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Effect of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) Infestation on Wheat Flour Quality at Different Temperatures

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Abstract *Tribolium castaneum* is one of the most destructive insects infesting cereals and their products worldwide. The quality changes of wheat flour infested by initial 0 (as a control), 2, 4, 6, and 8 adults of *T. castaneum* in 500 g wheat flour were investigated at 15, 25, and 30°C after different storage periods. The quality of wheat flour changed little during 150 d storage at 15°C, but it significantly decreased with increasing initial insect density and storage time during 150 d storage at 25 and 30°C. The moisture content, fatty acid value, falling number, and protein content of wheat flour were 12.21±0.19%, 190.08±3.51 mg KOH $(100\ g)^{-1}$, 208.67±7.23 s, and 14.25±0.02% after 30 d storage at 30°C, respectively. The wet gluten content and dry gluten content of wheat flour could not be detected after 90 d storage at 25 and 30°C. These results provide useful information for maintaining wheat flour quality.

Keywords: *wheat flour, Tribolium castaneum, quality, infestation, safe storage*

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1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops widely grown around the world. It contributes between 20% and 50% of the total calories in the human diet [1]. China, the largest producer and consumer of wheat, planted 24.27 million hectares of wheat in 2018, with a total production of about 130 million tons, amounting to as much as $858 million [2,3]. As an important raw material for common food processing, wheat flour plays an important role in people's daily diets.

Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) is a common damaging insect that is often found in wheat flour processing facilities and storage spaces [4]. When wheat flour is stored at 25-35°C, stored grain insects rapidly multiply and their population growth rate can increase 20-60 fold per month, which can cause serious quantity and quality loss [5]. The irritant substances secreted by *T. castaneum* usually make the wheat flour stink and clump, resulting in contamination with its exuviae and feces [6]. The fatty acid value and the falling number of high gluten wheat flour stored at 15, 25, and 35°C increased, while the wet gluten content and gluten index decreased with increasing storage time [7]. When native starch and heat damaged starch (treated at 60 and 70°C) were added into wheat flour at different proportions (3, 6, and 9 %), the falling number of wheat flour decreased with increasing amount of native starch and heat damaged starch [8]. When the wheat flour was infested by high density of insects (180 g of wheat flour initially infested by 10-200 *T. castaneum* adults) for 15-45 d, the moisture content and protein content of wheat flour increased, and the falling number decreased significantly [9].

Usually, the insect density of insects (180 g of wheat flour initially infested by 10-200 *T. castaneum* adults) is too high to happen frequently under common storage conditions. The tolerance to live insects in wheat flour is zero in most countries. However, a very small number of eggs sometimes remain in wheat flour and become a potential threat, or a few of insects occasionally invade stored wheat flour. This often results in customer complaints [10]. Particularly, the insects with initial low population density can rapidly build up their large population in wheat flour at favorable conditions. However, little is known about the impact of initial low density of adult *T. castaneum* infestation on the quality of stored wheat flour so far. Therefore, this present research aims to investigate the effect of initial low density of adult *T. castaneum* infestation on the quality of wheat flour stored at different temperatures for different times, which is in favor of implementing reasonable measures for maintaining wheat flour quality.

2. Materials and Methods

2.1. Insects

Tribolium castaneum population was maintained on food medium (wheat flour: yeast = 9: 1, by weight) for several generations at 27±2°C, 70±5% r.h. and 12:12 (L: D) h at the Institute of Stored Product Insects of Henan University of Technology, Zhengzhou, China. The healthy *T. castaneum* adults (1 week old) were selected for the experiment. The males and females of *T. castaneum* were determined at pupal stage under a microscope [11], then separately reared at 27±2°C, 70±5% r.h. and 12:12 (L: D) h.

