MIXOLAB

A New Approach to Rheology

Edited by

Arnaud Dubat, Cristina M. Rosell, and Eimear Gallagher

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Arnaud Dubat
CHOPIN Technologies
Laboratoire d'Applications
Villeneuve la Garenne, France

Cristina M. Rosell
Instituto de Agroquímica y Tecnología de Alimentos
Consejo Superior de Investigaciones Científicas
Paterna, Spain

Eimear Gallagher
Teagasc Food Research Centre
Ashtown, Dublin, Ireland

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Contents

TABLE 5.4
Mixolab Data for Blends of Soft Wheat Flour (SWF) with Starch from Three Different Sources

	Mixolab Data								
Sample Name	C1	C2	C3	C4	C5	Alpha	Beta	Amplitude (Nm)	Stability (s)
SWF	1.39a	0.73ab	0.41a	2.93b	4.16c	–0.061b	0.020a	0.10a	271b
SWF with 5% wheat starch	1.62bc	0.65ab	0.33a	2.85ab	3.94b	–0.055bc	–0.007a	0.14a	181ab
SWF with 10% wheat starch	1.82c	0.50a	0.29a	2.78a	3.64a	–0.053c	–0.009a	0.13a	117a
SWF with 5% corn starch	1.71bc	0.70ab	0.37a	2.93b	3.94b	–0.061bc	0.038a	0.19ab	173ab
SWF with 10% corn starch	2.03d	0.45a	1.58ab	2.79a	3.71a	–0.065ab	0.135a	0.20ab	118a
SWF with 5% potato starch	1.67bc	0.93b	0.43a	3.08c	4.04b	–0.055bc	0.010a	0.24b	159ab
SWF with 10% potato starch	1.59ab	0.47a	3.08b	3.02c	4.02b	–0.073a	0.145a	0.18ab	204ab

[a] Values not sharing a common letter are significantly different ($P \leq 0.05$).

5. CONCLUSION

The Mixolab, a new quality control tool, has recently been developed to analyze flour samples. In real time, it measures the torque produced by the dough between the two blades. After a dough is formed, the device measures its behavior as a function of temperature, time, and mixing.

In the present study, the effect of added starch on Mixolab flour profiles was investigated. In the first part of the study, an experimental waxy wheat line (NDSW0481) was compared with control flours. As expected, waxy wheat flour displayed a very distinct Mixolab profile in comparison to that of control flours. It had the lowest C4 and C5 values, in agreement with previous findings. Previous studies with the Rapid Visco Analyser have shown that waxy wheat has lower setback values, which are correlated with the C5 value from Mixolab data. In this study, the C5 value was lower in waxy flour relative to that in nonwaxy genotypes. Therefore, the Mixolab could be used in breeding programs for specialty wheat to screen varieties based on amylose and amylopectin content. The low amylose percentages in waxy lines provide unique Mixolab profiles, with C4 and C5 values that are significantly lower than those of control flours (Fig. 5.1).

In the second part of the study, blending was performed. Starch from three different sources was added to two different flours at two different levels, 5 and 10% of flour weight. Since C3, C4, and C5 values have been reported to be correlated with starch properties, these were the values discussed in this study. Overall, addition of 5% wheat, corn, or potato starch did not significantly change the C3 values. Significant changes were observed by addition of 10% in blends. Potato starch seemed to have the least effect on Mixolab profiles except in the case of the C3 value of SWF with a 10% potato starch blend. Based on this data, one can conclude that the effect of starch on Mixolab profiles may be investigated at addition levels ≥5%. Corn and wheat starch seem to produce a more dramatic effect on Mixolab profiles than potato starch.

