

Effect of moisture content, nitrate and nitrite on TSNA accumulation in burley tobacco after high temperature storage

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Introduction

- ❖ Tobacco specific nitrosamines (TSNAs) are important **toxic components** in tobacco and maintain their status as important main stream smoke toxicants due to its specificity in tobacco and tobacco products.
- ❖ **Reduction of TSNA formation** and accumulation in tobacco leaves and their products have been important topics for many researchers around the world.

- ❖ TSNAs are formed from the nitrosation of tobacco alkaloids. They could form during both **air-curing** and **leaf storage**, nevertheless, research has been conducted mainly on TSNA formation during air-curing.
- ❖ Previous studies revealed that **during air-curing**, the high humidity environment could promote TSNA formation. **On the contrary**, TSNAs formed more under low humidity environment **during leaf storage**.

In this research :

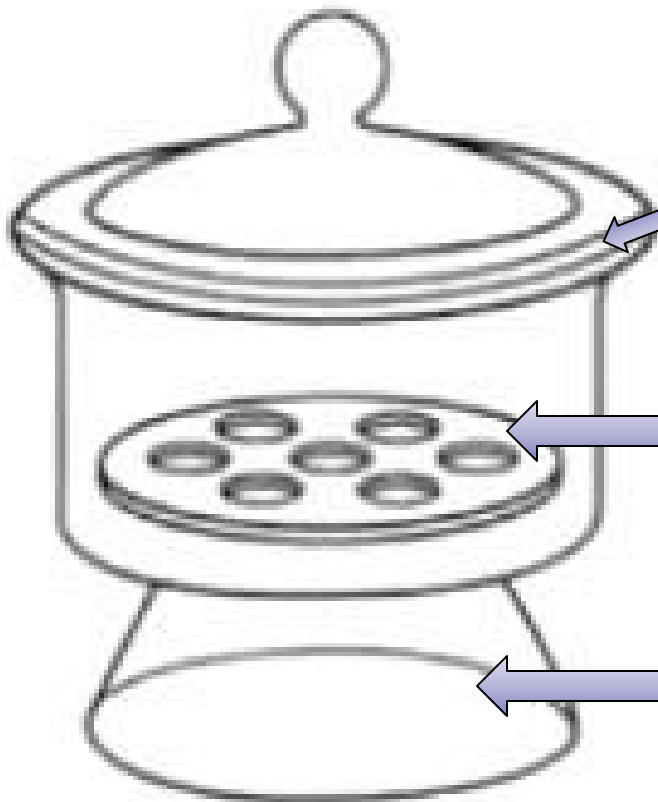
Three experiments were designed to produce burley tobacco samples with **varied moisture content levels**, and then to investigate the effects of various treatments on TSNA formation in burley tobacco leaves during high temperature storage.

Materials and Methods

All the burley tobacco samples were TN86 from Yunnan, produced in 2012.

At 24.5°C, the saturated aqueous solution of potassium carbonate (K_2CO_3) within an enclosed space could keep the air humidity at 43%. Based on this rule, the burley tobacco leaves with different moisture content were obtained by using solutions of K_2CO_3 with different saturation degrees.

Glass Dryers



Seal glass dryers with vaseline

Burley tobacco (sealed cut rag)
20 g

Solution of potassium carbonate
with different saturation levels
(300 mL)

Water balance — store the glass dryers in the temperature-controlled chamber at 24.5°C for 4 d.

Chemical Measurements

- ❖ **TSNA measurement:** Tobacco samples were sent to the University of Kentucky and Beijing Cigarette Manufacturer to run for TSNA. Solid Phase Extraction – Liquid Chromatography - Mass Spectrometry (**SPE-LC-MS/MS**) method was used to conduct the analysis.

Results-Part 1

Effect of different moisture content on TSNA formation in burley tobacco after high temperature storage

Tobacco were treated with five moisture contents and stored in the temperature-controlled chamber at 45°C for 15 d.

Saturation level of K_2CO_3 solution	1	3/4	1/2	1/4	0
Moisture content / %	7.71	11.23	17.53	25.12	31.52

The freshly cured burley tobacco leaves and all the samples after high temperature storage were measured for TSNAs.

