


 Cite this: *RSC Adv.*, 2020, 10, 14581

Effects of deterioration and mildewing on the quality of wheat seeds with different moisture contents during storage

 Ruolan Wang,^{ID}*^a Lulu Liu,^a Yapeng Guo,^a Xin He^a and Qian Lu^{*ab}

Deterioration and mildewing caused by moisture of wheat seeds are serious problems that should be addressed for safe storage. In this work, simulated storage facilities were employed to preserve wheat seeds with different moisture contents (16%, 18%, 20% and 22%). The temperature changes in grain bulk, the mildewing situation, and the biochemical properties of wheat seeds were analyzed. Microstructures of endosperm and embryos were observed under electron microscopy. The results showed that with the increase of moisture content in wheat seeds, the deterioration and mildewing became serious. In the storage of wheat seeds with high moisture content, serious damage to the internal structure of endosperm and embryo was observed. Besides, the temperature increase and the fungal growth in grain bulk are attributed to the moisture of wheat seeds. Particularly, with the nutrient loss and cell damage, germination efficiency decreased with the extension of the storage period and the biochemical properties of wheat seeds are negatively impacted. Based on the results of this study, to prevent the deterioration and mildewing of wheat seeds and protect the biochemical properties in long-term storage, moisture should be controlled during the storage.

 Received 18th January 2020
 Accepted 26th March 2020

DOI: 10.1039/d0ra00542h

rsc.li/rsc-advances

1 Introduction

Wheat seeds are a staple feedstock for both food and feed industry.¹ To alleviate the conflict between continuous food/feed consumption and seasonal wheat production, a large number of facilities have been constructed for wheat seed storage.¹ Large-scale grain storage could not only prevent a food shortage crisis, but also maintain the prices of grain products in the market, playing a key role in the development of modern society.² However, a detrimental result of the large-scale grain storage is the grain deterioration and mildewing, which can be caused by temperature fluctuation, insect activity, air humidity, fungal growth, and many other factors.^{1,3} To reduce the economic loss of grain storage, the deterioration and mildewing under different storage conditions should be fully understood.

According to previous studies, a variety of mechanisms are involved in the grain deterioration and mildewing during storage. Firstly, temperature increase, which can be caused by the grain cell respiration and solar radiation, widely occurs during the grain storage.⁴ The high temperature can further create a favorable environment for grain cell respiration, causing the nutrients loss and weight loss in grain seeds.

Secondly, insects' activities can damage the structure of grain seeds and accelerate the deterioration process during storage.⁵ Although fumigation can limit the insects' activities or densities, it is not possible to reduce the number of insects to zero in the large-scale grain storage facilities. Hence, the damages caused by insects' activities are along with the grain storage. Thirdly, in the storage conditions with high humidity, fast deterioration of wheat seeds has been reported by previous studies.^{6,7} One of the main mechanisms is that the humid environment can favor the fast growth of fungi, which damage the surface of wheat seeds and consume the nutrients.

Moisture is an important concern during the storage of wheat seeds. On one hand, moisture content is important to the edible properties of wheat seeds. On the other hand, moisture may change the storage environment and further impact the properties of wheat seeds. Reed *et al.* (2007) reported that moisture can accelerate the respiration of wheat seeds during storage and promote the spoilage.⁸ Previous studies also reported the promotional effects of moisture on the respiration and spoilage of many other types of grains.^{9,10} However, effects of moisture gradients in wheat seeds on the temperature fluctuations of grain bulk have been rarely studied. In addition, previous studies mainly focused on the cell respiration while neglected the relation between moisture and fungal growth.¹¹ To our knowledge, during the storage, moisture transfer always occurs in the grain bulk, resulting in the dramatic increase of moisture content in a portion of wheat seeds.^{12,13} Also, microbial activities, particularly fungal activities, in grain bulk might damage the external

^aSchool of Food Science and Technology, Henan University of Technology, Zhengzhou, 450001, China

^bSchool of Resources, Environmental & Chemical Engineering, Key Laboratory of Poyang Lake Environment and Resource Utilization, Ministry of Education, Nanchang University, Nanchang 330031, China. E-mail: wangruol@163.com


structure of wheat seeds and cause the spoilage.¹⁴ Therefore, it is necessary to fully understand the effects of high moisture content and fungal growth on the storage of wheat seeds.

The main aim of this work was providing answers to four important questions: (1) What are the temperature changes in the three-dimensional grain bulk during storage? (2) Is fungal activity contributing to the temperature increase of wheat seeds with high moisture contents? (3) What are the effects of storage on biochemical properties of wheat seeds with different moisture contents? (4) What are the microstructures of endosperm in wheat seeds before and after storage? With the solutions of aforementioned questions, effects of moisture content on the biochemical properties of wheat seeds can be fully understood. By the end of this work, strategies for wheat seeds storage and the evaluation of storage cost are provided.

2 Materials and methods

2.1 Wheat seeds and storage facility

The wheat seeds used in this work were produced in Henan Province (China) and harvested in June, 2017. The storage facility used for the experiment was designed by our research team. The size of storage facility, of which the length, width, and height are 1.5 m, 0.95 m, and 1.2 m, respectively, is made of carbon steel. Specific structures of the storage facility are shown in Fig. 1. There are vents for temperature-measuring cables, which can monitor the actual temperature of the grain bulk, on the sides of storage facility. Ventilation system is equipped with the storage facility to prevent the temperature increase caused by the wheat seeds

respiration. In this work, the wheat seeds were stored at room temperature (15–25 °C).

To evaluate the effects of moisture contents on the biochemical properties of wheat seeds during storage, the moisture contents of wheat seeds were set as 16%, 18%, 20% and 22%. The storage periods of wheat seeds with 16%, 18%, 20% and 22% moisture contents were set as 103 days, 49 days, 28 days, and 24 days, respectively.

2.2 Experimental design

The experiments in this study were carried out in four steps. Firstly, the temperature changes of wheat seeds with different moisture contents were measured to confirm the effects of moisture content on the storage conditions. To improve the reliability of the research results, both vertical temperature and horizontal temperature of the grain bulk were measured. Secondly, effects of moisture content on the mildewing of wheat seeds during storage were studied by quantifying the number of fungi. Thirdly, microstructures of endosperm and embryo of wheat seeds before and after storage were demonstrated. The changes of large starch granules, small starch granules, protein matrices, broken starch, and broken protein in the endosperm were observed at different storage stages. In the embryo, changes of cell wall, mitochondria, nucleus, nucleolus, and nucleus membrane were observed. Fourthly, biochemical properties, including the germination efficiency, farinograph properties, and extensograph properties, of wheat seeds before storage and after storage were measured. By the end of this