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CHAPTER 6

Amylase Activity and the Mixolab

G. Sinnaeve,[1] J. Le Brun,[2] and A. Dubat[3]

[1]Centre Wallon de Recherches Agronomiques, Département Valorisation des Productions, Gembloux, Belgium
[2]ARVALIS Institut du Végétal, Boigneville, France
[3]CHOPIN Technologies, Laboratoire d'Applications, Villeneuve la Garenne, France

1. OVERVIEW

In northern regions, the bread-making quality of wheat lots can be largely affected by sprout-damaged kernels. Sprout damage occurs when the wheat is quite mature and cannot be harvested because of a long period of rain. As the seed is starting its germination, huge amounts of enzyme are produced, with possible degradation to the starch and to proteins, which can lead to a loss of quality and economical losses either for the producers or for the milling and bread-making industries (Walker-Simmons and Ried 1992). The Hagberg falling number (FN) method (Hagberg 1960, 1961), which is widely used, is accepted for estimating the sprout damage level and α-amylase activities (Derera 1989, Feillet 2000). The Hagberg FN is an indirect measurement of α-amylase activities (Feillet 2000). A low value is theoretically linked to high amylase activity, which is linked to sprout-damaged kernels. In most European countries, the Hagberg value is the first criteria considered for the acceptance and payment of wheat lots. A low Hagberg value can lead to a refusal of the lot without consideration of any other quality criteria such as protein content, Zeleny index, or Chopin-Alveograph values.

In recent harvests, northern countries have had to deal with low Hagberg values. These were often, but not always, linked to sprout damage. In some cases, low Hagberg values were observed with little effects on the α-amylase activities and on the bread-making quality. Some of the present wheat breeds cultivated with new agricultural techniques (producing higher yield, using fungicides such as strobilurines) may not fully fit the relationship between FN and α-amylase activity. It is worth considering that the FN method developed in the early 1960s is perhaps not as able to estimate the true level of α-amylase activity due to sprout damage (Sinnaeve 2001) as it was earlier.

1.1 Economic Aspects

The economic consequences of high α-amylase activities can be very important. In Australia, 22.5% of the 20.3 million tonnes (t) were degraded from food to feed grade, with an estimated loss of 30%. The huge problems due to the sprout damage of crop year 1983-84 led to the development of new analytical methods such as the WheatRite test kit (Skerritt and Heywood 2000) or the Rapid Visco Analyser stirring number for the assessment of amylase activities (Bason 1996).

The prevention of sprout-damage risk is of major concern in the European Union. It is estimated that sprout-damaged grains occur one year in every five, with a loss of 10% of the yield and 50% of the lots downgraded due to poor bread-making quality; the annual costs for the European Union were estimated by Autran et al (1995) at 50–60 million Ecus (50–60 million Euros).

1.2 Technological Aspects

Endogenous α- and β-amylases are necessary to convert starch into maltose and glucose that can be fermented by the yeast to ensure proper CO_2 production. Amylase activity is required for CO_2 production, for bread volume and for the color of the crust (Feillet 2000). An excess of amylase activity leads to excessive hydrolysis of the starch, resulting in a significant release of maltose and glucose. The fermentation step is excessively rapid, leading to an irregular crumb structure with holes, to sticky bread crumb, and to excessive crust color due to Maillard reactions (Chamberlain et al 1981, Meredith and Pomeranz 1985, Viot 1992).

2. METHODS AVAILABLE FOR THE DETERMINATION OF AMYLASE ACTIVITIES

Different methods are available to assess the amylase activities of wheat and flours. These methods are based on the measurement of viscosity, on colorimetric reactions, or on specific antigen-antibody reactions.

2.1 Viscosimetric Methods

2.1.1 Hagberg Falling Number

The FN method was developed in the early 1960s for the determination of amylase activities of wheat and flours (Hagberg 1960, 1961). This method is widely used in cereal analysis and is published as ICC and ISO standard methods (ICC 1995, ISO 2009). The FN is an indirect measurement of α-amylase activities (AAA) (Feillet 2000), in which a low FN value theoretically reflects high enzymatic activity linked to sprout-damaged kernels. However, in working with waxy wheats (which have very low amylase contents), researchers from the University of Nebraska (Graybosch et al 2000) noticed that the relationship between FN and α-amylase activity was not accurate. There is evidence that, beside the enzymatic activity, the FN also reflects the structure of the starch and the starch damage (Ringlund 1983, Feillet 2000). In 1983, Ringlund noticed that samples with similar amylase activities showed different Hagberg levels. In 2001, Sinnaeve also demonstrated that the relationship between FN and amylase activities shows some discrepancies (Fig. 6.1).