TSNA contents in freshly cured tobacco leaves and high temperature stored leaves with different moisture content

	Moisture Content (%)	NNN ($\mu\text{g}\cdot\text{g}^{-1}$)	NAT ($\mu\text{g}\cdot\text{g}^{-1}$)	NAB ($\mu\text{g}\cdot\text{g}^{-1}$)	NNK ($\mu\text{g}\cdot\text{g}^{-1}$)	Total TSNA ($\mu\text{g}\cdot\text{g}^{-1}$)
Before storage	13.60	0.1137	0.3328	0.0102	0.0635	0.5202
After storage	7.71	1.5452 ^a	2.9974 ^a	0.0524 ^b	0.3704 ^a	4.9654 ^a
	11.23	1.0867 ^b	2.6562 ^b	0.0802 ^a	0.3692 ^a	4.1923 ^b
	17.53	0.4549 ^d	1.2017 ^c	0.0416 ^c	0.1598 ^b	1.8580 ^c
	25.12	0.4561 ^d	1.0780 ^d	0.0337 ^d	0.1573 ^b	1.7251 ^d
	31.52	0.5457 ^c	1.0711 ^d	0.0433 ^c	0.0909 ^c	1.7510 ^d

The results showed that after 45°C storage, for all treatments the TSNA contents significantly increased than that of freshly cured leaves.

After storage, the TSNA contents decreased with the increment of moisture content. While the moisture content was higher than 17.53%, the total TSNA content tended to be stable.

Results-Part 2

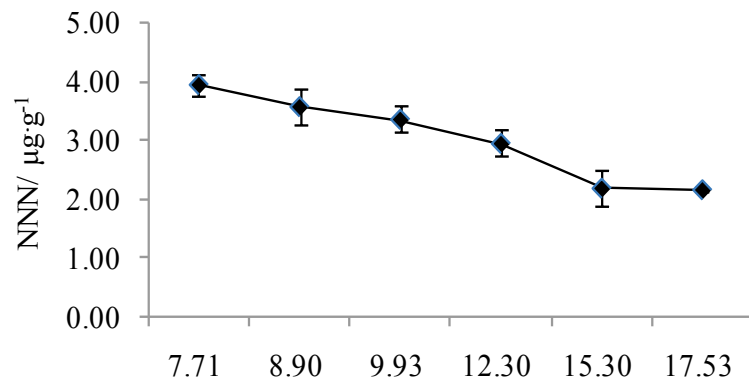
Effect of detailed moisture content on TSNA formation in burley tobacco after high temperature storage

Tobacco were treated with six moisture contents and stored at 45°C for 15 d.

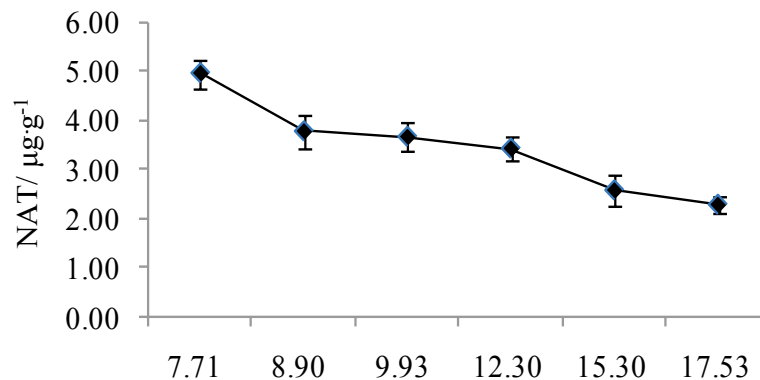
Saturation level of K_2CO_3 solution	1	9/10	4/5	7/10	3/5	1/2
Moisture content / %	7.71	8.90	9.93	12.30	15.30	17.53

The freshly cured burley tobacco leaves and all the samples after high temperature storage were measured for TSNA.

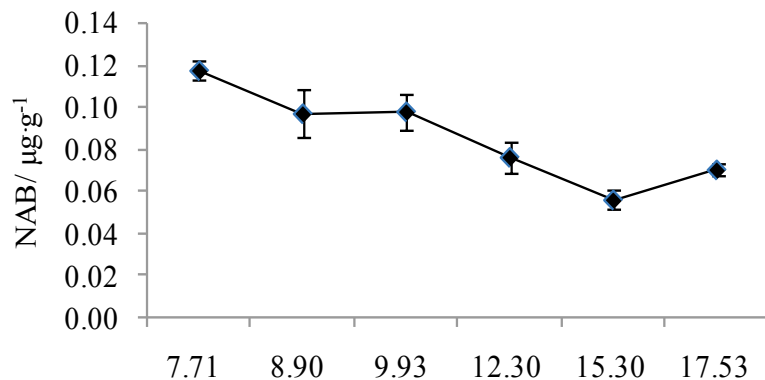
TSNA contents in burley tobacco leaves with detailed moisture content after high temperature treatment



