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The relationship between the catalase activity and physicological and quality changes of stored wheat

Feng Jia, Jin-Shui Wang*
College of Bioengineering, Henan University of Technology, Zhengzhou 450001, (CHINA)
E-mail: jinshuiw@163.com

ABSTRACT

Conventional detection methods of microbes and quilty of wheat are very time-consuming and labor-intensive not to meet the demands of the food industry. In this study, we successfully built the relationship between the catalase and protease activities in stored wheat samples. There was a simple linear relationship between the catalase activities and temperature during the storage in wheat (R^2=0.9699). What's more, the closely correlation (R^2 = 0.9536) exists between catalase and protease activity of wheat samples. Moreover, the results showed inverse relation between mechanical properties of dough and gluten except negative area index. The aging of wheat suggested that the storage conditions affected the endogenous enzymes activities of wheat to change the physiological and mechanical properties. Therefore, the changes of the catalase activity could suggest the physicological and quality changes of stored wheat.

KEYWORDS

Wheat;
Catalase activity;
Protease activity;
Temperature;
Moisture.

INTRODUCTION

Conventional methods rely on the cultivation of microbes for their multiplication to visible colonies, which are very time-consuming and labor-intensive not to meet the demands of the food industry[1]. Moulds, kinds of fungi, are pervasive in nature, which are currently crucial in the production of food (i.e., cheese, bread, sausage, soy sauce and alcoholic beverages) and medications (i.e., penicillin and cyclosporine). They do not utilize photosynthesis to obtain energy and recycle nutrients within ecosystems. However, a few of mould species, which can secrete mould toxicity, has a causative link with human disease being established, such as infection, allergic, and toxicity[2].

The growth cycle of winter wheat is long, almost over the fall autumn, winter and spring. In the winter, wheat might be inoculated with snow mould [3, 4]. In high temperature and humidity autumn, fungal contamination results in terrible problem for wheat storage. The most common fungus in Chinese wheat are *Aspergillus*[5], *Penicillium*[6] and *Fusarium*[7], which may produce the mycotoxins aflatoxin (AFL), ochratoxin (OTA), fumonisin (FUM) and deoxynivalenol (DON) under the suitable condition for fungi in wheat storage. These toxins threaten to human health, such as anorexia, vomiting, diarrhea,

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fever, hepatocyte poisoning, carcinogenic and teratogenic.

During the wheat storage, the temperature and moisture are the primary factors influencing the growth and development of mould[8]. Normally, the mould will develop slowly at low temperature and low humidity conditions. Insteadly, at high temperature and high humidity conditions, it will grow quickly. In fact, the conditions of low temperature and low humidity will cost a lot of energy. Wheat will deteriorate during storage, thus results in decrease in processing quality and eating quality of wheat. There were closely correlation between the microbial counts and catalase activity (R^2=0.98)[9]. Therefore, the catalase activity can be used to indicate the microbial counts in the storage wheat.

The protease of wheat could digest the proteins of gluten to water soluble protein, and the reduction in viscosity and foam stability were related to the extent of hydrolysis of high molecular mass glutenin protein[10]. EP3 protease was suggested to be a metal-dependent serine protease, because its activity was inhibited by serine protease inhibitors PMSF, AEBSF and metal related protease inhibitor EGTA [11]. Moreover, heat stress-induced decrease in catalase and increase in protease activities can be used efficiently as biochemical markers to assess the relative heat stress tolerance of wheat genotypes at the seedling stage[12].

Viscoelasticity of the dough is a crucial property that related to dough sheeting characteristics, [13]and dough viscoelasticity increased at 4 °C storage[13]. Cooked gluten viscoelasticity (CGV) was expressed as relative recovery calculated from the creep curve of the Viscoelastograph[14]. Gama-gliadins was evidenced for a direct causal effect of low molecular weight subunits of glutenins on gluten viscoelasticity in durum wheats[15]. The viscoelasticity of gluten was evaluated by determining the number of significant crossover interactions between pairs of genotypes and environments[16].

In this study, we aim to investigate the relationship of the microbial counts, catalase activity, protease activity, and the viscoelasticity properties of the dough and gluten under various temperature or moisture, in order to understand the mechanism of microbes affected grain quality during wheat aging

MATERIALS AND METHODS

Samples, samples preparation and characterization

Wheat (*Triticum aestivum, Zhengmai 366*), used in this work without visual signs of insect damage, were obtained from the seed company in 2012-2013. Wheat characteristics were the crude protein 11.6 (based on 9.6% moisture content). Wheat moisture contents were determined by AACC Method 44-15 [17]. The wheat was regulated by suitable sterile water at 10°C for 48 h to the different moisture contents (12.0%, 15.0% and 18.0%).

