



# Development of Certified Reference Materials for the visual inspection of unsound wheat kernels

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## Abstract

Unsound wheat kernel content is important in the wheat trade and is generally determined by sensory detection. To assist laboratory quality control, inspection personnel training and unsound wheat kernel inspection, visual Certified Reference Materials (CRMs) for unsound wheat kernels were developed. Unsound wheat kernels were collected from wheat fields or laboratory prepared and then screened and embedded in epoxy resin with high light transmittance. The CRMs were developed according to GB 1351-2008, a national wheat standard of the People's Republic of China. Five CRM items, including injured kernels, spotted kernels, broken kernels, sprouted kernels and mouldy kernels, were developed, and each item contained 10 kernels that demonstrated varying levels of unsoundness. The CRMs possessed excellent homogeneity, and stability monitoring demonstrated that the CRMs were stable at a low temperature of -20°C and a high temperature of 45°C for 14 days and at least 18 months at room temperature out of direct sunlight and strong light. All the CRMs were assessed by authoritative laboratories and specialists, and the results showed that they conformed to the morphological characteristics described in the textual standard.

**Keywords:** Unsound wheat kernel; Certified Reference Materials (CRMs); qualitative; sensory inspection

## Introduction

Wheat is one of the most consumed grains in the world, and it is the main and most important commodity and reserved grain in China. Unsound kernel detection is an important quality control criterion in wheat production, purchasing, circulation and consumption. Actually, there are many parameters for judging wheat quality, such as unit weight, impurity percentage, moisture, and unsound kernels, among others. Unsound wheat kernels, which include injured kernels, spotted kernels, broken kernels, sprouted kernels and mouldy kernels, are defined as damaged, but still useful kernels in the National Standard of the People's Republic of China GB 1351-2008 [1]. Unsound kernels affect the processing quality and edible properties of wheat. Currently, the determination of unsound wheat kernels in China is based on text descriptions of GB 1351-2008 [1], which are sensory-based and very subjective, in addition to being time-consuming and laborious. Furthermore, the results are greatly influenced by detector's experience. Inspection results were often inconsistent

between different inspection authorities or laboratories, therefore, CRMs are vital for accurate and rapid inspection of unsound wheat kernels, especially spotted kernels, sprouted kernels and mouldy kernels. During our 3-year experimental period, identification and comparison experiments of inter and intra laboratories by inspection bodies in China were repeated to set detection criteria and realize continuous improvements of accuracy and reliability for wheat unsound kernel detection.

Unsound wheat kernels are mainly produced during production, harvest, storage, and packaging. Of the unsound kernels, injured kernels are mainly formed during storage, and this can be avoided by improving storage facilities; broken kernels are generally produced during mechanical operations [2]. Spotted kernels usually result from disease. *Helminthosporium* spp. and *Alternaria* spp. are the infectious sources of black germ kernel; when the infected area is limited only to the end of embryo, a small black spot occurs on the embryo, while when the infected area occupies a certain area on kernel surface, a large black blob is formed. *Fusarium* head blight (FHB) or scab, caused by different species of *Fusarium*, is a serious worldwide problem in wheat, barley and other small grain cereals, and it generally leads to decreased grain yield and quality [3]. Gibberella damaged kernels are usually crinkled and shrivelled, and moreover, they can be contaminated with mycotoxins, which are secondary metabolites produced by some *Fusarium* species [4]. Sprouted kernels and mouldy kernels are generally caused by improper temperature and humidity conditions during storage.

To assist sensory wheat inspection, a national standard GB 22504.1-2008 was issued by the Standardization Administration of the People's Republic of China in 2008 [5], which conveys textual descriptions that can easily cause ambiguity with intuitive and clear pictures that make the judgment criterion

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uniform and standardized. Similarly, an analogous standard was released in the USA, which was called the Grain Inspection Visual Reference Images (VRIs) and was approved and published by United States Department of Agriculture (USDA) and the Grain Inspection, Packers and Stockyards Administration (GIPSA). This system consists of Interpretive Lines (IL), General Appearance (GA) prints, and Other VRIs. VRIs are auxiliary visual resources for cereal, rice and beans detection personnel. The VRIs are digitally generated, with grain gradation information included and routinely updated related data and images. VRIs are used to ensure the consistent and uniform application of grading interpretations. The visual grading aids system represents the foundation for the national inspection system's subjective quality control program, providing an effective management tool for inspectors to assist them in making proper and consistent subjective grading decisions.