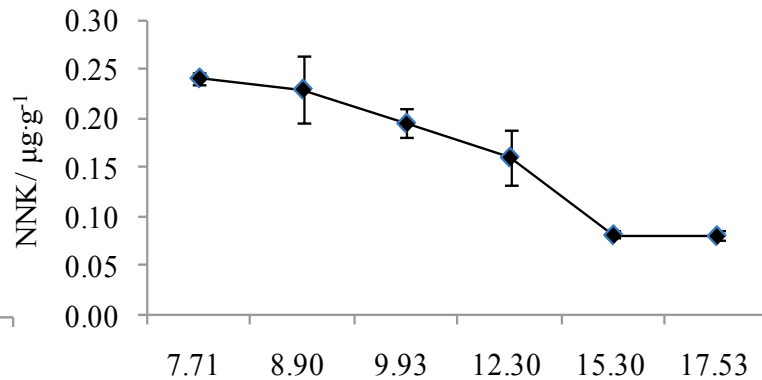
A moisture content/%



B moisture content/%



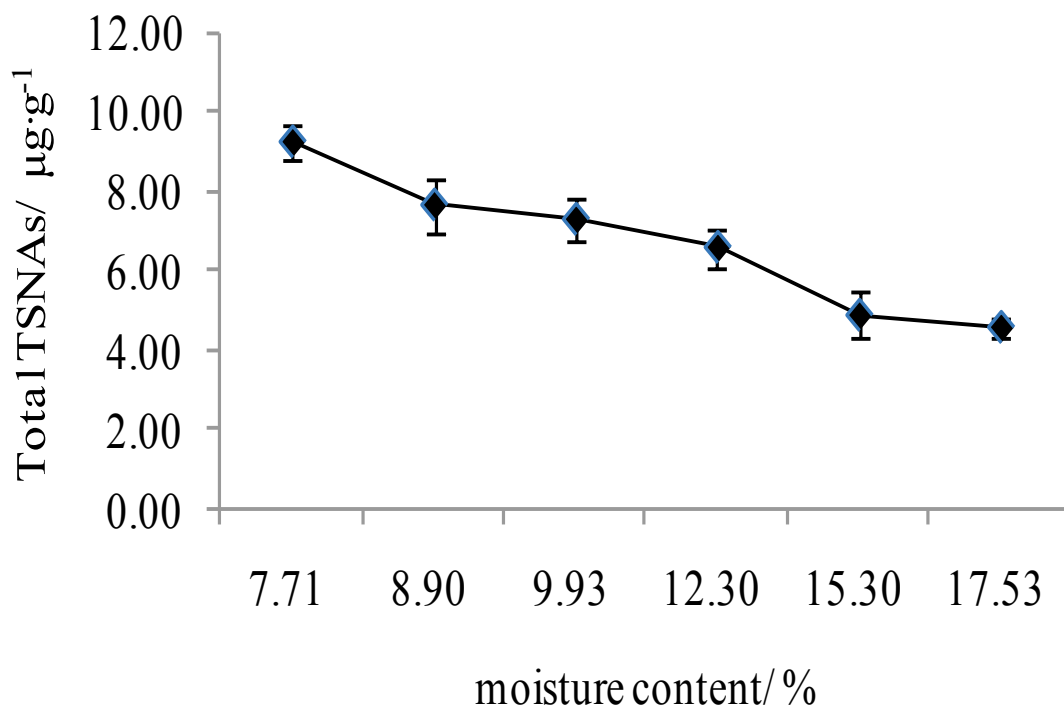
C moisture content/%



D moisture content/%

After 45°C storage for 15 d, TSNA contents were all significantly higher than that of before storage. NNN, NAT, NNK and NAB decreased as the moisture content increased from 7.71% to 15.30%.