2.1.2 Brabender Amylograph

Using the standard procedure ISO 7973 (ISO 1992), the Brabender Amylograph determines the amylase activity of flours in order

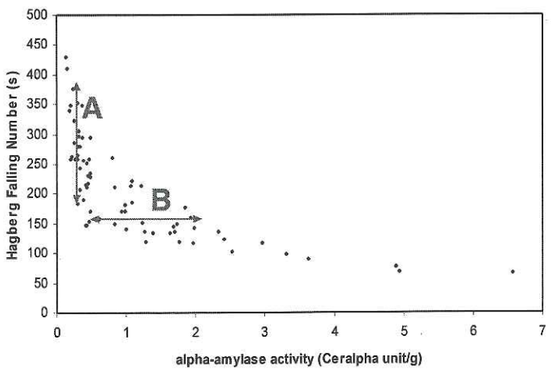

Fig. 6.1. Relationship between Hagberg falling number (FN) and true amylase activity as obtained by the Ceralpha method. A constant α-amylase activity can correspond broadly to the FN measurement (A), or a constant FN value can correspond to a variation in amylase activity (B). (Reprinted from Sinnaeve 2001)

to detect sprout-damaged wheat. It can also be used to produce enzymatically balanced flour mixes (Mercier and Tollier 1984). More recently, Brabender developed the Micro-Visco-Amylograph to study starch gelatinization and retrogradation as well as amylase activity.

2.1.3 Newport-Perten Rapid Visco Analyser (RVA)

The RVA monitors the viscosity while a flour or a starch slurry is heated and cooled (Feillet 2000). The amylase activity is assessed by the loss of viscosity of the slurry. A specific method was developed and standardized (No. 161, ICC 1996) to give the stirring number (SN) (Ross et al 1987). The same device can be used for a longer protocol (13 min) for a better understanding of the rheological properties of flours and starches (Batey 2007). To be able to dissociate the enzymatic activities and the intrinsic properties of the flour, a double measurement is proposed, one using the standard protocol in water and one replacing the water with a silver nitrate 2 m*M* solution to block enzymatic activities (Crosbie et al 1999, Bason and Blackeney 2007).

2.1.4 Chopin Mixolab

The Mixolab unit, graphs, and results are extensively presented in Chapter 1. It measures the consistency of dough during mixing and the heating and cooling of the mixer bowl. Research has compared the Mixolab to existing pieces of equipment such as the Farinograph (Lebrun et al 2007) and the RVA (Collar et al 2007). Previous studies (A. Dubat, *unpublished*) have shown that the second part of the curve may be a good indicator of wheat or flour amylase activity. Other than in the Mixolab method, all measurements concerning starch hydrolysis by amylases in cereal are made on batters or liquid suspensions. However, the real conditions in industry are different, with a limited amount of free water affecting both the starch gelatinization and the enzyme mobility. Assessing amylase activity of dough with the Mixolab is therefore an interesting possibility.

TABLE 6.1
Details of the Parameters Measured with the Mixolab on White-Flour and Ground-Wheat Samples

Consistency	Units	Mixolab
C1	Nm	Torque C1
C2	Nm	Torque C2
C3	Nm	Torque C3
C4	Nm	Torque C4
C5	Nm	Torque C5
C3 – C4	Nm	Difference C3 – C4
WA	%	Water absorption

TABLE 6.2
Assessments of α-Amylase Activities on White-Flour and Ground-Wheat Samples

Measurements Compared	Units
Hagberg falling number	s
Hagberg liquefaction number	...
RVA[a] maximum viscosity in silver nitrate	cP
RVA maximum viscosity in water	cP
Megazyme Ceralpha α-amylase activities	Ceralpha µm/g

[a] Rapid Visco Analyser.