Although VRIs or an assistant atlas contribute to the sensory inspection of unsound wheat kernels, they may not be as intuitive or clear as physical samples. Therefore, the objective of this study is to develop Certified Reference Materials (CRMs) of unsound wheat kernels, which will be used to visualize the specific explanations in the textual standard GB 1351-2008 in China [1]. In addition, these CRMs will contribute to reducing subjective error and serve as a visual and physical standard. The unsound wheat kernel CRMs could be employed as an important means of quality control, in personnel training and appraisal for cereal and related products agencies, and in inspection laboratories to ensure accurate and reliable measurement results.

## Materials and methods

### Textual standard for unsound wheat kernels in China

In the Chinese National Standard GB 1351-2008 [1], unsound wheat kernels were defined as follows: 1) injured kernel impaired by insects, with its embryo or endosperm damaged; 2) spotted kernel damaged by various diseases with disease spots on its surface and with embryo or endosperm damaged. Spotted kernels were further divided into (a) black germ kernel with a dark brown or black coloured embryo, and with embryo or endosperm damaged, and (b) Gibberella damaged kernel, which is shrivelled, in some cases with a purple coloured kernel surface, or with a visible pink substance like mildew, or occasionally with black perithecia on the kernel surface; 3) broken kernel, which is squashed or broken with embryo or endosperm damaged; 4) sprouted kernel in which the bud or radicle have not broken through the testa, but the testa of the embryo is ruptured or visibly swollen and separated from the embryo, or if the bud or radicle have broken through the testa, they do not exceed the length of kernel itself; 5) mouldy kernel with its kernel surface mildewed. The development of the visual CRMs in this study was based on these definitions in the GB 1351-2008 [1].

### Collection and laboratory preparation of candidate unsound wheat kernel

**Collection of injured kernels, spotted kernels and broken kernels:** We collected approximately 5000 wheat

samples, covering more than 300 wheat cultivars produced from 14 provinces in the main wheat-growing areas of China in recent years. Injured kernels, broken kernels and spotted kernel (mainly black germ kernel) were picked and collected from these samples and stored in dryers at room temperature for screening of unsound wheat kernels.

Meanwhile, field collection of Gibberella damaged kernels was conducted in the cities of Huai'an, Yangzhou, and Xuzhou of Jiangsu Province; the cities of Hefei and Suzhou of Anhui Province; the cities of Nanyang, Xinyang, and Luohe of Henan Province; and the cities of Xiangyang and Suizhou of Hubei Province in China from late May to early June 2012, where there was significant FHB. *Fusarium*-infected wheat panicles were cut off in the field, then air-dried and threshed. The Gibberella damaged kernels were then separated manually based on colour and the degree of shrivelling, and then stored in dryers at room temperature for screening of unsound wheat kernels.

### Laboratory preparation of sprouted and mouldy kernels

Wheat sprouted kernels and mouldy kernels were prepared in the laboratory. Wheat kernels of normal size were cleaned and any impurities were removed. They were routinely placed on water saturated filter paper inside a culture dish with the lid on and cultivated in an incubator at 20°C. The kernels were observed, collected, and air-dried over 1-2 days according to their different budding levels. The culture dishes, filter paper, experimental appliances and water were sterilized before use.

For the preparation of mouldy wheat kernels, clean and impurity-free kernels were dampened by spraying with water and then stirred constantly to reach a moisture content of approximately 14%-15%, followed by bottling in a jar with lid and placing in an incubator at 45°C. When mildew on the wheat kernel surface was observed, the wheat kernels were collected, air-dried, and stored in dryers at room temperature for screening.

### Screening of candidate unsound wheat kernels

The collected and laboratory prepared unsound kernels were screened in white trays and stored in dryers at room temperature for CRM production.

### Screening of injured kernels

Kernels with insect-damaged wormholes, tunnels, or eroded embryos were included. Severe wormholes, tunnels and eroded embryos are easy to discriminate, however, less obvious injury characteristics are a test of a detector's level of expertise, as some wormholes are very tiny and the detector must observe the kernel surface meticulously when testing. Additionally, whether the embryo or endosperm is damaged should also be determined and if not, the kernel should be classified as normal. Finally, when necessary, the kernel should be split for testing.