2.2. Experimental Procedure

Wheat (variety: Zhengmai 583) provided by Henan Academy of Agricultural Sciences, was put into a freezer at -20°C for 72 h, then processed into whole wheat flour with a lab mill. Every 500 g of wheat flour (13.5 ± 0.5% moisture content) was put into a wide-mouthed mason jar (2500 ml). Then, 0 (as a control), 2, 4, 6, and 8 *T. castaneum* adults (males: females =1:1) were respectively transferred into the jars. Subsequently, the jars were covered with cotton cloth and maintained at 15, 25, and 30 °C and 75% r.h. The quality of the wheat flour was determined every 30 d during the following 150 d of storage. The numbers of *T. castaneum* populations in the infested wheat flour were respectively countered after 150 d of storage. Three replicates were conducted.

The moisture content of the wheat flour was determined before the experiment by drying triplicate 10-g samples at 130°C for 21 h inside a conventional oven [12]. Protein content, gluten, fatty acid value, falling number value were measured according to the methods approved by American Association of Cereal Chemists (AACC) [13].

2.3. Statistical Analysis

Means and standard errors of wheat flour quality were calculated by using SPSS Statistics 20. Data on the wheat flour quality were subjected to analysis of variance (ANOVA) with storage temperature and time as fixed variables, and quality indices (the moisture content, protein content, gluten content, fatty acid value, and falling number value of wheat flour) as response variables. Treatment means were compared and separated by Tukey's test at p = 0.05.

3. Results

3.1. Effect of *T. castaneum* Infestation on the Moisture Content of Wheat Flour

The different storage temperatures significantly influenced the moisture content of wheat flour infested by *T. castaneum* over 150 d storage (df=2,89, F=16.879, P<0.01) (Figure 1). The moisture content of wheat flour initially infested by 0, 2, 4, 6, and 8 *T. castaneum* adults were not significantly different for the same storage time at the same temperature (df=4,89, F=23.687, P=0.15) (Figure 1). At 15°C, the moisture content of wheat flour increased in the first 60 d, then gradually tended to an equilibrium state. The moisture content of wheat flour initially infested by 0, 2, 4, 6, and 8 *T. castaneum* adults reached 13.10±0.10, 13.18±0.06, 13.33±0.31, 13.11±0.06, and 13.21±0.07% after 150 d storage, respectively. At 25°C, the moisture content of wheat flour changed little in the first 30 d, but decreased rapidly after 30 d, then gradually tended to an equilibrium state after 60 d. The moisture content of wheat flour initially infested by 0, 2, 4, 6, and 8 *T. castaneum* adults reached 12.12±0.04, 11.86±0.09, 12.13±0.23, 12.29±0.06, and 12.21±0.19% after 150 d storage, respectively. The moisture content of wheat flour changed little during 150 d storage at 30°C.