Nine treatments were conducted according to orthogonal experiment with three factors and three levels including temperature, moisture and their interactions. The factors and levels, and nine treatments of the orthogonal experiment were showed in TABLE 1 and TABLE 2. The wheat was kept in different temperature and humidity chambers for 30 days. Moreover, five treatments were conducted in this experiment with 15.0% moisture content wheat for 5, 10, 15, 20, 25 d at 30°C temperature, respectively.

Determination of catalase activities

The catalase activities were performed with the methods based on the SK. WJH - 1 Microbial activity detector by Cai et al.[8]. And determination of microbiological analysis was carried out from wheat using the method of Zhang et al.[9]. Briefly, the 100 g of treated wheat were rinsed with 500 ml distilled water and detected the catalase activity with the Microbial activity detector.

Determination of protease activity of storage wheat

TABLE 1 : Factors and levels of orthogonal experiment of the catalase activity

levels	Temperature/°C	Moisture/%
1	20	12.0
2	30	15.0
3	40	18.0

TABLE 2 : Design of orthogonal experiment of the catalase activity

Treatments	A Temperature/°C	B Moisture/%	C Temperature×Moisture	D Vacancy
1	1	1	1	1
2	1	2	2	2
3	1	3	3	3
4	2	1	2	3
5	2	2	3	1
6	2	3	1	2
7	3	1	3	2
8	3	2	1	3
9	3	3	2	1

Protease activity was determined by the casein digestion assay described by Drapeau [18] and Hameed et al.[12]. In this method 1 U is indentified as the amount of enzyme that releases acid-soluble fragments equivalent to 0.001 A_{280} per minute at 37°C and pH 7.8. Enzyme activity was expressed on a grain weight basis.

Determination of viscoelasticity properties of the dough and gluten

Water was added as required to give a Brabender Farinograph consistency of 500720BU (ICC Standard 115). The dough mixing was performed in a recording National Mixograph with 20 g of flour. Viscoelasticity properties of the dough and glutens were determined by TA-XT2i texturometer (Stable Microsystems, Surrey, UK) with probe code P/ 0.5R. The performance was assessed under the following conditions: pre-test speed, 2.0 mm/s; test speed, 2 mm/s; post-test speed, 10.0 mm/s; Strain : 70%; trigger force, auto-5g; data acquisition rate, 200 pps.

Statistical analysis

Analysis of variance (ANOVA), using the DPS 6.05 with orthogonal contrasts, was performed to detect the data obtained of treatments in this paper. Fisher's least significant difference (LSD) test was used to describe the means at 5% significance level (P<0.05).

RESULTS AND DISCUSSION

The results of catalase activity after different treatments

The catalase activity of wheat had a significantly difference in the nine treatments Figure 1. The catalase activities of wheat in 40°C were the highest in this experiment. The catalase activities of wheat in 20°C were lowest. The catalase activities and the temperature of wheat during storage were not in a linear relationship. The results of the catalase activity showed that it increased with temperature increase and it maybe reach the highest activity at 40°C Figure 1, whereas, its expression was the lowest at 20°C. The catalase activity would suggest the microbial counts in the storage wheat, because there were good correlation between the microbial counts and catalase activity (R^2=0.98)[9].

The changes of the catalase activity after different treatments

Their levels of catalase activities are shown in TABLE 3. Results of range analysis showed that effects of various treatments on wheat catalase activ-

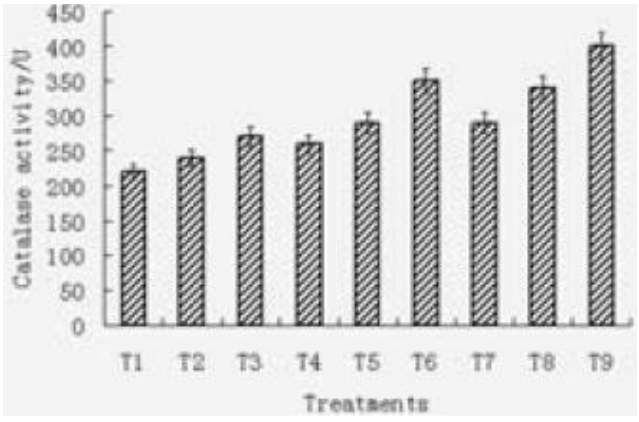

Figure 1 : The catalase activity of wheat after different treatments Lines from 1 to 9 are treatments, as showed in TABLE 2

