Multiple Criteria for a Sprouted Whole Grain

Oldways Whole Grains Council / Sprouted Grains Working Group
Phase I Summary Report • October 2017

Background: In mid-2015, the Oldways Whole Grains Council launched a project to explore standards and definitions for sprouted grains. We planned to reach out to companies already sprouting grains and gather information on all the different processes and standards already existing within these companies, to learn about common approaches that might evolve into standards.

We started this project for educational purposes. We wanted to provide solid information to three groups:

- **Millers and sprouters**, so they could be sure they were following conventional methods for safe sprouting.
- **Manufacturers**, to help them write clear specs for purchasing sprouted grains and better incorporate sprouted grains into their products.
- **Consumers**, so they could trust the meaning of "sprouted" on a package.

Over a period of two years, we worked with 47 individuals from 28 different companies, through a series of conference calls, emails and individual interviews. While we gathered a tremendous amount of useful information, the useful answers we found also led to additional questions, since the intentional sprouting of grains is a fairly new endeavor for nearly all Sprouted Grains Working Group (SGWG) participants.

The following pages sum up the information gathered to date. It must be stressed that defining standards for sprouted grains is still a work in progress. Although we're concluding Phase I of this project with the dissemination of this summary, the Oldways Whole Grains Council expects to continue informally gathering information on some of the additional questions we discovered; we hope to provide an updated status report in the future, to support the growing category of sprouted grains.

This summary report includes:

A. The foundation: First, establish and document viability of the grain
B. Multiple criteria for documenting that a grain has indeed sprouted
   1. Four principle methods for documenting the presence of a sprouted grain.
   2. Notes on each of the four methods.
C. Future possibilities and considerations for additional methods of documenting sprouted grains

First: Establish Viability, Then Use Intentional Processes

Grain kernels have, in the past, fallen into two broad categories: those destined for our food supply and those destined to be planted as seeds.

Viability – the ability of the grain to grow into a new plant – is of course essential for seeds. In our food supply, however, viability has generally been considered unimportant or even detrimental, and grains that will be ground into flour or eaten as grain side dishes are often subjected to heat treatment. While this treatment quickly brings the grains to a uniform moisture content – much to be desired for standard manufacturing and milling processes– it can also destroy the viability of the kernel. With the growing popularity of sprouted grains, a third category arises: grains destined for our food supply, but in sprouted form.

All documentation of sprouted grains should therefore begin with verification that the grain is sound and viable. One SGWG member estimates that only about 60% of grains on the market are capable of germination; many have been rendered un-viable by "aggressive" grain driers bringing down high moisture levels or by heat treatment to stabilize unsaturated lipids (common with oats). Although these grains are suitable for grinding into flour, or for other ingredient purposes, they can't be sprouted. Older seeds, or seeds that have already seen field sprouting also cannot be intentionally sprouted in a controlled environment.

Genetics may matter too. Many varieties of grain have been bred to *resist* sprouting in the field, and these varieties may not be as well suited to intentional sprouting procedures as grains that have retained their natural, optimal sprouting capacity.

Possible Parameters. Some SGWG members suggested possible parameters for defining viability. One said, "Sound seeds are to be defined as certified pathogen-free seeds possessing a minimum embryo viability of ninety-five percent (95%) based on standardized official methods and preferably by the Tetrazolium (TZ) Index or equivalent bio-chemical test." A second suggested that percents may need to vary for different grains: "A good germination rate for hard red spring wheat is 95% while good germination in buckwheat is 85%." A third suggested germination rates of 85-90%.

Testing for viability needn't be high-tech or expensive; the process can be as simple as putting the seeds between wet paper towels or blotting paper and soaking for a prescribed period to verify that all or virtually all of that batch is capable of sprouting.

Since buying "dead" (un-viable) seed is generally much cheaper, it's important to prove viability. Otherwise companies could compete unfairly by buying dead seed, soaking it in water, drying it out and calling it sprouted.

Another key factor for sprouted grains is that **they must have been sprouted intentionally, through a controlled process**; grains that have accidentally field-sprouted should not be part of the food supply, as their safety cannot be documented.

