JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2014. Vol. **8**(3), p. 2135-2139

The Influence of Different Storage Conditions on the Cured Tobacco Leaves Mildew

Jianhua Lu[1], Huina Zhang[1], Xinhong Su[2], Dan Ma[1], Xuguang Bai[1] and Yueguang Du[3]

[1]School of Food Science and Technology, Henan University of Technology, Zhengzhou, Henan, China.
[2]Henan Tobacco Company, Zhengzhou, Henan, China.
[3]Tianchang International Tobacco Co., Ltd., Xuchang, Henan, China.

(Received: 03 January 2014; accepted: 26 March 2014)

The effect of different temperatures, relative humidities, and packaging materials on cured tobacco leaves mildew was investigated in laboratory at 20, 30 and 40 °C, and at 65, 75, 85 and 94% relative humidity respectively. Two types of common packaging materials, kraft paper and high-density polythene film, were tested. The cured tobacco leaves mildew degree developed significantly with increasing relative humidities at 30 °C. The cured tobacco leaves quality could be kept well at 65% relative humidity. However, the severe spoilage rapidly appeared after 3 d and 6 d exposure at 94% relative humidity, respectively. Different temperatures had significant effect on the cured tobacco leaves mildew at 75% relative humidity. The cured tobacco leaves mildew happened after 11 d and 3 d of exposure time at 20 °C and 30 °C, respectively. The favorable temperature for mould development is 30 °C, and the cured tobacco leaves mould could not developed during the whole exposure time at 40 °C. Kraft paper pouches were better than high-density polythene film pouches for keeping the cured tobacco leaves from mildew during the whole exposure time. These results suggested that the cured tobacco leaves mildew could be prevented by rationally controlling environment condition.

Key words: Cured tobacco leaves; Mildew; Storage environment condition.

Cured tobacco (*Nicotiana tabacum* L.) leaves are very valuable stored product and vulnerable to mould during storage period[1, 2]. About 12.9% of cured tobacco leaves can not be used for cigarette manufacturing due to storage fungi spoilage, and 11.3% of cigarette loses commercial value as a result of mildew in China[3]. Usually, the cured tobacco leaves should be aged for 1~3 years for improving aroma, taste and quality characteristics before being used for cigarette manufacturing[1,4]. Many microorganisms can rapidly develop during the aging time when the environmental temperature and moisture is suitable, because the cured tobacco leaves provide an abundant source of nutrients, such as proteins, lipids, soluble carbohydrates, organic acids, nicotine, mineral substances, and so on[5].

Cured tobacco mildew during storage period results in huge loss every year. The storage conditions, for example, temperature, relative humidity and packaging material, significantly affect cured tobacco leaves mildew. Empirically, the higher temperature and relative humidity, the higher risk of mildew. However, little is known about how these storage conditions determine the stored tobacco leaves mildew. Thus, we evaluate the effect of different temperatures, relative humidities, and

* To whom all correspondence should be addressed.
E-mail: jianhlv@163.com

packaging materials on the cured tobacco leaves mildew in laboratory.

MATERIALS AND METHODS

Effect of different relative humidities on cured tobacco leaves mildew

Every five gram of high quality cured tobacco leaves were laid in a petri dish which was then put in a 10 l desiccator. There was about 500 ml saturation solution of chemical at the bottom of the desiccator. A series of relative humidities of 65, 75, 85 and 94% were maintained with saturation solution of different chemicals as Table 1. The desiccators with tobacco leaves and saturation solution of chemical were kept in chambers at 30 °C, and the cured tobacco leaves mildew degree was observed and recorded after 3, 6, 9, 12, 15, 18, 21 and 30 d. Four replicates were conducted.

Effect of different temperatures on cured tobacco leaves mildew

Each five gram of high quality tobacco leaves were also laid in a petri dish which was then put in a 10 l desiccator. There was about 500 ml saturation solution of sodium chloride at the bottom of the desiccators for maintaining 75% relative humidity. The desiccators with tobacco leaves and saturation solution of sodium chloride were kept in chambers at 20, 30 and 40 °C, and the cured tobacco leaves mildew degree was observed and recorded after 3, 7, 11, 15, 19, 23, 27 and 31 d. Four replicates were conducted.

Effect of different packaging materials on cured tobacco leaves mildew

Kraft paper and high-density polythene film are two types of common packaging materials in China. Kraft paper (0.2 mm thickness) and high-density polythene film (0.2 mm thickness) were respectively cut into 10 cm × 20 cm pieces, and afterwards 8 cm × 15 cm pouches were prepared with gluewater for the kraft paper pouches and with the aid of press plastic machine for the polythene film pouches. These pouches were carefully checked without any pores. Each pouch contained 10 g of high quality cured tobacco leaves, then was sealed and kept in chambers at 75% relative humidity and 30 °C. The cured tobacco leaves mildew degree was observed and recorded after 5, 10, 20, 30, 40, 50 and 60 d. Four replicates were conducted.

