Canadian Grain Commission | Commission canadienne des grains

RAPID ASSAY FOR FUNCTIONAL PROTEIN FRACTIONS IN WHEAT FLOUR OR WHOLEMEAL

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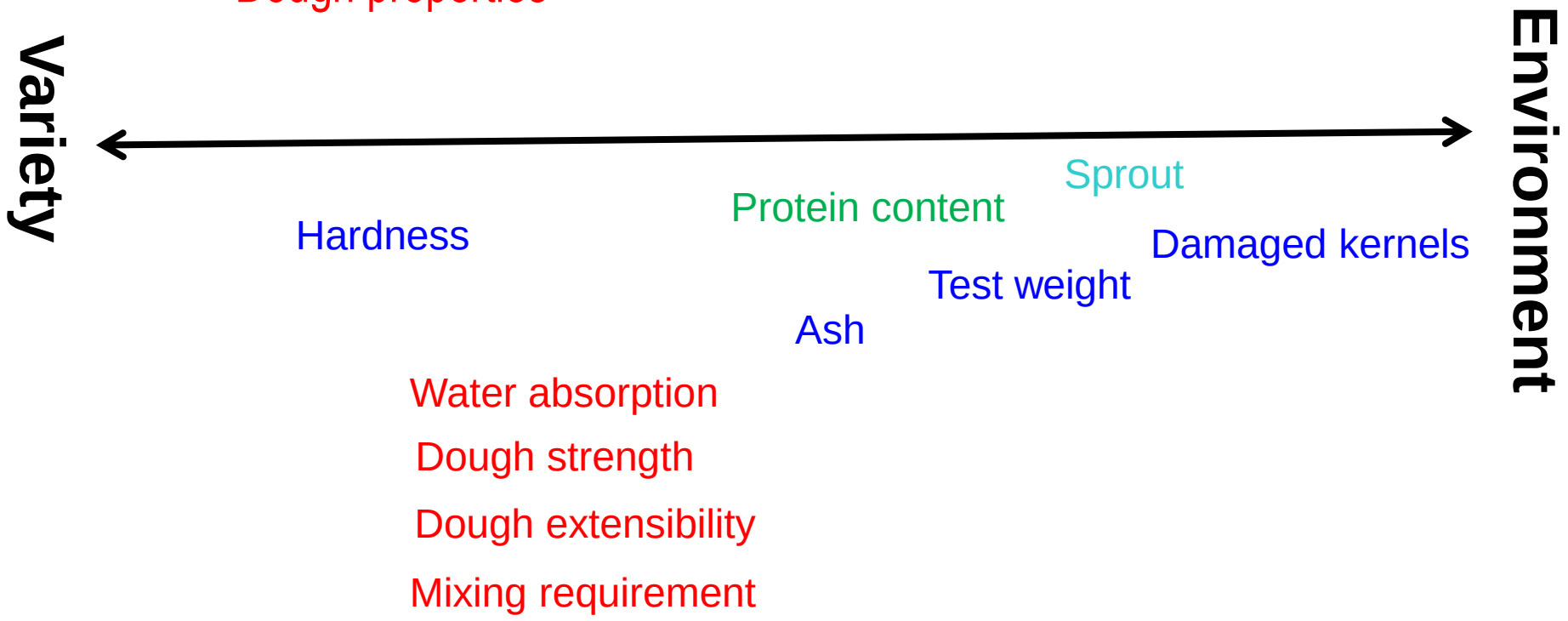
Canada

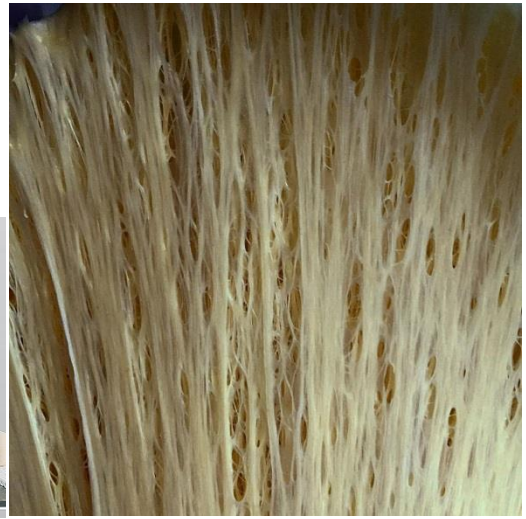
The wheat functional quality package

- **Functionality:**
  - Soundness
  - Protein content
  - Milling performance
  - Dough properties



Why are dough properties important?

- **Water absorption:**
  - Amount of water required to develop functional dough (not sticky not dry)
  - Profitable
- **Mixing requirement:**
  - Energy input to develop optimum gluten network
  - Mixing time important part of automation



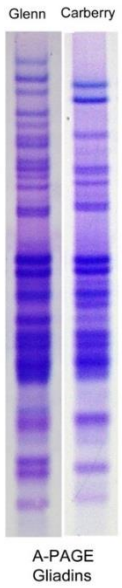
Gluten with Balanced Viscoelasticity

- Dough has two major physical properties derived from gluten, i.e., extensibility and elasticity.
- The balance between dough extensibility and elasticity determines the end-use quality
  - Highly elastic and inextensible dough is difficult to develop and handle during mixing and molding, and might not rise to its full potential during baking
  - Overly extensible with low resistance dough can be sticky for handling, and will collapse during fermentation and baking

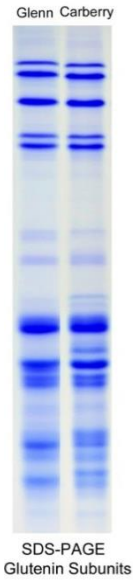
Dough viscoelasticity in relation to gluten protein composition

- Some types of gluten protein contribute to elasticity, while others are responsible for extensibility.
- The gluten protein types and their amounts are key factors determining dough properties.