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TABLE 3 : Analysis of the catalase activity of wheat after different treatments

Means	The catalase activity of wheat		
	Temperature	Moisture	Temperature×Moisture
K_1	243.33 a	256.67a	303.33 a
K_2	300.00 a	290.00 a	300.00 a
K_3	343.33 a	340.00 a	283.33 a
Range	100.00	83.33	20.00

Notes : K_is are the means of treatments. An i shows the different levels of treatments; Mean values followed by the same letter are not significantly different at $P < 0.05$.

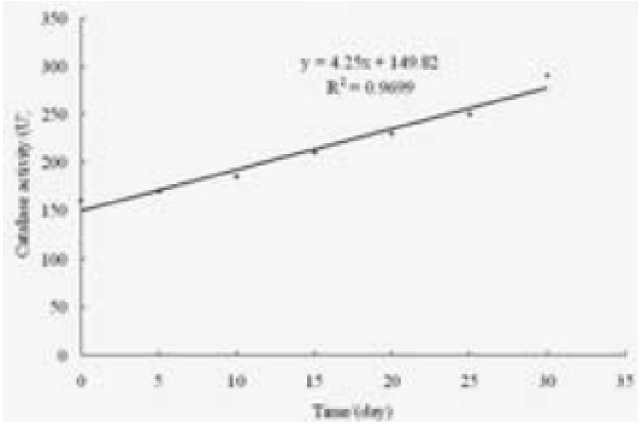

Figure 2 : The catalase activity of storage wheat with 15.0% moisture at 30°C

ity was: Temperature>Moisture>temperature ×moisture. The analysis of variance results showed that the temperature was the crucial factor to affect the catalase activity. However, its effect was not significant at $P < 0.05$ in three levels using Duncan's test TABLE 3. Moreover, the result also revealed that the effect of interaction between temperature and moisture was significant than the effect of moisture. Moreover, The results of temperature treatments showed that there was a simple linear relationship between temperature and catalase activities in wheat (R^2=0.9699) Figure 2. The results of catalase activities may be associated with species of mould.

The results of protease activity after different treatments

Protease activity significantly increased with different temperature treatments Figure 3. The highest level of protease activity was observed in T9 and the lowest was in T1. A significant positive correlation (R^2 = 0.9536) was observed in the increase of percentages in protease activity and catalase activity Figure 1. This indicated that more proteolytic

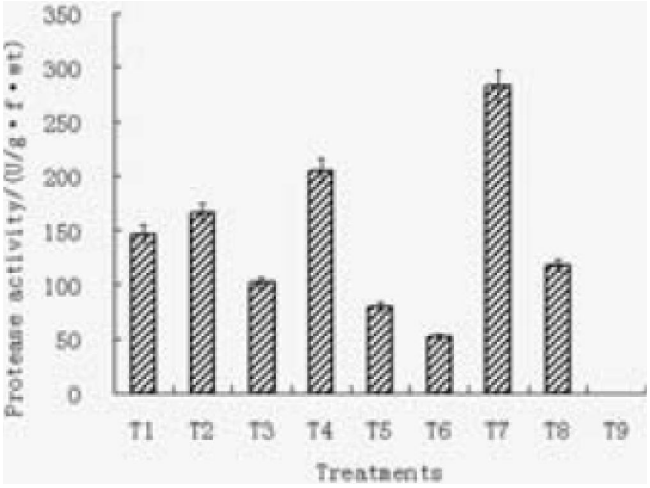

Figure 3 : Effect of temperature on protease activity in different treatments

enzymes were produced in higher temperature.

On the one hand, previous investigations have shown that the different temperature (warm-cold alternating) could change the activities of guaiacol peroxidase (GPX), superoxide[19]. The α-amylase activity of wheat had dramatic change in after-ripening stage after storage at room temperature[20]. Moreover, some studies showed that the electrical conductivity of wheat grain increased during artificial aging[21, 22].