2.2 Colorimetric Reactions

Most of the methods for the determination of α-amylase activities are based on colorimetric reactions measuring the time required by the amylases in the sample to bring a "β-limit dextrin" substrate (one that cannot be degraded by β-amylase) to a degree of hydrolysis leading to a fixed coloration with iodine (No. 108, ICC 1968). In the Ceralpha assay, a wheat flour extract is incubated with an artificial substrate (*p*-nitrophenyl maltoheptaoside) under defined conditions. The reaction is stopped by a reagent, and the absorbance measured at 410 nm is directly related to the α-amylase activity of the sample (No. 303, ICC 1998; Megazyme 2001a). One Ceralpha unit is defined as the quantity of enzyme that, in the presence of an excess of thermostable α-glucosidase, is required to hydrolyze 1 µmol of *p*-nitrophenyl maltoheptaoside in 1 min (under the conditions of the assay). Megazyme has also developed a kit to assess amylase activity using an azurine–cross-linked amylase as substrate (Amylazyme assay, Megazyme 2001b).

2.3 Kit Based on Immunochromatography

The WheatRite kit (Bayer Crop Science AG, Germany) uses an immunochromatography method that detects amylase activity and gives results in traditional FN values. It is suited for screening on-farm or at the elevator or as a fast laboratory test. Results can be evaluated visually by comparison with a color chart to estimate the FN equivalent. The test allows a four-group classification of the samples according to the amylase activity (Skerritt et al 1999, Skerritt and Heywood 2000).

3. EXPERIMENTAL DETAILS

Samples from the 2007 harvest were collected in northern France to get a broad variation in α-amylase activity. In 2007, the harvest conditions were rather wet, leading to sprout-damaged wheat lots.

Mixolab data on α-amylase activity were compared with data from other existing tests performed on white flour (WF) obtained with a Brabender Quadrumat Senior mill. A similar approach was conducted on ground wheat (GW), i.e., whole meal.

For both samples, the Mixolab data (Table 6.1) were compared with some other tests devoted to α-amylase analysis (Table 6.2). Mixolab parameters were obtained following the Mixolab Standard protocol or Chopin+ procedure. Finally the relationship between the Mixolab measurements performed on GW and those performed on WF were studied.

The Mixolab data (C2, C3, C4, and C5) were related to the direct measurement of AAA (Ceralpha), to the classical Hagberg FN (s), and to the liquefaction number (LN; LN = 6,000/[FN – 50]). The latter criterion shows a more linear fit with the Ceralpha AAA. The Mixolab data were also compared with the RVA data using the water and silver nitrate protocol. The comparison is based on the ratio determined as

$$(PVs - PVw)/PVw$$

where PVs is the peak viscosity measured in silver nitrate and PVw the peak viscosity in water. The loss of viscosity is associated with AAA.

TABLE 6.3
White Flour: Determination Coefficients (r^2) Observed Between Consistency Measured with the Mixolab and Data from Other Methods for Assessing Amylase Activity

	Mixolab Consistency				
Measurements Compared	C2	C3	C4	C5	C3 – C4
Hagberg falling number	0.63	0.67	0.59	0.77	0.38
Hagberg liquefaction number	0.57	0.61	0.63	0.69	0.49
RVA,[a] (PVs – PVw)/PVw	0.51	0.60	0.62	0.68	0.50
Megazyme Ceralpha α-amylase activity	0.45	0.54	0.60	0.60	0.52

[a] Rapid Visco Analyser. PVs = peak viscosity measured in silver nitrate, PVw = peak viscosity measured in water.