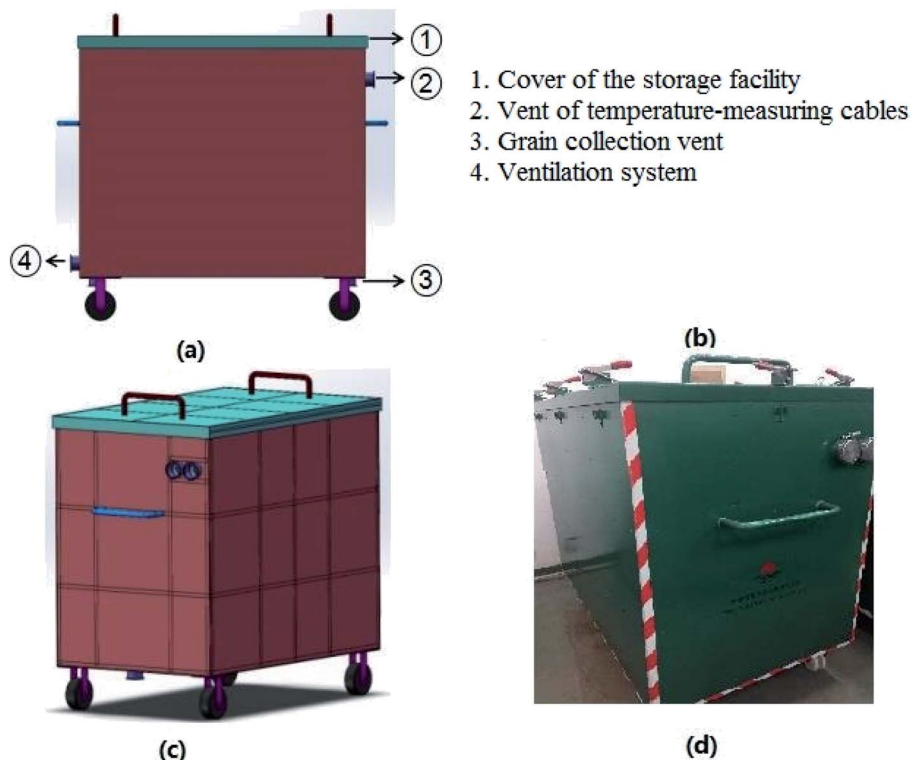


Fig. 1 Specific structures of wheat seeds storage facilities.



work, strategies to alleviate the negative effects of moisture on the quality of wheat seeds during storage were discussed.

In this work, all the experiments and tests were performed in triplicate and the experimental results were expressed as mean or mean \pm standard deviation.

2.3 Analysis methods and experimental procedures

2.3.1. Changes of storage temperatures. Temperature-measuring cables (Zhejiang, China) were employed to monitor the changes of temperature in grain bulk and ambient environment. In this work, fifteen cables used for temperature measurement were placed evenly in the storage facility. The distance between two cables in the same row was set as 25 cm while the distance between two cables in the same column was set as 30 cm. The temperatures at upper layer, middle layer and lower layer of the grain bulk were measured by the cables. Therefore, the temperature changes at vertical level and horizontal level could be obtained.

2.3.2. Measurement and identification of mildewing. To identify the mildewing of wheat seeds, the amount of fungi on the seed surface was quantified. The samples were collected from three layers (upper layer, middle layer, and lower layer) of five sampling points in the grain bulk. About 300 g samples were collected from each point for further analysis.

The amount of fungi on wheat seeds was expressed as colony-forming unit (CFU) ($10^4/g$). The measurement of CFU was performed according to the national standard (GB 4789.15-2016) of China. Specific procedures are listed as follows: (1) 25 g samples are mixed with 225 mL sterile distilled water and then the

solution is diluted by 10 times; (2) 1 mL diluted samples are sprayed on Petri dish with Bengal red agar medium; (3) the samples were preserved in incubator at 28 ± 1 °C for 5 days; (4) optical microscope is used to quantify the number of fungal colonies on the agar medium; (5) the CFU is calculated.

In this work, the mildewing degrees of wheat seeds were identified according to the CFU. When the values of CFU fell in the scopes of 4.4×10^4 – 1.2×10^5 , 1.2×10^5 – 7.1×10^5 , and $>7.1 \times 10^5$, the wheat seeds would be considered as mild mildewing, moderate mildewing, and severe mildewing, respectively.¹⁵

2.3.3. Observation of microstructure. The wheat seeds with 20% moisture content were preserved in storage facilities at room temperature for 24 days. Samples collected on Day 0, Day 6, Day 9, Day 12, and Day 24 are collected for further microstructure analysis. Since the severe mildewing of wheat seeds with 20% moisture content occurred on Day 12 (Table 3), the analysis of samples collecting from the 24 day storage can exactly reflect the changes of microstructures of wheat seeds in the process of mildewing. Endosperm and embryo of wheat seeds were observed by using scanning electron microscope and transmission electron microscope (Hitachi, Japan), respectively.^{1,2} The observation of endosperm was performed at $2000\times$ and $3000\times$ magnification according to the instruction manual. In the observation of endosperm, starch granules, broken starch, protein matrices, and broken protein could reflect the deterioration of wheat seeds.^{16–18} The embryo was observed at $3000\times$, $5000\times$, and $20\,000\times$ magnifications with focus on the cell wall, intercellular space, mitochondria, nucleus, nucleolus, and nucleus membrane.

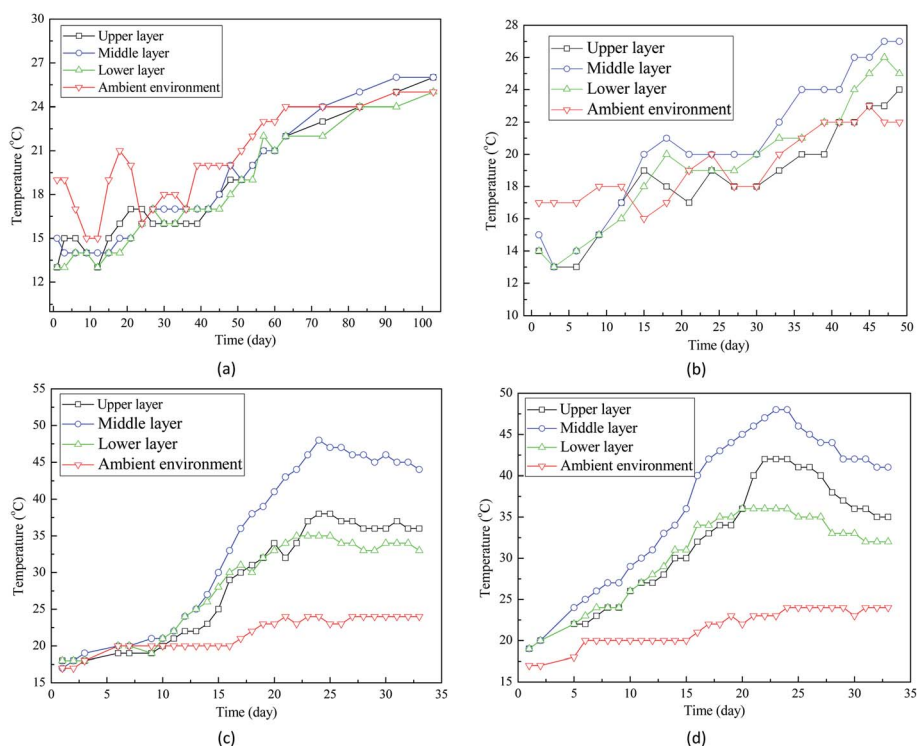


Fig. 2 Temperature changes (vertical level) of wheat seeds during storage: (a) 16% moisture content; (b) 18% moisture content; (c) 20% moisture content; (d) 22% moisture content.



