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Effects of controlled atmosphere storage with nitrogen on quality of soybean (*Glycine max*) with different moisture content

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ABSTRACT

Qualities of different m.c. CH (10, 12, and 14%) soybeans [*Glycine max* (L.) Merr.] during controlled atmosphere storage with N_2 (nitrogen concentration: 96% to 98%) were compared with soybeans in conventional conditions at 26°C after 60 days storage. The result showed that controlled atmosphere storage with N_2 could effectively inhibit the decrease of soybean germination rate and delay the increase of acid value and peroxide value. For soybeans with 10% m.c. controlled atmosphere with N_2 had significant effects on germination rate ($F = 9.000$, $P<0.05$). For soybeans with 12% m.c. controlled atmosphere with N_2 showed significant effect on germination rate and peroxide value ($F = 16.363$, $P<0.05$; $F = 251.722$, $P<0.05$). For soybeans with 14% m.c. controlled atmosphere with N_2 had significant effects on peroxide value ($F = 68.435$, $P<0.05$) also showed highly significant effects on germination rate and acid value ($F = 352.899$, $P<0.01$; $F = 512.000$, $P<0.01$). Controlled atmosphere with N_2 did not show significant effects on the crude fat content ($F = 6.327$, $P<0.05$). After 60 days storage, the total count of mould changed. Compared with the number before storage, for soybeans with 10% m.c., the total count of mould reduced, for the soybeans with 12% m.c., but the total count of mould increased, for the soybeans with 14% m.c.. For soybeans with 10% and12% m.c., there was no dominant mould. For soybeans with 14% m.c., *Aspergillus glaucus* (L.), was the dominant mould. The detection rates of *Aspergillus glaucus* were 92% and 96% for soybeans with 14% m.c. in controlled atmosphere storage with N_2 and in conventional conditions.

Key words: *Aspergillus albicans, A.glaucus*, Moisture content, Nitrogen, Soybean

Soybean [*Glycine max* (L.) Merr.] is an important grain, and is grown in different areas of China. The main soybean producing area is north-east China. In north China, soybean fields are common in the middle and lower Yangtze River region and part of north-west area (*Grain Dictionary* Editional Board, 2009). Because of the high contents of protein and fat, soybean is prone to absorb moisture, oxidize, lose germination. The storage stability is poor (Lu Xiyu, 2003;Tao Cheng, 2004).

Controlled atmosphere (CA) storage as a green storage technology has been applied commercially at home and abroad (Lan Shengbin et al., 2007). Different researches showed that CA storage could kill grain insects, reduce the respiration of grain and keep grain quality (Lu Yujie, 2008; Dale Jude, 2008). In Australia, America, Russia, CA storage has become the first choice of replacing chemical fumigants (Wu Zidan, 2011).

With the development of nitrogen-making technology and the optimization of nitrogen charging process in grain storage, the cost of CA storage with nitrogen has reduced. Controlled atmosphere storage with nitrogen has become a low-cost pest controlling method (Bu Cunhai et al., 2013). In recent years, Sino-grain has realized CA storage with nitrogen extensively in south of China. At present, CA storage with nitrogen is mainly used on wheat (*Triticum* sp.), paddy (*Oryza sativa* L.) and corn (*Zea mays* L.). However, there are few reports about CA storage with nitrogen on soybeans. This study was conducted to find out soybean qualities and mould changes in soybean having different m.c. in CA storage with nitrogen and conventional conditions, to provide the basis for CA storage with nitrogen on soybeans.

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MATERIALS AND METHODS

Soybean

Heinong 38, harvest on 2009, provided by Sinograin Dunhua depot. 98% nitrogen: Sichuan Qiaoyuan Gas Ltd; petroleum ether (30°C~60ºC boiling range), isooctane, absolute ether: Kermel Chemical Reagent Ltd.

Main instruments

ZRX-1000ESM intelligent cultivation cabinet: Hangzhou Qianjiang instrument and equipment Ltd; AL204 electronic scales: Mettler Toledo; R206 rotary evaporator: Shanghai Senco Science and Technology Ltd; SHB-III vaccum pump: Zhengzhou Greatwall Ltd; DHG-9146A Electric constant temperature drying oven: Shanghai Shengxin Scientific Instrument Ltd; XMTD-204 thermostat water bath: Shanghai Meixiang instrument Ltd. The concentration of nitrogen (N_2) was calculated by deducting the oxygen (O_2) concentration from 100%. The nitrogen monitor equipment was: P860 made by Chengdu Chang-ai Electric Technology Co., Ltd.

Methods

Samples: The original m.c. of soybean is 12%, one-third of soybeans were dried by sunshine to reduce the m.c. to 10%, one-third of the soybeans were raised to m.c. 14% by wet-film.

