Hydrolysis of wheat starch and its effect on the Falling Number procedure: experimental observations†

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Abstract: The underlying mechanisms of starch hydrolysis in cereal grains, as measured by the Falling Number procedure, were studied. Wheat starch, spiked with barley malt α-amylase, was used as a model experimental system. A non-invasive and real-time monitoring system was developed to simultaneously record the sample temperature and stirrer velocity profiles, which are critical parameters that affect Falling Number readings. Experimental results show that the repeatability of the Falling Number procedure can be improved by slowing the heating rate, which is accomplished by increasing the quantity of sample components (ie starch and water) while maintaining the same proportion as currently specified in standard methodology. Among the tested range of heating rates, the best repeatability of Falling Number readings was obtained with 8.4 g starch and 30.0 g water. The experimental findings of this study also serve as basic information for the development of mathematical models on the hydrolysis of starch.

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INTRODUCTION

Sprout damage adversely affects wheat quality and hence trade value. Indicated by a high level of α-amylase, such damage changes the starch structure and therefore affects many processing properties of grain meals, such as dough handling and finished product texture.[1] Various methods have been developed to monitor the level of sprout damage in wheat, rye, and barley.[2] The Falling Number (FN) method has been approved by the American Association of Cereal Chemists[3] and is used widely in the regulation, trade, and processing of wheat and other grains. The principle of this method is that enzyme activity can be indirectly measured by the rheological properties of heated starch suspensions. The procedure involves the agitation by a stirring rod of a meal–water mixture within a precision test tube immersed in a boiling water bath. After 1 min, the stirring rod is released at the top of the tube and falls by its own weight through the viscous suspension. The time, in seconds, needed for the stirrer to travel through a fixed depth of suspension, plus 60 (from the agitation period) is the FN. Although limited by its single value output (ie rheological properties during starch gelatinisation are not measured), the advantages of the FN method include (1) simplicity – suitable for grain receiving-point applications; (2) reliability – well accepted by most wheat trading countries; and (3) practicability – heating, agitation, and gelatinisation during the FN procedure also occur in many food processing procedures.

Since first introduced by Hagberg[4] and commercialised by Perten[5] in the 1960s, the FN method has been investigated to improve its performance. Möttönen[6] studied the factors that affect the readings of FN, such as entrapped air bubbles and non-homogeneous heating. Brümmer[7] explored what effect changing the water bath temperature had on FN values. The AACC method was revised in 1982 to incorporate an altitude correction.[8] Recently, research has been directed toward the application of FN as a tool for food processing investigations. Holmes[9] used it to study the malting potential of barley and malt. Raschke *et al*[10] combined the outputs of the FN and Rapid Visco Analyser (RVA, Newport Scientific, Warriewood, NSW, Australia) to estimate the sorghum malt diastatic power.

Summarising these papers, most research has been

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based on empirical measurement. A comprehensive study and modeling of the fundamental mechanisms underlying the FN procedure are still missing. A model to describe the dynamic properties of starch under common food processing conditions is needed. Kokini *et al*[11] summarised the current knowledge of starch rheological properties and modelling studies. They concluded that there is a similar need from the industrial point of view. Because of the similarity of FN procedures to common food processing conditions, a model describing FN fundamentals is potentially applicable to general starch processing operations.

The broad objectives of the current study have been to examine the dynamics of starch hydrolysis and the changes in the rheological properties of starch samples during FN measurement, and to eventually represent these mechanisms by a comprehensive model framework. In this report, emphasis has been placed on experimental observations of the FN procedure as enhanced by temperature and velocity measurement instrumentation.

EXPERIMENTAL

The basic experimental design of this study was to control sample α-amylase content and monitor changes to the apparent variables associated with the samples under FN conditions. These variables included temperature, falling stirrer velocity and final paste viscosity. To obtain more reproducible data, wheat starch spiked with varying concentration of α-amylase was used instead of wheat flour. A non-invasive monitoring system was developed to follow the temperature and stirrer velocity profile during the FN procedure.