2.3.4. Biochemical properties of wheat seeds. Since wheat seeds after storage can be used for food production or seeding, properties of the wheat seeds are of great importance.^{1,19} In this work, the germination efficiency, farinograph properties, and extensograph properties of wheat seeds were measured. Farinograph properties, including development time, maximum consistency, water absorption, stability time, and softening, were measured according to the national standard (GB/T 14614-2006) of China. Extensograph properties, including area, resistance, extensibility, maximum resistance, *R/E*, and max *R/E*, were measured according to the national standard (GB/T 14615-2006) of China. Germination efficiency of wheat seeds was measured by using the national standard (GB/T 5520-2011) of China.^{15,20}

3 Results

3.1 Effects of moisture contents on storage heating

3.1.1. Temperature changes at vertical level. As shown in Fig. 2, temperatures of wheat seeds at middle layer and lower layer only increased by 10–13 °C during the 100 day storage. It is noteworthy that the temperature change of wheat seeds with 16% moisture content was in accordance with the change of ambient temperature, suggesting that ambient temperature fluctuation mainly contributed to the temperature increase in storage facility. Similar phenomenon was observed during the storage of wheat seeds with 18% moisture content.

Fig. 2(c) and (d) demonstrated that the temperatures of grain seeds at three layers were much higher than the ambient temperature. For example, after 30 day storage, ambient temperature increased to around 22 °C while the final temperatures of grain bulk ranged between 30 °C and 44 °C (Fig. 2(c)). Such a difference suggests that in addition to the ambient temperature increase, some other factors contributed to the temperature increase of grain bulk. Interestingly, the difference between ambient temperature and grain bulk temperature is related with the moisture content of wheat seeds. Fig. 2 indicated that the higher the moisture content, the larger the temperature difference. Therefore, it is hypothesized that high moisture content accelerated the temperature increase of grain bulk.

3.1.2. Temperature changes at horizontal level. To verify the aforementioned hypothesis, temperature changes at horizontal levels of storage facility are measured. As shown in Fig. 3, wheat seeds with higher moisture content had much faster temperature increase. For example, temperature of wheat seeds with 16% moisture content increased by around 11–13 °C in the 103 day storage while the temperature increase of wheat seeds with 22% moisture content could reach 20 °C after 24 day storage. Therefore, high moisture content of wheat seeds accelerated the temperature increases at both vertical level and horizontal level.

Not only the temperature fluctuation, but also the temperature gradient was related with moisture content of wheat seeds. Fig. 3 indicated that the temperature gradient in grain bulk was only around 2 °C before storage while the temperature gradient of some grain bulks increased dramatically after storage. For example, the final temperature gradient of wheat seeds with 16% moisture content was still around 2 °C after 103 day storage. However, the temperature gradients of wheat seeds

with 20% and 22% moisture contents were over 10 °C. Interestingly, temperature of the central part of grain bulk was much higher than that of surrounding part (Fig. 3(d) and (h)). The most possible reason for this phenomenon is that self-heating caused by the moisture of wheat seeds contributed to the temperature increase in grain bulk.

Two theories have been proposed to explain the moisture-related temperature increase in the storage of grain seeds. Firstly, semi-closed grain bulk creates a favorable environment for fungal activities, which can generate heat and further increase the temperature. Secondly, it has been fully documented that high moisture can accelerate the respiration of wheat seeds, releasing heat and increasing storage temperature. Ghazavi and Houshmand (2014) reported that the over intensive respiration of grain seeds during storage can consume the grain nutrients and even damage the cell structures.²¹ To verify if the endogenous activity (wheat seeds' respiration) or the exogenous activity (fungal growth) contributed to the temperature increase in grain bulk, more studies have been conducted in this work.

3.2 Effects of moisture contents on mildewing

3.2.1. Changes of CFU. As shown in Fig. 4, the increase of CFU is in positive correlation with the temperature increase in grain bulk. The number of fungi on wheat seeds with 16%, 18%, 20% and 22% moisture contents increased with the extension of storage periods. This result suggests that the fungal activities are along with the wheat seeds at different bulk layers during storage. Comparison of Fig. 4(a)–(d) showed that the increase rate of the CFU is closely related with the moisture content. For example, the CFU of wheat seeds with 16% moisture content only increased to around $100 \times 10^4/g$ after 103 day storage while the CFU of wheat seeds with 22% moisture content could reach over $500 \times 10^4/g$ after 24 day storage. Therefore, to prevent the growth of fungi during storage, moisture content of the wheat seeds after harvesting should be strictly controlled.

Interestingly, the CFU of wheat seeds at upper layer was much lower than those of wheat seeds at middle layer and lower layer. This result suggests that the wheat seeds at middle layer and lower layer are more likely to be impacted by the deterioration caused by fungal activities. This is in accordance with the study of White *et al.* (2010) that explored the relationship between fungal growth and wheat seeds deterioration in storage facilities.²² Therefore, during on the long-term storage, appropriate technologies should be applied to hinder the fast growth of fungi at the middle layer and lower layer of grain bulk.

As reported by Awasthi *et al.* (2014), the microbial activities can generate heat, further causing temperature increase in the closed grain storage facilities.²³ Therefore, based on the observation of a large quantity of fungi on wheat seeds, the first hypothesis that fungal growth and activity contributed to the temperature increase in grain bulk is confirmed.

3.2.2. Identification of the critical point of mildewing. According to the CFUs of mildewed wheat seeds, the critical points of mildewing were identified for the wheat seeds with different moisture contents. As shown in Table 1, the mild, moderate, and severe mildewing of wheat seeds with 16%



