Low Falling Numbers in the Pacific Northwest Wheat Growing Region: Preharvest Sprouting, Late Maturity Amylase, Falling Number Instrument, or Low Protein?

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A kernel of wheat is an edible product as a result of years of selection pressure by humans, which has led to kernels that when milled produce flour with the properties required for proper mixing and baking. A kernel, left to its own devices, is predisposed only to produce another wheat plant. As such, kernels are storehouses of calories and nutrients that not only enable germination, but also have the secondary ability to be used to make tasty products people enjoy eating.

The energy and nutrients within a kernel are stored primarily as large polymers of glucose in the form of starch (endosperm), amino acids in the form of proteins (endosperm), and lipids in the form of triacylglycerols and other fats (scutellum and embryo, which collectively make up the germ). Minerals and other compounds necessary to propagate the plant also exist within the kernel. However, none of the nutrient and caloric components of the kernel can be accessed by a sprouting, growing plant without the presence of enzymes that are synthesized as part of the germination process.

As nutrient-containing compounds are converted from polymers to their monomeric constituents by enzymes, these polymers become less useful for baking and other food processing needs. Chief among the detrimental impacts of enzymatic activity, from a human point of view, is the hydrolysis of starch into glucose by α-amylase, an endo-acting enzyme that randomly hydrolyzes starch polymers and with amyloglucosidase produces glucose monomers used by the growing plant for energy.

From the perspective of cereal grain utilization as a food product, starch is necessary to allow for proper baking profiles, in terms of water and heat, to make a functional end-use product. Starch granules swell, imbibing water that is present in the baking system at specific temperatures and controlling the amount of water available for other reactions within the system. Removal of water during this gelatinization process initially creates viscosity and then, through the process of starch recrystallization or retrogradation, “sets” the baked product as the system cools postbaking.

Without large, intact starch polymers, neither gelatinization nor retrogradation can occur sufficiently well to produce an acceptable product—sticky doughs and collapsed and gooey end products are the result (8). The presence of α-amylase, an endo-acting enzyme that specifically acts on α1-4 glycosidic bonds, deleteriously affects starch gelatinization and retrogradation processes through starch polymer hydrolysis. The result is serious economic losses if the presence of amylase is not discovered prior to utilization of the grain.

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https://doi.org/10.1094/ CFW-63-1-0012

The need to characterize grain lots for the presence of amylase was recognized many years ago (9,14). The most direct test method was to measure the ability of flour, or ground grain, to form a gel sufficiently robust to produce acceptable products. If amylase had hydrolyzed starch beyond a certain arbitrary point, the grain was judged to be of inferior quality. Hagberg (9) and Perten (14) developed the falling number test to quantify the soundness of wheat grain lots relative to the presence of α-amylase (i.e., low enzyme activity) (AACCI Method 56-81.03 [1]). The falling number test involves suspending a measured amount of ground grain or flour in a long cylindrical tube with water and then mixing the suspension in a boiling water bath for 60 sec, past the point of gelatinization and denaturation of α-amylase. Intact starch polymers produce a more robust gel than granules affected by enzymic hydrolysis, and longer times are required for a standardized, weighted plunger positioned on top of the gelatinized starch to fall through the gel. The number of seconds it takes for the plunger to fall through the gel is recorded from the start of the test. Because starch that has been degraded by α-amylase produces a less firm gel, the plunger falls more quickly, resulting in a lower falling number.

The falling number test actually measures the impact of α-amylase on starch pasting properties, as well as other factors, and not the amount of enzyme itself. This falling number test has been adopted by the grain trade industry as a rapid and useful test to determine starch quality and, therefore, the suitability of a grain lot for baking. Milling and baking companies also utilize falling number in their specifications. Economic value is assigned to wheat as it is traded and utilized as a result of this test.

To properly place falling number results into a useful end-use context for estimation of performance and establishment of functional value placed on a grain lot, it is necessary to understand the falling number test. The instrument itself, how it operates, the cereal chemistry components and factors that confound results, and sources of amylase all need to be discussed to understand the falling number results that were obtained in the U.S. Pacific Northwest wheat growing region in 2016.

The Falling Number Test

The falling number test indirectly measures α-amylase by evaluating its impact on starch pasting properties. The test is a widely accepted standard for detection of degradation of starch due to amylolytic action. It is important to remember, however, that the falling number method does not test for amylase itself, but rather tests the results of enzymatic hydrolysis of starch and other starch properties linked to starch pasting.