4. ALPHA-AMYLASE ACTIVITY FROM MIXOLAB DATA ON WHITE FLOUR

In this part of the study, the comparison was performed on the WF samples, and the aim was to relate the Mixolab parameters (C2, C3, C4, and C5) to the results obtained with other techniques. The determination coefficients (r^2) are given in Table 6.3. The best r^2 was found with the classical Hagberg FN measurements. Linearization of the Hagberg measurements through the calculation of the LN or the use of RVA data or even the true AAA measurements did not improved the r^2. Subtracting C4 from C3 did not improve the r^2 values. It is thus possible to have an estimate of the FN on the basis of the Mixolab curve. Using the C2 was too delicate, as a large variation in the Hagberg FN corresponds to a small variation in the C2 values. The most significant r^2 values are logically observed with the final section of the curve (C4 and C5), which is affected by amylase activities (Fig. 6.2). Although the data set was reduced, multivariate estimates of the FN were developed on the basis of the Mixolab data. A first regression based on the consistency measured at C2, C4, and C5 (Est FN1 = 336.8 × [C2 – 129.84] × [C4 + 133.58] × [C5 – 6.32]) shows an r^2 of 0.85 compared to the true Hagberg FN value. Another estimate of the FN based on the water absorption (WA) and the consistency measured at C4 and C5 (Est FN2 = 12.389 × [WA – 108.68] × [C4 + 161.21] × [C5 – 611.9]) shows an r^2 of 0.90 with the true Hagberg FN value (Fig. 6.3).

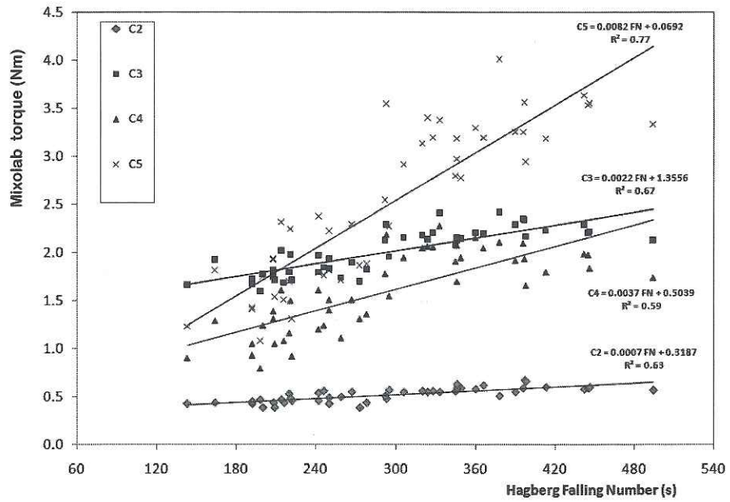

Fig. 6.2. White flour: relationship between Hagberg falling number (FN) and Mixolab consistency measurements (C2, C3, C4, and C5).

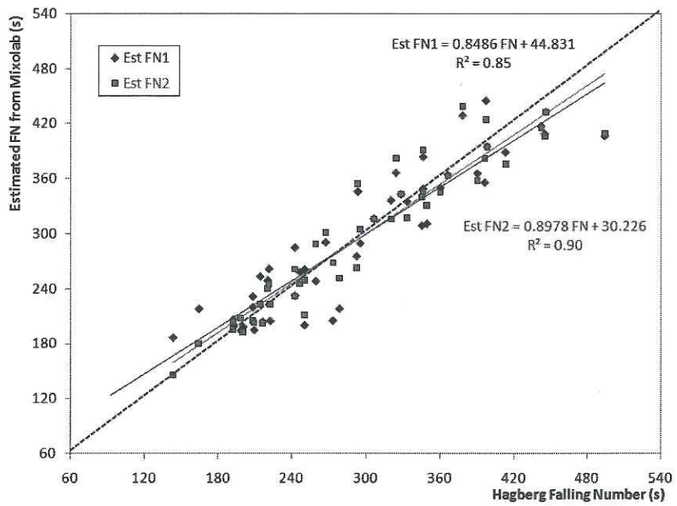

Fig. 6.3. Relationship between Hagberg falling number (FN) and estimated FN values based on Mixolab data. Est FN1 = 336.8 × (C2 – 129.84) × (C4 + 133.58) × (C5 – 6.32), where C2, C4, and C5 are consistencies measured with the Mixolab. Est FN2 = 12.389 × (WA – 108.68) × (C4 + 161.21) × (C5 – 611.9), where WA is the water absorption and C4 and C5 are consistencies measured with the Mixolab.