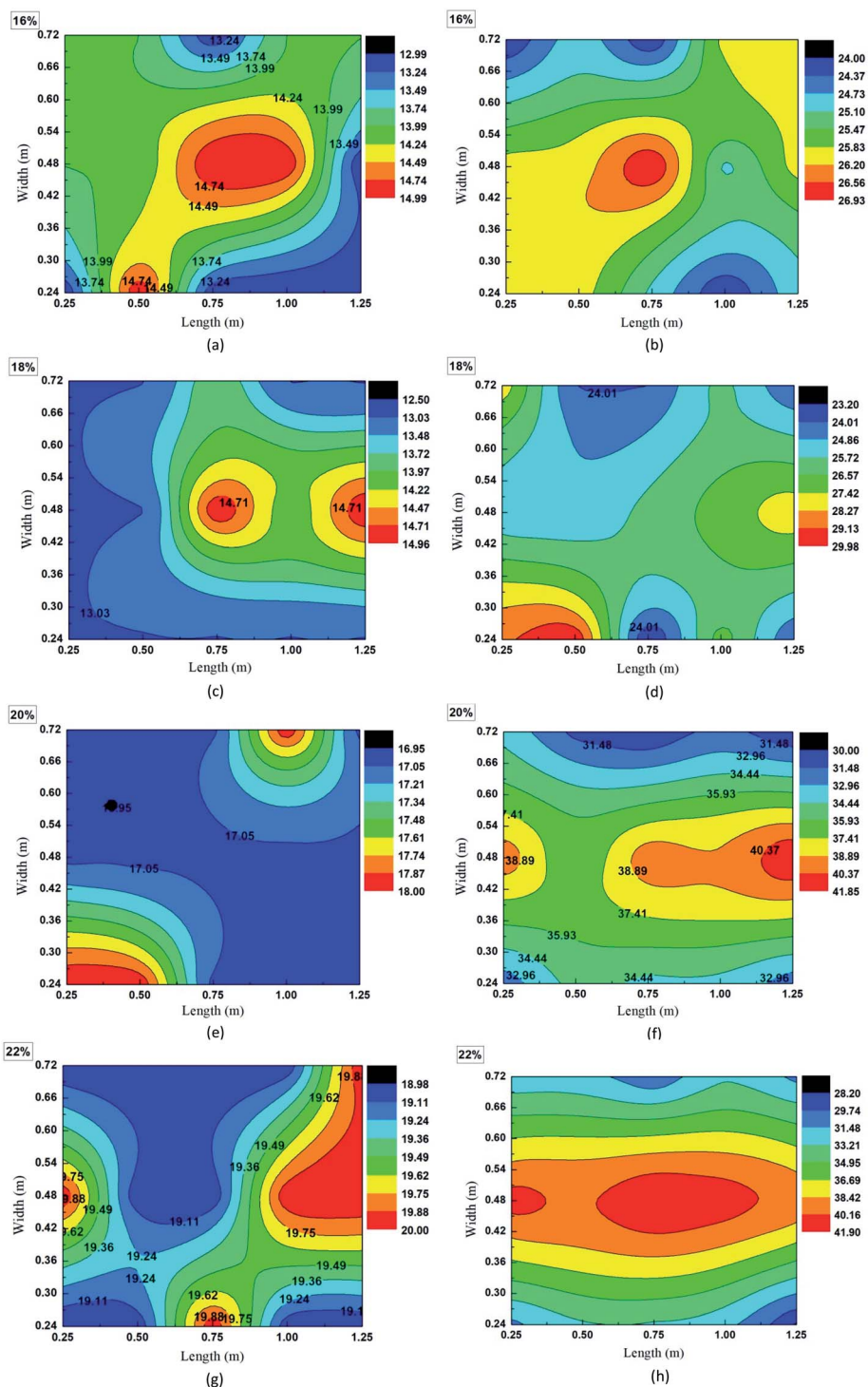


Fig. 3 Temperature distribution (horizontal level) of wheat seeds at middle layer during storage: (a) initial temperature distribution (16% moisture content); (b) final temperature distribution (16% moisture content); (c) initial temperature distribution (18% moisture content); (d) final temperature distribution (18% moisture content); (e) initial temperature distribution (20% moisture content); (f) final temperature distribution (20% moisture content); (g) initial temperature distribution (22% moisture content); (h) final temperature distribution (22% moisture content).

moisture content occurred on Day 33, Day 60, and Day 93, respectively. However, the mild, moderate, and severe mildewing of wheat seeds with 22% moisture content were observed from Day 6, Day 9, and Day 12, respectively. Such a big difference suggests that the moisture content of wheat seeds can dramatically impact the mildewing process during storage. This

result also suggests that it is a practically-feasible way to extend the storage period by appropriately reducing the moisture content of wheat seeds.

The mechanisms for the correlation of moisture content and mildewing are complex. Firstly, the moisture is favorable to the growth of fungi in grain bulk since most fungal metabolisms



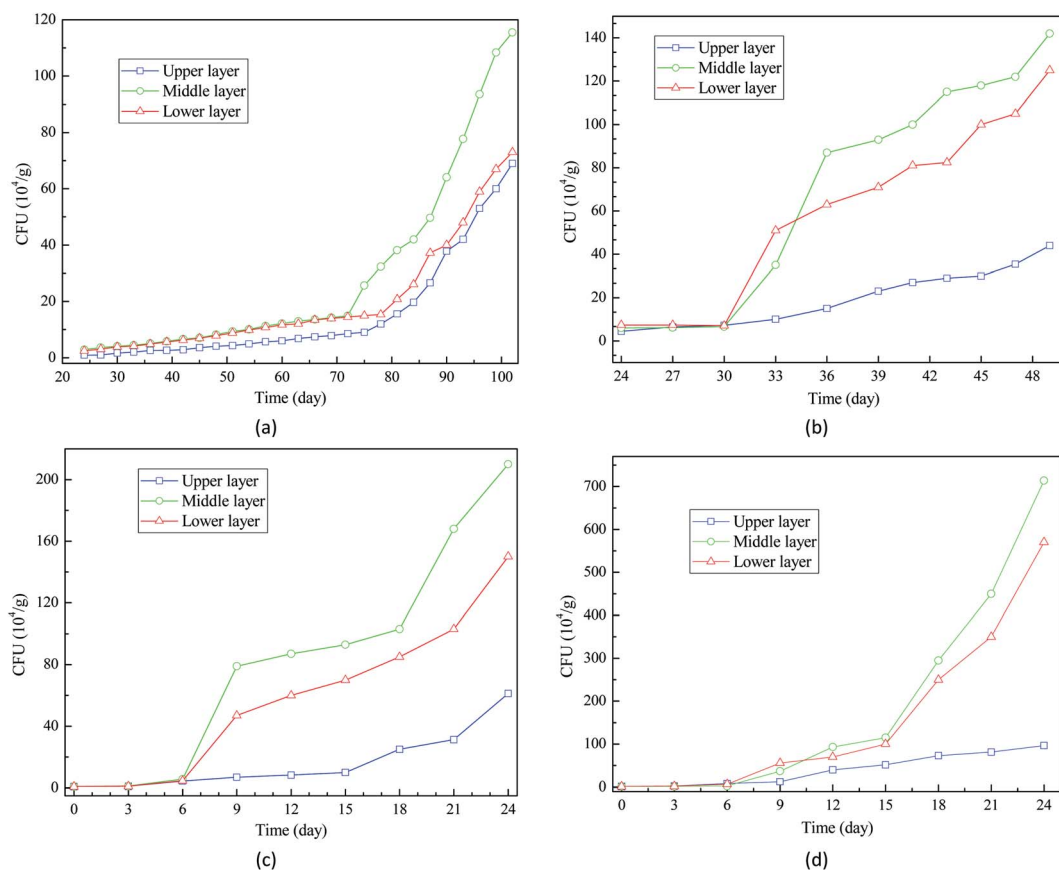


Fig. 4 Measurement of CFU for the analysis of wheat seeds mildewing: (a) 16% moisture content; (b) 18% moisture content; (c) 20% moisture content; (d) 22% moisture content.

Table 1 Critical points of mildewing for wheat seeds with different moisture contents

Mildewing degree	Moisture contents of wheat seeds			
	16%	18%	20%	22%
Mild	Day 33	Day 24	Day 6	Day 6
Moderate	Day 60	Day 33	Day 9	Day 9
Severe	Day 93	Day 36	Day 12	Day 12

require moisture.^{24,25} The wheat seeds with high moisture content can continuously supply extra moisture to the fungi surviving in grain bulk. Secondly, wheat seeds with high moisture content are prone to having higher respiration rate, generating more heat and increasing the temperature in storage facility. The warm and humid environment would accelerate the growth of fungi, resulting in mildewing of wheat seeds during storage.²⁶

If the wheat seeds with severe mildewing are considered unsuitable for further storage, the storage periods of wheat seeds with 16%, 18%, 20%, and 22% moisture contents should be no longer than 93, 36, 12, and 12 days, respectively (Table 1). This result confirmed that it is a practically feasible way to extend the storage period of wheat seeds by lowering the moisture content before storage. In most cases, to ensure the food security for the society, wheat seeds need to be preserved in

the storage facilities for even over two years. Therefore, moisture content of wheat seeds should be lowered below 16% to avoid severe mildewing. Additionally, some other advanced technologies, such as fumigation, ventilation, and temperature control, should be adopted to maintain the quality of wheat seeds during the long-term storage.