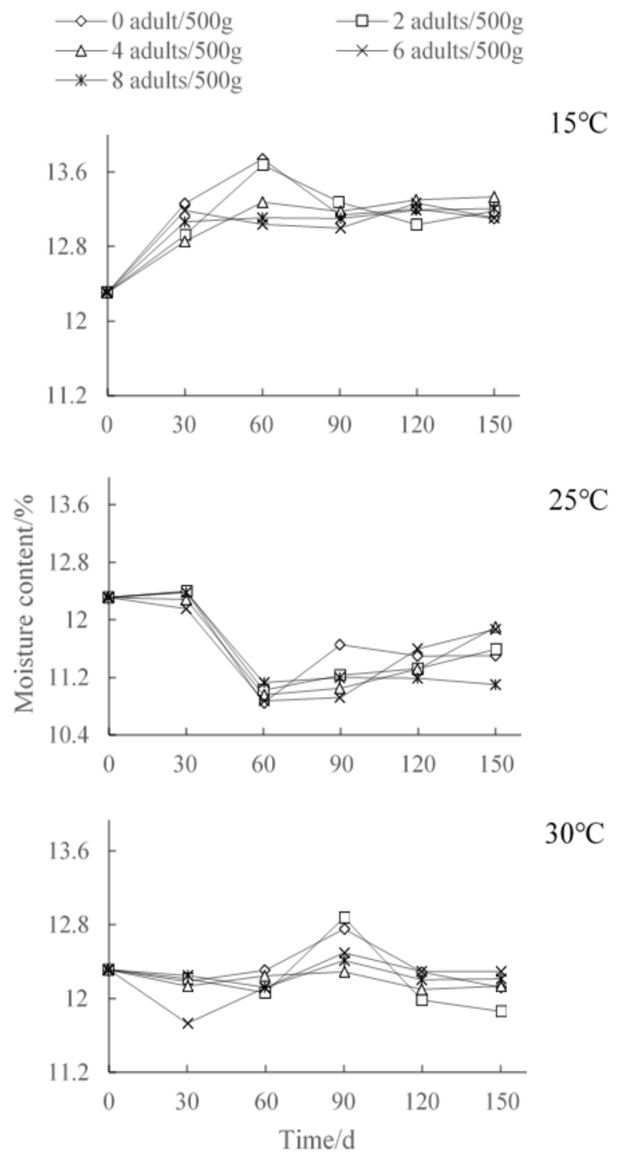

Figure 1. Changes of moisture content of wheat flour infested by initial 0 (as a control), 2, 4, 6, and 8 *T. castaneum* adults in 500 g of wheat flour over time at 15, 25, and 30°C

3.2. Effect of *T. castaneum* Infestation on the Fatty Acid Value of Wheat Flour

The fatty acid value of wheat flour initially infested by 0, 2, 4, 6, and 8 *T. castaneum* adults increased with increasing storage time, temperature, and initial density of insects (Figure 2). In general, the fatty acid value of wheat flour changed little at 15°C, and increased greatly at 25 and 30°C (df=2,89, F=242.702, P<0.01). The fatty acid value of wheat flour initially infested by 0, 2, 4, 6, and 8 *T. castaneum* adults reached 146.18±1.06, 164.65±11.29, 177.25±2.14, 186.28±2.62, and 190.08±3.51 mg KOH $(100\ g)^{-1}$ after 150 d storage at 30°C, respectively.