The changes of viscoelasticity of dough and gluten after different treatments

Figure 4 showed that the maximal area (force) in dough was T9, and T7 in gluten. The integrated areas of the dough by the force increased with the increase of water at 20°C and 40°C, and the integrated area of the force of the dough was maximum at moisture of 15% at 30°C Figure 4. The treatment of T9 could not elute the gluten Figure 5. In the whole nine processing, the largest force integral area of the dough was at 40°C and 20% moisture Figure 5.

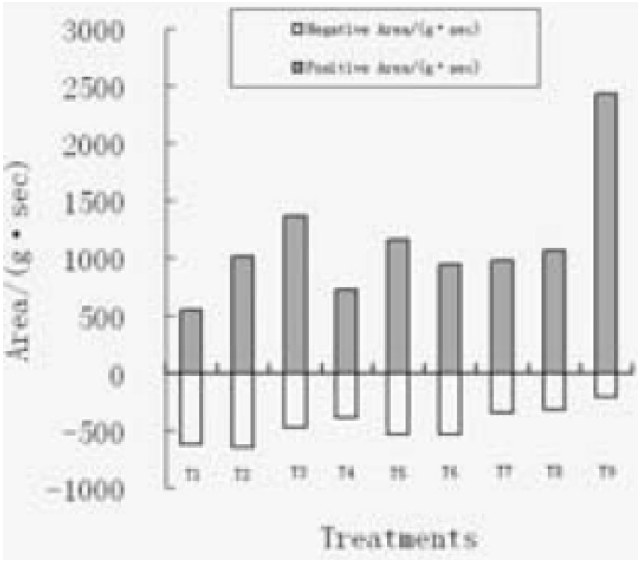

Figure 4 : Effects of temperature and moisture on dough stickiness

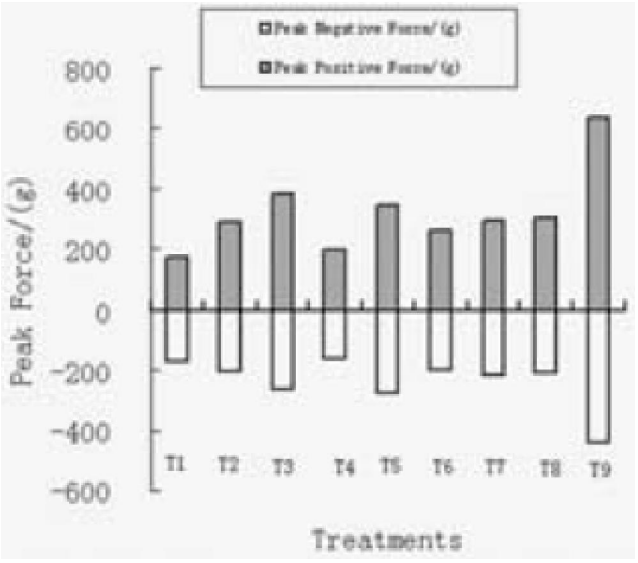

Figure 6 : Effect of temperature and on the hardness of the dough

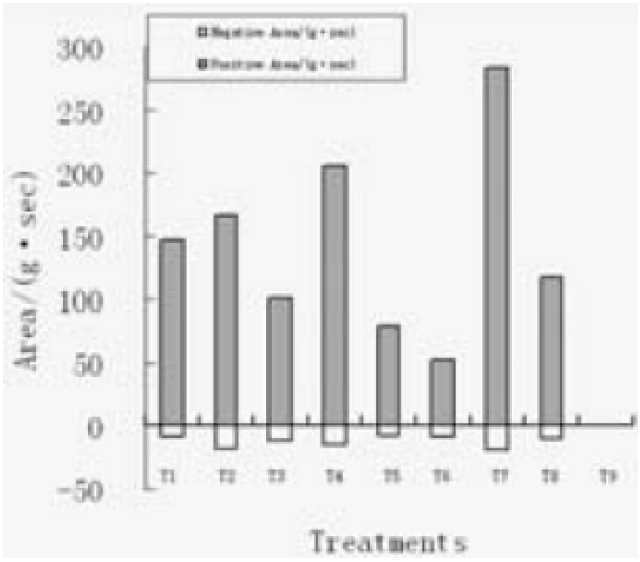

Figure 5 : Effects of temperature and moisture gluten stickiness

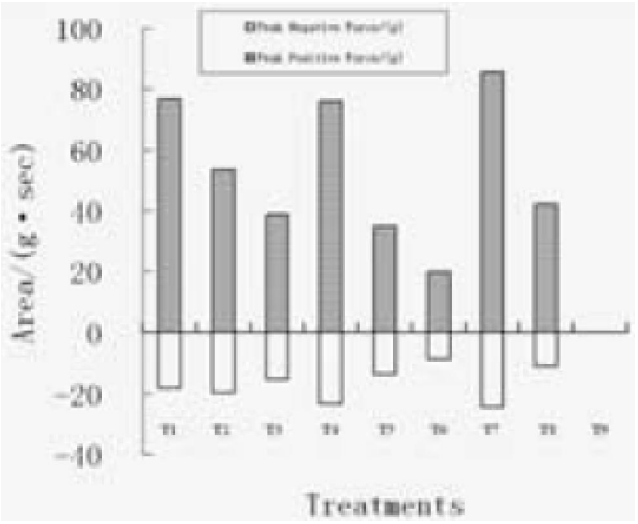

Figure 7 : Effect of temperature and moisture on the hardness of the gluten

Figure 6 showed the positive pressure (Hardness) changes of wheat dough, which the positive pressure of the dough increased with increasing moisture in the storage temperature at 20°C and 40°C. However, the temperature of wheat in storage is at 30°C, the positive pressure of the dough reached to maximum in moisture content of 15%. And in the processing of dough in a negative pressure (suction) was basically consistent with the positive pressure change, but the values were relatively small. Figure 7 showed that the biggest pressure on the wheat gluten was at 40°C. And the processing of gluten in a negative pressure (suction) in wheat during storage when temperature is 30°C and 40°C, with basically the same positive pressure (hardness) changes of only relatively small values.