3.3 Microstructure of endosperm and embryo during storage

3.3.1. Microstructure of endosperm. As shown in Fig. 5(a), the endosperm contained a large quantity of small starch granules, which mainly stored the nutrients of wheat seeds, before storage. Also, some protein matrices and large starch granules are included in the endosperm. According to previous studies, the sizes of starch granules can impact the edible and processing properties of wheat seeds.^{27,28} For example, wheat seeds with a larger quantity of small starch granules contain more amylopectin, further lowering the peak viscosity.²⁹ The complete endosperm structure indicated that the wheat seeds are well protected before storage, having no obvious nutrients loss or internal structure damage.

With the extension of storage period, the abscission of small starch granule was observed firstly (Fig. 5(b)). More holes caused by the abscission of small starch granules were observed in Fig. 5(d) and (f), suggesting that when the storage period was



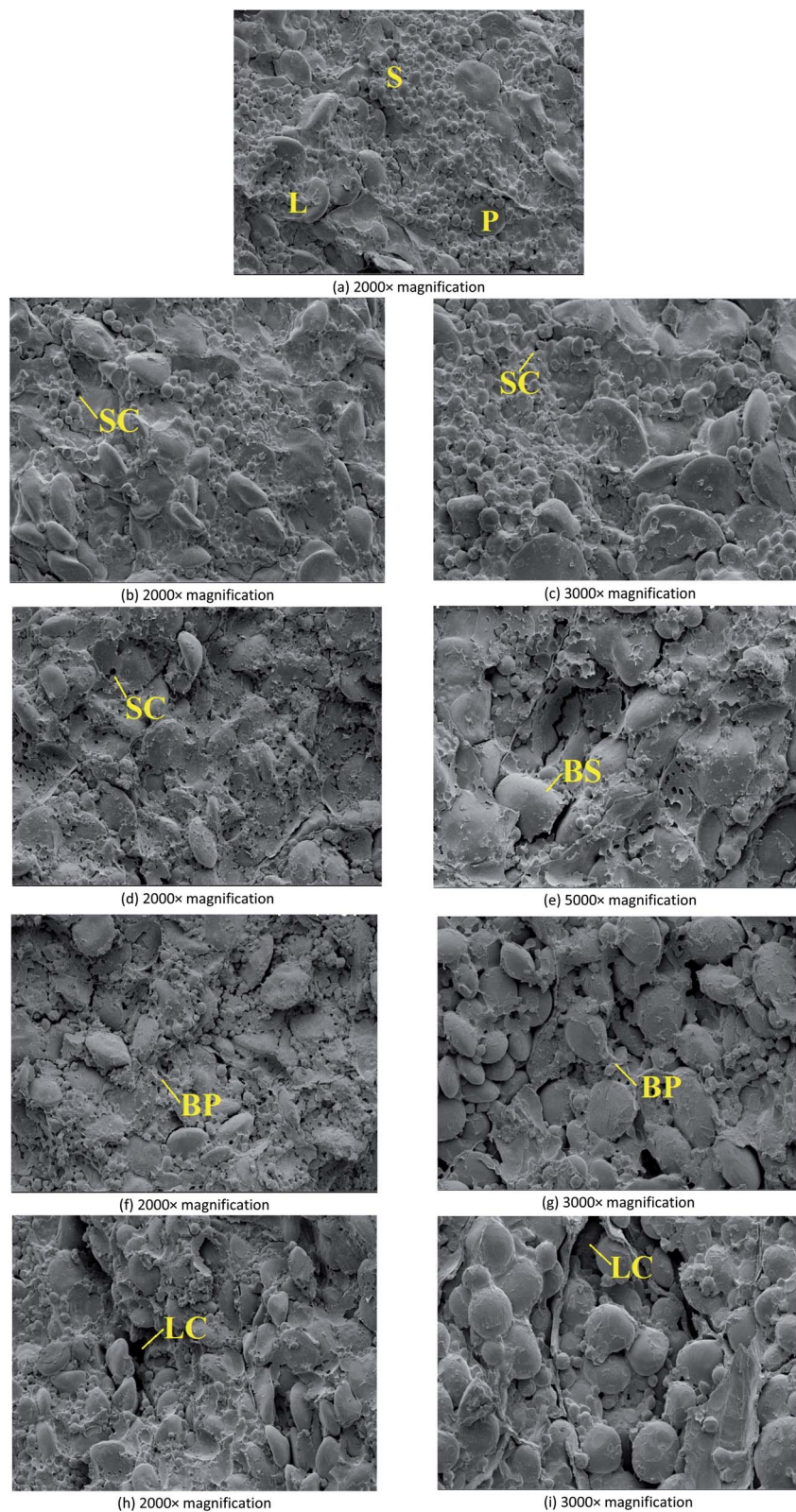


Fig. 5 Microstructure of endosperm of wheat seeds before and after storage: (a) Day 0; (b) Day 6; (c) Day 6; (d) Day 9; (e) Day 9; (f) Day 12; (g) Day 12; (h) Day 24; (i) Day 24. (L, large starch granules; S, small starch granules; P, protein matrices; BP, broken protein; SC, pits after the drop of small starch granules; LC, pits after the drop of large starch granules).