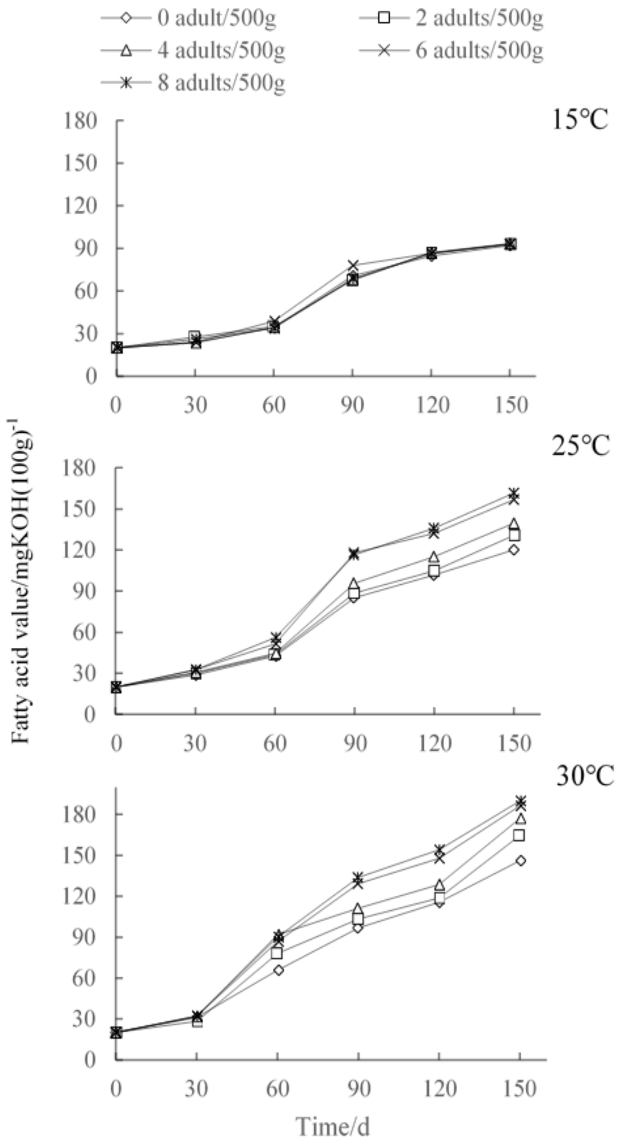

Figure 2. Changes of fatty acid value of wheat flour infested by initial 0 (as a control), 2, 4, 6, and 8 *T. castaneum* adults in 500 g of wheat flour over time at 15, 25, and 30°C

3.3. Effect of *T. castaneum* Infestation on the Falling Number of Wheat Flour

The falling number of wheat flour was significantly affected by storage temperature and initial density of *T. castaneum* adults (df=2,89, F=15.603, P<0.01) (Figure 3). At 15°C, the falling number of wheat flour increased a little during 150 d of storage period. At 25 and 30°C, the falling number of wheat flour increased with increasing storage time in the first 30 d, then greatly decreased with increasing storage time and initial density of *T. castaneum* adults. The falling number of wheat flour initially infested by 0, 2, 4, 6, and 8 *T. castaneum* adults reached 450.33±3.06, 259.33±3.51, 242±3.61, 220.67±4.04, and 208.67±7.23 s after 150 d storage at 30°C, respectively.

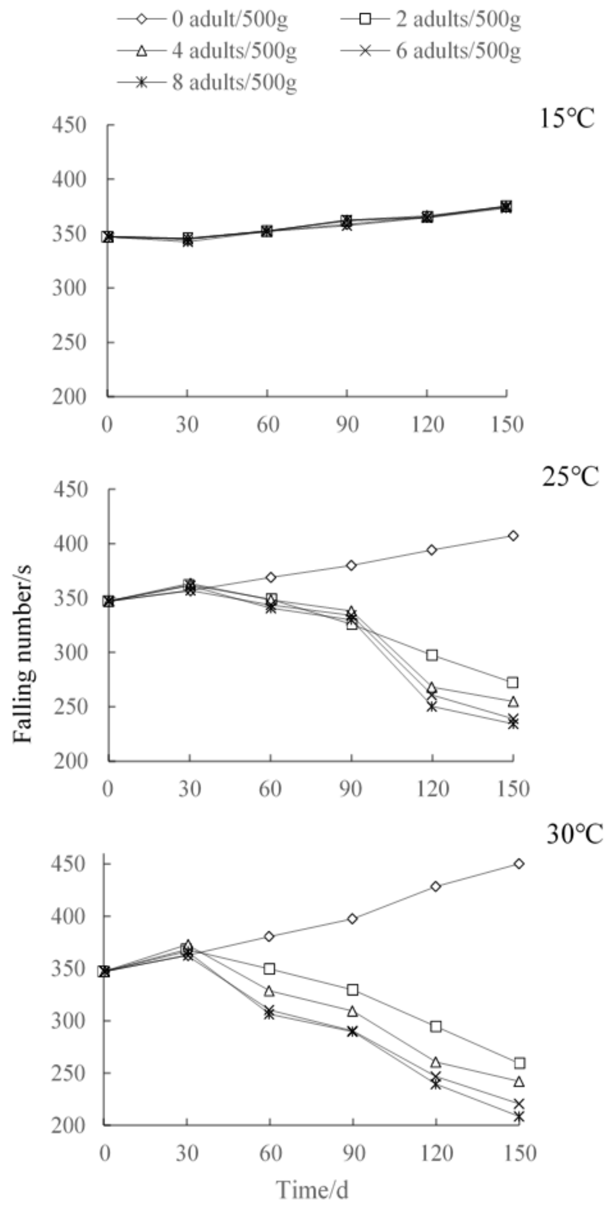

Figure 3. Changes of falling number of wheat flour infested by initial 0 (as a control), 2, 4, 6, and 8 *T. castaneum* adults in 500 g of wheat flour over time at 15, 25, and 30°C