The test itself involves placing a sample of ground grain in a long cylindrical tube with distilled water, suspending the sample, and then placing the tube and its contents in a boiling water bath where it is mixed for 60 sec. At the end of mixing, a standardized, weighted plunger is positioned on top of the gelatinized starch, and the number of seconds (in addition to the initial 60 sec) it takes for the plunger to fall through the gel is recorded. Therefore, 60 sec is the lowest falling number that can be recorded.

Different grading systems have different, somewhat arbitrary, cut-off points established for the characterization of grain as sound or as negatively affected by amylase. In the U.S. system, as established by the U.S. Department of Agriculture Grain Inspection, Packers and Stockyards Administration and Federal Grain Inspection Service (GIPSA/FGIS), samples with falling number values greater than 300 sec are considered sound (6,16). In France, the standard is 250 sec. The characterization of wheat as sound or sprouted would not be as problematic if economic value were not rigidly applied based on falling number test results. For example, discounts are applied in the U.S. Pacific Northwest at a rate of $0.01/sec between 300 and 275 sec, and greater discounts exist for wheat with falling numbers values below 275 sec. This discount schedule can result in enormous losses for growers who are already in financial peril due to preexisting low grain prices.

Broadly, as amylase concentrations increase in flour, issues associated with starch functionality increase dramatically. As starch from soft white wheat, the predominant class of wheat produced in the Pacific Northwest, is hydrolyzed into shorter chain lengths and eventually monosaccharides, water absorption increases, gelatinization temperature decreases, and retrogradation of starch after baking, frying, or boiling is hindered, resulting in collapsed, wet, structurally inadequate end products. In dry products such as crackers or cookies and noodles, postproduction structure is compromised due to starch chain lengths that are insufficient for adequate retrogradation: crackers and cookies tend to fall apart too easily due to the poor structure, and noodles snap apart during drying after production.

Further, a falling number lower than 250 sec for ground wheat creates difficulties, because as falling number decreases issues with product quality, especially in batter-based products, increase. Cakes, doughnut batters, and other similar products are likely to have deficient quality characteristics as a result of amylase activity. Arabic-style flat breads and some baguettes are the most resilient products when it comes to issues caused by amylase and can withstand lower falling number values than can other products.

Clearly, millers and bakers want to purchase only sound grain for their respective processes. Although enzymes are added during bread production to reduce falling number and enhance bread quality, bakers prefer to utilize enzymes in a controlled fashion, starting with a sound flour that is unaffected by amylase. Commercial purchasers want a falling number value that provides assurance that the grain is unaffected by amylase activity, including a "buffer" for within-test variation produced by the analysis itself. Therefore, purchase specifications often include falling number values greater than 300 sec and, in some cases, 350 sec.

Falling number for ground wheat does not always correlate well with actual amylase concentrations measured by AACCI Methods 22-02.01 and 22-05.01 (1) because falling number measures the impact of the enzyme on starch (Fig. 1). There is great variation in actual amylase in the 300–250 sec falling number range. Above 300 sec, and especially 350 sec, amylase and falling number value are in good agreement. Similarly, below 250 sec the two tests are in considerable agreement.

In addition, falling number does not always correlate well with product quality (Fig. 2). Even with falling number values of 200 sec, acceptable cake volume can be achieved, depending on the wheat cultivar. Falling number values above 300 sec do not guarantee good cake performance either. Clearly, other factors, in addition to the presence of amylase, are involved in grain quality. Although falling number provides information on the extent of amylase-induced degradation of starch, it does not provide as concrete an estimate of end-use functionality as is sometimes imagined.

End users, such as bread bakers, prefer falling number values to be 350 sec or higher to assure the grain is sound. Subsequently, bakers frequently treat wheat flour with malt (a source of amylase) to reduce the falling number value to around 250 sec to provide glucose residues for yeast consumption in bread baking. Additionally, malt (and preharvest sprouted wheat) contains an array of other enzymes that modify other grain storage polymers, such as proteins, lipids, and nonstarch carbohydrates (e.g., arabinoxylan). Enhanced control over the amount and performance of dough-modifying enzymes present in a formulation is the primary reason for the seemingly contradictory practice of adding malt or amylase back into sound flour. The assurance provided to mills and bakeries by high falling number results also takes into consideration any implicit variation within the test itself.

