Journal of Stored Products Research 72 (2017) 161–165

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Physiochemical changes in wheat of different hardnesses during storage

Shuai-Bing Zhang, Yang-Yong Lv, Yu-Li Wang, Feng Jia, Jin-Shui Wang, Yuan-Sen Hu*

College of Biological Engineering, Henan University of Technology, Zhengzhou 450001, China

ARTICLE INFO

Article history:
Received 26 August 2016
Received in revised form
11 May 2017
Accepted 11 May 2017
Available online 19 May 2017

Keywords:
Wheat storage
Physiochemical properties
Wheat hardness
Artificial aging

ABSTRACT

We compared physiochemical changes in wheat cultivars of different hardnesses during long-term storage. Respiration, ATP content, germination ability, expansin gene expression, and the activities of dehydrogenase, catalase, and peroxidase were studied in soft wheat ('Zhengmai 004'), medium hard wheat ('Aikang 58'), and hard wheat ('Zhengmai 9023'). Wheat cultivars were stored under natural conditions for one year or harvested fresh and then stored for eight weeks either naturally or with artificial aging. Enzymatic activities decreased about 10% during one year of natural storage. Physiochemical properties of fresh and naturally aged wheats did not change significantly in eight weeks of further storage at 12% moisture content (MC) and 30 °C, which suggests that wheat stores well. However, physiochemical quality deteriorated rapidly in artificially aged wheat stored at 12% MC and 30 °C. Changes were relatively small in the soft wheat cultivar, with low basal metabolism and slow reduction of physiological enzymes indicating that soft wheat may have better storage tolerance than harder cultivars. This study provides important data for determining storage tolerances of different wheat cultivars.

1. 1Introduction

Wheat (*Triticum aestivum*) is one of the main staple cereal grains used in the preparation of food products worldwide (Lim, 2013). In China, post-harvest wheat is usually stored long-term, 3–5 years, in large warehouses to guarantee food supply in the event of crop failure on a massive scale (Zhang et al., 2014). During long-term storage, the physiochemical properties of wheat change naturally even under standard safe storage conditions, which negatively affects the edibility and processing quality of the wheat (Dell'Aquila, 1994; Varzakas, 2015). Therefore, understanding physiochemical deterioration of wheat is of great importance to minimize the deterioration in quality of stored wheat.

Stored wheat is a living, albeit dormant, organism. During storage, metabolism continues and the plant consumes its own nutrient stores to produce energy (Matthews et al., 2012). Meanwhile, physiochemical properties change due to free radicals and other cytotoxic metabolites that cause a deterioration in quality during long-term storage (Walters, 1998). Wheat has characteristics of relaxation of tissue structure, loss of nutrients, and reduction of enzymatic activities and seed vigor after long-term storage, collectively called "aging" (Kibar, 2015). Kernel hardness is an important characteristic of wheat that has a profound effect on overall quality and end-uses. The kernel texture and biochemical composition, including starch granule texture, protein content, pentosan content, and especially puroindoline content vary among wheat of different hardnesses (Pasha et al., 2010). Although it is obvious that physiochemical changes occurs in stored wheat, the details and differences in these changes among wheat cultivars are still unclear.

In this study, we investigated the physiochemical changes in stored wheat cultivars of different hardnesses. We focused on critical enzymatic activities, CO_2 production, ATP content, germination ability, and the level of expression of the expansin gene. These characteristics were compared in three cultivars under short- and long-term natural storage and artificial-aging storage. The results provide valuable information for understanding the physiochemical deterioration of stored wheat.

* Corresponding author. College of Biological Engineering, Henan University of Technology, 1 Lianhua Street, Zhengzhou, Henan 450001, China.
E-mail address: hys308@126.com (Y.-S. Hu).

http://dx.doi.org/10.1016/j.jspr.2017.05.002
0022-474X/

2. Material and methods

2.1. Materials and chemicals

Three wheat cultivars of different hardnesses—'Aikang 58' (soft), 'Zhengmai 9023' (medium hard), and 'Zhengmai 004' (hard)—were purchased from Fengle Seed Company in Zhengzhou, China. Wheat that was harvested in 2013 and stored at ambient temperature for one year was used as our naturally aged wheat. Fresh wheat was newly harvested just before the experiment began in 2014. ATP content test kits were purchased from Cominbio (Suzhou, China). All other reagents were obtained from general commercial suppliers and used without further purification.

2.2. Grain storage

The three fresh and three naturally aged wheat cultivars were all adjusted to 12.0% moisture content and stored in a constant-temperature and -humidity chamber (KBF720, Binder, Germany) at 25 °C and 60% relative humidity. To accelerate the aging process, 200 g samples of the three fresh cultivars were adjusted to 12.0% moisture content, loaded into 1.5 L plastic bottles, and stored at 50 °C and 40% relative humidity. Subsamples of each wheat were withdrawn and their physiochemical properties analyzed once a week for eight weeks.