Wheat starch, flour and malt amylase

Wheat starch (no S5127, Sigma Chemical Co St Louis, MO, USA) was from one sample lot. Wheat flour was obtained from a local grocery store. Barley malt α-amylase was obtained from Sigma (EC 3.2.1.1, Type VIII-A) and stored in a freezer (~ −15°C). α-Amylase activities of the wheat starch and the malt α-amylase were assayed in triplicate by kit directions (CERALPHA method, Megazyme International Ltd, Wicklow, Republic of Ireland), and reported in international units per gram starch or (IU g^{-1}). Briefly, IU is a measurement scale for α-amylase activity defined by the Nelson–Somogyi reducing sugar procedure.[12] A multiplication factor of 3.0, as defined by the kit manufacturer, was used to convert from the kit's units of activity to IU. Stock solutions of malt α-amylase in deionised water were prepared at various concentrations. These solutions were used in place of pure water during the FN tests. Spiking of starch and water mixtures with the malt α-amylase was necessary to bring FN values within the typical range for wheat flour. Likewise, spiked flour and water mixtures were used to represent the conditions expected in commercial wheat flour monitoring.

FN instrument

The FN instrument used throughout the study was a Perten Instruments model FN 1600 (Perten Instruments, Huddinge, Sweden). The instrument was equipped with two slots for duplicate simultaneous measurements. Wheat starch (7.0 g) and 25 ml stock solution were loaded into each test tube. The tubes were vigorously shaken by hand until the sample and water were well mixed. A stirrer was then inserted in each tube, the tubes were placed in the boiling water bath, and the agitator was turned on. The instrument agitated the samples for 60 s with a rate of 1 stroke s^{-1} (down and up is one stroke). After this period the stirrers were released from the top of the tubes. When the stirrers traveled the fixed length of the tubes, the test was concluded and the total time needed, including the first 60 s of stirring, was reported as the FN, in units of seconds. Each reported FN value was an average of the two readings. Runs that had readings that disagreed by more than 10% of the average were discarded.

Temperature profile

A FN stirrer was modified by replacing the solid core shaft with a stainless steel hollow tube of the same OD, into which a copper–constantan (ANSI type T), 0.255 mm conductor diameter, insulated thermocouple (no 5TC-TT-T-30, Omega Engineering Inc, Stamford, CT, USA) was inserted (Fig 1). Leading away from the thermocouple junction located at the bottom of the tube, the wire leads passed through the tube's shaft, then through a hole drilled along the axis of the cylindrical magnetic weight located at the top of the tube. Welded steel was added to the weight to restore the mass of the assembly to original conditions. Downward movement of the modified stirrer, with thermocouple wire

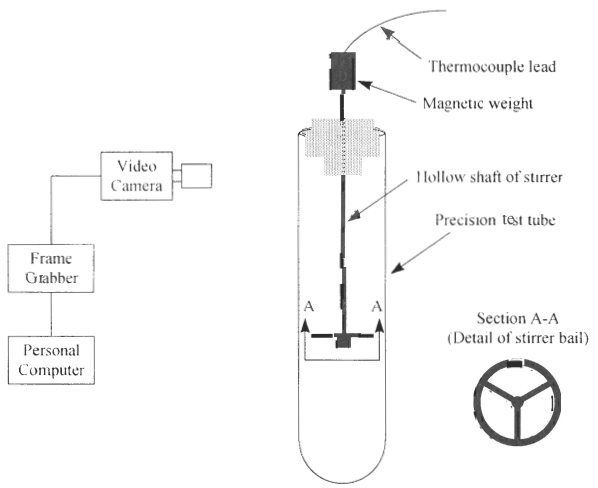

Figure 1. Details of instrumented Falling Number apparatus (not to scale).

in place, was checked using wheat starch suspensions. Movement was identical to that of an unmodified stirrer run simultaneously in the duplicate slot, which indicated that the modified assembly did not affect the FN procedure. During actual testing, the thermocouple was referenced to ice water, and emf values were read by a digital multimeter that was controlled by a personal computer. The temperature readings were recorded every second during the 1 min stirring phase and first 3 min of the release phase, beyond which, 5 s intervals were used.

Velocity profile

The position of the descending stirrer was recorded non-invasively at 1 s intervals by a 384 × 485 pixel colour video camera (Model JE-3012, Javelin Electronics Inc, Torrance, CA, USA) operated in black and white mode (Fig 1). Equipped with a 50 mm 1 : 1.2 lens, the camera was mounted on a tripod ~0.6 m from the stirrer. Video images were captured by a frame grabber (Model DT2851, Data Translation Inc, Marlborough, MA, USA) controlled by a 386-based 33 MHz personal computer. The position of the stirrer during freefall was determined in real time based upon a high contrast between the upper edge of the black magnetic weight and the whitened front panel of the FN instrument. Descent velocity of the stirrer was determined as distance traveled between successive readings divided by the reading time interval.