Multiple Criteria for a Sprouted Whole Grain

When grains begin to germinate, they undergo numerous biochemical reactions that cause changes (many beneficial) in the nutritional composition of the grain. When these changes have taken place – **using viable, intact grain as described above and an intentional process** – the grain can be called a "sprouted grain."

Sprouted grains are now growing in popularity for three main reasons:

a. They are thought to enhance the established health benefits of whole grains, with increased bioavailability of some nutrients and easier digestion for some people.
b. They offer additional flavor and texture options to manufacturers and consumers.
c. They can (especially for baking) offer manufacturing advantages over traditional whole grains, such as better dough volume, less need for added gluten, shorter proofing time, etc.

Ideally, the nutritional changes would be measured, to stop the sprouting process at the peak of this nutritional transformation and to document the value-added proposition of sprouted grains. These changes, however, are elusive and difficult to measure, and some changes peak earlier than others, making it impossible to pinpoint optimum overall nutrition.

So, for now, proxy measurements are generally used. While it may be difficult to measure most nutritional changes directly, these changes happen at the same time as other changes that may serve as markers/proxies for the desired nutritional changes. Below are some possible ways to document the presence of a sprouted grain.

Note: With all testing methods, it's important to do both *before* and *after* testing to document the changes that accompany sprouting. Such testing should be done on a dry-matter basis (or results extrapolated to control for any moisture changes). Ideally, tests can be identified that can be done inline during production, with high consistency and low costs.

Four Ways to Document a Sprouted Grain

A grain shall be considered sprouted if it meets one or more of these criteria:

1. **Visible Sprout**
   Sprouting activity is often demonstrated by the presence of a visible chit/rootlet or (usually later) a visible acrospire/sprout. If sprouting is visible, a grain shall be considered sprouted.

   However, it is common for grains to have undergone changes consistent with sprouting/germination without showing a visible sprout for reasons including (but not necessarily limited to):
   • Many beneficial changes take place *before* a visible sprout is evident.
   • Seeds may sprout at different rates in the same batch.
   • Seeing a sprout on smaller seeds like amaranth may be difficult.
   • The sprout may have grown but then fallen off during drying.

   Therefore, while a visible chit or acrospire definitely *confirms* sprouting, the lack of a visible chit or acrospire should not *disqualify* a grain as sprouted if sprouting can be documented in other ways.

2. **Alpha-Amylase Increase**
   One enzyme produced early in the germination process is alpha-amylase, which catalyzes the conversion of starch into sugars to nourish the growing plant embryo. Since the level of alpha-amylase in sound grain is very low compared to that in germinated grain, alpha-amylase levels in grain can be a ready marker of germination.

   Two methods are widely used to indirectly estimate the amount of alpha-amylase in grains, by measuring the viscosity of the dried and ground grain in water. For both these methods, it is *essential* to compare values for the original grain to values for the sprouted grain, to ensure that unintentional field sprouting has not taken place.

   a. **Falling Number (FN) Test… for gluten grains, especially wheat**
      The grain's Falling Number has decreased by a specified percentage when compared to the Falling Number of the grain before sprouting.

   b. **Rapid Visco Analyzer (RVA) Test… for all grains, including gluten-free**
      While FN machines are largely "hard-wired" for wheat (but may work for barley and rye also), RVA machines allow for different parameters to be set to assess the viscosity of different grains. As with FN testing, RVA testing will measure the change between unsprouted grain and the grain after sprouting, to document that specific parameters for that grain have been met.

3. **Amino Acid Increase (GABA in rice or other grains)**
   As grains germinate, protein changes take place as well as starch changes. When rice has sprouted, for instance, GABA (gamma amino butyric acid) levels will have increased by 300% or more. Other grains, including wheat, also see an increase in GABA with sprouting. It may also be possible to measure overall total free amino acids, which increase in many sprouted grains.

4. **Phytate Breakdown and/or Phytase Increase**
   The enzyme phytase catalyzes a breakdown and reduction of phytate in many grains as they undergo the germination process. A specified percent reduction of phytate can indicate a sprouted grain.

Notes on Multiple Criteria for Sprouted Grains

The information below documents the specific experiences of SGWG members and expands on additional issues related to each of the criteria described above.