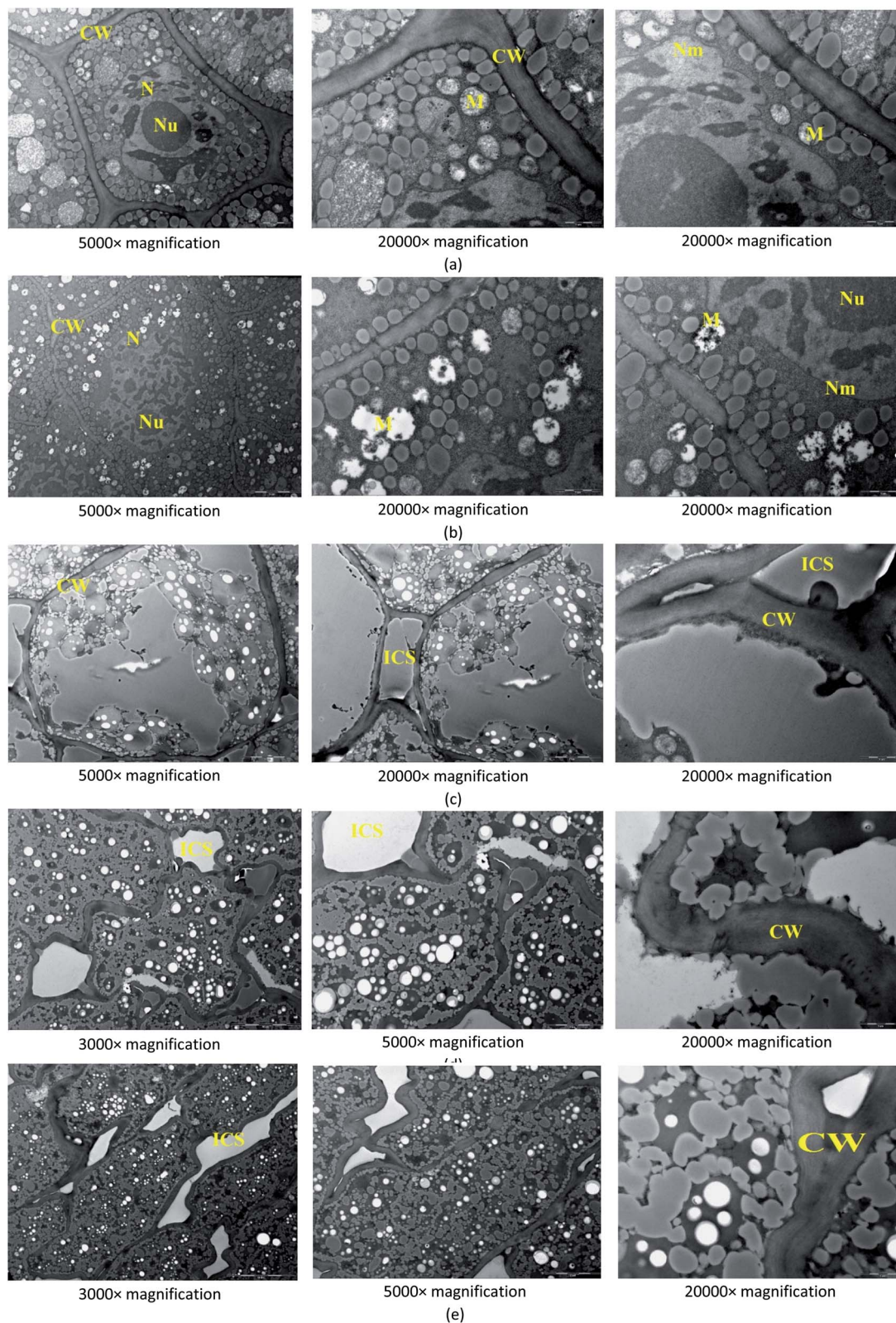


Fig. 6 Microstructure of embryo of wheat seeds before and after storage: (a) Day 0; (b) Day 6; (c) Day 9; (d) Day 12; (e) Day 24. (CW, cell wall; ICS, intercellular space; M, mitochondria; N, nucleus; Nu, nucleolus; Nm, nucleus membrane).

extended, the internal structure of endosperm can be seriously damaged. As shown in Fig. 5(f), on Day 12, the damages of protein matrices in endosperm were observed, resulting in

some broken protein matrices. Fig. 5(h) demonstrated that large starch granules dropped from the endosperm when the storage period was extended 24 days. Therefore, in the storage of



wheat seeds with high moisture content (20%), serious destruction of endosperm was observed during the 24 day storage. Fig. 5(c), (e), (g), and (i), which reflected the internal structures of endosperm at 2000 \times magnification, also confirmed the results concluded based on Fig. 5(b), (d), (f), and (h).

3.3.2. Microstructure of embryo. Fig. 6(a) showed that wheat seeds before storage had complete structures, containing cell wall, nucleus, nucleolus, nucleus membrane, and mitochondria. The cell organs were compacted closely in the intracellular environment, suggesting that the cells of wheat seeds were well protected before storage. After 6 day storage, the compaction of cell organs became loose (Fig. 6(b)). When the storage periods were extended to 12 days and 24 days, some intracellular spaces were observed (Fig. 6(d) and (e)). The main reason for this phenomenon is that some cell organs degraded under the unfavorable conditions during storage, resulting in large intracellular spaces.^{1,2} Comparison between Fig. 6(a) and (e) showed that the complete cell structure of wheat seed was transformed into disordered status after 24 day storage. In addition, the embryo of wheat seeds after 24 day storage did not have complete cell wall, suggesting that the structure of embryo was seriously destroyed. Consequently, the damage of embryo, which determines the germination capacity, may result in the low germination efficiency and low bioactivity of wheat seeds.

Generally, a variety of factors can contribute to the damage or deterioration of wheat seeds during storage.^{30,31} Firstly, the fungal activities can damage the cell structure of wheat seeds and further cause the nutrients loss in endosperm. Secondly, the internal deterioration caused by the intensive respiration of

wheat seeds may cause the nutrients loss in endosperm. As a result, with the damage of endosperm and embryo, the biochemical properties of wheat seeds would be negatively impacted. To further explore the negative effects of storage on the biochemical properties of wheat seeds with different moisture contents, germination efficiency, water absorption of gluten, farinograph properties, and extensograph properties were measured.

3.4 Effects of moisture contents on the biochemical properties of wheat seeds

3.4.1. Germination efficiency and water absorption of gluten. Germination efficiency, which reflects the biological activity, is an important parameter that should be considered during the storage of wheat seeds.³² Fig. 7(a) showed that from Day 0 to Day 30, the germination efficiency of wheat seeds with 16% moisture content ranged between 80% and 100%. However, after Day 30, dramatic drop of the germination efficiency was observed. The main reason for this phenomenon is that wheat seeds with low moisture content in short-term storage had good status owing to the mild respiration and inactive fungi. However, with the extension of storage period, the fungal growth and continuous respiration would damage the internal structures of wheat seeds, resulting in the decrease of germination efficiency. Interestingly, wheat seeds collected from the upper layer had higher germination efficiency than those collected from the lower layer.

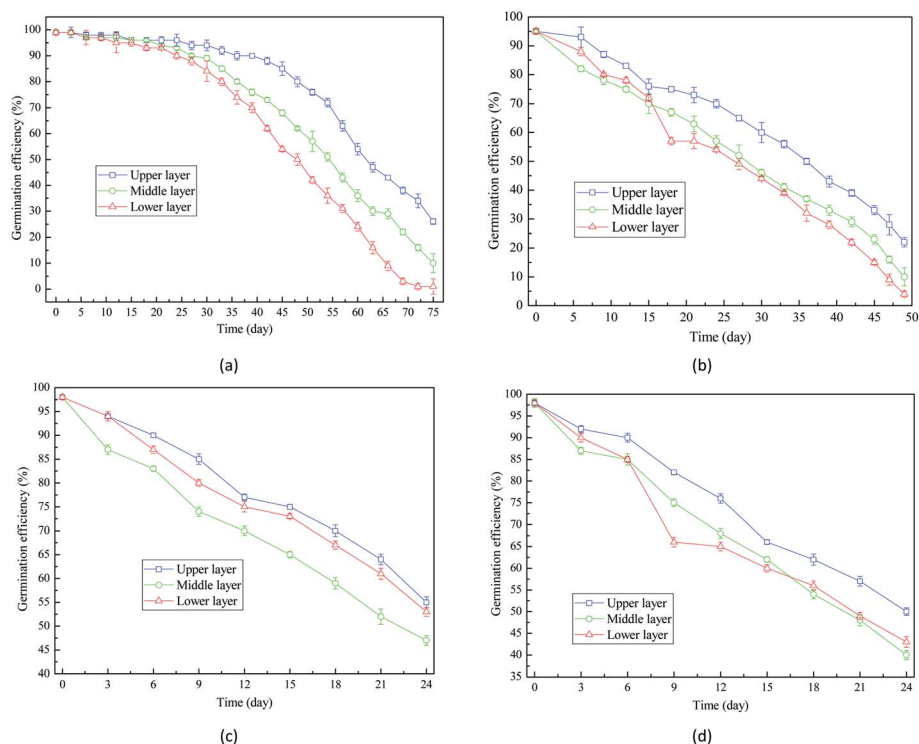


Fig. 7 Germination efficiency of wheat seeds at different time during storage: (a) 16% moisture content; (b) 18% moisture content; (c) 20% moisture content; (d) 22% moisture content.



