starch chains into shorter starch chains and eventually into sugars. If a batch of wheat produces too much alpha-amylase before being milled into flour, the enzyme in the dough degrades the starch and results in bread and cakes that collapse or noodles that are mushy.

The Hagberg-Perten Falling Number test is used to measure damage to the wheat kernel's starchy endosperm as a consequence of amylase enzyme action. The method is based on the fact that a slurry of wheat flour and water will ge-

latinize upon boiling—just like making gravy.

To perform the FN test, a mixture of flour and water is heated

and stirred for

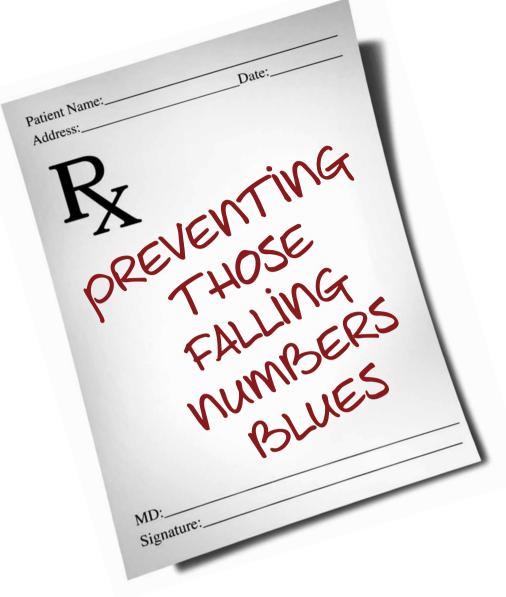


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60 seconds inside a test tube before measuring how long in seconds it takes for a plunger to fall to the bottom of the tube. Starch chains sliced by alpha-amylase activity fail to gelatinize well and are therefore thinner, meaning the more alphaamylase in a sample, the faster the plunger falls, the lower the falling number.

In terms of baking, alpha-amylase is a three bear's story. A falling number below 140 is much too low (too much alpha-amylase), while a falling number above 400 can be too high. A falling number of 250 to 300, meanwhile, is just about right, although the marketplace has determined 300 is the minimum acceptable value. Bakers can add alpha-amylase to their dough if the FN is high, but they cannot remove the alpha-amylase that's already there. Also, bakers like to have a consistent product. Thus, having a



## By Camille M. Steber, Arron H. Carter and Michael O. Pumphrey

Let's say you're a farmer who has planted a first-rate variety with an excellent disease resistance package, the correct nutrients have been applied, insects have been kept at bay, and Mother Nature has cooperated with fine weather.

At this point, only one last obstacle lies between the farmer and a healthy bottom line: the Falling Numbers (FN) test which measures whether incipient sprout has started to develop in an otherwise bountiful crop. In the market-place, a falling number below 300 can result in stiff discounts.

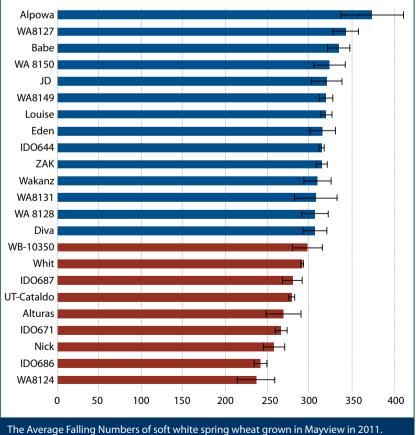
The Washington Grain Commission-funded project, "Developing Washington wheat with Higher Falling Numbers" is working to reduce the risk of low falling numbers by breeding for tolerance to environmental triggers. First, some background.

Every grain of wheat carries a tiny plant embryo seated atop a large starchy endosperm, which is 70 to 75 percent of the seed by mass. This endosperm is intended to feed the plant as it germinates. To turn this starch into usable food, the germinating embryo induces production of an enzyme called alpha-amylase.

Similar to an enzyme found in human spit, alpha-amylase cleaves long



If you want to study falling numbers, you've got to study sprout, and doing that requires an apparatus which initiates the process. The wheat head mister seen here is located in the wheat greenhouse on the Washington State University campus.



The Average Falling Numbers of soft white spring wheat grown in Mayview in 2011 Error bars indicate standard deviation.

minimum FN of 300 improves the marketability of Washington wheat.

Keep in mind that a small increase in alpha-amylase content has a big effect on FN and quality, so it is unwise to mix sprouted grain with sound grain because it will only compromise the quality of the sound grain. For example, if you mix equal amounts of grain with an FN of 100 and an FN of 400, you will get an FN lower than 250.

FN is a test farmers love to hate. The reason is inconsistency. Wheat that is below the 300 cutoff on the first try may be above 300 the second time.