FN variability

To examine the variability of FN readings, a series of FN measurements with different amounts of starch and stock solution was conducted. The measurements were made at four different sizes, while maintaining the same starch to solution ratio: the standard 32 g (7 g starch and 25 ml solution), 38.4 g (8.4 g + 30 ml), 44.8 g (9.8 g + 35 ml), and 51.2 g (11.2 g + 40 ml). At each size, six solution concentrations were examined: 0.115, 0.345, 0.575, 1.15, 2.30, and 5.75 IU ml^{-1}. Each combination of size and concentration was represented by six FN readings. The average of the coefficients of variation (CV) at each size was then calculated to represent the overall variability of FN at that sample size.

Final viscosity

The final viscosity of each sample was measured by a Rheometrics RFS II rheometer (Rheometric Scientific Inc, Piscataway, NJ, USA) within 2 min of the conclusion of the FN procedure. The starch paste was removed from the FN test tube and placed in a sample pan with parallel plate geometry to measure the shear stress under a steadily increased shear rate. Because of the limited heating capacity of the instrument, the temperature was controlled to approximately 85°C while the apparent viscosity was measured and then corrected to 100°C by using the Arrhenius Law.

RESULTS AND DISCUSSION

The α-amylase levels of the native wheat starch and barley malt α-amylase, as measured by Megazyme kit, were 1.2 and 1150 IU g^{-1}, respectively. Figure 2 shows the FN readings obtained for the standard size of wheat starch in malt solution (7 g in 25 ml) at various concentrations of malt α-amylase. Also shown are the FN values from the locally purchased commercial wheat flour, to which α-amylase stock solutions were similarly used. Values for the x-axis represent the enzyme level of added α-amylase and do not include the naturally occurring α-amylase. FN values ranged from that which is considered high for commercial processing (>300 s) to ~150 s for wheat starch and <130 s for wheat flour, both of which would be indicative of severe sprout damage.

The upper graph of Fig 3 shows a temperature profile recorded during FN measurement for a typical sample of wheat starch (7 g) with added malt α-amylase solution (25 ml at 1.61 IU ml^{-1}). The dotted vertical line at 60 s marks the cut-off of mechanical agitation. Another dotted vertical line at 164 s corresponds to the FN of the sample. During the agitation period, a change in slope occurred at approximately 65°C. The slope change was attributed to the onset of gelatinisation, which is in general agreement with values reported from differential scanning calorimetry or from loss of birefringence measurement.[13] During the initial stage of gelatinisation, starch granules start to swell and the suspension becomes a dispersion of swollen granules and partially disintegrated granules. This endothermic gelatinisation process slows down the rate of temperature increase. Reasoning that the heat conduction coefficient for a starch gel is probably lower than that for a dispersion of swollen and partially

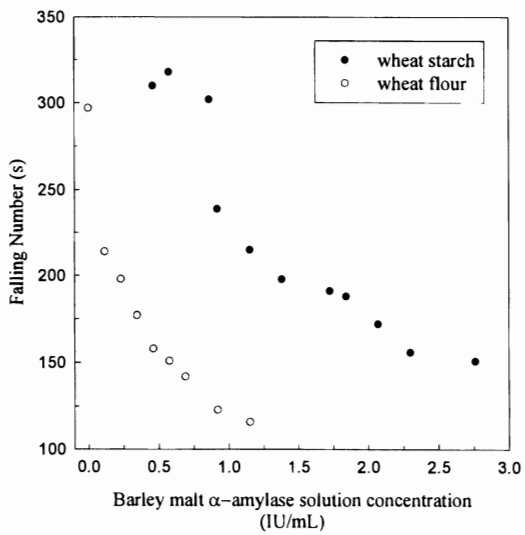

Figure 2. Falling Number values for a wheat starch sample (7.0 g) and a commercial wheat flour sample (7.0 g), at various barley malt α-amylase solution (25 ml) concentrations. (IU, international units; Nelson–Somogyi measurement scale; per Megazyme kit directions.)

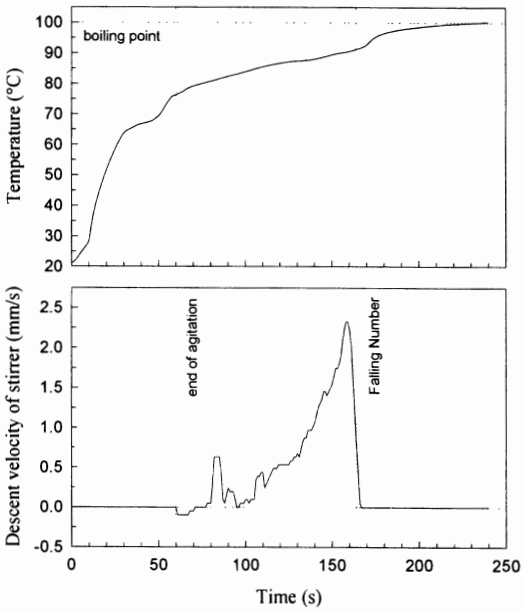

Figure 3. Upper graph: Temperature profile of wheat starch (7 g) in barley malt α-amylase solution (25 ml at 1.61 IU ml^{-1}) during a typical Falling Number run. Lower graph: Velocity profile of the stirrer during the same run. (IU defined in Fig 2).