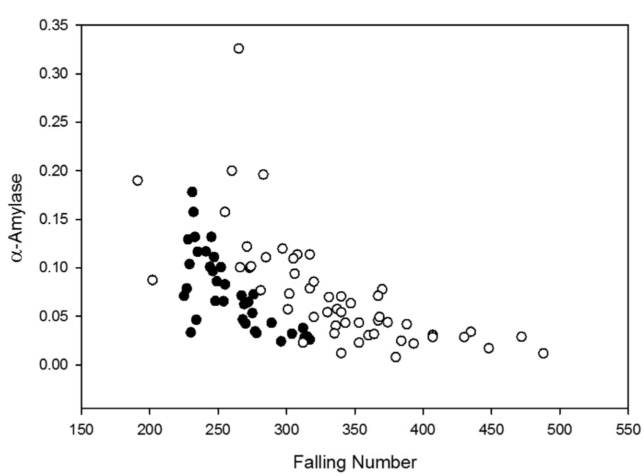

Fig. 1. Falling number (sec) versus α-amylase content (U/g). Black dots (●) and open dots (○) represent soft white wheat samples from two different sources—both from the 2016 Pacific Northwest harvest. (Graph courtesy of A. Kiszonas, USDA-ARS-WWQL)

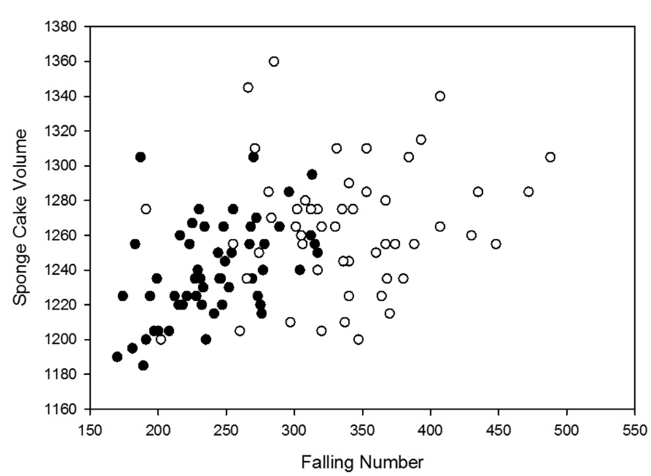

Fig. 2. Falling number (sec) versus Japanese sponge cake volume (cm^3). Black dots (●) and open dots (○) represent soft white wheat samples from two different sources—both from the 2016 Pacific Northwest harvest. (Graph courtesy of A. Kiszonas, USDA-ARS-WWQL)

Falling Number Test Variation

The falling number test itself can contribute to variation in the results that are used to place an economic value on a grain lot based on a fixed falling number value (300 sec). Any test will produce variation in measurements. An examination of variation in falling number results was performed by the AACCI Food Safety, Regulatory, and Quality Task Force Specifications Working Group (2). In the case of falling number, for soft white wheat (the predominant wheat class grown in the Pacific Northwest), variation was measured (with outliers removed) as average standard deviation. A critical value was calculated from the results of collaborative testing in the study (2). When results were pooled and outliers removed, the critical value at $P = 0.05$ was 51.1 sec for falling number. In other words, when comparing two falling number measures at that probability level, if two measures are less than 51.1 sec apart, they can be considered to be the same. Only when two values are greater than 51.1 sec apart can they be considered different at $P = 0.05$.

To base an economic value on a hard-and-fast falling number cutoff value of 300 sec when variation of this magnitude exists may lead to incorrect devaluation of a functional grain lot, resulting in loss of income to farmers. On the other hand, a falling number value of 300 to 350 sec provides insurance that a sample is in fact unaffected by enzyme activity. This provides confidence to the buyer and end user of the grain lot that the wheat will function properly. However, if one is a farmer, the economic impact of a sample that is graded as having a falling number of 275 sec is great, even though it is not significantly different than a sample graded as having a falling number of 325 sec.

Much of the variation in the falling number test can be attributed to a combination of factors, including operator error, poorly calibrated equipment, worn or dirty equipment, and other strictly mechanical factors. However, other factors also contribute to variation.