Any test will have some sample-tosample variation. Physical factors during the test can influence the FN, such as temperature and altitude (FN is higher at higher elevations due to lower atmospheric pressure). The FN test has been an industry standard since 1968. Buyers like it because they know what the values mean. Alternative methods include the rapid visco analyzer (RVA) based on viscosity; color-based assays that measure alpha-amylase enzyme activity such as the Phadebas® Amylase Test; and ELISA assays that directly measure the presence of alpha-amylase protein rather than its effects. Unfortunately, the ELISA assay developed in Australia is not yet commercially available.

Steber, Pumphrey and Carter have launched a project to improve FN numbers by improving the genetic resistance of Washington wheat varieties. When the FN test was performed on wheat from multiple locations using the 2011 WSU Cereal Variety Trials, problems with low FN were location dependent. For instance, Mayview and Lind showed lower FN than Moses Lake or Walla Walla.

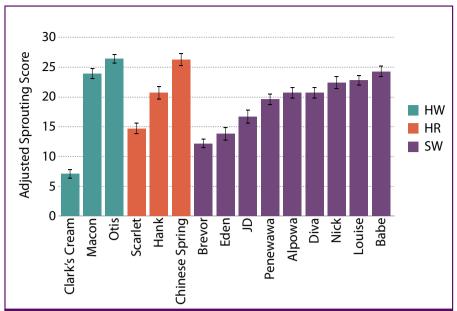
A tendency for an FN below 300 was observed in 2011 and 2012 in the soft white spring wheat varieties Nick, WA8124, UI-Cataldo and Alturas. Waxy wheat cultivars, like Alturas, generally tend to have a lower FN regardless of environmental conditions. Among soft white winter wheat cultivars, Bruehl and Xerpha both showed FN below 300 in 2011.

Several environmental conditions can cause low FN. The most obvious is preharvest sprouting that occurs when it rains before harvest. Cool and rainy conditions break seed dormancy, leading to germination and alpha-amylase production. Some suggest that even a heavy and persistent mist may be sufficient to trigger mild sprouting in susceptible cultivars.

Alpha-amylase production can occur even before there is visible evidence of germination, a symptom that grain inspectors grade for visually. The most dormant varieties stand up best to conditions that cause preharvest sprouting. White-hulled wheat tends to be less dormant than red, which tends to make it more susceptible to low FN. Too much seed dormancy, however, causes problems with poor seedling emergence—especially in fall-planted winter wheat.

Wheat breeders must strike a balance between sufficient dormancy at maturity to prevent sprouting against the ability of the seed to lose its dormancy quickly enough to obtain good emergence when planted. Farmers can help with this postharvest dormancy loss, called after-ripening, by storing dry grain under warm conditions (80 to 90° F).

Susceptibility to preharvest sprouting is characterized using the spike wetting test. Wheat spikes harvested from the field are placed under a misting system to create artificial rain, and differences are scored over five days. This test must be conducted over multiple years to confidently characterize sprouting susceptibility, because grain dormancy varies from year to year depending on environmental con-



Spike wetting tests on spring wheat show variation for preharvest sprouting resistance. The y-axis shows a standardized sprouting score based on the area under the curve over 5 days.

ditions during grain maturation. For example, Alpowa was more sprouting resistant in 2011 than in 2012.

Preharvest sprouting cannot explain every case of low FN—especially in years when there is no rain. Fertilizer application rates, fungicides and Fusarium infection can all influence FN. Cold temperature shock during late grain maturation (25 to 35 days after pollen shedding) can also induce low falling numbers in varieties susceptible to Late Maturity Alpha-Amlyase (LMA) induction. Whereas the alpha-amylase produced during preharvest sprouting is localized mainly to the embryo end of the seed because it is embryo induced, LMA results in spotty areas of alpha-amylase throughout the aleurone layer surrounding the endosperm. Greenhouse LMA assays to determine whether there is an LMA susceptibility in Washington wheat germplasm are currently underway.

To induce LMA in the greenhouse, plants are grown (80° F day/65° F night), and then tagged when pollen is shed. After 25 days, these plants are placed in a cold chamber with a 64° F day and 45° F night for 8 days to induce a cold shock. Once treated plants reach maturity, alpha-amylase levels are measured using the Phadebas<sup>®</sup> assay. The soft white spring line, WA8124 that showed low FN in 2011, also showed alpha-amylase induction in initial LMA greenhouse assays. This would indicate there is some LMA susceptibility in Washington germplasm.

Based on annual falling number tests, LMA and preharvest sprouting tests of the Cereal Variety Trials and breeding lines, the goal of the WGC-sponsored project is to remove susceptible germplasm from the winter and spring wheat breeding programs and provide information about released cultivars. By breeding wheat with a reduced risk of low FN without compromising emergence, we can avoid falling cakes, mushy noodles and farmers' falling number blues.