Gliadins



Glutenin subunits

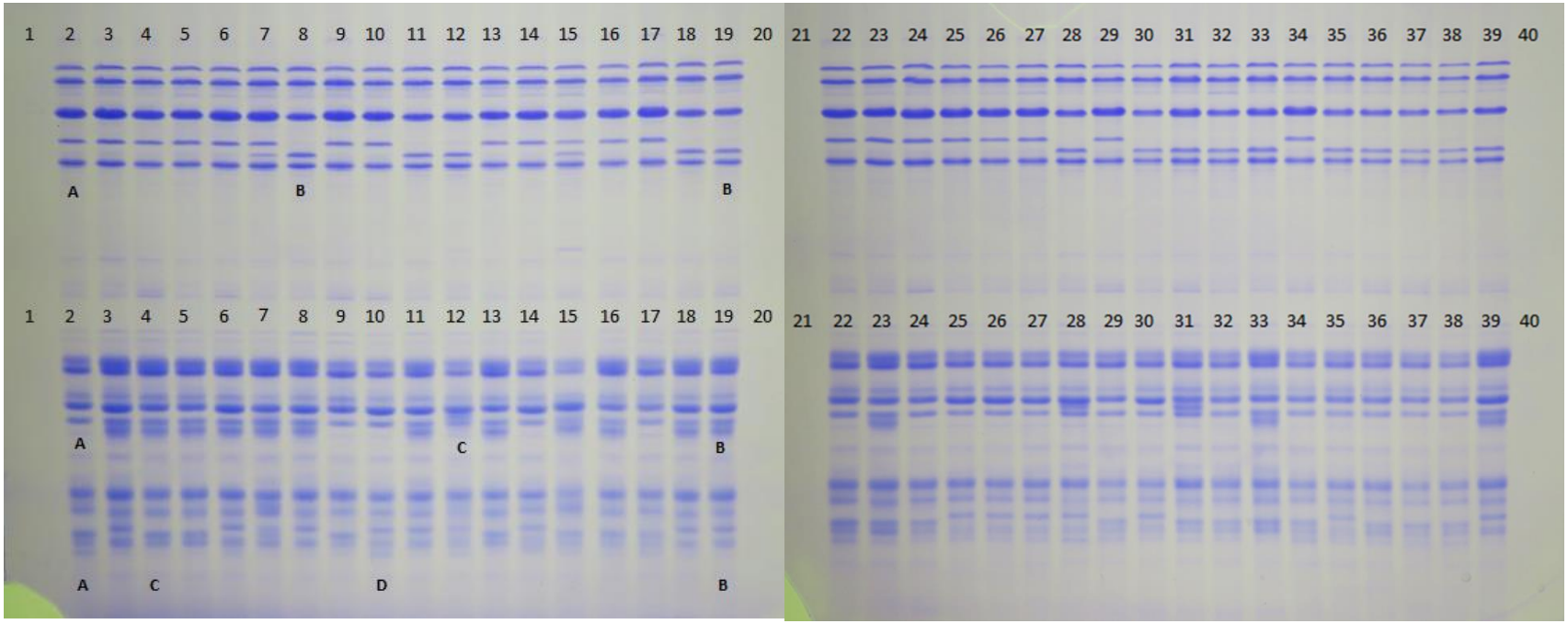


Individual glutenin subunits are not functional, loss of dough strength

Variation in dough properties of offsprings

	Lines	R_{max}	Extensibility	Mixing Time
Rmax: 800-1200	12H071	1112	14.2	5.0
	12H025	1098	12.6	7.2
	12H037	998	13.7	6.1
	12H079	984	15.6	4.9
	12H060	949	15.0	5.8
	12H044	884	16.8	4.5
	12H046	855	16.8	5.8
	12H015	831	18.1	3.7
Rmax: 600-799	12H013	784	16.3	4.4
	12H068	769	16.6	4.1
	12H078	709	18.0	4.8
	12H082	686	15.8	4.8
	12H002	669	18.0	4.0
	12H020	654	17.8	5.2
	12H085	644	17.9	3.9
	12H014	617	18.6	4.5
Rmax: 400-599	12H035	573	19.8	3.3
	12H007	546	19.7	3.2
	12H016	536	18.2	3.7
	12H012	511	18.0	3.5
	12H097	477	20.5	2.9
	12H090	476	17.9	3.3
	12H074	447	17.7	2.7
	12H076	424	16.0	2.9
Rmax: 200-399	12H100	388	18.5	2.8
	12H047	381	19.0	3.4
	12H056	372	20.8	2.5
	12H075	316	19.6	2.1
	12H033	288	18.7	2.7
	12H010	258	20.4	2.0
	12H017	231	19.3	2.3
	12H065	223	18.3	2.1
Rmax: ~550	AC Cadillac	573	19.4	2.9
	Carberry	547	18.3	3.7

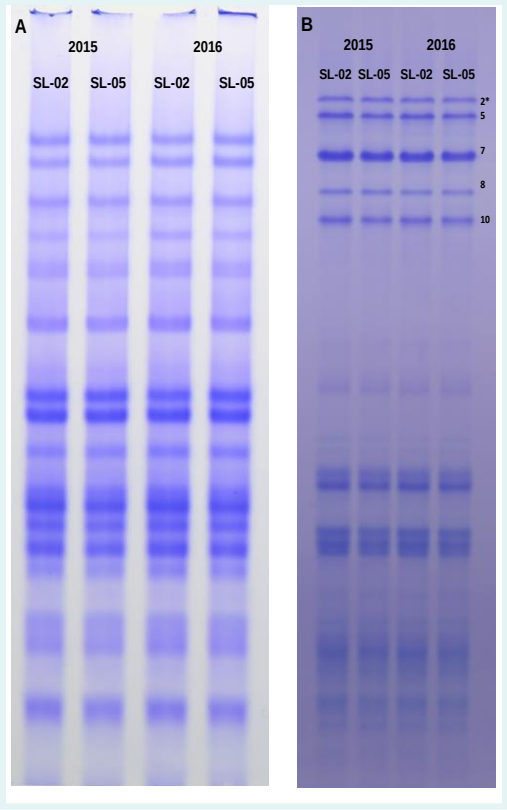
Allelic variation & combination in relation to strength

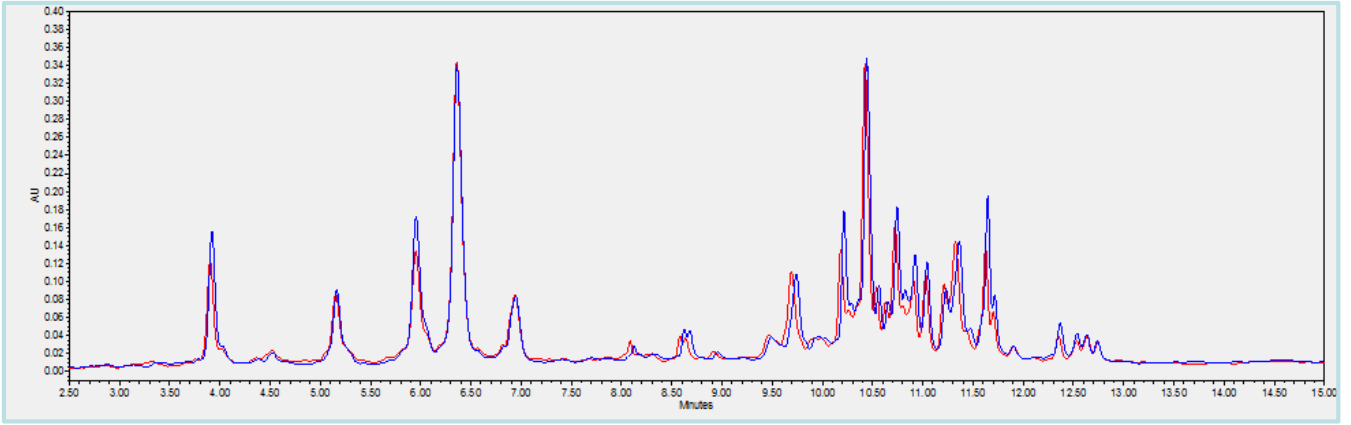


Strong, 1200 BU AC Cadillac x Carberry Weak, 200 BU

- All have Glu-1D 5 + 10
- Combination patterns of various subunit appeared to be more important than the presence or absence of individual subunits.
- Performance of a particular subunit depends on the rest of team members.

Sister lines with identical gliadin and glutenin fingerprints but diverse dough strength





	SL-02	SL-05
$R_{max,}$ BU	548	349
Insoluble/soluble P. glutenin	3.3	2.1
Glu-1Dx 5, %	20.0	15.7

Difference in the expression of Glu-1Dx can explain the contrasting dough strength between sister lines which have identical gliadin and glutenin fingerprints.

Why rapid assay for functional protein fractions?

"Rapid, cost effective quality tests for screening quality in early generation lines and at point of delivery"

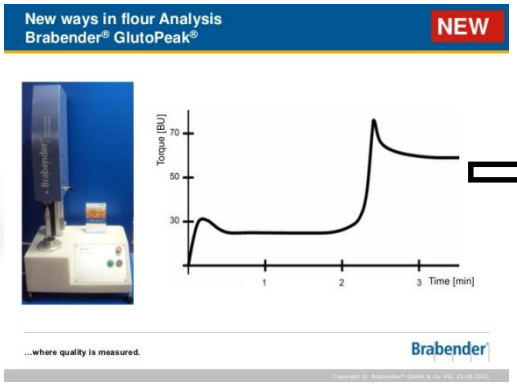
AAFC and Cereals Canada, Canadian Wheat Research Priorities - 2017-2022 Outlook

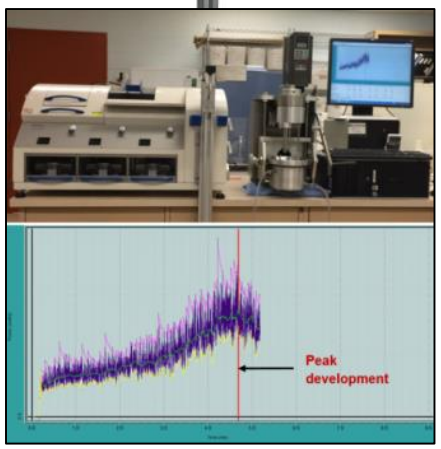
- Multi-mechanisms/genes for dough properties
- Environment has significant impact on gluten strength
- Regulation mechanisms for polymerization of functional glutenin polymers from subunits (not functional) largely unknown
- Rapid methods for dough strength at micro-scale (mg level)

Evaluation of Gluten Strength for Variety Registration and Advanced Line Selection

Registration Trials
(5 – 10 Kg)

Selection of advanced Lines
(0.2 – 0.5 Kg)





- Dough viscoelasticity of a new variety has to be within the defined range for Canada Western Red Spring (CWRS)
  - Carberry as minimum (floor)
  - Glenn as the maximum (ceiling)

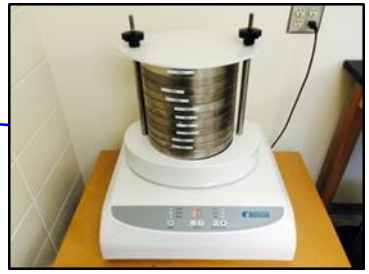
Flour Preparation

Grain (100 - 200 g)