### Screening of spotted kernels

In GB 1351-2008 [1], spotted kernels include Gibberella damaged kernels and black germ kernels. A Gibberella damaged



kernel can be observed as a shrivelled and pale kernel, or a kernel with a purple surface, or a kernel with a visible pink substance like mildew, occasionally with black perithecia. Gibberella damaged kernels can be slightly, moderately or severely damaged. Kernels shrivelled but not pale should be considered as sound kernels, as immature wheat kernels that are classified as normal kernels may shrivel after harvest. Black germ kernels can be further divided into black point kernels or smudge kernels. Black point kernels have dark brown or black spots that present in the embryo and surrounding tissues and are damaged slightly or severely. Smudge kernels have dark brown or black plaques that present in the embryo and on the endosperm surface. The cumulative area of the plaques exceeds more than 1/2 of the area of kernel surface or exceeds more than 1/2 of the area of the abdominal groove of the wheat. Dark brown or black plaques may occur at any point on the kernel surface, but they usually occur on the embryo and abdominal groove of the wheat. No matter where the plaques appear, kernels with distinct plaques and embryo or endosperm damage should be classified as unsound wheat. Sometimes, kernels should be split to observe if the colour of the embryo or endosperm has changed or not, and it is classified as a spotted kernel if there is a colour change.

### Screening of broken kernels

Broken kernels include squashed kernels with embryo or endosperm damaged and broken kernels with embryo or endosperm damaged. It should be noted that kernels with only a tiny crack on the surface and undamaged embryo or endosperm should be classified as sound kernels.

### Screening of sprouted kernels

Sprouted kernels include kernels with the embryo testa ruptured, kernels with a bud or radicle that has broken through its testa but not exceeded the length of kernel itself, and kernels with testa that are swollen and separated from the embryo.

One of the characteristics of a sprouted wheat kernel is testa that has swollen and separated from the embryo, which is difficult to understand and identify correctly. Similarly, it is difficult to prepare in the laboratory, because if the kernel is taken out of the incubator too early, these characteristics will not be evident and representative after air-drying, while if the kernel is removed too late, the testa of the embryo ruptures; thus, mastering the degrees of wheat testa swelling and the precise time to remove kernels from the incubator is very important. To identify these characteristics, the detector can press on the swollen part with tweezers, and if the embryo depresses, such as if a cavity is present, it should be classified as a sprouted kernel.

### Screening of mouldy kernels

Slightly mildewed kernels are those with sporadic mildew on their surface. Moderately mildewed kernels have approximately 1/2 of their kernel surface mildewed and severely mildewed kernels have approximately 3/4 of their kernel surface mildewed. Completely mildewed kernels have their whole kernel surface mildewed.

It should be noted that mouldy kernels are kernels in

which the surface is mildewed, but the embryo or endosperm is not damaged. It should be classified as an organic impurity if the embryo or endosperm is damaged. In addition, dust and microorganisms can easily lurk in the pappus of wheat, and the detector should carefully distinguish contamination of the pappus to avoid misjudgement.

### Production of the unsound wheat kernel Certified Reference Materials

Epoxy resin with high light transmittance was used to embed unsound wheat kernels that were later certified as CRMs in this study. Epoxy resin is liquid and because of this excellent characteristic it can fully flood the surface of the wheat kernel and cannot be dissolved in water after drying. The high transparency of the epoxy resin contributes to the observation of the fine structure of unsound wheat kernels. Epoxy resin also has some additional desirable attributes such as flame-retarded, age-resistant, non-poisonous, antioxidative, thermotolerant, and insulative.

The detailed embedding process was as follows: A clean and dust free mould of 88×55×20 mm was prepared in advance. Epoxy resin and curing agents (3:1) were poured into a container and stirred in a clockwise direction until they were thoroughly mixed. In some cases, the epoxy resin was heated in advance and then the curing agents were added and stirred at a low temperature. Bubbles were removed using a vacuum, or by letting the samples stand for 10-20 min. The standing time cannot be too long, or the epoxy resin can harden and deform due to the rising temperature. Half of the epoxy resin and curing agent mixture was poured into the mould for hardening, or the mould was heated at 50-60°C to accelerate the process. Unsound wheat kernels were evenly placed onto the hardened epoxy resin, then the other half of the epoxy resin and curing agent mixture was poured on top. After the second layer of epoxy resin hardened, the epoxy resin and curing agents were paved layer by layer, and then the bubbles were removed using vacuum, or by waiting for them to naturally disappear. The last layer of epoxy resin should extend from the mould by 2-3 mm, after which the mould was allowed to harden for 12 hours. The epoxy resin block was polished using a sander or manually (80-120-200-280-360-400.....5000 mesh).

### Homogeneity testing

Homogeneity testing was conducted to investigate whether 10 kernels in each epoxy resin block were correctly labelled as unsound wheat kernels or not based on the definitions of GB 1351-2008 [1]. This homogeneity testing was called within-bottle homogeneity testing. Between-bottle homogeneity testing was conducted with 100 epoxy resin blocks containing the same type of CRMs to investigate if they were correctly labelled as unsound wheat kernels or not.