TABLE 6.4
Ground Wheat: Determination Coefficients (r^2) Observed Between Consistency Measured with the Mixolab and Data from Other Methods for Assessing Amylase Activity

Measurements Compared	Mixolab Consistency				
	C2	C3	C4	C5	C3 – C4
Hagberg falling number	0.41	0.73	0.81	0.81	0.79
Hagberg liquefaction number	0.33	0.64	0.66	0.64	0.59
RVA,[a] (PVs – PVw)/PVw	0.35	0.67	0.67	0.65	0.58
Megazyme Ceralpha α-amylase activity	0.41	0.73	0.71	0.72	0.88

[a] Rapid Visco Analyser. PVs = peak viscosity measured in silver nitrate, PVw = peak viscosity measured in water.

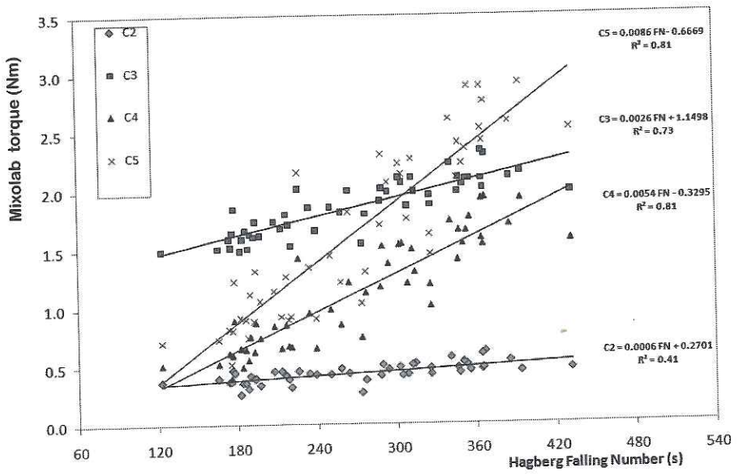

Fig. 6.4. Ground wheat: relationship between Hagberg falling number (FN) and Mixolab consistency measurements (C2, C3, C4, and C5).

5. ALPHA-AMYLASE ACTIVITY FROM MIXOLAB DATA ON GROUND WHEAT

For this section of the study, a comparison was performed on the corresponding ground wheat samples with the aim of relating the consistencies measured with the Mixolab (C2, C3, C4, and C5) to the results obtained with other techniques. The determination coefficients (r^2) are given in Table 6.4. The most significant r^2 values were noticed between the classical Hagberg FN measurements and Mixolab consistency values C4, C5, and the difference C3 – C4. The linearization of the Hagberg measurements through the calculation of the LN or the use of RVA data or even the true AAA measurements did not particularly improve the r^2. The most significant r^2 values are logically observed with consistency measured in the final part of the curve (C4 and C5), which is affected by amylase activities (Fig. 6.4). A good correlation was noticed between the AAA and the difference between C3 and C4 ($r^2 = 0.88$). The r^2 between the FN and the consistency measured at C2 is too small to allow a correct estimate of the Hagberg FN. A small variation in the C2 values corresponds to a large variation in the FN values.