Experimental treatments: Soybeans with 10, 12, 14% m.c were located into 3 L glass bottles with rubber plug. Nitrogen 98% nitrogen was passed into glass bottles. Nitrogen first passed through different saturated salt solutions to keep different m.c. soybean. The control treatments were soybeans without CA storage. All samples were placed in 26 ± 0.5°C intelligent cultivation cabinet. After 60 days, all samples were tested. The nitrogen concentration detector was used to test nitrogen concentration. In the process, the concentration of nitrogen ranged from 96 to 98%.

Maintain the moisture content of soybean

Nitrogen supplied to soybean with 10, 12, 14% m.c. was first passed through K_2CO_3, NaBr and KI saturated salt solutions respectively. For control treatments, rubber plugs were used to seal glass bottles. After 60 d, the m.c. of soybean in CA storage with Nitrogen was 10.2, 12.1 and 14.0%. The m.c. of soybeans in the control treatments was 10.0, 11.8 and 13.9%.

Indicator measuring method

MC: refer to GB/T 14489.1-2008; Crude fat: refer to GB 5512-2008; Germination rate: refer to GB/T3543.4-1995; Mould colony number: refer to GB/T 4789.15-2010; Mould phase: put soybean samples into triangular flask, add 1% NaClO solution, soak soybean in 1% NaClO solution for 30 min, then discard the 1% NaClO solution and pour sterile water into the flask, wash for 1 min and then discard the sterile water, repeat 5 times. Put 10 soybean kernels in CD Czapek agar in each plate, each sample for 5 plates. At last, put these plates in 28°C incubator for 7 d; Crude fat acid: refer to *GB/T 5530 2005 Animal and vegetable fats and oils-Determination of acid value and acidity*; Crude fat peroxide value: refer to *GB/T 5538-2005 Animal and vegetable fats and oils-Determination of peroxide value*.

Statistical analysis

Excel 2007 and SPSS 12.0; significant tests were conducted with ANOVA and multiple comparing.

RESULTS AND ANALYSIS

Germination

Before storage, germination rates had no significant difference ($F = 0.600$, $P > 0.05$) for all treatments. After 60 d storage, germination rate decreased in all treatments (Fig. 1). Compared with conventional treatments, germination rate decreased slowly for CA storage with nitrogen treatments. For treatments with CA storage, germination rate had no significant difference ($F = 2.214$, $P > 0.05$), compared with germination rates before storage, they still kept high germination rates. For treatments in conventional conditions, germination rates did not show significant difference ($F=124.564$, $P<0.05$), for 10 and 12% m.c. treatments in conventional conditions, germination rate had significant difference ($F = 13.016$, $P < 0.05$). In

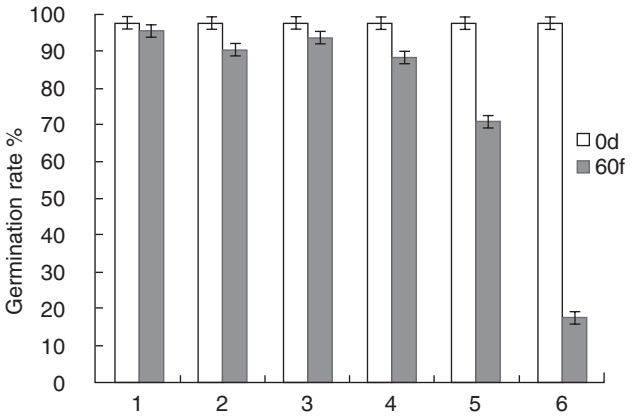

Fig. 1. Germination rate (1-3 represent 10%, 12%, 14% m.c. soybeans with CA storage; 4-6 represent 10%, 12%, 14% m.c. soybeans with conventional storage)

case of 14% m.c. treatment in conventional storage condition, germination rate was 17%, obviously lower than the other treatments. For treatments having the same m.c., germination rate showed significant difference (F = 9.000, $P < 0.05$) for 10% m.c., so did germination rate for 12% m.c. For treatments having 14% m.c., germination rates had highly significant difference (F = 352.899, $P < 0.01$). Therefore, CA with nitrogen could inhibit germination rate decreasing, especially for soybeans having m.c.

Crude fat acid value

Acid value is an important indicator to evaluate soybean oil quality. The higher the acid value, higher the free fatty acid content in oil. After 60 d storage, for all treatments, acid value increased obviously. Higher the m.c., the more obvious acid value increased. After 60 d storage, for the 10%, 12% and 14% m.c. treatments with CA storage, crude fat acid values were 1.3 mg KOH/g, 1.5 mg KOH/g and 2.0 mg KOH/g, respectively, which showed highly significant difference (F = 706.500, $P < 0.01$). For treatments with 10% m.c., acid value had no significant difference (F = 7.377, $P > 0.05$), neither was the treatments with 12% m.c. (F = 0.089, $P > 0.05$). For treatments with 14% m.c., high significant difference (F = 512.000, $P < 0.01$) was observed. Therefore, m.c. had an important effect on acid value. Under same storage condition, higher the m.c., faster the increase in acid value. Nitrogen had little effect on acid value in soybeans having low m.c., however, it had obvious effect on inhibiting acid value increase for soybeans having high m.c.