Overall Issues

Which criterion makes the most sense may be heavily context-dependent according to:

- Which grain is being sprouted (including which cultivar of that grain, etc.)
- What the end use of the sprouted grain will be. Different testing and standards can be important for sprouted flour for baked goods, for instance: once a visible sprout appears, some sprouted grains may no longer work well for baking bread. Other criteria may be important for functionality in extruded products or for sprouted intact grains used as inclusions in products or eaten as side dishes.

Visible Sprout

a. **Defining visible.** "Visible" will need to be further defined. Visible to the naked eye? With magnification? One SGWG member says "Magnification depends on the seed. Only a few are so small that you need magnification. We use our iPhones with an add-on $20 40x magnifier."
b. **What percent must be visible?** What percent of a batch needs to show visible evidence of sprouting to consider the sprouting process complete? Will this vary by grain? One person suggests a visible sprout on >80% of kernels. Another cites 60% sprouting as typical of quinoa, for instance. Another suggests that sorting seeds by size before sprouting is important, as size can determine germination time and only similarly-sized seeds will sprout at the same time.
c. **Can sprout be longer than seed length?** AACCI in February 2008 stated that sprout growth of sprouted grains should not exceed kernel length. (Longer sprouts would result in classification as a plant/vegetable instead of a grain, in USDA's eyes.) SGWG members in general cautioned *against* a maximum length limit, as some seeds (quinoa, millet and amaranth were cited) quickly grow a very long sprout. At *some* point, however, a sprouted grain crosses the line from grain to new plant, and nutritional values would diminish – another topic for future documentation.

Alpha-Amylase: Falling Number (FN)

a. What is the desired decrease in falling number? Experience of SGWG member companies shows that for wheat the desired percentage change to document sprouting varies by type of wheat.

Company #1: from a low of 25% (for soft wheats with a low initial FN of 275) to a more typical 50-60% (for wheats with an initial FN of 350-500).
Company #2: 35-60% decrease depending on type of wheat.
Company #3: minimum of 50%.

b. **For which grains can Falling Number testing be used to document sprouting?** FN is most often used for wheat, as the [device] is basically calibrated / hard-wired for wheat. The manufacturer of a leading instrument used to measure FN (Perten), says that "anyone handling wheat, barley, rye or sorghum" will benefit from the FN system.

c. **How is Falling Number measured/tested?** FN instruments have long been used to measure unintentional sprout damage. The instruments are readily available, and tests can be done quickly and inexpensively in-house (as shown in this short YouTube or this longer USDA one with more explanation), so there's a high level of familiarity with and confidence in this test.

Alpha-Amylase: Rapid Viscose Analyzer (RVA)

a. **What is the desired change in RVA?** Only one SGWG member reports currently using RVA to document sprouting; the company has purchased an RVA machine and is currently exploring protocols and establishing ranges for different grains; they cannot yet suggest any standards or benchmarks. Others see RVA as valuable but too costly and with too many unknowns. However, because most companies trust the FN test and see RVA as the comparable approach for gluten-free grains, there is heavy interest in this option.

Amino Acid Increase / GABA

a. **Is GABA only found in rice?** Though GABA is most associated with rice ("GABA rice" is widely sold in Asia), this amino acid is also found in other grains. Various research studies document that GABA and/or free amino acids increase not only in rice but in other grains including sorghum.

b. **How much does GABA increase with sprouting?** One member reports a 300-400% increase in GABA as rice germinates; this increase takes place before a sprout is visible. She also reports a 500-600% increase in GABA in hard white wheat that has been sprouted. Another member reports GABA increases of up to 700-900% in wheat, though he cautions that GABA amounts – though larger than other free amino acids – are small to begin with, so large percentage increases may not indicate large absolute increases. Results even on the same batch are variable; altitude may also play a role.

c. **How is GABA increase measured/tested?** Liquid chromatography can be used, using the Medallion ion-exchange chromatography AOEC method, for about $260-$350 per test. In Japan, a machine called a GABAlyzer is available.

Phytate Breakdown and/or Phytase Increase

a. **How much does phytate decrease?** The amount of phytate (phytic acid) in different grains (and legumes) differs widely, and the decrease during sprouting also ranges widely according to the grain, the soaking medium, and time elapsed.