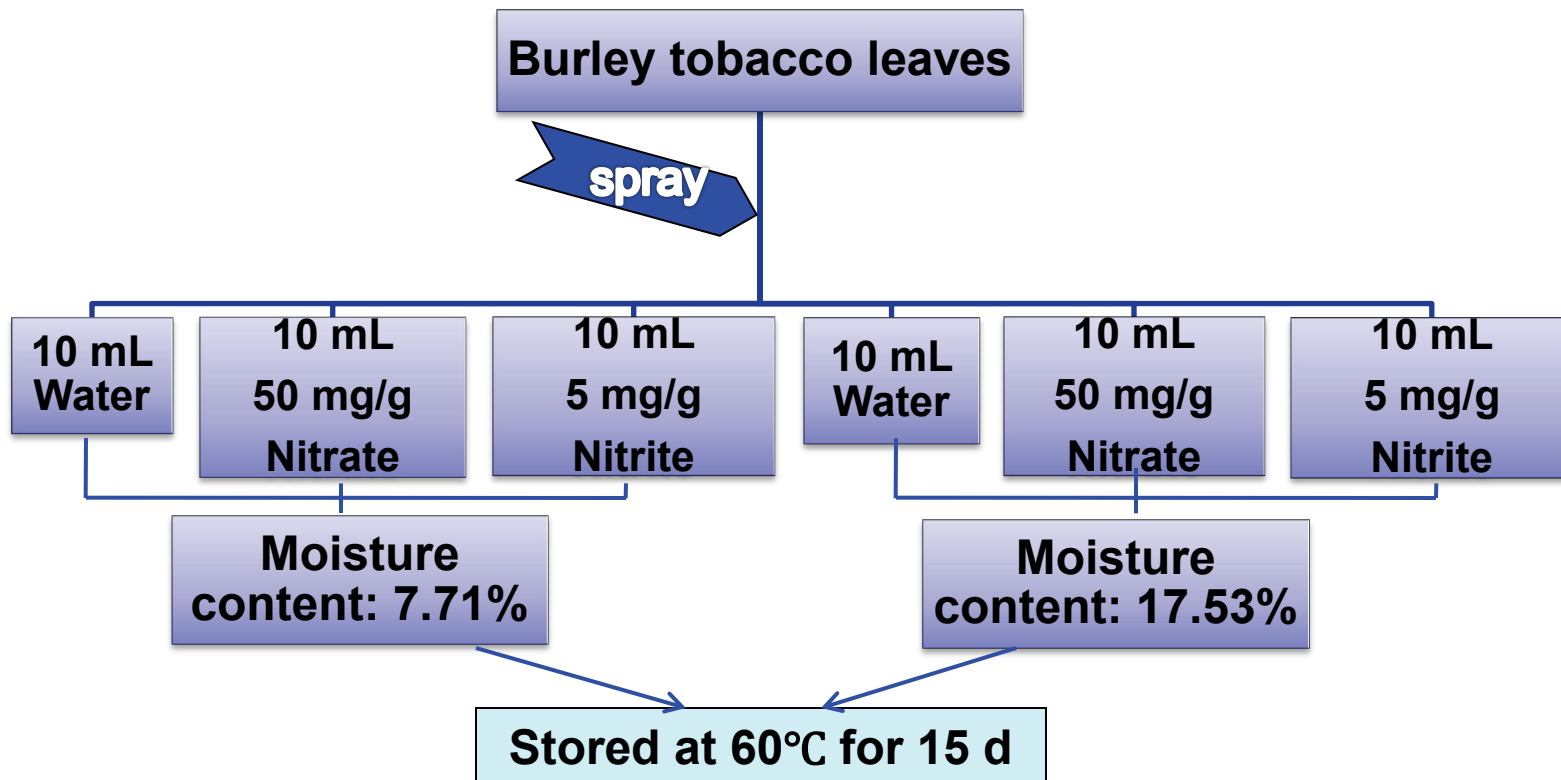
Total TSNA content in burley tobacco leaves with detailed moisture content after high temperature treatment