Tempering

Milling
Brabender Jr. Mill

Sifting Material



Flour

Whole Meal Preparation

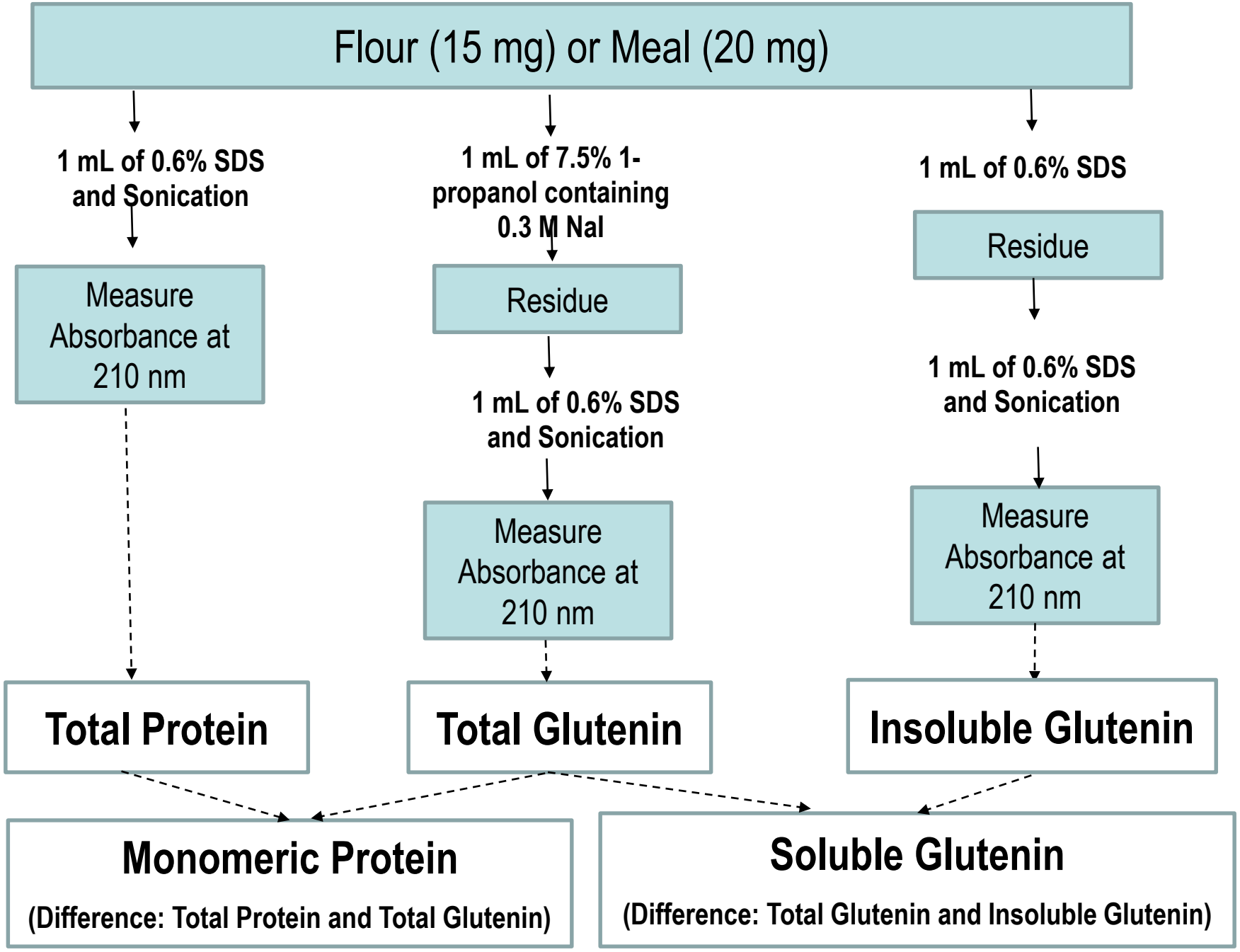
Grain (1 - 10 g)

Grinding (0.5 mm)
Retsch Mill

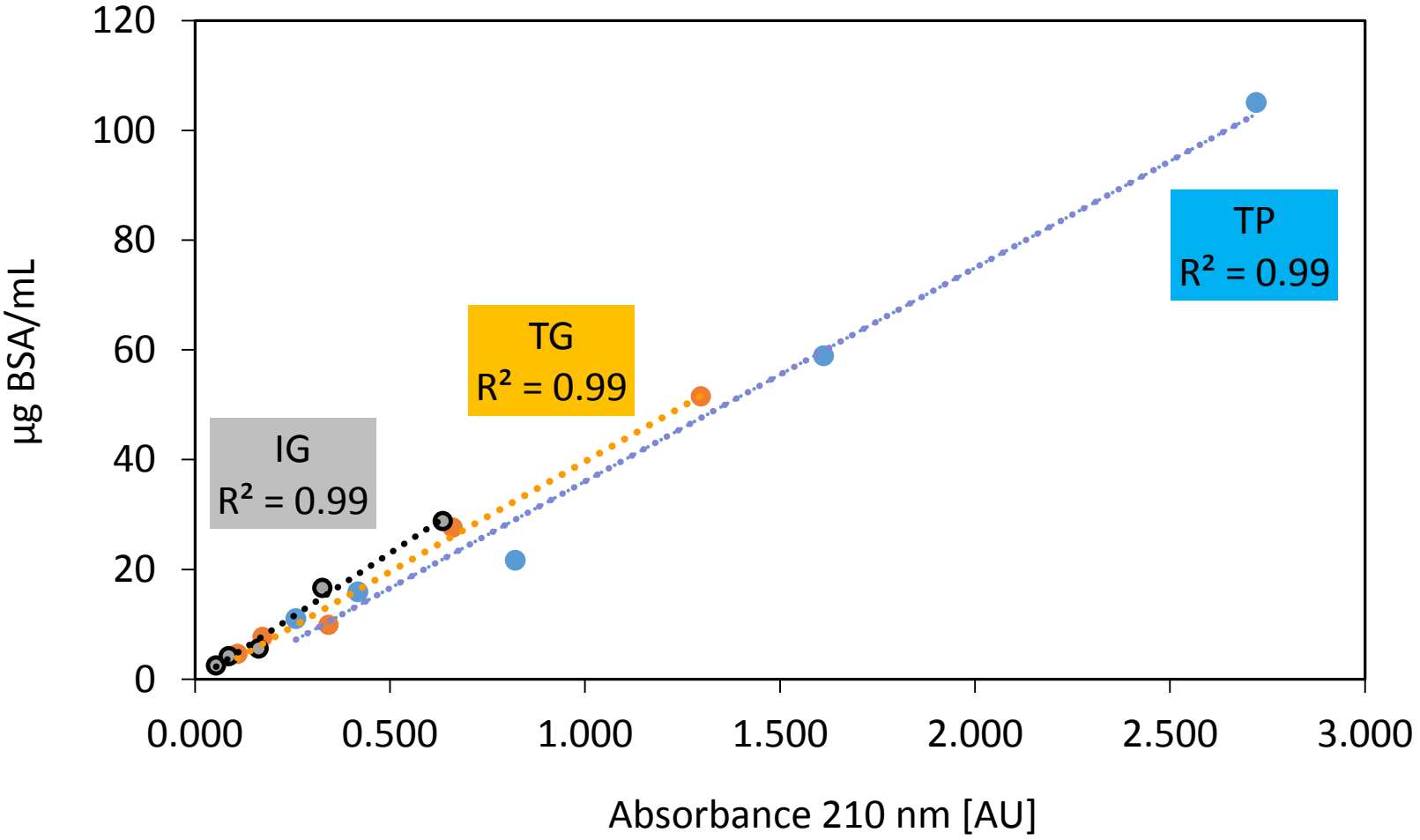
Whole Meal

Gluten strength of the selected wheat set (n=29)

	R_{max} [BU]	Extensibility [cm]	Mixing Time [min]	Energy Input [Wh/kg]
Max	1098	21.5	7.2	20.9
Min	231	12.6	1.9	5.4
Mean	577	17.8	3.8	10.3
SD	226	1.9	1.3	4.0
RSD (%)	39.3	10.5	34.3	38.6


Flour (15 mg) or Meal (20 mg)
1 mL of 0.6% SDS and Sonication
1 mL of 7.5% 1-propanol containing 0.3 M NaI
1 mL of 0.6% SDS
Measure Absorbance at 210 nm
Residue
Residue
1 mL of 0.6% SDS and Sonication
1 mL of 0.6% SDS and Sonication
Measure Absorbance at 210 nm
Measure Absorbance at 210 nm
Total Protein
Total Glutenin
Insoluble Glutenin
Monomeric Protein
(Difference: Total Protein and Total Glutenin)
Soluble Glutenin
(Difference: Total Glutenin and Insoluble Glutenin)