3.4. Effect of *T. castaneum* Infestation on the Protein Content of Wheat Flour

The protein content of wheat flour was also greatly affected by storage temperature and initial density of *T. castaneum* adults (df=2,89, F=34.811, P<0.01) (Figure 4). The protein content of wheat flour changed little in the first 30 d storage, but changed significantly after 30 d at different temperatures. At 15°C, the protein content of wheat flour decreased a little during 150 d storage. At 25 and 30°C, the protein content of wheat flour decreased slowly in the first 30 d storage, then decreased markedly until 30 d storage, subsequently increased with increasing storage time and initial density of *T. castaneum* adults. The protein content of wheat flour without insect infestation gradually decreased. The protein content of wheat flour initially infested by 0, 2, 4, 6, and 8 *T. castaneum* adults reached 13.14±0.02, 13.45±0.02, 13.60±0.07, 13.78±0.03, and 14.25±0.02% after 150 d storage at 30°C, respectively.

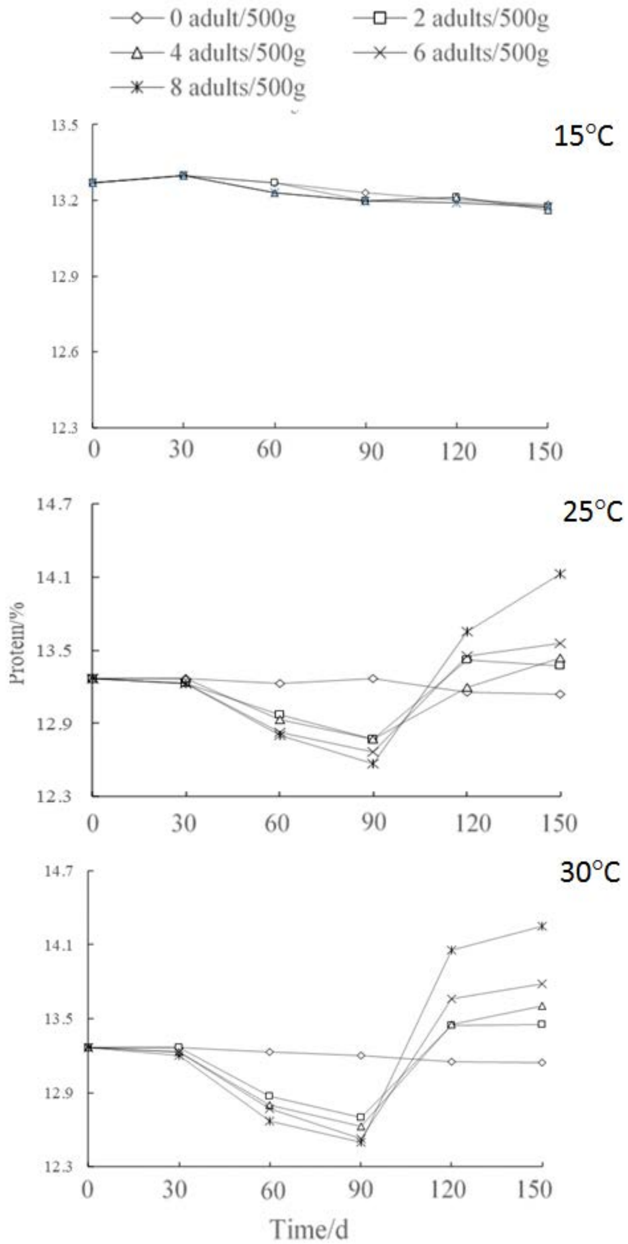

Figure 4. Changes of protein content of wheat flour infested by initial 0 (as a control), 2, 4, 6, and 8 *T. castaneum* adults in 500 g of wheat flour over time at 15, 25, and 30°C