6. RELATIONSHIP BETWEEN FLOUR AND GROUND WHEAT DATA

When dealing with wheat, the Chopin Mixolab is the only rheological instrument that can measure either the GW or the corresponding WF. From the set of data available, it is worth measuring the characteristics of the GW using the Mixolab and then applying the resulting curve to estimate the characteristics of the final WF. This could be of major interest to the top of the chain (seed selection, producers, grain traders) to fulfill the expectations and requirements of the bottom of the chain (millers, bakers etc.). The Hagberg FN values measured on the WF samples were highly correlated with the measurements on the corresponding GW samples ($r^2 = 0.93$). Therefore, from the Mixolab measurements performed on GW samples, it is possible to derive the amylase activities of the corresponding WF samples. The best correlations were observed between the C3, C4, and C5 consistencies and the Hagberg FN ($r^2 = 0.73$, 0.79, and 0.79, respectively) (Fig. 6.5).

7. CONCLUSIONS

In the cereal chain, dealing with endogenous amylase activity can be a major concern, depending on the harvest conditions. As the Chopin Mixolab was designed to measure either ground wheat samples or white flours, it can be used along the whole chain from the seed breeder to the end-user. A trial conducted on white flour samples from the 2007 harvest produced the following conclusions:

- The Mixolab curves obtained on white flours and especially the end of the curves (C4 and C5) are related to endogenous α-amylase activities.
- The best correlations are obtained with the Hagberg falling number. Calculation of the liquefaction number did not improve the correlation.
- Good correlations were also observed with other techniques such as analysis by the Newport Rapid Visco Analyser (in water and in silver nitrate) or measurement of true α-amylase activity by the Megazyme Ceralpha method.
- A first attempt to use multiple linear regressions of the Mixolab data led to a good estimation of the Hagberg falling number. More samples are required to validate this approach.

Similar observations were made using the corresponding ground wheat. The best correlations were observed between the Hagberg falling number and the C4 or the C5 of the Mixolab and between the

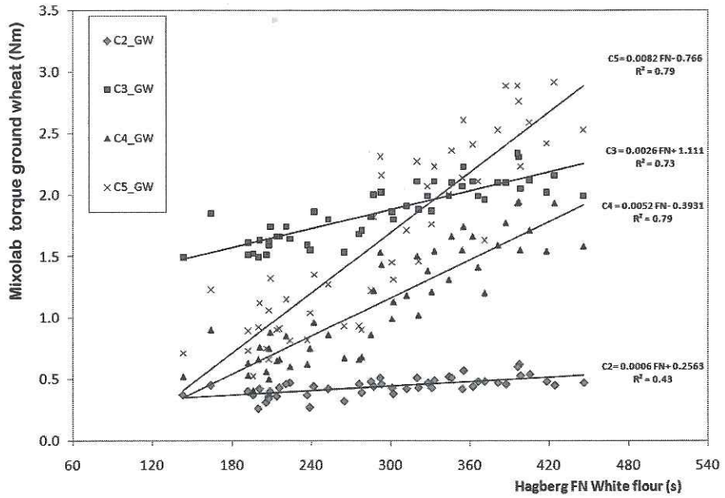

Fig. 6.5. Relationship between the Hagberg falling number (FN) measured on white flours and Mixolab consistency measurements (C2, C3, C4, and C5) obtained on the corresponding ground wheat samples.

Ceralpha α-amylase activities and the difference C3 – C4 measured with the Mixolab.

Using the Mixolab on ground wheat samples, it was possible to estimate the Hagberg falling number of the corresponding white flours. This is very important for seed breeders, as the Mixolab can provide information about the protein, starch, and enzymes on a small quantity of grain. Using the same procedure, grain traders have access to a tool that can help them to decide the lots for the final users. They may also use the Mixolab either on the ground wheat to select their lots or on white flours as a quality control tool.

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CHAPTER 7

Mixolab Analysis of Vital Wheat Gluten

Scott R. Frazer

Research & Development Manager, Cargill, Inc., Wayzata, Minnesota, U.S.A.