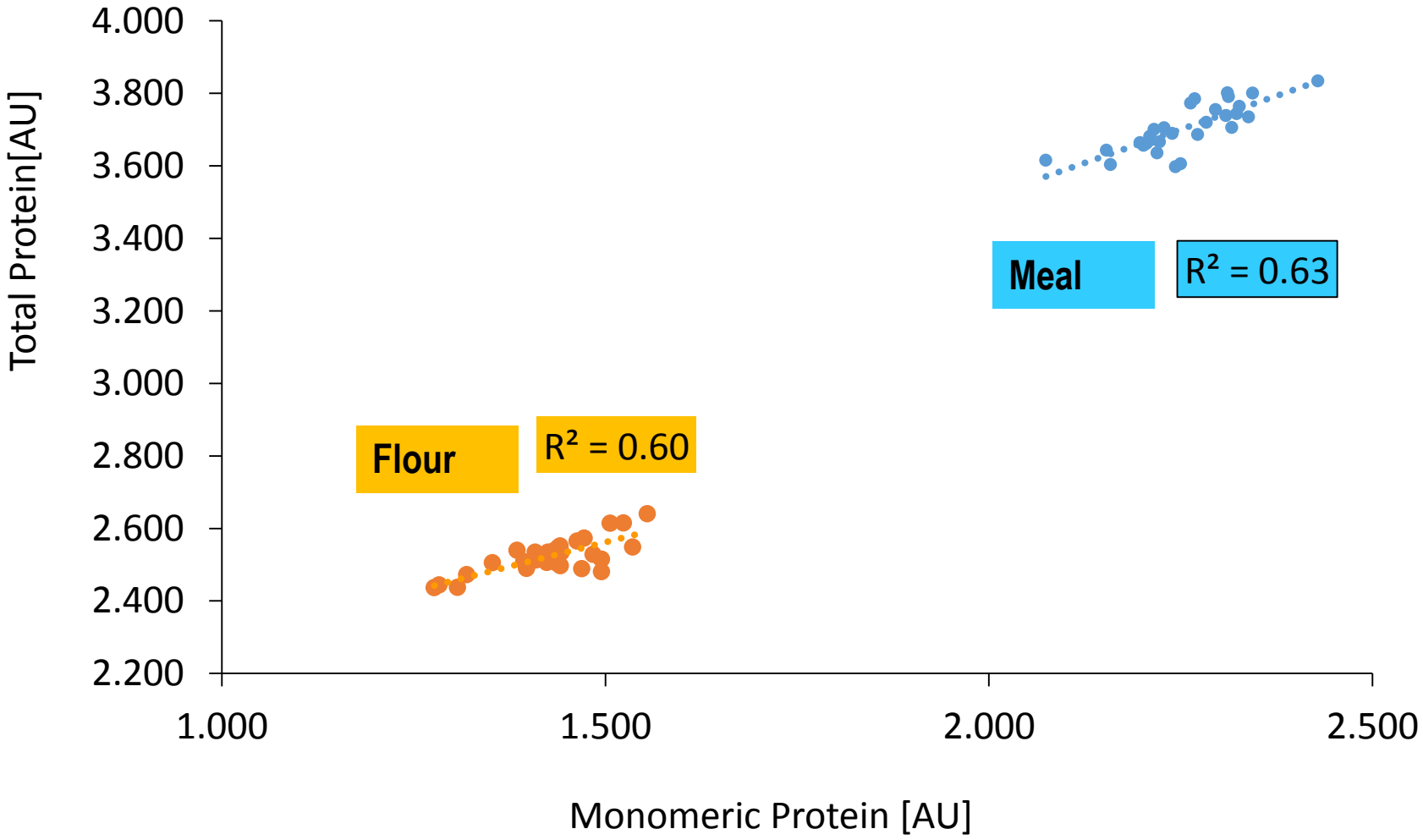
Relationship between quantity of protein fractions measured by spectrophotometer at 210 nm [AU] and BCA method [µg BSA/mL]



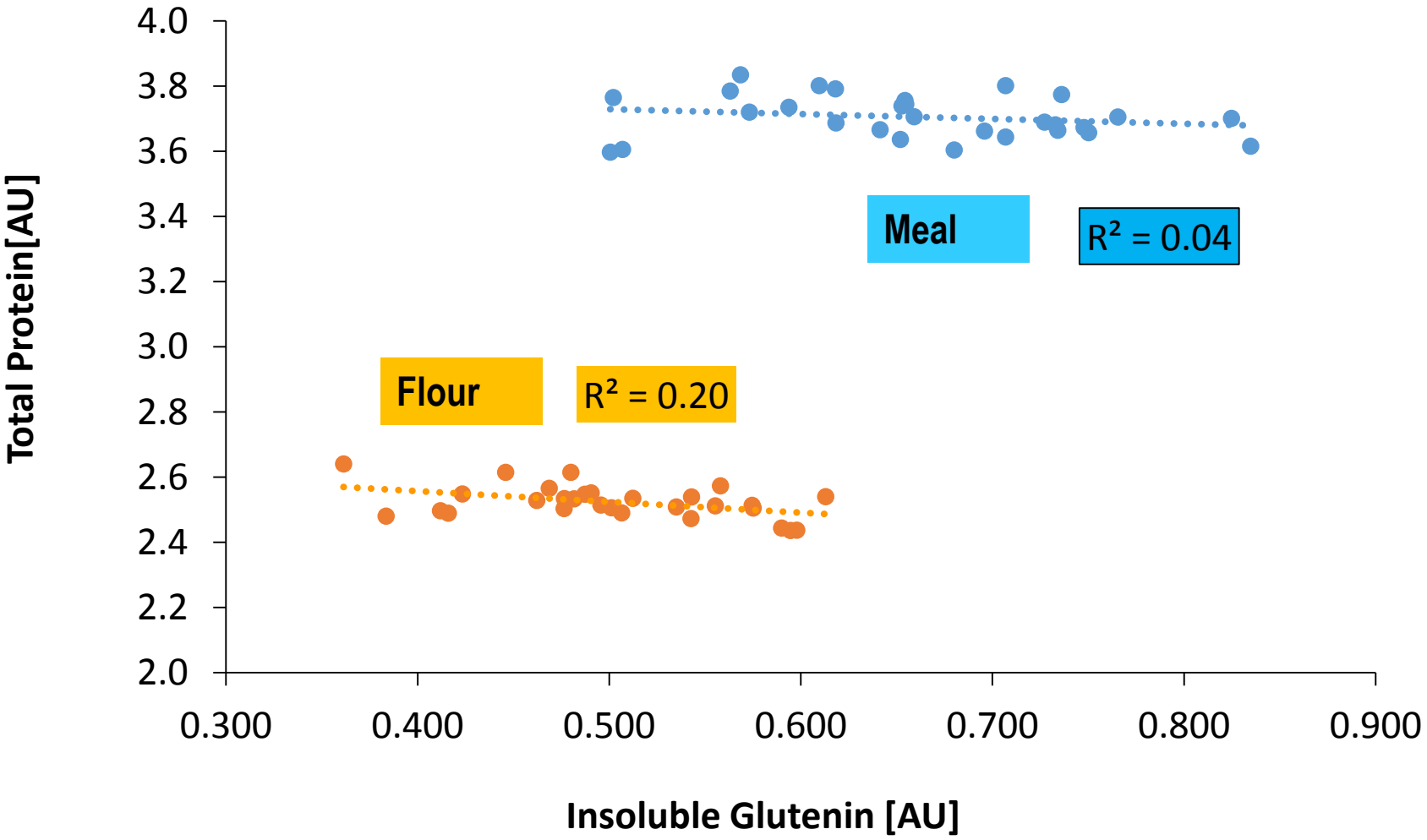
Proportion of protein fractions over total protein

	Monomeric Protein [%]		Polymeric Protein [%]		Soluble Glutenin [%]		Insoluble Glutenin [%]	
	Flour	Meal	Flour	Meal	Flour	Meal	Flour	Meal
Max	60.3	63.3	47.6	42.6	27.4	25.2	24.5	23.1
Min	52.4	57.4	39.7	36.7	20.9	17.9	13.7	13.3
Mean	56.6	60.9	43.4	39.1	23.5	21.2	19.9	17.9
SD	2.1	1.2	2.1	1.2	1.5	1.7	2.8	2.5
RSD (%)	3.7	2.0	4.9	3.2	6.4	8.2	14.2	13.9