### Stability monitoring

**Short-term stability:** To ensure their stability under transportation conditions, ten blocks of CRMs were chosen randomly and stored at -20°C, 4°C, 25°C, 37°C and 45°C for 1, 3, 7 and 14 days. After storage, the blocks were inspected for epoxy



resin changes, changes in the identification characteristics of unsound wheat kernels, or changes that affected unsound wheat kernel classification at different preservation conditions.

### Long-term stability

The long-term stability of the CRMs after storage at room temperature and out of direct sunlight or strong light for 1, 2, 4, 6, 12, 18 months was investigated. The blocks stored under different preservation conditions were inspected for epoxy resin changes, changes in the identification characteristics of unsound wheat kernels, and for changes that affect the classification of unsound wheat unsound kernels.

### Co-laboratory confirmation

According to National Standard of the People's Republic of China GB/T 15000.3-2008 [6], co-laboratory confirmations of the CRMs were organized and conducted, and eight laboratories throughout China were selected and invited based on their reputable and highly technical experience in the field of unsound wheat kernel detection.

Three set of CRMs were provided to each laboratory, and the identification characteristics of each unsound wheat kernel item were confirmed according to GB 1351-2008 [1].

## Results and Discussion

### The CRMs

One hundred sets of unsound wheat kernel CRMs were produced in this study; each set included 5 items (injured kernels, spotted kernels, broken kernels, sprouted kernels and mouldy kernels), and each item contained 10 kernels with varying levels of damage. Pictures of the developed CRMs are shown in Figures 1-5. Unsound wheat kernels were embedded in epoxy resin, such that they could not be removed from the epoxy resin once it dried. The surface of the CRMs should not be coagulated with moisture or contaminated with dust or fingerprints when used, and as such the user should hold the CRM in their hand, face the direction of a light source, keep the CRM at eye level, and observe the CRM with the naked eye under natural or incandescent light in alliance with GB 1351-2008 [1]. Some characteristics, such as tiny wormholes or mildew, may not be very obvious, therefore, the CRMs should be observed from different directions and angles.

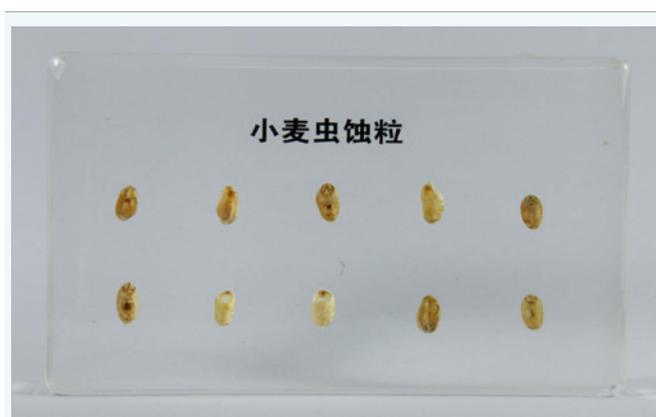
Figure 1 shows wormholes of different sizes representing the different levels of damage of an injured kernel. Of note, wormholes can occur at any part of the kernel, including in the centre, the abdominal groove, and at both ends and sides of the kernel. On some kernels, tunnels formed by worm consumption could be observed on the surface or under the kernel layer. Kernels with wormholes or tunnels frequently presented as transparent under light. Another type of kernel injury shown is an eroded embryo (demonstrated by the second and third images of the lower row (from left to right, the same below) in Figure 1), which appears as a round area of surface damage and presents with a white endosperm.

Spotted kernel CRMs are showed in Figure 2, with the upper row showing Gibberella damaged kernels and the lower row

showing black germ kernels. The first and second kernel of the upper row are representative Gibberella damaged kernels; the first kernel appears to have a purple surface, and the second kernel is shrivelled and presents as a pale colour. The first three kernels of the lower row are smudge kernels of black germ kernels, whose cumulative area of dark brown or black plaques exceeded more than 1/2 of the kernel surface area or more than 1/2 of the area of the abdominal groove of the wheat. The fourth and fifth kernel of the lower row are black point kernels, which presented with dark brown or black in their embryos and surrounding tissues.

Broken kernels were further divided into squashed kernels and broken kernels, both of which display characteristics of embryo or endosperm damage. The first kernels shown in the upper and lower rows of Figure 3 are representative broken kernels of differing levels, of which approximately 1/2 and 1/4 of the kernels broke and disappeared, respectively. The fifth kernel of the lower row is a representative squashed kernel, which was squashed along abdominal groove.