3.5. Effect of *T. castaneum* Infestation on the Gluten Content of Wheat Flour

In general, the wet gluten content and dry gluten content of wheat flour decreased with increasing storage time and initial adult population density of *T. castaneum* (Figure 5 and Figure 6). The wet gluten content (df=2,89, F=14.229, P<0.01) and dry gluten content (df=2,89, F=6.221, P<0.05) of wheat flour decreased slowly when stored at 15, 25, and 30°C for the first 30 d, then decreased rapidly at 25 and 30°C after 30 d (Figure 5 and Figure 6). The wet gluten content and dry gluten content of wheat flour could not be detected after 90 d of storage at 25 and 30°C. The higher the initial density of *T. castaneum* adults and storage temperature, the faster the wet gluten content and dry gluten content of wheat flour decreased.

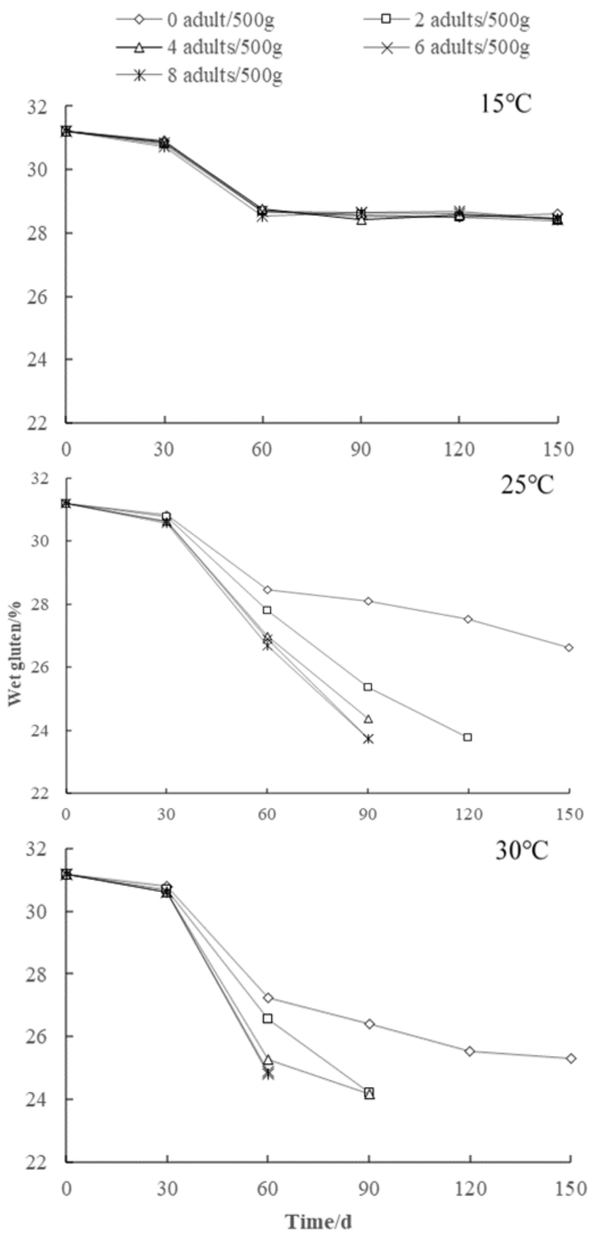

Figure 5. Changes of wet gluten of wheat flour infested by initial 0 (as a control), 2, 4, 6, and 8 *T. castaneum* adults in 500 g of wheat flour over time at 15, 25, and 30°C

3.6. The Number of *T. castaneum* Populations in the Wheat Flour after 150 d Storage

The number of *T. castaneum* populations in the wheat flour initially infested by 0, 2, 4, 6, and 8 *T. castaneum* adults significantly increased after 150 d of storage at 25 and 30°C (df=4,89, F=25.328, P<0.01). The numbers of *T. castaneum* populations in the wheat flour initially infested by 0, 2, 4, 6, and 8 *T. castaneum* adults reached 0.00±0.00, 91.86±0.09, 211.13±3.33, 312.29±2.67, and 515.21±5.33 after 150 d of storage at 30°C (Table 1).