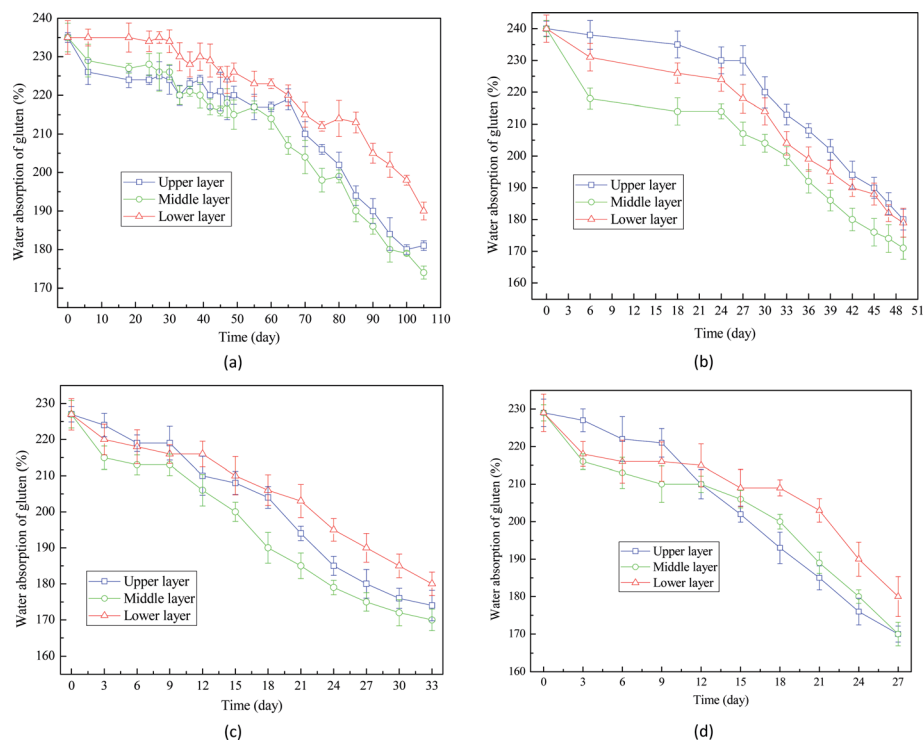


Fig. 8 Water absorption of gluten at different time during storage: (a) 16% moisture content; (b) 18% moisture content; (c) 20% moisture content; (d) 22% moisture content.

Comparison between Fig. 7(a) and (b) indicated that when the moisture content increased from 16% to 18%, the decrease rate of germination efficiency was improved. Fig. 7(d) showed that the germination efficiency of wheat seeds with 22% moisture content decreased to around 40% after 24 day storage. Therefore, the extension of storage period is detrimental to the

germination capacity of wheat seeds and the high moisture content accelerates the decrease of germination efficiency.

Similar trends were also observed in the analysis of water absorption of gluten (Fig. 8). It took around 103 days, 49 days, 28 days, and 24 days to reduce the water absorption of gluten of wheat seeds with 16%, 18%, 20% and 22% from 230% to 170%,

Table 2 Farinograph properties of wheat seeds with different moisture contents during storage

Moisture content	Storage time (day)	Development time (min)	Maximum consistency (FU)	Water absorption (%)	Stability time (min)	Softening (FU)
16%	0	3.19 ± 0.25	493 ± 12	66.3 ± 3.5	2.40 ± 0.16	80 ± 2.9
	24	3.34 ± 0.19	487 ± 24	60.1 ± 4.9	2.82 ± 0.08	84 ± 4.5
	48	3.71 ± 0.26	502 ± 21	66.0 ± 1.5	3.26 ± 0.10	87 ± 3.8
	72	4.03 ± 0.09	507 ± 16	66.3 ± 1.9	3.47 ± 0.05	93 ± 2.7
	96	3.85 ± 0.12	501 ± 19	66.1 ± 2.5	3.79 ± 0.09	102 ± 5.9
18%	0	2.86 ± 0.33	507 ± 30	63.8 ± 2.7	2.61 ± 0.11	80 ± 4.2
	12	3.89 ± 0.31	483 ± 24	66.7 ± 2.6	4.05 ± 0.21	71 ± 1.5
	24	4.04 ± 0.28	492 ± 38	66.8 ± 3.3	5.57 ± 0.15	75 ± 1.9
	36	4.68 ± 0.15	513 ± 36	67.3 ± 3.8	5.71 ± 0.16	82 ± 2.8
	48	4.52 ± 0.17	495 ± 24	67.9 ± 1.5	5.13 ± 0.32	78 ± 2.6
20%	0	3.14 ± 0.12	467 ± 11	65.3 ± 2.4	2.73 ± 0.15	64 ± 3.5
	6	3.23 ± 0.22	485 ± 18	65.8 ± 3.6	2.85 ± 0.22	68 ± 3.9
	12	3.60 ± 0.25	494 ± 26	70.5 ± 1.1	3.73 ± 0.27	75 ± 4.1
	18	6.26 ± 0.16	488 ± 42	67.5 ± 2.8	4.61 ± 0.16	118 ± 4.8
	24	5.09 ± 0.31	483 ± 31	66.6 ± 4.2	4.31 ± 0.24	124 ± 3.8
22%	0	3.21 ± 0.32	502 ± 25	66.9 ± 4.1	2.98 ± 0.26	62 ± 3.5
	6	3.44 ± 0.22	506 ± 27	67.0 ± 3.8	3.02 ± 0.30	71 ± 2.9
	12	4.99 ± 0.28	493 ± 11	67.0 ± 3.2	4.96 ± 0.27	87 ± 3.1
	18	4.45 ± 0.26	508 ± 15	66.4 ± 1.5	5.37 ± 0.17	92 ± 1.5
	24	6.76 ± 0.40	482 ± 22	66.2 ± 2.8	4.82 ± 0.22	120 ± 4.9



suggesting that low moisture content has positive effects on the water absorption of gluten. This result is in accordance with the changes of microstructure of endosperm shown in Fig. 5. The main mechanism for the decrease of water absorption of gluten is that the broken protein observed in wheat seeds during storage could damage the properties of gluten.^{33,34}

3.4.2. Farinograph properties. As shown in Table 2, farinograph properties of wheat seeds with different moisture contents at different storage periods had obvious difference. When the moisture contents of wheat seeds were 16% and 18%, the stability time increased to 3.79 min and 5.13 min, respectively, after 96 day and 48 day storage. The increase of stability time indicated that the storage did not negatively impact the gluten strength of wheat seeds. However, the stability time started to dramatically decrease when the storage periods of wheat seeds with 22% moisture content were over 18 days, suggesting that long-term storage and high moisture content are not favorable to the gluten strength. At the end of the storage, owing to the activities of fungi, carbohydrate and protein in wheat seeds were consumed and the stability time and development time dropped to some extent.

No obvious changes were observed in the measurement of water absorption, suggesting that the water absorption capacity of wheat flour remained stable during the storage. The softening of wheat flour is a factor that reflects the softness of wheat dough. As shown in Table 3, softening values increased with the extension of storage periods and the increase of moisture content. For example, the softening value of wheat seeds with 22% moisture content increased from 62 FU to 120 FU when the storage period was extended to 24 days. In a real-world application, wheat flour with high softening values may not be suitable for the food production.

3.4.3. Extensograph properties. Table 3 showed that extensograph properties are also impacted by the storage period and moisture content of wheat seeds. Firstly, with the increase of moisture content and storage time, the area increased. Besides, the resistance and the maximum resistance of wheat dough were improved during the storage. These results indicated that with the increase of dough strength, the wheat seeds might not be suitable for the food production since the high strength of dough could hinder the fermentation process. Secondly, extensibility of wheat seeds with low moisture contents remained stable while high moisture content promoted the increase of extensibility during the storage. For example, when the fermentation time of wheat seeds with 16% moisture content was 45 min, extensibility of wheat dough increased from 117 mm to 119 mm in the 96 day storage. However, when the moisture content of wheat seed was 22%, extensibility of wheat dough increased from 107 mm to 121 mm in 24 days. Thirdly, the values of *R/E* and max *R/E* increased with the extension of storage periods. This result showed that resistance capacity of the dough made of wheat seeds after storage increased.

