:

- Dr. James Anderson and his research group at the University of Minnesota Agronomy/Plant Genetics Department
- Dr. Lee DeHaan (The Land Institute)
- North Dakota State University Wheat Quality and Carbohydrate Laboratory under Dr. Senay Simsek
- USDA-ARS Cereal Crops Research Unit under Dr. Jae-Bom Ohm

Thank You