Crude fat peroxide value

Peroxide value is mainly used to evaluate the hydroperoxide content. During the initial period of oil oxidation, Peroxide value is an important indicator to evaluate the degrees of oil oxidation. All treatments had low peroxide values and had no significant

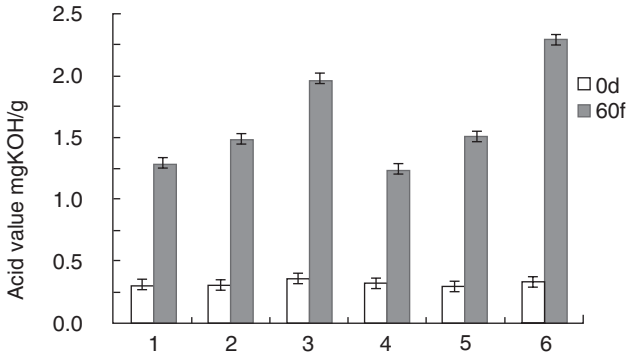

Fig. 2. Crude fat acid value (1-3 represent 10%, 12%, 14% m.c. soybeans with CA storage; 4-6 represent 10%, 12%, 14% m.c. soybeans with conventional storage)

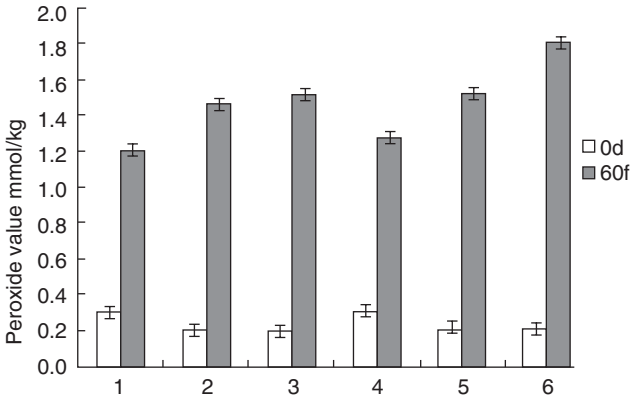

Fig. 3. Crude fat peroxide value (1-3 represent 10%, 12%, 14% m.c. soybeans with CA storage respectively; 4-6 represent 10%, 12%, 14% m.c. soybean with conventional storage respectively)

difference (F = 0.500, $P > 0.05$) before storage. After 60 d storage, in all the treatments crude fat peroxide value increased obviously. The three treatments with CA storage and without CA storage had significant difference F = 61.325, $P < 0.05$, and F = 375.440, $P < 0.05$, respectively. Therefore during the soybean storage, m.c. had a great effect on crude fat peroxide value. Higher the m.c., quicker the crude fat peroxide value increased. For treatments with 10% m.c., crude fat peroxide value had no significant difference (F = 4.749, $P > 0.05$). For treatments with 12% m.c., crude fat peroxide value showed significant difference (F = 251.722, $P < 0.05$), so was the treatments with 14% m.c. (F = 68.435, $P < 0.05$). Therefore, CA storage with nitrogen had no obvious effect on inhibiting peroxide value increase for low m.c. soybean. However, it had obvious effect on inhibiting peroxide value increase for high m.c. soybean.

Crude fat content

Crude fat content affects soybean ranks, for oil processing; crude fat content of soybean has direct influence on economic benefit. In all treatments, crude fat content increased a little after 60 d storage; however, the range was narrow (19.8% – 21.4%). All treatments had revealed significant difference (F = 6.327, $P > 0.05$).This indicated that m.c. and nitrogen had little effect on crude fat content. Also, crude fat content did not change easily with storage time. Our results are consistent with those of (RenZhiqiu et al., 2002; Jin Wen et al., 2010, and Wan Zhongmin et al., 2012.