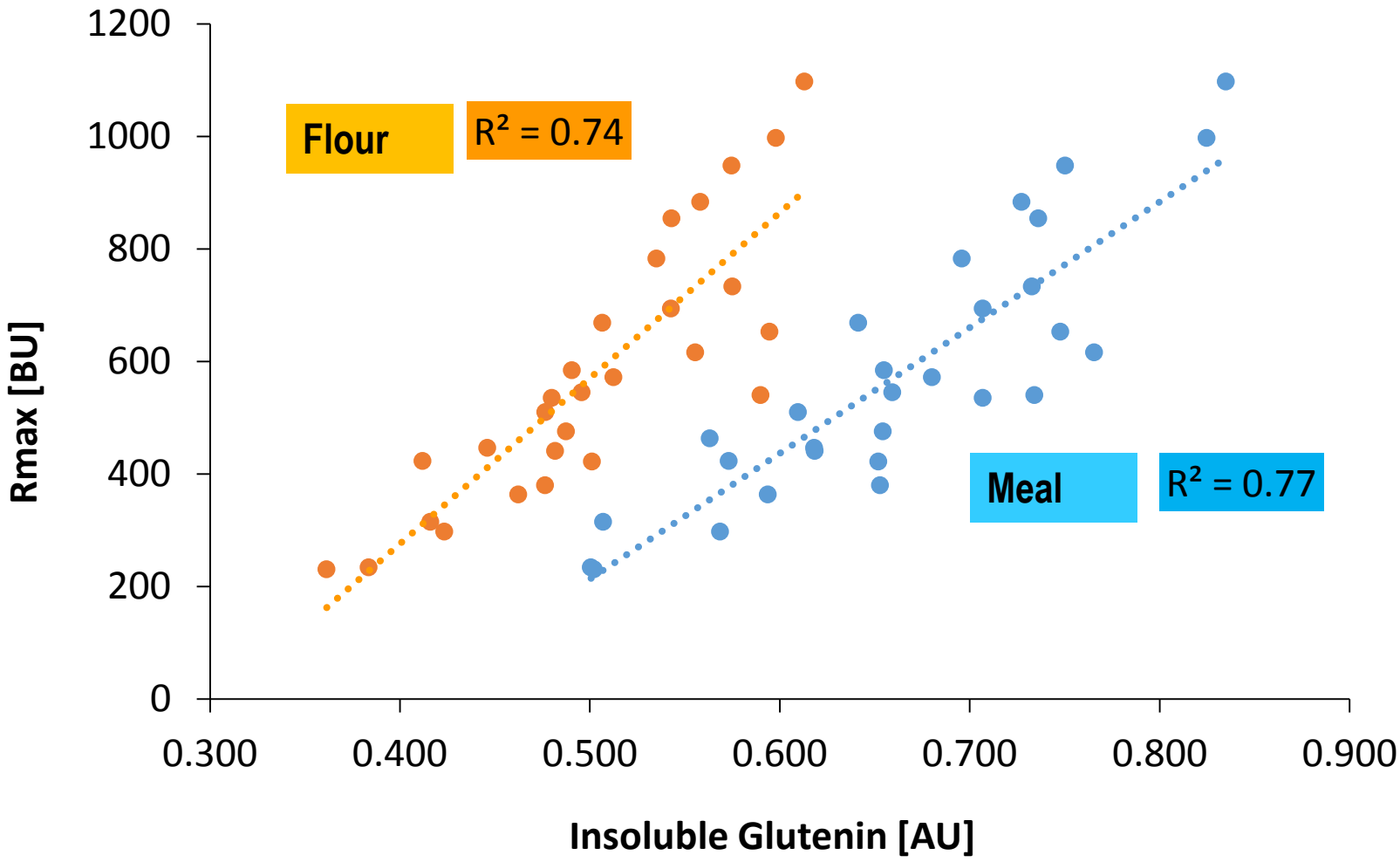
Relationship between total protein and monomeric protein in grain and flour



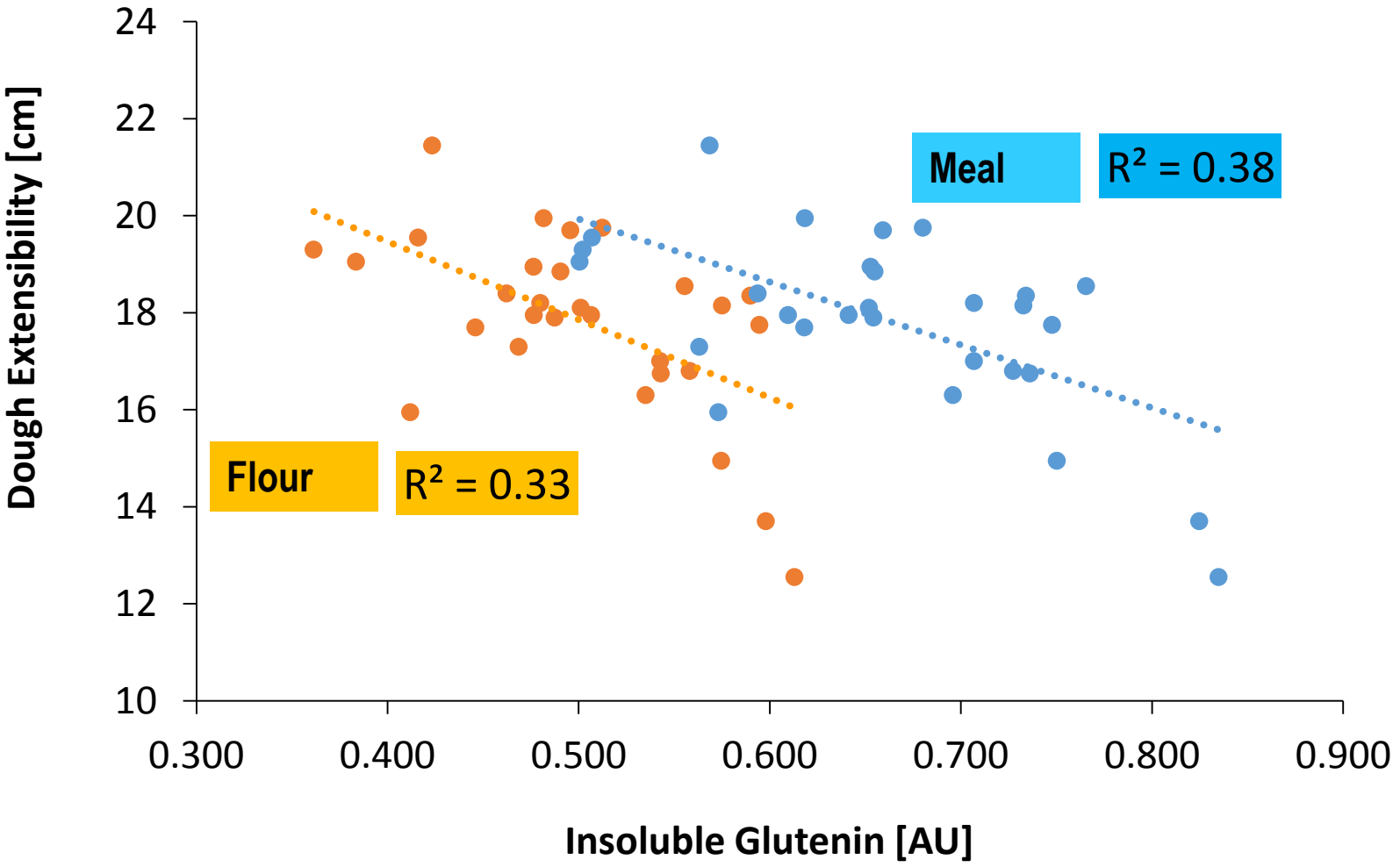
Relationship between total protein and insoluble glutenin in grain and flour



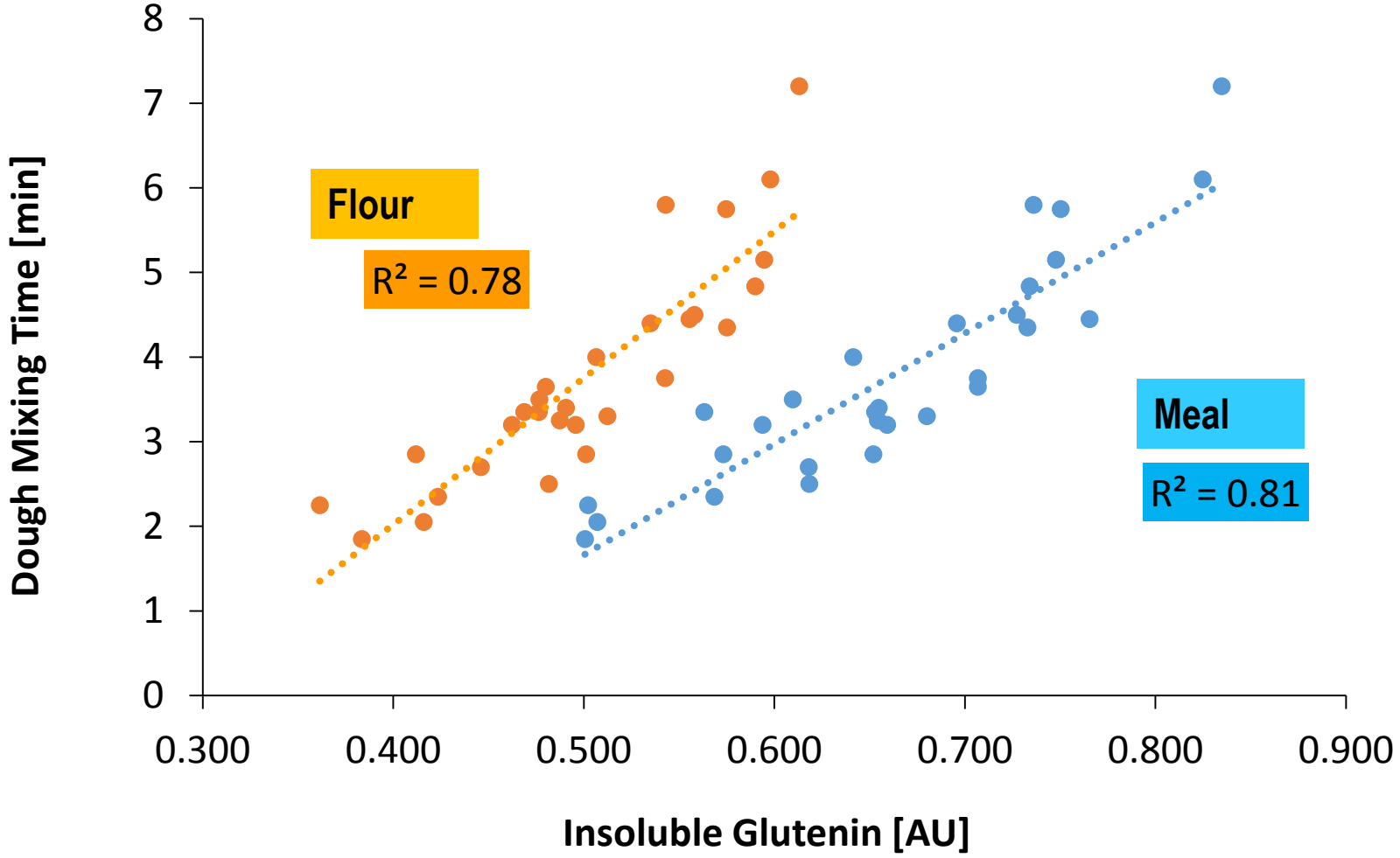
Dough R_{max} in relation to insoluble glutenin