Correlation analysis of physiological and biochemical indexes and the mechanical properties of dough and gluten

Figure 8 showed that there were negative correlation between the peak positive force, positive area, and negative area of dough and the physiological properties of wheat. And it has shown positive correlation between the peak negative force of dough and the physiological properties of wheat. However,

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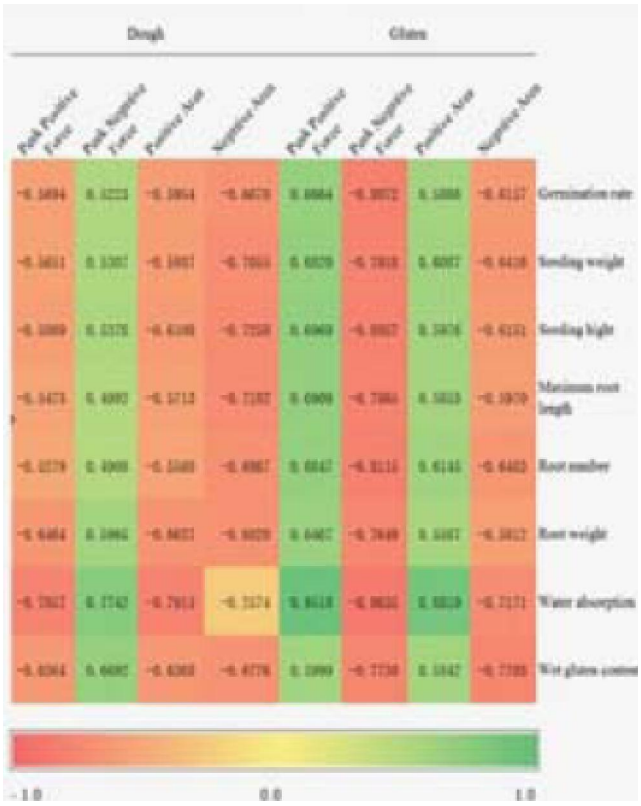

Figure 8 : The correlativity table of mechanical properties of dough/gluten and physiological properties of wheat

there was positive correlation between the peak negative force and positive area of gluten and the physiological properties of wheat. These results showed that there was inverse relation between mechanical properties of dough and gluten except negative area index.

CONCLUSIONS

In this study, we successfully built the relationship between the catalase and protease activities in stored wheat samples. There was a simple linear relationship between the catalase activities and temperature during the storage in wheat (R^2=0.9699). What's more, the closely correlation (R^2 = 0.9536) exists between catalase and protease activity of wheat samples. Moreover, the results showed inverse relation between mechanical properties of dough and gluten except negative area index. The aging of wheat suggested that the storage conditions affected the endogenous enzymes activities of wheat to change the physiological and mechanical properties.

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