Table 1. The numbers of *T. castaneum* populations in the wheat flour infested by initial 0 (as a control), 2, 4, 6, and 8 *T. castaneum* adults after 150 d of storage at 15, 25, and 30°C

Temperature (°C)	Initial insect density (adults/500 g of wheat flour)				
	0	2	4	6	8
15	0.00±0.00Ea	2.00±0.00Dc	4.00±0.00Cc	6.00±0.00Bc	8.00±0.00Ac
25	0.00±0.00Ea	63.67±4.04Db	113.00±1.00Cb	245.33±1.33Bb	413.00±3.67Ab
30	0.00±0.00Ea	91.86±0.09Da	211.13±3.33Ca	312.29±2.67Ba	515.21±5.33Aa

Each datum in the table is mean ± SE of three replicates. The data in a column followed by different lower case letters and the data in a raw followed by different capital letters indicate significant differences tested by Scheffe's test at p = 0.05 level.

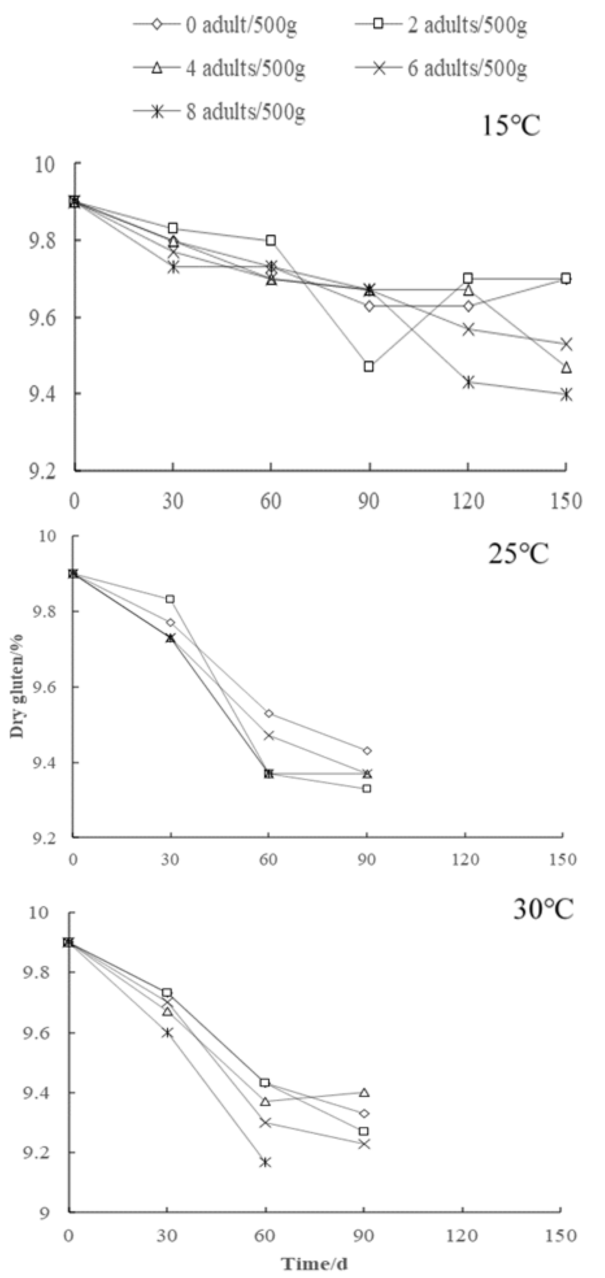

Figure 6. Changes of dry gluten of wheat flour infested by initial 0 (as a control), 2, 4, 6, and 8 *T. castaneum* adults in 500 g of wheat flour over time at 15, 25, and 30°C