2.3. Enzymatic assays: dehydrogenase, catalase, and peroxidase

Dehydrogenase activity was determined using 2,3,5-triphenyltetrazolium chloride as a substrate (Jensen et al., 1951). The reaction was carried out as follows: 3 g wheat kernels were immersed in 50 mL distilled water for 8 h at ambient temperature. The germs of the kernels were peeled off and stained in 10 mL 1% 2,3,5-triphenyltetrazolium chloride (TTC) solution for 12 h. Stained wheat germs were washed three times with distilled water. Red-colored triphenyltetrazolium formazan (TTF), the reduction product of TTC, was extracted with 10 mL 95% ethanol for 12 h at 37 °C. The TTF was quantified by measuring absorbance at 490 nm wavelength. The enzymatic activity of dehydrogenase was defined as the absorbance at 490 nm under the reaction condition.

Catalase activity was determined by the potassium permanganate ($KMnO_4$) titration method, following Chinese National Standard protocol BG/T 5522-2008. Wheat kernels were milled and the moisture content of the resulting powder was measured. One gram of wheat powder was dissolved in 50 mL pH 7.7 phosphate buffered saline. The wheat powder solution was shaken for 5 min and allowed to stand for 3 h, and then the supernatant was filtered through a 0.22 nm MF-Millipore membrane (Millipore, Bedford, MA, USA). A reaction solution containing 20 mL filtered solution, 20 mL distilled H_2O, and 3 mL 2% H_2O_2 was held in a 30 °C water bath for 15 min. To stop the reaction, 5 mL 10% sulfuric acid was added, then 0.2 mol/L $KMnO_4$ was added to titrate the residual H_2O_2.

A control experiment was performed as above, except that the filtered solution was boiled for 1 min to inactivate the catalase. Catalase activity was calculated by $\boldsymbol{X = (V_0 - V_1) \times c / (m - (100 - M)) \times 17 \times (50/20) \times 100}$. **X** is the amount of catalase (mg). $\mathbf{V_0}$ is the volume of $KMnO_4$ consumed in the control experiment (mL). $\mathbf{V_1}$ is the volume of $KMnO_4$ consumed in the reaction (mL). **c** is the concentration of the $KMnO_4$ (mol/L). **m** is the weight of the wheat powder sample (g). **M** is the moisture content of the sample (%). Last, **17** is the volume of H_2O_2 consumed per 1 mL $KMnO_4$ consumed. The remaining terms are conversion factors.

Peroxidase activity was determined using a reaction mixture (3 mL final volume) containing 13 mM H_2O_2 and 124 mM guaiacol in 50 mM Tris, pH 7.0, at 30 °C for 10 min. The reaction was started by adding the enzyme. The increase in absorbance at 470 nm that was caused by the formation of tetraguaiacol was measured throughout the reaction. One unit of enzymatic activity was defined as the amount of the enzyme required for the production of 1 mmol of tetraguaiacol per minute in the reaction conditions (Carusoa et al., 2001).

2.4. Evaluation of CO_2 production and ATP content

The concentration of CO_2 produced by wheat respiration was measured using a portable infrared CO_2 detector (GXH-3010E, Huayun, Beijing, China) and previously reported methods (Zhang et al., 2014). For ATP determination, the wheat germs were peeled off the kernels as described above and milled into powder in liquid nitrogen. The ATP was extracted with an ATP content test kit according to the manufacturer's directions. In brief, 0.1 g germ powder was mixed with 1 mL 0.6 mol/L perchloric acid and centrifuged for 15 min at 4000 rpm, and then 1 mol/L NaOH was added to adjust the pH of the supernatant to 7.0. The solution was centrifuged again for 15 min at 4000 rpm and filtered through a 0.22 nm MF-Millipore membrane. The ATP content was measured with HPLC using a Luna C18 reverse-phase column (Phenomenex, Torrance, CA, USA). A mobile phase of phosphate buffer saline acid was used and absorption at 295 nm was recorded using a PDA detector.

2.5. Quantitative analysis of expansin gene expression

Total RNA was extracted from wheat germs using TRIZol reagent (Invitrogen, Carlsbad, CA, USA), then subjected to DNase I (New England Biolabs, Ipswich, MA, USA) digestion and purification. The purified RNA was used to synthesize the first-strand cDNA using a RNA PCR kit (TaKaRa, Dalian, China) according to the manufacturer's directions. Real-time PCR was performed with the ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using a SYBR® Premix EX-Taq™ II kit (Takara, Japan). An ADP-ribosylation factor was used as an internal control (Paolacci et al., 2009). The primers EXPB2F: 5′-TGTTCAAGGACGGCAAGGG-3′, EXPB2R: 5′-CCGCTCAGGTCGAAGTGGT-3′, and ADP-F: 5′-GCTCTCCAACAACATTGCCAAC-3′, ADP-R: 5′-GCTTCTGCCTGTCACA-TACGC-3′ were used for the amplification of the expansin gene and the internal control gene, respectively.

2.6. Seed germination test

Two layers of sterile filter paper (Whatman No. 1) were saturated with sterilized water and placed on the bottom of a petri dish (9 cm diameter). Fifty seeds were chosen randomly, placed on the filter paper, and incubated in an artificial climate chamber at 20 °C and a light intensity of 900 Lux. The seed germination rate was calculated as the percentage of seeds geminated after 7 days of incubation.

3. Results

3.1. Physiological enzymatic activity

Three critical enzymes—dehydrogenase, catalase, and peroxidase—are thought to be closely related to the physiological properties of crop seed (Shaban, 2013). The activities of the three enzymes in naturally aged wheat cultivars were about 10% lower than their activities in fresh wheat. The sole exception was that the