Vital wheat gluten (VWG) is produced when wheat flour is extracted with water and the soluble starch components are removed. The insoluble gluten product is then dried and ground into a fine powder. The quality and functionality of gluten are determined by both the conditions of the extraction process and the quality of the source wheat used in that process. Several good publications have been written on the subject of gluten analysis (Shewry and Lookhart 2003), and some work has been done on its rheological analysis (Tronsmo et al 2003). However, there is still a real need for improved analysis of gluten functionality. The amount of protein in most gluten products runs around 80%, but protein content alone is not a good measure of gluten quality (Popper et al 2006). The gluten index essentially measures the ability of gluten to agglomerate, but this test does not always correlate well with baking performance.

1. ANALYZING GLUTEN USING THE MIXOLAB

The Chopin Mixolab is designed for flour analysis, but other applications of the instrument have also been developed. Analysis of VWG is a natural application for the Mixolab, as the strength and quality of gluten are often judged by its ability to build and maintain viscosity in dough during mixing. This chapter examines some of the progress that has been made in the application of the Mixolab instrument to assessing the quality of VWG by determining its functionality. It does not review the basic operation of the Mixolab, as that was covered in previous chapters.

2. ANALYSIS OF NATIVE VITAL WHEAT GLUTEN

The simplest way to analyze VWG is to weigh out the required weight of gluten, place it directly into the mixing bowl, and take a viscosity profile. The Chopin applications manual (Chopin 2006) recommends that the sample be run using the standard Chopin+ protocol (Table 7.1), at a water absorption value of 120% and without moisture correction ("as is"). An example of such an analysis on two gluten samples is given in Figure 7.1. By using such an analysis, one can compare the water-absorption capacities of the gluten samples. As all samples are run with the same water addition, the sample that has the highest viscosity (usually occurring between 2 and 8 min) has the highest water absorption.

An approximation of the quantity of residual starch in the sample may be made by assessing the size of the slight peak at ~22 min, which results from the gelatinization of that starch. However, the height or area of such a small peak is difficult to measure, and actually quantifying the amount of starch present by this method is rather difficult.

Running straight gluten samples, although a simple process, can present several problems. First, it is often difficult to get powdered gluten to hydrate when water is applied to it. When a gluten sample is hydrophobic, a gluten film can form around a central core of dry gluten, and a continuous "gluten dough" never forms. In such cases, the data are completely worthless. Second, even when this difficulty occurs only in the in the early stages of the analysis, profiles can be very inconsistent. As seen in Figure 7.1, even when wetting of the gluten sample does occur, the data are often very noisy and difficult to analyze. Finally, even comparing water-absorption capacities for different samples can be difficult. When the water-absorption kinetics for two samples differ, the shapes of the viscosity peaks in the profiles for those samples are also different. This makes comparison of

TABLE 7.1
Instrumental Settings Defined in the Mixolab Software

Mixolab Standard	Settings
Mixing speed	80 rpm
Tank temperature	30°C
Temperature, first plateau	30°C
Duration, first plateau	8 min
Heating rate	4°C/min
Temperature, second plateau	90°C
Duration, second plateau	7 min
Cooling rate	4°C/min
Temperature, third plateau	50°C
Duration, third plateau	5 min
Total analysis time	45 min

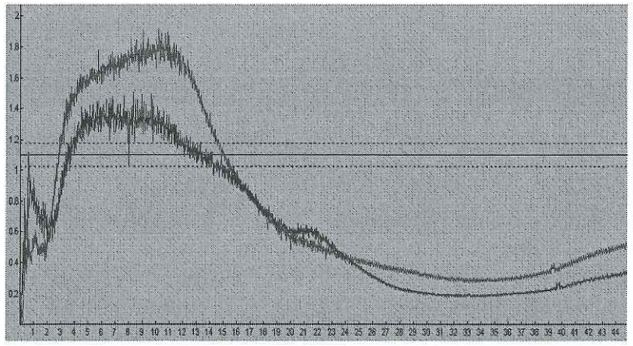

Fig. 7.1. Native vital wheat gluten (VWG) analysis. Profiles of two typical native VWG samples by using the Chopin+ protocol. *Y*-axis = torque (Nm); *x*-axis = time (min).