Mould colony number

Moisture is an indispensable factor for micro-organic growth. Dryness could cause water loss

Table 1 Mould colony number*(CFU/g)

storage	1	2	3	4	5	6
0 d	(2.4±0.14) $\times10^2$a	(3.0±0.28) $\times10^2$a	(2.8±0.21) $\times10^2$a	(2.4±0.14) $\times10^2$a	(3.0±0.28) $\times10^2$a	(2.8±0.21) $\times10^2$a
60 d	<10b	(1.5±0.14) $\times10^2$a	(2.4±0.07) $\times10^3$c	45±4.24b	(2.4±0.71) $\times10^2$a	(2.7±0.13) $\times10^3$c

(*Different superscript letters within a column indicate statistical differences $P\leq0.05$)(1-3 represent 10%,12%,14% MC soybeans with CA storage respectively; 4-6 represent 10%, 12%, 14% m.c. soybeans with conventional storage, respectively)

Table 2 Mould phase (%)

*Detection rate**	*Cladosporium* sp.	*Fusarium* sp.	*Penicillium* sp.	*Aspergillus albicans*	*Aspergillus glaucus*	*Alternaria* sp.
1	0*	6	0	0	0	0
2	0	0	4	0	0	0
3	2	0	0	0	92	0
4	0	0	0	2	6	0
5	0	0	2	2	2	2
6	2	0	0	0	96	0

* The detection rate; 1-3 represent 10%,12%,14% m.c. soybeans with CA storage respectively; 4-6 represent 10%,12%,14% m.c. soybeans with conventional storage, respectively

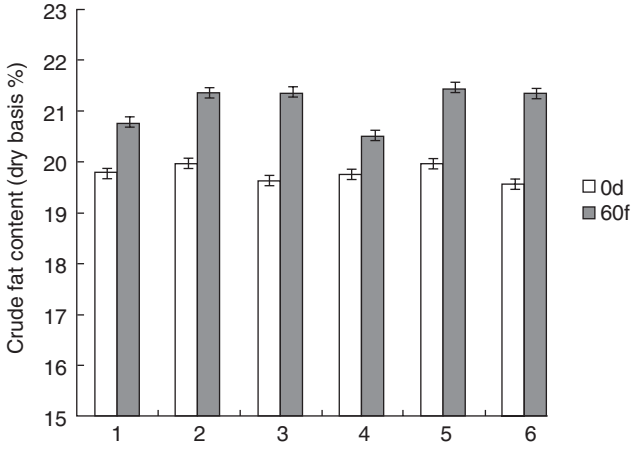

Fig. 4. Crude fat content (1-3 represent 10%,12%,14% m.c. soybean with CA storage respectively; 4-6 represent 10%,12%,14% m.c. soybeans with conventional storage respectively)

and even death (He Guoqing, 2002). From Table 1, after 60 d storage, mould colony numbers changed obviously for different treatments. Mould colony numbers decreased for soybeans having 10% m.c. For the soybeans of 12% m.c., mould colony numbers changed a little. For soybeans of 14% m.c., mould colony numbers increased. After 60 d storage when the treatments have the same m.c., mould colony numbers had no significant difference. When the treatments have the different m.c., mould colony numbers showed significant difference. Controlled at morpheme with nitrogen had little effect on mould colony number, m.c. had great effect on mould colony number.

Mould phase

After 60 d storage, for soybeans of 10 and 12% m.c., mould species were few and there was no dominant mould. For soybeans of 10% m.c. with CA storage, *Fusarium* sp. was detected; for soybeans of 12% m.c. with CA storage, *Penicillium* sp.was detected; for soybeans of 10% m.c. in conventional storage, (*Aspergillus albicans*) and *Aspergillus glaucus* were detected; and for soybeans of 12% m.c. in conventional storage, *Penicillium* sp., *A.albicans* and *A. glaucus* were detected. For soybean of 14% m.c., *A. glaucus* was the dominant mould, and the detection rates were 92% and 96% respectively. Some researches showed *A. glaucus* hadantihypoxia capacity. It could grow in 0.2% oxygen content condition (Yin Weishen, 1983). So in this study, 96%-98% nitrogen could not inhibit the growth of *A. glaucus*. Therefore, m.c. was an important factor of affecting mould phase. Controlled atmostphere with nitrogen had no significant impact on mould phase.

CONCLUSION

Soybeans with different m.c. were stored by CA with nitrogen. The results showed that the germination rate was reduced, the crude fat acid value and peroxidation value increased after 60 d storage. Higher the m.c., faster was the increase of crude fatty acid value and peroxidation value. The m.c.

had significant impact on mould colony number and mould phase. When the m.c. of soybean was under 12%, mould colony number was less than 10^2 CFU/g and there was no dominant mould; however, when the m.c. of soybean was 14%, mould grew rapidly, *Aspergillus glaucus* being the dominant mould. The m.c. and storage time had no significant impact on crude fat content.

Controlled atmosphere with nitrogen could inhibit the decline of germination rate, the rise of crude fat acid value and peroxidation value. When the m.c. was 10%, CA with nitrogen had significant impact on germination rate. When the m.c. was 12%, CA with nitrogen had significant impact on germination rate and crude fat peroxidation value, while with 14% m.c., CA with nitrogen had highly significant impact on germination rate, crude fat acid value and crude fat peroxidation value. Controlled atmosphere with nitrogen had no significant impact on crude fat content and mould colony number.

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