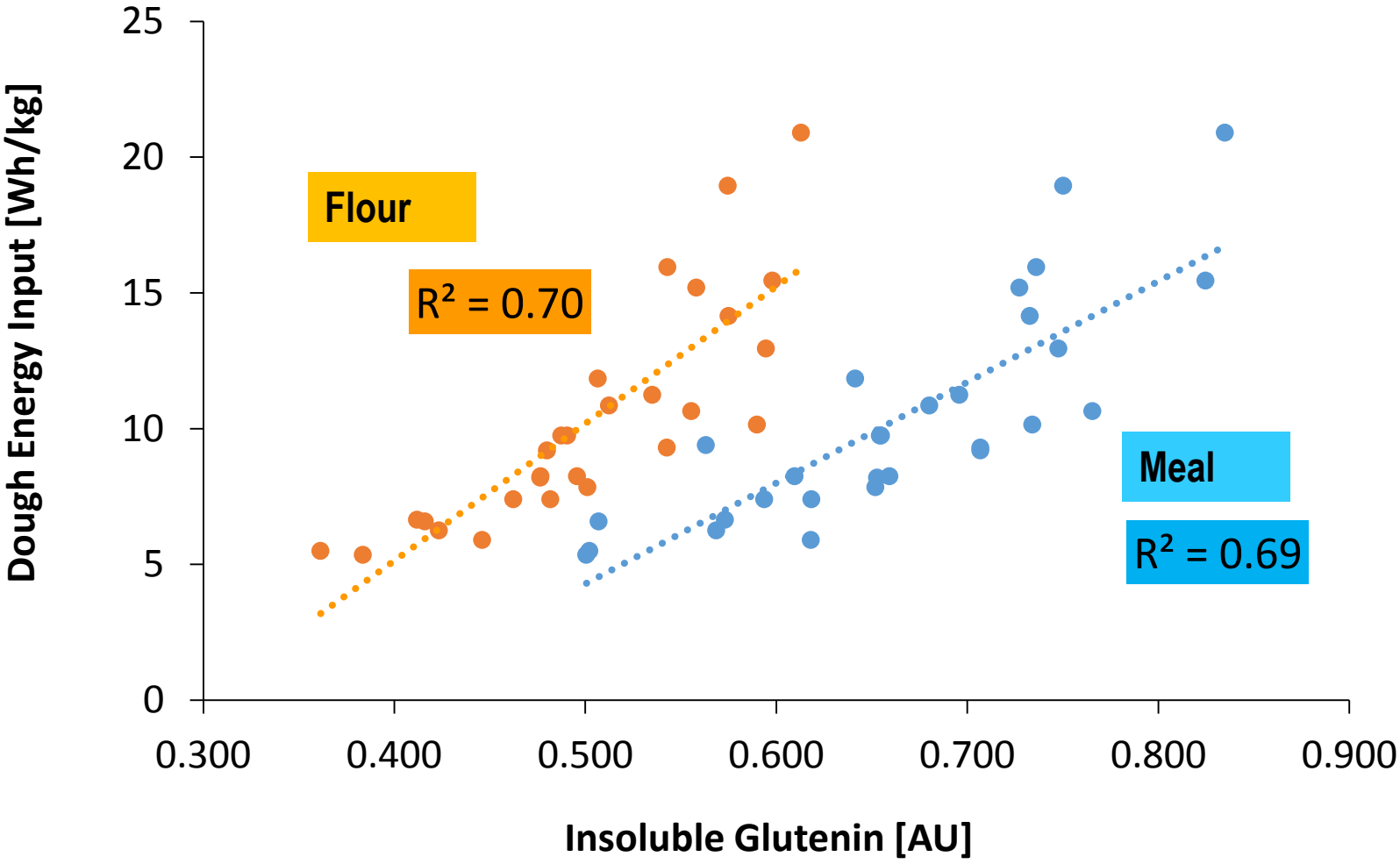
Dough extensibility in relation to insoluble glutenin



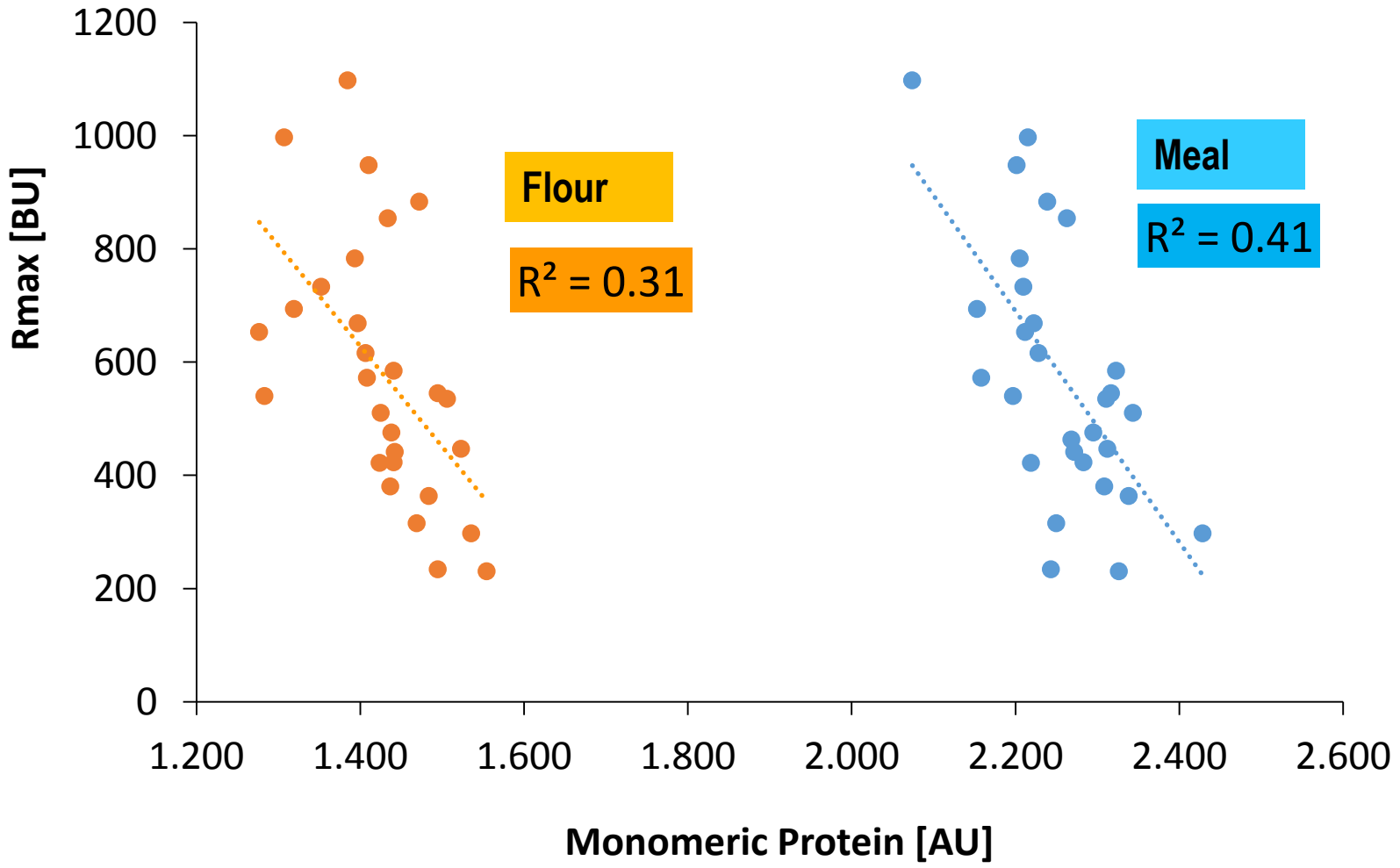
Dough mixing time in relation to insoluble glutenin