Sprouted kernel CRMs are presented in Figure 4, and three types could be observed: testa that are swollen and separated



**Figure 1** Certified Reference Materials of unsound wheat kernels (1) —injured kernel.



**Figure 2** Certified Reference Materials of unsound wheat kernels (2) —spotted kernel.



from the embryo (representative kernel: the first kernel in the upper row), testa of embryo ruptured (representative kernel: the fifth kernel in the lower row), and testa broken through by the bud or radicle but not yet exceeding the length of kernel itself (representative kernel: the third kernel of the upper row).

Figure 5 shows CRMs of mouldy kernels with varying mildewed levels. We observed that mildew could occur at any part of the kernel and sometimes should be viewed from the side of the CRMs

### Homogeneity assessment

In the development of unsound wheat kernel CRMs, each kernel embedded in the epoxy resin blocks was screened and identified specifically, the characteristics of which were in accordance with the descriptions in textual standard. Thus, essentially all kernels in the 100 CRMs were tested for homogeneity and therefore, the CRMs were homogeneous.

### Stability assessment

Short-term and long-term stability studies of unsound wheat kernel CRMs were performed under varying transportation



**Figure 3** Certified Reference Materials of unsound wheat kernels (3)  
—broken kernel.



**Figure 4** Certified Reference Materials of unsound wheat kernels (4)  
—sprouted kernel



**Figure 5** Certified Reference Materials of unsound wheat kernels (5)  
—moldy kernel.

and storage conditions. The results of the short-term stability assessment demonstrated that the epoxy resin did not splinter at  $-20^{\circ}\text{C}$ , or melt at  $45^{\circ}\text{C}$ , which might have affected the morphological characteristics of each unsound kernel. No changes or conversions among the different types of unsound wheat kernels were observed. For example, the damage levels of each unsound kernel did not get worse, get better, or disappear. Additionally, the epoxy resin and the wheat kernels within remained unchanged at  $4^{\circ}\text{C}$ ,  $25^{\circ}\text{C}$ , and  $37^{\circ}\text{C}$ .

The 18-month storage stability assessment results showed that the characteristics of each unsound kernel in the epoxy resin blocks did not change over time when kept at room temperature and out of direct sunlight and strong light. Continued long-term stability monitoring will prolong the CRM expiry date.

Unsound wheat kernels inlaid in epoxy resin avoid oxygen, mildew and mechanical damage, so their morphological characteristics will not change over time and are not affected by storage conditions. The epoxy resin used in this study could tolerate temperatures from  $-50^{\circ}\text{C}$  to  $180^{\circ}\text{C}$ , which are beyond the transport and storage temperature of CRMs.

### Certification of CRM

All of the results from the certification laboratories showed that the CRMs are in accordance with the morphological characteristic descriptions in GB 1351-2008 [1].

### Conclusion

We report the development of unsound wheat kernel CRMs in this article. Candidate unsound wheat kernels were collected or laboratory prepared, screened, and embedded in epoxy resin. The homogeneity testing and stability studies demonstrated that the CRMs were homogenous and stable for at least 18 months at room temperature and out of direct sunlight and strong light and for 14 days at delivery conditions between  $-20^{\circ}\text{C}$  and  $45^{\circ}\text{C}$ . These CRMs, after certification by Chinese authorities, will be a useful auxiliary tool for quality control, method validation and personnel training and appraisal in unsound wheat kernel inspection.



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## References

1. National Standards of the People's Republic of China. GB 1351-2008 wheat. Beijing: China Standard Press, 2008.
2. Liu Z, Qian YL, Zhao L, He XF, Wang JL. Impact of Fungicide Treatments on the Constitution of Unsound Wheat Kernels. *Agricultural Biotechnology*. 2018; 7: 46-50, 53.
3. Parry DW, Jenkinson P, Mcleod L. Fusarium ear blight (scab) in small grain cereals, a review. *Plant Pathol*. 1995; 44: 207-238.
4. Aleš K, Andrej S, Stanislav V, Avrelija C, Mario L. Fate of deoxynivalenol and nivalenol during storage of organic whole-grain wheat flour. *J. Stored Prod. Res.* 2010; 46: 66-71.
5. National Standards of the People's Republic of China. GB 22504.1-2008 Inspection of grain and oils-assistant atlas of grain sensory inspection-part 1: wheat. Beijing: China Standard Press, 2008.
6. National Standards of the People's Republic of China. GB/T 15000.3-2008 Directives for the work of reference materials (3)Reference materials—General and statistical principles for certification (ISO Guide 35: 2006, Reference materials—General and statistical principles for certification, IDT). Beijing: China Standard Press, 2008.