disintegrated granules (as true for polymer melts vs non-melts, eg polystyrene[14]), the forming gel may also contribute to the decreased rate of temperature gain. At ~240 s, the suspension reached the boiling point (100°C) and temperature recording ceased.

The lower graph of Fig 3 contains the velocity profile of the stirrer, which was recorded simultaneously with the temperature profile. Changes in the starch suspension viscosity during stirrer freefall are apparent as changes in recorded velocity. In this graph, a delay period in downward movement is evident between the end of the agitation period (60 s) and 72 s. The delay was attributed to viscous forces in the starch suspension being sufficiently large to resist the force of gravity, thus keeping the stirrer stationary. Small upward movements of the stirrer (yielding negative velocities) were mainly attributed to the swelling of starch granules and the upward movement of air bubbles that were entrapped during agitation. Within 10–20 s, hydrolysis of the starch was sufficiently advanced to cause a drop in viscosity with concomitant initiation of the downward movement of the stirrer. By observation, this delay time changed with α-amylase concentration; a smaller delay occurred for the higher α-amylase concentrations.

Fluctuations in the velocity curve were attributed to the random occurrence of entrapped air bubbles near the stirrer that were observed during spot checks of runs (in which temperature and position were not recorded). Similar observations were reported by Möttönen.[6] The FN reading corresponds to the time, within a few seconds, past the point of peak velocity. We reason that while viscosity is still being reduced by hydrolysis and rise in suspension temperature, the velocity of the stirrer decreases as it approaches the bottom of the test tube, where it is affected by a change in flow pattern of fluid between the stirrer and tube wall.[15]

The effect of sample size on FN at the six malt α-amylase solution concentrations mentioned previously is shown in the upper graph of Fig 4. These concentrations corresponded to a range in the ratio of naturally occurring α-amylase (in wheat starch) to added α-amylase (in stock solution) of 0.333 to 17.1 IU IU^{-1}. Because water bath temperature is constant, the change in sample size results in a change in heating rate. This change in heating rate is expected to affect the kinetics of gelatinisation, enzymatic reactions, and enzyme deactivation. With the exception of the lowest concentration (0.115 IU ml^{-1}), an increase in sample size from standard (7 g + 25 ml) to largest (11.2 g + 40 ml) resulted in an increase in FN by no more than 83 s. The FN value of 392 s for the standard size at lowest concentration is very near the upper limit of the instrument's useful operating range, typically regarded as 400 s.[3] At standard size, the concentrations 0.345, 0.575, and 1.15 IU ml^{-1} had FN values (299, 242 and 187 s, respectively) that were typical for wheat meal. When the size was increased to the next larger (8.4 g + 30 ml), the corresponding increases in FN averaged 38, 42, and 33 s, respectively.

The lower graph of Fig 4 shows the corresponding coefficients of variation (CV). At 0.115 IU ml^{-1}, the 8.4 g + 30 ml size produced the smallest CV. At

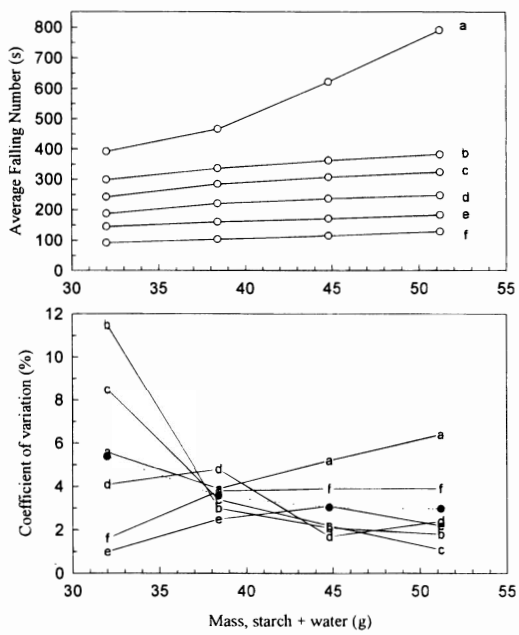

Figure 4. Upper graph: Averages of Falling Number as a function of sample size, maintaining a constant mass proportion of starch to solution. Lower graph: Corresponding coefficients of variation (CV). Solid circle represents the average at each size. Each average and CV is based on six replicate analyses at one level of α-amylase. Concentrations of α-amylase, in IU ml^{-1}, are as follows: a = 0.115, b = 0.345, c = 0.575, d = 1.15, e = 2.30, f = 5.75. (IU, as defined in Fig 2).