Definition of mildew degree of cured tobacco leaves

High quality cured tobacco leaves with 12.3 % moisture content were used in the above experiments. According to Chinese national standard GB/T 23220-2008: Tobacco Leaves Storage Method, mildew degree of cured tobacco leaves can be defined as follows: class 0 (namely no mildew, sound and no mouldy odour), class I (namely slight mildew, very slightly mouldy odour, mildewy leaves less than 0.5%), class II (namely moderate mildew, apparently mouldy odour, mildewy leaves between 0.5% and 5%), class III (namely severe mildew, strong mouldy odour, mildewy leaves more than 5%).

RESULTS

Effect of different relative humidities on the cured tobacco leaves mildew

The cured tobacco leaves mildew degree at different relative humidities and 30 °C was showed in Table 2. Different relative humidities significantly affected the cured tobacco leaves mildew. The mildew degree significantly aggravated with increasing relative humidities in the environment. The cured tobacco leaves mildew did not appear during the whole exposure time at 65% relative humidity, and the cured tobacco leaves quality still was kept well. However, the cured tobacco leaves mildew rapidly appeared after 3 d

Table 1. The relative humidities maintained by saturation solution of different chemicals at different temperatures

Relative humidity	Tested temperature (°C)		
(%)	20	30	40
65	Ammonium nitrate	Ammonium nitrate	Ammonium nitrate
75	Sodium chloride	Sodium chloride	Sodium chloride
85	Potassium chloride	Potassium chloride	Potassium chloride
94	Potassium nitrate	Potassium sulfate	Potassium sulfate

of exposure time at 94% relative humidity. Strong mouldy odour was emitted, and severe spoilage happened after 6 d of exposure time. The cured tobacco leaves mildew happened after 6 d of exposure time at 75% and 85% relative humidity.

Table 2. Effect of different relative humidities on cured tobacco leaves mildew

Observation time (d)	Relative humidity (5) 65	75	85	94
3	-	-	-	+
6	-	+	+	+++
9	-	++	++	+++
12	-	+++	+++	+++
15	-	+++	+++	+++
18	-	+++	+++	+++
21	-	+++	+++	+++
30	-	+++	+++	+++

Note: "-" class 0; "+" class I; "++" class II; "+++" class III. The same as table 3 and table 4.

Table 3. Effect of different temperatures on cured tobacco leaves mildew

Observation time (d)	Temperature (°C) 20	30	40
3	-	+	-
7	-	++	-
11	+	+++	-
15	++	+++	-
19	+++	+++	-
23	+++	+++	-
27	+++	+++	-
31	+++	+++	-

Table 4. Effect of different packaging materials on cured tobacco leaves mildew

Observation time (d)	Packaging materials Kraft paper	High-density polythene film
5	-	-
10	-	-
20	-	-
30	-	-
40	-	+
50	-	+
60	-	+

Effect of different temperatures on the cured tobacco leaves mildew

Different temperatures had significant effect on the cured tobacco leaves mildew at 75% relative humidity (Table 3). At 20 °C, the cured tobacco leaves mildew took placed after 11 d of exposure time, and strong mouldy odour and severe spoilage emerged after 15 d and 19 d respectively. It is the favorable condition at 30 °C for mould development. The cured tobacco leaves mildew rapidly occurred after 3 d of exposure time. Strong mouldy odour was smelled after 7 d of exposure time, and severe spoilage arised after 11 d of exposure time. The cured tobacco leaves quality was maintained well without mildew during the whole exposure time at 40 °C.

Effect of different packaging materials on the cured tobacco leaves mildew

Different packaging materials exhibited significant effect on cured tobacco leaves mildew (Table 4). Kraft paper pouches could keep the cured tobacco leaves from mildew during the whole exposure time at 75% relative humidity and 30 °C. The cured tobacco leaves mildew occurred in high-density polythene film pouches after 40 d of exposure time.

DISCUSSION

The cured tobacco leaves are subject to attack by storage fungi which can result in huge spoilage loss during the aging period. Particularly, mold growth has detrimental effects on the quality of the cured tobacco leaves and may result in mycotoxin contamination[6].

The dominant spoilage fungi usually include *Aspergillus flavus*, *A. niger*, *A. versicolor*, *A. repens*, *A. ruber*, *Eurotium* spp., *Peronospora hyoscyami* de Bary f. sp. *tabacina*, *Penicillium chrysogenum*, *P. viridicatum*, *Mucor* spp.[7-14]. The mycotoxin-producing fungi *A. flavus*, *P. tabacina and* so forth can occur widely, on inadequately dried cured tobacco leaves, in sub-tropical and tropical climates throughout the world. Specially, the moulds develop very quickly when the environmental relative humidity is suitable[15].

In the present research, the moulds could not grow on the cured tobacco leaves when the environmental relative humidity is at 65%, but they grew very rapidly on the cured tobacco leaves when

the environmental relative humidity is at 85% or above. The presence of blue and green spoilage moulds on the surface of the cured tobacco leaves indicates that the higher moisture conditions suitable for molds development, and musty odors soon were emitted. At 75% relative humidity, the spoilage fungi grew rapidliest at 30 °C. However, they could not develop at 40 °C. These results showed that the tobacco spoilage fungi growth can be effectively prevented by controlling reasonable environmental relative humidity and temperature during storage period, and the cured tobacco leaves mildew can be avoided. Ideal storage environmental conditions for cured tobacco leaves are a clean place with constant low temperature and low relative humidity.