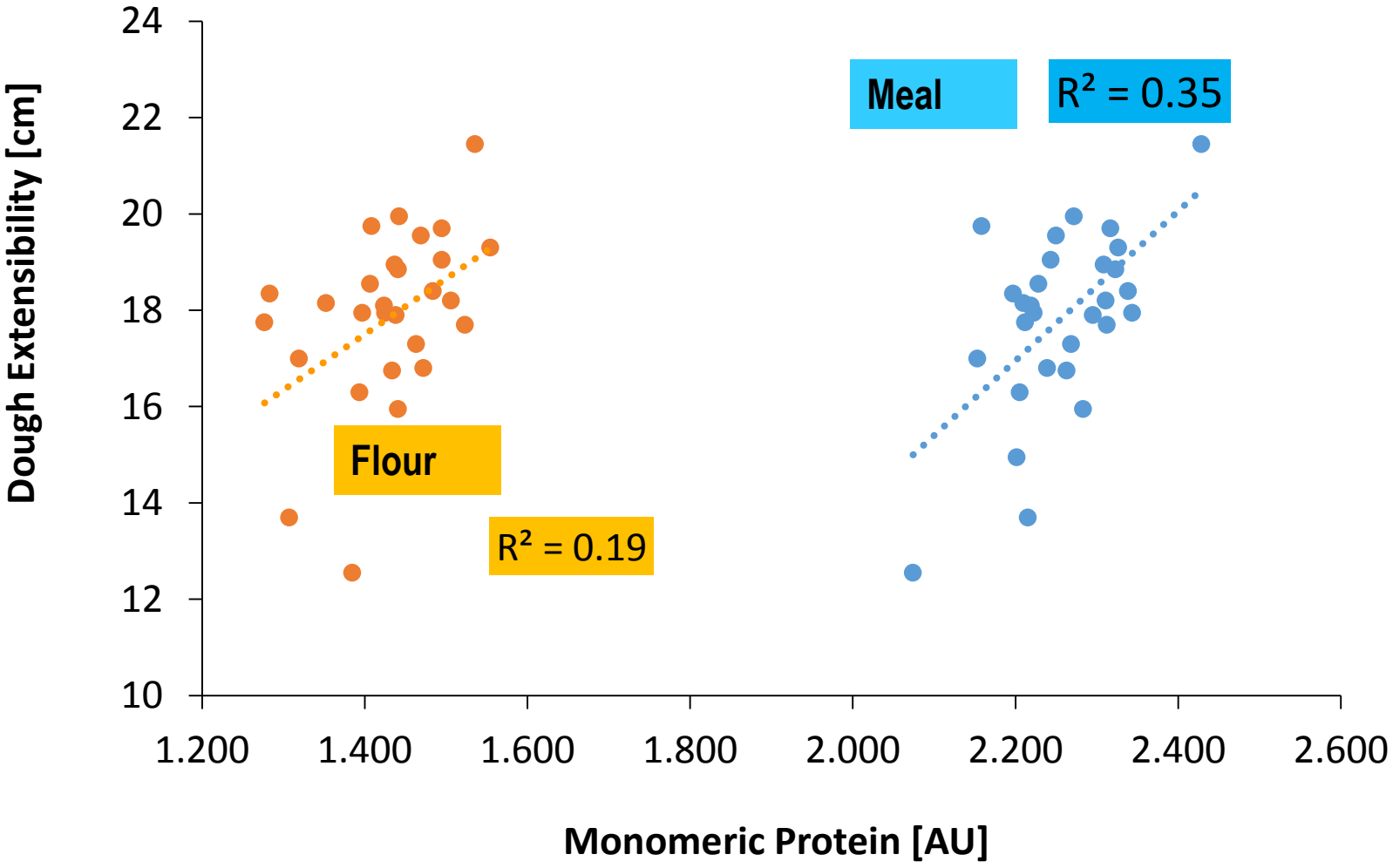
Dough mixing energy input in relation to insoluble glutenin



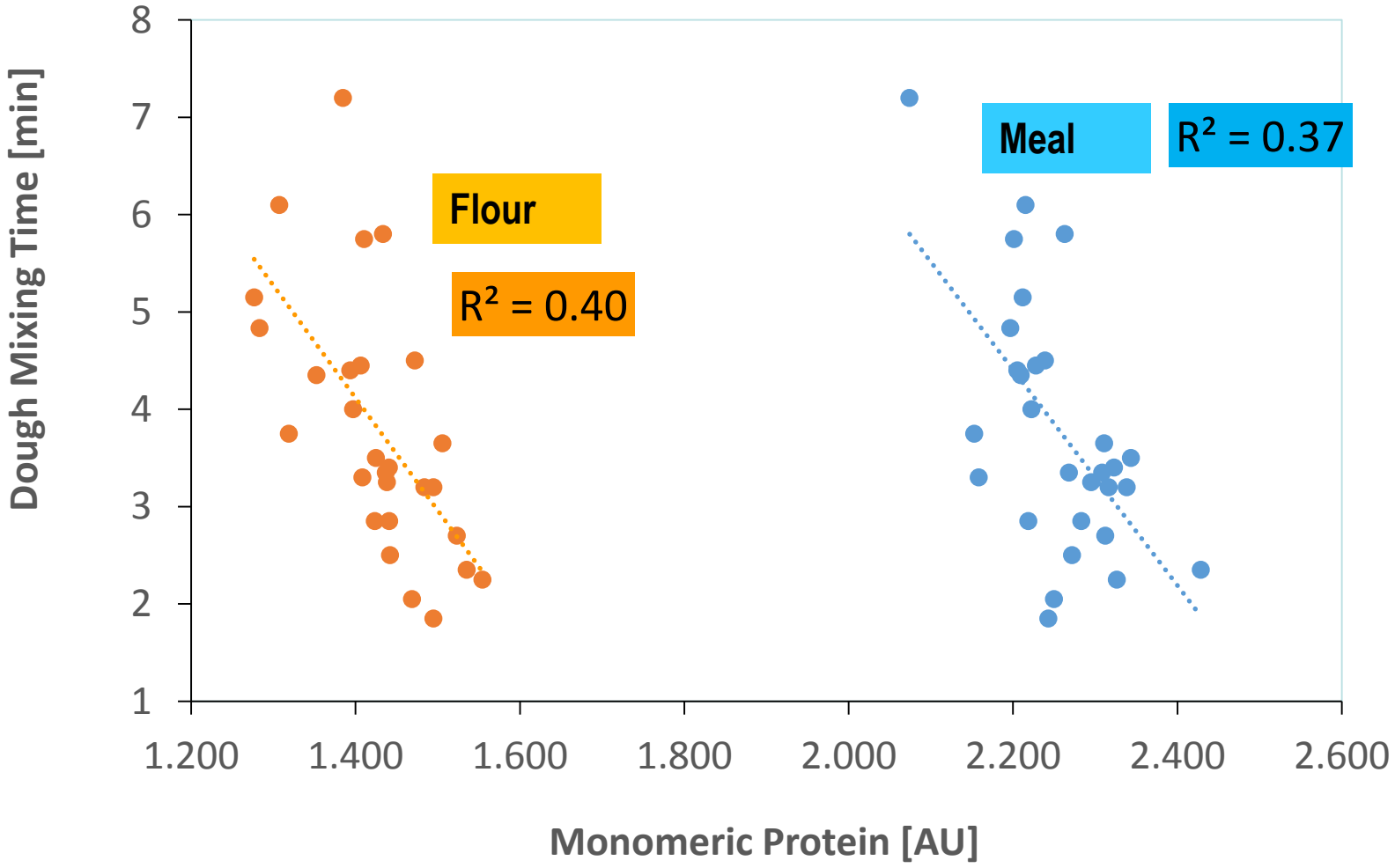
Dough R_{max} in relation to monomeric protein