Table I. A partial list of partial waxy wheat varieties grown in the United States

Variety	Class[a]	Waxy State[b]
Snow Crest	HWS	2
WB7328	HWS	1
BR7030	HWS	1
Dayn	HWS	1
Klasic	HWS	1
Lolo	HWS	1
Macon	HWS	1
Otis	HWS	1
Star	HWS	1
Alturas	SWS	1
Whitebird	SWS	1
Treasure	SWS	1
Jubilee	SWS	1
Cataldo	SWS	1
Centennial	SWS	1
Penewawa	SWS	1
Edwall	SWS	1
Nick	SWS	1
Whit	SWS	1
WaxyPen	SWS	3
Parshal	HRS	1
Mattern	HRW	3
Mattern (sibling)	HWW	3
Ike	HRW	2

[a] HWS: hard white spring wheat; SWS: soft white spring wheat; HRS: hard red spring wheat; HRW: hard red winter wheat.

[b] Waxy state indicates the number of granule-bound starch synthase (GBSS) deletions—increasing waxy state implies greater amylopectin percentage in total starch.

Other Contributors to Falling Number Variation

Falling number, because it measures starch pasting properties in a heated, aqueous environment, is subject to physical and biochemical factors that influence the result and need to be considered. Starch pasting properties are modified, as discussed earlier, by α-amylase. The source of amylase can differ, however, and low falling number results can surprise farmers and grain elevator operators. Both preharvest sprouting (PHS) and late maturity amylase (LMA) play prominent roles in reduction of falling number results. These sources of amylase are discussed in more detail below.

The altitude at which the falling number test is performed also has an effect on results. In an unsealed container, such as is the case with falling number testing, water boils at a lower temperature as altitude increases (6). Within the falling number protocol is an equation used to remove the influence of altitude on falling number results. This equation is to be used only for testing at elevations above 2,000 ft (600 m). There exists, however, a significant difference in water boiling point between sea level and 2,000 ft (100°C versus 98°C) that can affect results through differences in rate of heating of starch and inactivation of amylolytic enzymes (6).

The ratio of the two polymeric components of starch, the amylose/amylopectin ratio, also plays a role in falling number results. Amylopectin, which typically makes up about 78% of starch composition, with the remaining 22% made up of amylose, swells to a greater extent than does amylose. The ratio of amylose to amylopectin is controlled by three granule-bound starch synthase (GBSS) enzymes from each of the three wheat genomes. With sequential deletion of each controlling *waxy* gene, less GBSS is synthesized, leading to a decrease in amylose content. One deletion leads to amylose content of about 20%, two deletions lead to an amylose content of 18%, and deletion of all three GBSS genes results in fully waxy wheat with almost 0% amylose content (18).

Because amylopectin swells to a greater extent than does amylose, a fixed weight of ground grain used in falling number testing results in a larger volume of gel when partial waxy wheat is used. The same weight of solids dispersed over a larger swollen pasted volume means the gel is less dense and, therefore, more easily penetrated by the falling number test probe, leading to a reduced falling number value. This is construed as indicating starch has been hydrolyzed by amylase when, in fact, no amylase is present, resulting in a lower falling number value and discounted prices offered to growers for otherwise sound grain.

The differential swelling phenomenon is the basis of AACCI Method 56-21.01 for measuring flour swelling volume (1). The test measures the relative degree of swelling and reflects the amylose/amylopectin ratio present in the grain lot. Although there are not many varieties commercially grown that exhibit these traits, they are grown in the United States (Table I) and may be inaccurately assessed as having issues with amylase when none exist. Most of these varieties are grown in the Pacific Northwest and, therefore, may be a contributor to the overall lower falling number values found in this region.

A more direct measurement of α-amylase through the use of specific substrates can be made using AACCI Methods 22-02.01 and 22-05.01 (1). The results of these methods provide a more robust analysis of the enzyme without other compounds, physical influences, or protocols introducing variation.

When grain is graded, falling number results for waxy wheat can lead to a reduction in wheat price if the phenomenon is not well-understood and documented. Because *waxy* is a genetic trait, identification of partial waxy cultivars (one or two *waxy* genes) should be possible if the cultivars are reported at the point of sale.

The rapid visco analyzer (RVA) provides a different, although equally indicative, assessment of PHS or LMA than does the falling number test. The RVA method is not influenced by altitude relative to temperature profile and provides a positive relationship between pasting viscosity and *waxy* gene state. The greater the amylopectin content, the greater the registered viscosity, in contrast to the decrease seen in falling number testing. If amylase is present in the sample, low viscosity is recorded, just as with falling number testing. The RVA, therefore, presents a potentially better assessment of PHS and LMA than does falling number and is widely used for this purpose in wheat producing areas such as Australia.