Sound packaging material is an important defence to prevent the cured tobacco leaves from mildew, which can keep the cured tobacco leaves at good conditions. In the current results, Kraft paper was better for keeping the cured tobacco leaves from mildew than high-density polythene film, which was similar to previous results. Shirai et al. reported that typical paper material can not effectively prevent moisture migration, and as environment-friendly cigarette packaging materials, wax-coated paper material could be as alternative to polymer film for use in cigarette packaging[16]. In the present research, the effect of different temperatures, relative humidities and packaging materials on the natural occurrence of tobacco spoilage fungi, not the inoculated tobacco spoilage fungi, on the cured tobacco leaves was investigated. Hence, the identification of the naturally occurred tobacco spoilage fungi need to be further studied.

CONCLUSIONS

In summary, the cured tobacco leaves can be kept high quality at 65% relative humidity or somewhat below, and lower temperature during the storage period, which can effectively prevent mildew. Kraft paper is an ideal packaging material for the cured tobacco leaves.

ACKNOWLEDGMENTS

This research was supported by the National Research Plan for the High-tech R & D Program during the Twelfth Five-year Plan Period (National 863 plan, No. 2012AA101705-2), Plan of Nature Science Fundamental Research in Henan University of Technology (No. 11JCYJ01), Key Technologies R & D Program of the Education Department of Henan Province (No. 12A210003) and Key Technologies R & D Program of Henan Tobacco Company (No. 201019).

REFERENCES

1. Ryan, L. (ed): Post-harvest Tobacco Infestation Control. Chapman & Hall. 1995.
2. Perez, L., Rodriguez, M.E., Rodriguez, F., Roson, C. Efficacy of acibenzolar-S-methyl, an inducer of systemic acquired resistance against tobacco blue mould caused by *Peronospora hyoscyami* f. sp. *Tabacina*. *Crop Prot*., 2003; **22**: 405–413.
3. Li, K., Li, T.S., Wang, P.Z., Li, H.Q., Bao, Y.R. Study on mycoflora in tobacco and the cause of its going midewed. *J. Zhengzhou Instit. of Technol.*, 2003; **24**(3): 20-24.
4. Kaelin, P., Gadani, F. Occurrence of *Bacillus thuringiensis* on cured tobacco leaves. *Curr. Microbiol*., 2000; **40**: 205–209.
5. Peng, Q.Y., Yi, T.Y. Analysis of tobacco mildew and its control measures. *Chinese Agr. Sci. Bull*., 2007; **23**(11): 146-150.
6. Forgacs, J., Carll, W.T. Mycotoxicoses: toxic fungi in tobaccos. *Science*, 1966; **152**: 1635-1634.
7. Welty, R.E., Lucas, G.B. Fungi isolated from damaged flue-cured tobacco. *Appl. Microbiol*., 1968, **16**: 851-854.
8. Welty, R.E. Lucas, G.B. Fungi Isolated from Flue-cured Tobacco at Time of Sale and After Storage. *Appl. Microbiol*., 1969; **17**(3): 360-365.
9. Welty, R.E. Lucas, G.B. Fungi Isolated from Flue-Cured Tobacco Inoculated in the Field with Storage Fungi. *Appl. Microbiol*., 1971; **21**(3): 552-554.
10. Mutasa, E.S. Effect of Water Activity and Potassium Sorbate on Growth of Deteriogenic Fungi from Tobacco. Cranfield Institute of Technology, 1988.
11. Zipper, R., Hammer, T.R., Spring, O. PCR-based monitoring of recent isolates of tobacco blue mold from Europe reveals the presence of two genetically distinct phenotypes differing in fungicide sensitivity. *Eur. J. Plant Pathol*., 2009; **123**: 367–375.
12. Ristaino, J.B., Johnson, A., Blanco-Meneses, M., Liu, B. Identification of the tobacco blue

mold pathogen, *Peronospora tabacina*, by polymerase chain reaction. *Plant Dis*., 2007; **91**(6): 685-691.

13. Moss, M. A., Main, C.E. The effect of temperature on sporulation and viability of isolates of *Peronospora tabacina* collected in the United States. *Phytopathology*,1988;**78**:110-114.
14. Trigiano, R.N., Van Dyke, C.G., Spurr, H.W., Main, C.E. Ultrastructure of sporangiophore and sporangium ontogeny of *Peronospora tabacina*. *Tob. Sci.*, 1985; **29**:116-121.
15. Waggoner, P.E., Pack, A.B., Reifsnyder, W.E. The climate of shade, a tobacco tent and a forest stand compared to open fields. *CAES Bull*., 1959; **626**: 39.
16. Shirai, A., Miyauchi, M., Nakanishi, Y. Evaluation method for paper materials used in wrapping cigarettes. *J. Jpn. Soc. for Food Sci. Technol*., 2006; **53**(11): 572-579.