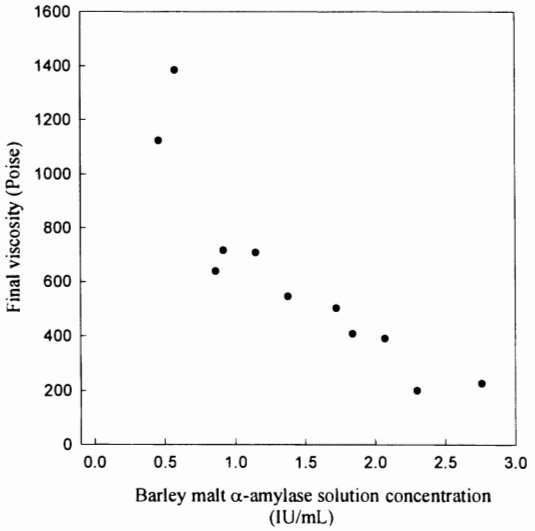

Figure 5. Viscosity upon conclusion of Falling Number runs of a wheat starch sample (7.0 g) and a commercial wheat flour sample (7.0 g), at various barley malt α-amylase solution (25 ml) concentrations.

intermediate concentrations (0.345 and 0.575 IU ml^{-1}), the CV decreased monotonically with increase in sample size. At 1.15 IU ml^{-1}, minimum variation occurred at the 9.8 g + 35 ml size, whilst at the two highest concentrations (2.30 and 5.75 IU ml^{-1}), the standard size produced the smallest CV. Thus, the degree of FN variability is dependent on the sample amount. On average, FN variability was smaller at the two largest sizes (CV = 3.03% and 2.97%, respectively) than at the standard (5.38%) or 8.4 g + 30 ml (3.57%) sizes. However, the most consistent variability occurred at 8.4 g + 30 ml size, which suggests that this amount might represent the optimal condition for instrument precision. We reason that due to a smaller swelling rate from lowered heating,[16] use of a 8.4 g + 30 ml sample would typically add less than 1 min to the FN procedure, yet would improve the ability to distinguish between minor differences in α-amylase activity.

The final paste viscosities of the wheat starch samples, which were originally described in Fig 2, are plotted in Fig 5. Viscosity was measured at a shear rate of 1 s^{-1} on the Rheometrics rheometer. As with FN, final viscosity is negatively correlated with α-amylase level. The fundamental principle underlying Figs 1 and 5 is that enzyme concentration and sample cooking history affect the rate of starch hydrolysis and therefore affect the molecular weight distribution and average molecular weight of the starch fragments.[17] From polymer rheology theory, the average molecular weight and molecular weight distribution of the dissolved polymer determines the solution properties, including viscosity.[14]

CONCLUSIONS

To explore the underlying mechanisms that determine the FN readings, we have investigated the FN measuring process by using a sample system of wheat starch spiked with malt α-amylase. A non-invasive, real-time, monitoring system was developed to record the starch suspension temperature and stirrer velocity profiles during the FN measurement.

The temperature profiles show that gelatinisation begins during the preliminary (1 min) agitation period. During the ensuing release period, the starch suspension typically reaches 90–100°C prior to the FN recording. The stirrer velocity profiles show that fluctuations in velocity occur during the initial stages of the release period. Such fluctuations can be attributed to starch granule swelling and randomly dispersed entrapped air bubbles in the test tube. Based on a comprehensive series of FN tests at varying heating rates, it is shown that FN reading consistency can be improved by slowing the heating rate. This can be accomplished by increasing the quantity of meal and water, while maintaining the same proportion as specified in standard methodology. The same data also indicate that the current FN procedure provides the highest consistency when the α-amylase level is high, which correspond to low FN readings (<180 s). For lower α-amylase levels, a larger sample quantity (38.4 g) gives FN readings with the lowest variation. The findings of this study are intended to aid in the development of current (Chang, unpublished) and future mathematical models on starch hydrolysis.

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