4 Discussion

4.1 Problems caused by high moisture content

High moisture content poses a serious threat to the storage of wheat seeds. Firstly, moisture can accelerate the cell respiration of wheat seeds during storage, resulting in the temperature increase of grain bulk. As a consequence, the high temperature of the grain bulk will increase the deterioration rate of grain and negatively impact their biochemical properties.³⁵ Secondly, with the continuous respiration of wheat seeds during storage, air

Table 3 Extensograph properties of wheat seeds with different moisture content during storage

Moisture content	Storage time (day)	45/90/135 min					
		Area (cm ²)	Resistance (BU)	Extensibility (mm)	Maximum resistance (BU)	<i>R/E</i>	Max <i>R/E</i>
16%	0	25/34/40	138/182/232	117/122/114	139/183/235	1.2/1.5/2	1.2/1.5/2.1
	24	29/33/39	141/181/186	113/117/119	142/181/188	1.2/1.5/1.6	1.3/1.5/1.6
	48	43/41/45	241/237/245	114/114/118	241/239/245	2.1/2.1/2.1	2.1/2.1/2.1
	72	44/46/51	267/267/279	109/116/120	263/269/281	2.4/2.3/2.3	2.4/2.3/2.3
	96	47/49/53	249/251/273	119/121/124	249/253/274	2.1/2.1/2.2	2.1/2.1/2.2
18%	0	24/31/37	134/176/213	114/117/114	141/176/213	1.2/1.5/1.9	1.2/1.5/1.9
	12	29/37/45	182/231/279	104/107/109	182/231/280	1.7/2.2/2.5	1.7/2.2/2.6
	24	27/33/45	156/195/233	112/109/110	156/196/234	1.4/1.8/2.1	1.4/2.8/2.1
	36	37/42/44	206/228/257	116/121/111	206/230/258	1.8/1.9/2.3	1.8/1.9/2.3
	48	31/38/43	174/210/234	116/117/121	174/211/236	1.5/1.8/1.9	1.5/1.8/1.9
20%	0	21/27/31	120/161/179	103/114/117	121/164/183	1.2/1.4/1.5	1.2/1.4/1.6
	6	21/33/39	122/183/236	108/120/108	129/184/237	1.1/1.5/2.2	1.2/1.5/2.2
	12	28/36/41	149/188/216	118/121/123	149/190/218	1.3/1.6/1.8	1.3/1.6/1.8
	18	43/46/48	222/241/264	126/126/124	226/247/268	1.8/1.9/2.1	1.8/2.0/2.2
	24	34/40/42	178/206/218	120/124/125	180/212/226	1.5/1.7/1.8	1.5/1.7/1.8
22%	0	27/31/39	162/207/221	107/109/111	162/208/223	1.5/1.9/2.0	1.5/1.9/2.0
	6	29/37/42	167/218/260	113/112/109	169/218/261	1.5/2.0/2.4	1.5/2.0/2.4
	12	37/46/47	224/268/280	108/116/119	225/268/283	2.1/2.3/2.4	2.1/2.3/2.4
	18	35/46/47	202/264/282	114/114/112	202/264/282	1.8/2.3/2.5	1.8/2.3/2.5
	24	45/46/45	221/245/252	121/124/118	225/253/257	1.8/1.8/2.1	1.9/2.3/2.2



humidity in storage facilities will be improved to a higher level. Both high moisture content in wheat seeds and high air humidity favor the growth of fungi in grain bulk. The metabolisms of some fungi can damage the complete structure of wheat seeds and obtain nutrients from wheat seeds. Also, the fungal activities can further promote the temperature increase in storage facility.³⁶

This study firmly confirmed aforementioned two explanations. According to the analysis of temperature changes, it was discovered that grain bulk with higher moisture content had higher temperature. The dramatic increase of temperature was partly attributed to the intensive cell respiration of wheat seeds during storage. In addition, the fast growth of fungi and the damage of cell structures were observed, confirming that the fungal activities are correlated with the deterioration of wheat seeds during storage.

The negative effects of moisture on the biochemical properties of wheat seeds indicate that the storage process should be strictly controlled. Otherwise, the deterioration and mildewing would jeopardize the utilization of wheat seeds. On one hand, the germination efficiency of wheat seeds with 20% moisture content decreased to about 50% after 24 day storage. In China, the storage period of wheat seeds can be as long as four years. As a result, after the long-term storage, the wheat seeds would not be used for seeding. On the other hand, due to the nutrients loss and microstructure damage, extensograph properties and farinograph properties of flour made of wheat seeds after storage are negatively impacted.³⁷ Consequently, the wheat seeds after storage may not be suitable for further processing in food industry.

4.2 Strategies for the wheat seeds storage

To mitigate the negative effects of moisture content on the wheat seeds during storage, some strategies are available. In a real-world application, these strategies could be adapted according to the actual situations.

Firstly, moisture content of wheat seeds can be reduced to a lower level before the storage by appropriate dehydration process. Wheat seeds with lower moisture content would be more suitable for storage since the fungal activities and the cell respirations could be hindered under the low-moisture condition. However, since the grain with low moisture content could not be directly used for food production, the wheat seeds after storage should be remoisten before further processing in food industry. This step may make the wheat seeds processing more complicated and cost-consuming.

Secondly, air conditioning can be applied to prevent the dramatic increase of temperature during the long-term storage. Under the low temperature, fungal growth and metabolism would be limited, lowering the threats to wheat seeds storage. According to previous studies, low-temperature maintenance has been proven to be a good way for long-term storage of wheat seeds or rice seeds.^{2,38} However, the cost and energy consumption of maintaining low temperature in the huge storage facilities are high. As a result, the large-scale grain storage may not be affordable.

Thirdly, ventilation is another feasible method to limit the fungal growth and reduce the risks caused by high moisture content.³⁹ It was reported that ventilation can accelerate the air flow in storage facilities and hinder the fast growth of fungi and insects.⁴⁰ In most cases, the internal circulation ventilation is integrated with the utilization of fumigation as an effective way to kill fungi and insects in the grain bulk.⁴¹ The disadvantages of this technology include the potential contamination by residual fumigants and the moisture loss by ventilation.

5 Conclusions

It is concluded that (1) moisture plays a key role in the storage of wheat seeds by impacting the biochemical properties, fungal growth, and temperature fluctuation. (2) High moisture content promoted the temperature increase in grain bulk and shortened the storage period of wheat seeds. (3) During the storage, microstructures of endosperm and embryo were damaged in the wheat seeds with high moisture content. (4) Fungal growth in grain bulk was observed to be correlated with the moisture content of wheat seeds. (5) With the damage of internal structures and the nutrients loss, wheat seeds had low germination efficiency and poor extensograph properties and farinograph properties. Thus, the utilization of wheat seeds in downstream industry would be seriously hindered. In a real-world application, therefore, strategies, such as moisture content reduction, low-temperature maintenance, ventilation, and fumigation, should be adapted appropriately for the storage of wheat seeds with high moisture content.

Conflicts of interest

There is no conflict to declare.

Acknowledgements

This work was supported by the funding from National Key R&D Program of China (Grant No. 2016YFD0401005).

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