4. Discussion

Stored product insect infestation has significantly adverse effect on the quality of wheat flour, which depends on various factors, for instance initial insect density, insect species, strain, development stage, grain species, storage temperature, and so on. The more serious the insect infestation is, the faster the quality of wheat flour deteriorates. The present results showed that the quality of wheat flour changed little in the first 30 d when initially infested by 2, 4, 6, and 8 adults of *T. castaneum*, but it decreased rapidly after 30 d storage. The result is somewhat different from Abdullahi et al. [14]. The quantity and quality of stored cocoa bean infested by *T. castaneum* were negatively affected after 30 d storage. This is due to the following two reasons. First, *T. castaneum* infestation has different effect on different kinds of grains: wheat flour tested in the present study and cocoa bean tested in Abdullahi et al. [14]. Second, the lower initial insect densities of 2, 4, 6, and 8 *T. castaneum* adults in 500 g wheat flour were tested in the present study, while the higher initial insect densities of 10, 20, and 30 *T. castaneum* adults in 250 g cocoa bean were tested in Abdullahi et al. [14].

In the present research results, the protein content, wet gluten content, dry gluten content, and falling number decreased, while the moisture content and fatty acid value increased with increasing storage temperature and time after 30 d of storage. The result is in accordance with Wang et al. [7]. Gluten is a kind of plant protein that consists of gliadin and gluten [15]. The wet gluten and dry gluten content of wheat flour infested by initially low density of *T. castaneum* adults changed little when stored at 15°C, showing that low temperature storage was beneficial to maintain the quality of wheat flour. This is because the developmental threshold temperature of most stored product insects is about 15°C [16]. The stored product insects can not grow and develop normally at ≤ 15°C. When wheat flour was infested by initial high density of *T. castaneum* adults (for instance, 180 g wheat flour containing 10-200 adults) for 15-45 d, the moisture content, protein content, and falling number of wheat flour were adversely affected [9].

Tribolium castaneum usually obtains nutrients from wheat flour and its products, which results in deterioration of flour quality. The physic-chemical properties of wheat flour significantly affected *T. castaneum* survivorship and development time. The protein, water, carbohydrate, ash, riboflavin, and size variation of flour particles had a significant effect on the development of *T. castaneum* [6,17]. There have been a lot of researches in the influence of different grains and their products on the development of *T. castaneum* [18-22]. Different plant diets, including *Phaseolus vulgaris*, *Vigna unguiculata* and wheat flours, significantly affect the development of *T. castaneum* [23]. Different rice cultivars flours have different feeding resistances to *T. castaneum* [4]. Different barley cultivars as food media markedly affected the demographic parameters of *T. castaneum* [24]. Different wheat lines have different resistance to *T. castaneum*, which can be used to breed insect resistant wheat varieties [25,26].

However, it is a long-term process to improve the insect resistance of wheat flour by cultivating insect resistant wheat varieties. Based our current results, the wheat flour quality can be guaranteed when stored at no more than 15 °C and insect free environment.

In addition, only the effect of adult *T. castaneum* infestation on wheat flour quality was investigated in the present study. The effect of *T. castaneum* larvae infestation on wheat flour quality, and the effect of *T. castaneum* infestation on the quality of wheat four products still deserve to be investigated in the future.

5. Conclusions

In summary, the initial low density of adult *T. castaneum* infestation had little effect on wheat flour quality within the first 30 d, but the quality deterioration of wheat flour was significantly accelerated with increasing storage time and temperature after 30 d storage. The higher the initial density of *T. castaneum* adult population in wheat flour and storage temperature, the faster the wheat flour quality deteriorates. Therefore, the wheat flour quality can be maintained when shored at no more than 15°C.

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Statement of Competing Interests

The authors declare that there is no conflict of interest.

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