The one SGWG member currently using this approach reports a 49%-60% decrease in phytate for oats. Research shared by another member [Omary et al. (2012) Cer Chem, 89(1) 1-14] also suggests that phytate decrease in sprouted grains and varieties thereof may vary widely: Rice ~15%, Millet ~18%-80%, Sorghum ~35%-85%, Corn ~72%. Also see this research on rice, corn, millet, sorghum, wheat.

Another member who had tried this approach to document sprouting said that "phytic acid is inconsistent. Sometimes you see it, sometimes you don't. It's really frustrating."

b. What do tests cost? One third-party lab charges just under US$100, making the cost about $200, since "before" and "after" tests must be performed to gauge the change.

Future Possibilities

The methods outlined above are actual approaches being used by companies already sprouting grains. A few SGWG members also mentioned other approaches that might be feasible in the future:

Enzyme Testing for Alpha Amylase. Rather than use FN or RVA instruments to measure starch viscosity changes, one member looked into direct testing of alpha amylase. This company reported, "It's not feasible in production. You can do it in-house but it's really involved and takes too long to get results. It's a super, super sensitive test; you have to be unbelievably accurate or the results are really skewed."

Other Enzyme Tests. Another member suggested that testing for a variety of enzymes (such as lipases, lipoxygenases, arabinoxylinases, proteases) could be possible; this company mentioned Megazyme in Europe as a source for such testing. "Some of this is not easy chemistry; it's unlikely that suppliers would do the wet chemistry required to do these tests. It's not like sticking a dipstick in and then checking it; there's no rapid assay.")

Additional Specific Biomarkers. One member found that a particular antioxidant in oats "phenomenally increases" upon sprouting. There may be other components – most likely specific to each grain – that can be used as reliable measures of sprouting.

Protein Tests. One member reported that "protein solubility is a pretty easy and cheap test that can be done on the spot with a combustion analyzer, which a lot of companies already have." Apparently, this approach is often used in the brewing industry.

Another company suggested testing for Protein Digestibility Corrected Amino Acid Score (PDCAAS) since this would document changes in the human body's ability to digest the grain.

Multi-criteria "Index of Sprouting." Two SGWG member companies suggested using "a combination of methods that capture the starch-degrading enzymes and protein-lipid changing enzymes" with a specific suggestion for an overall Index of Sprouting that would include:

- Protein Index (PI) measuring protein hydrolyses
- Starch Index (SI) measuring starch hydrolyses
- Micronutrient Index (MNI) measuring vitamins, etc.

Under this proposed system, grains would be given a combined score of all three indices – PI x SI x MNI – and characterized at three levels, perhaps as "minimally sprouted," "intermediate sprouted," and "optimally sprouted." This approach needs to be fleshed out with more information about which tests would be used for each of the three components.

Neither company is actually using this approach at this time.

A Comment on Methods vs. Markers / Protocols vs. Policies

Time, temperature, and moisture level are the three factors that contribute to intentional sprouting of grains proven to be viable. While these factors alone cannot reliably document

that a sprouted grain has been produced, there was widespread agreement that sprouting cannot occur without these factors.

While almost all members of the SGWG cautioned against using moisture level alone as proof of sprouting, these three factors – time, temperature and moisture – can potentially be used to supplement testing methods such as those above. For example, if a company relies on RVA testing to document sprouting, it may find that a certain combination of time, temperature and moisture reliably lead to the same RVA results over and over. The company could then use these methods with confidence, doing only occasional RVA testing to confirm the ongoing validity of its approach.

The Oldways Whole Grains Council would like to thank all members of the Sprouted Grains Working Group for freely and generously contributing their companies' experience to this report. Their willingness to share information for the good of all – even though many of these companies compete with one another in the marketplace – is greatly appreciated.

If you work with a company sprouting grains or making sprouted grains products, and would like to participate in future work of the Sprouted Grains Working Group, please contact Kelly Toups, Director of Nutrition for Oldways and the Oldways Whole Grains Council, at 617-896-4884 or Kelly@oldwayspt.org.