To further assist in determination of whether amylase is present using RVA testing, comparing pasting characteristics between samples run with water and samples run with 1 mM silver nitrate ($AgNO_3$), an amylase inhibitor, can help separate which grains are affected by amylase and which grains with low falling number values may be affected by other processes (5).

Additionally, it has been shown that soft white wheats with very low protein contents (≲7% protein) may also have low falling number values (16). This observation would seem to contradict prevailing theories on the impact of starch on falling number results, in that greater functional (not affected by amylase) starch content in grain should imply greater falling number values. In a study conducted by Ross et al. (16), grain with no amylase but a low falling number was associated with low grain protein content. No theory or explanation for this phenomenon is known. Low protein content occurs with some regularity in the Pacific Northwest, so further study of this phenomenon would be useful, since low-protein wheat can lead to a reduction in crop price due solely to low protein content without the presence of amylase.

In another study, high grain protein content in bread wheat was associated with low falling number results (4). In the case of high protein content, the reduction in starch content due to high protein content might reduce falling number values.

Sources of Amylase

Sources of variation in accuracy and precision of the falling number test notwithstanding, results do successfully reflect the presence of amylase in grain through the impact of the amylase on starch. The most common source of amylase in grain is PHS. PHS occurs when mature grain, postripening and postdormancy, is wetted. Under damp conditions, kernels begin to sprout.

PHS induces production of a large array of enzymes, not just amylase, during germination. Lipase, protease, xylanase, phytase, etc. are all produced in large quantities (10,13). PHS enzymes begin migrating from the germ end of the kernel and work their way up and around the kernel, through the aleurone layer toward the distal end of the kernel, diffusing into the endosperm from the outside to the inside. If grain dries out after being wetted, the chance of hydrolytic damage to the starch by amylase is somewhat deferred. Due to the need for moisture to allow amylase to migrate through the endosperm, amylase can be present but not have a deleterious effect on starch until the grain is dampened a second time, during tempering or dough or batter formation during processing, at which time the amylase will begin to hydrolyze starch immediately.

It has been noted that small amounts of PHS may increase flour functionality (15) in terms of product quality, especially loaf and cake volume, due to gluten structure modifications promoted by proteases and xylanases produced during sprouting that enable increased volume. It is a fine line, however—too much sprouting or amylase will result in to unacceptable end-product quality. Commercial bakers prefer to perform their own malting to having uncontrolled amylase and other enzymes present.

Once grain kernels have broken their dormancy and the presence of amylase may be suspected due to untimely rain at harvest, falling number can provide useful information about both the potential disposition of the grain and the economic value of the grain. As mentioned earlier, however, placing a hard-and-fast falling number value on sound versus sprouted wheat may do a disservice to wheat growers economically.

A more insidious form of amylase that occasionally presents itself is LMA. LMA production requires a genetic predisposition and a narrow set of environmental circumstances to be manifested. Recessive genes are purported to be present on chromosomes 3BL and 7BL (11), although loci on chromosomes 6A, 6B, and 6D, as well as others, are also implicated (17). Other genes and interactions may also be factors in genetic predisposition to LMA, including recombination with dwarfing genes that confer gibberellic acid insensitivity (*Rht1*, *Rht2*, and *Rht3*) (11). Some of the LMA genes appear to be derived from synthetic hexaploid germplasm used to introgress additional D-genome genes into normal hexaploid wheat. The LMA predisposition is on the B genome of the tetraploid contributor to the synthetic hexaploids that were generated for use in breeding (12). LMA genes may have been inadvertently incorporated in wheat germplasm generated through breeding approaches using this genetic source.

LMA is very difficult to screen for in breeding programs and poses a series of very difficult problems in commercial production. Wheat with a genetic predisposition to LMA only produces amylase under certain environmental conditions. Rapid temperature swings, either up or down, at 25–30 days postanthesis during grain development is needed to activate LMA in susceptible varieties (4,7). The environment that leads to production of LMA does not occur naturally very often. Therefore, LMA may go undetected in breeding programs, or even in grain production channels, for years, until the exact, required circumstances arise. LMA then manifests itself when these circumstances are present.

In addition, environmental conditions leading to production of LMA may be quite localized. For example, the temperatures at the top of a hill may be relatively stable, but at the bottom of the same hill, cold air may accumulate on a calm night, leading to LMA synthesis. Temperature swings of ±5 degrees Celsius are sufficient to initiate amylase production in susceptible varieties (4). The amount of LMA produced depends on how many recessive genes are inherited—one or two (12).