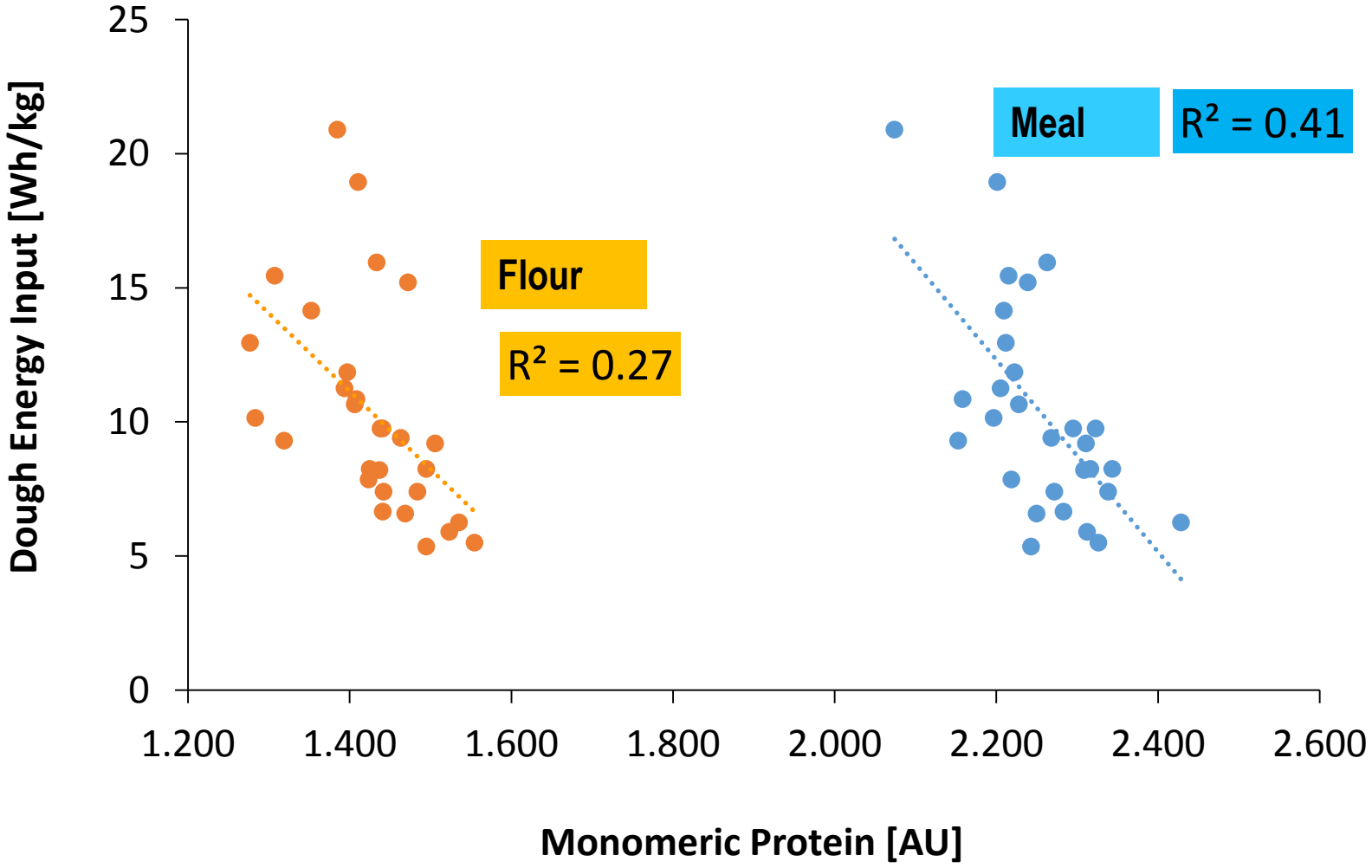
Dough extensibility in relation to monomeric protein



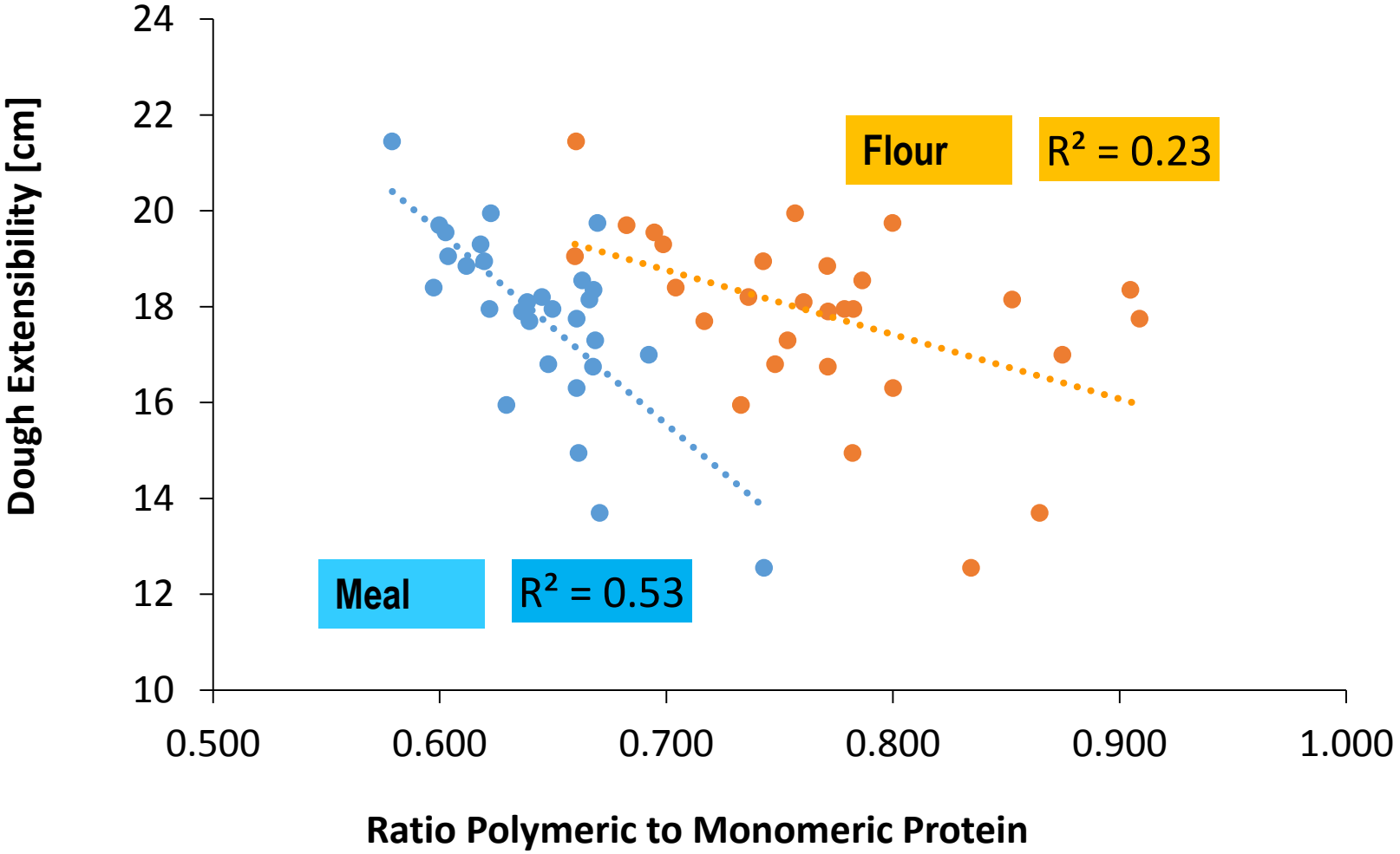
Dough mixing time in relation to monomeric protein



Dough mixing energy input in relation to monomeric protein



Dough extensibility in relation to the ratio of polymeric to monomeric protein

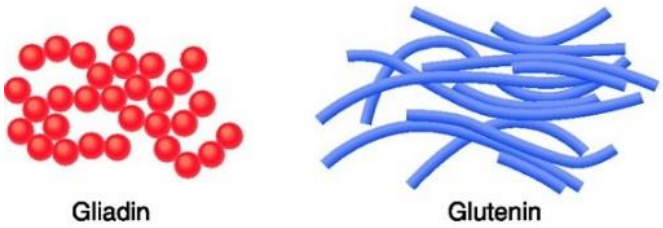


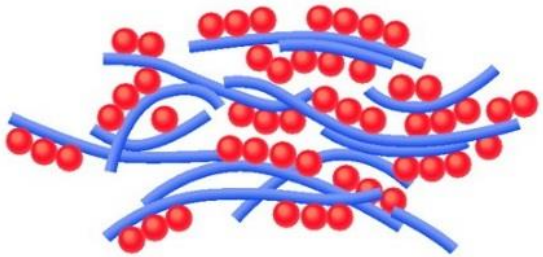
Conclusions

- The protocol proposed in this study for quantification of functional protein fractions only requires milligrams of wholemeal, and is simple, fast and cost effective.
- It can be used as a tool for screening in breeding programs or to investigate the effects of genotype, environment, and their interactions on dough functionality.

Conclusions

- **More work required to investigate dough extensibility**
  - Gliadins: amount and type
  - Glutenin: chain length and degree of branching
  - Other components: e.g., polysaccharides
  - Water absorption





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