Unlike PHS, LMA produces only amylase, not the rest of the suite of enzymes produced during PHS. LMA also appears to be synthesized with a higher pI than that of PHS amylase. In all other respects, the grain appears to be normal. There is no physical impact on the kernel; test weight and visual grading characteristics all appear normal. If there is no rain or wetting of the grain postmaturity, there is no suspicion that the grain may have issues with amylase content. If the LMA-affected wheat is subjected to a falling number test during the grading process, however, a low falling number will result, providing a shock to the grower who would have no idea that LMA had occurred, and will greatly reduce the value of the wheat in the marketplace.

The effects of LMA are as detrimental to end-use quality as the effects of PHS when measured by falling number. However, although there is an impact on starch from LMA, there may be less impact on functional quality compared with PHS (3).

It is exactly this scenario that played out in the Pacific Northwest during the 2016 harvest. Rapid temperature fluctuations of up to 7 degrees Celsius occurred several times during the critical 25–30 day postanthesis window. Because the Pacific Northwest has such a diversity of environments and planting dates, LMA manifested only spottily throughout the region. However, millions of dollars were lost due to the LMA phenomenon that did appear in a limited number of cultivars. Growers were taken by surprise and unable to adjust their marketing and sales strategies to mitigate the fiscal impact, and bankruptcies occurred as a result. The susceptible cultivars that were identified are shown in Table II.

Conclusions

Falling number is a rapid test that measures the impact of amylase on starch. Falling number results are used to place an economic value on wheat in the U.S. marketplace, which uses a cutoff of 300 sec. There are multiple factors beyond amylase that influence falling number values that are not accounted for in this grading system, however, and the test itself has inherent variation. Other tests may be more precise and robust but are not widely used in grain grading or the marketplace. The falling number test serves both the buyer and end user well in providing assurance that a grain lot is truly sound if falling number results are above 300 sec, or even 350 sec.

When amylase results from the presence of LMA, the condition is concealed and goes unnoticed until the grain is harvested and graded. At that time, low falling number test results occur. Clearly, the low falling number results in 2016 in the U.S. Pacific Northwest were due to LMA—significant damage to the crop had occurred early in grain development and was not known until an amylase test was performed.

In other crop years, low falling number values for very low protein wheat or partial waxy wheat may lead to downgraded grain lots even though amylase is absent. The unanticipated loss of revenue for farmers is great and has catastrophic impacts on their business.

Reexamining grain grading standards relative to their use in establishing grain prices, with an emphasis on the degree of variation implicit in falling number testing, should be considered. Alternative tests may assist in better interpreting falling number results, including direct amylase testing or alternative starch pasting tests.

With regard to the presence of LMA as an unpleasant surprise at harvest due to sporadic required weather conditions at the critical moment of grain development, removal of LMA genes from breeding germplasm and released cultivars is the only sure way to rectify the situation.

Table 2. Wheat cultivars suspected of predisposition to late maturity amylase in the Pacific Northwest

Hard White Spring	Soft White Winter	Club
Blanca Grande	SY Ovation	Bruehl
Klasic	Mela CL+	
Express	Curiosity CL+	
	Xerpha	

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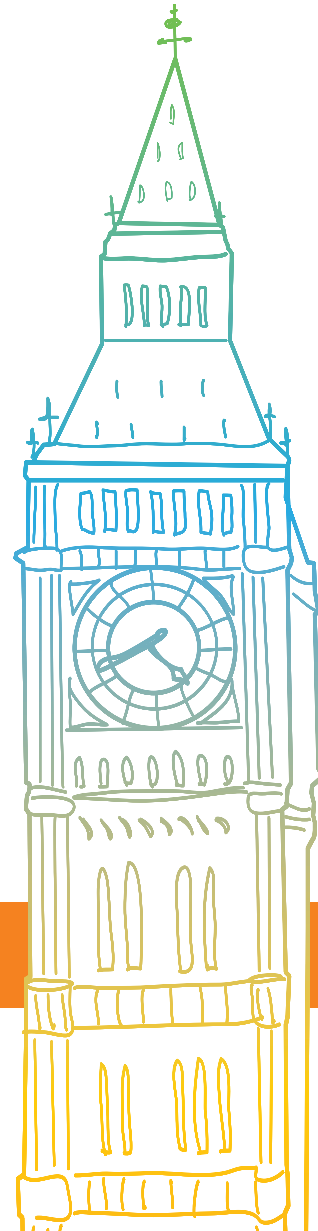

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