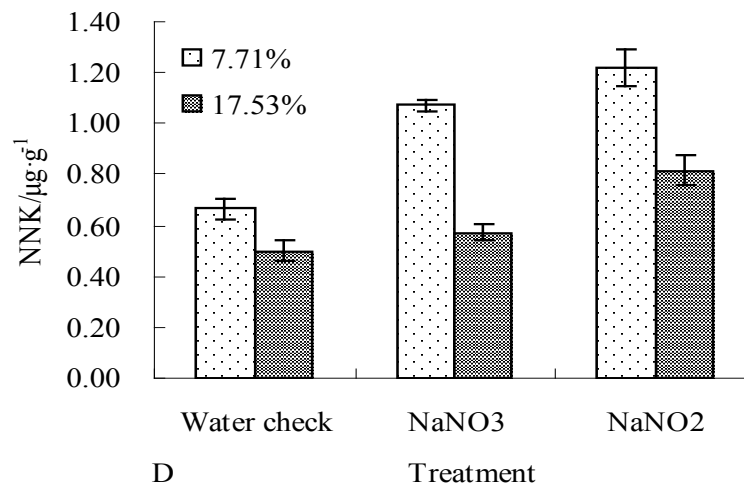
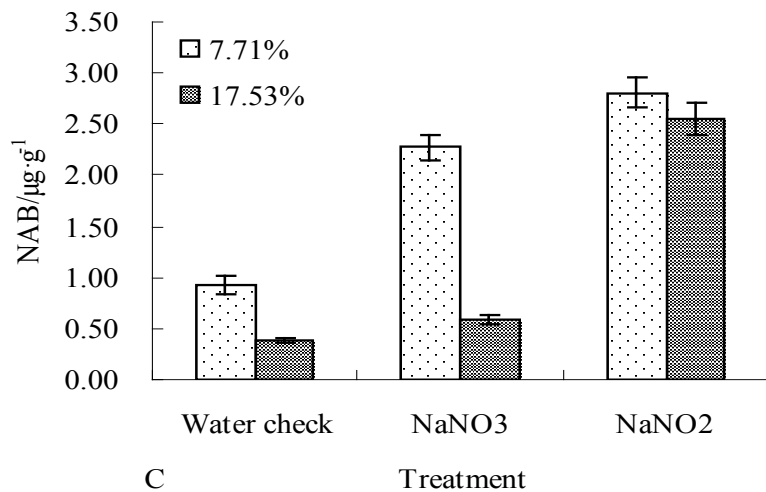
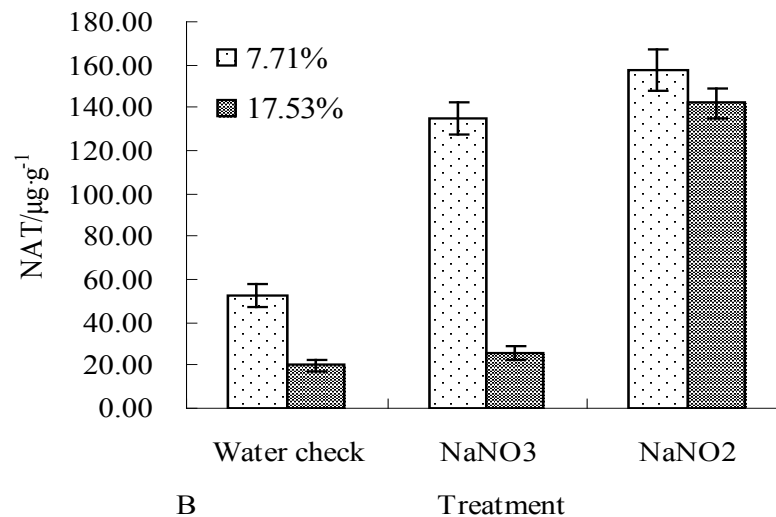
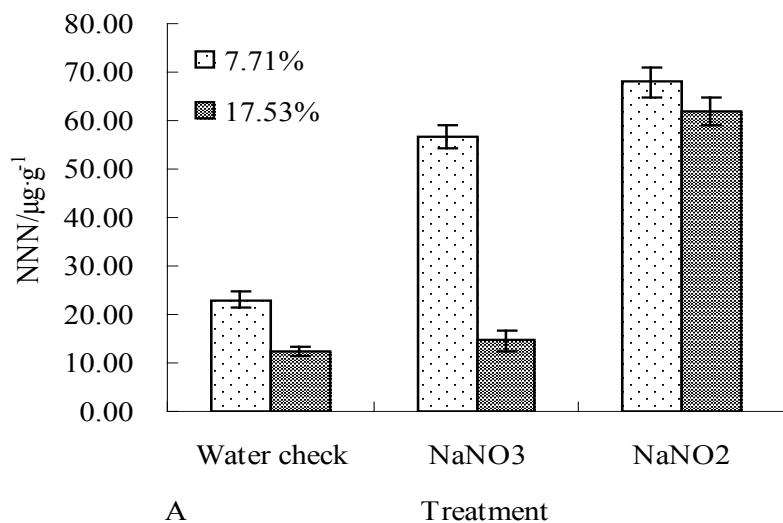
From the initial level of 1.2 ppm, the total TSNA content in burley tobacco leaves with detailed moisture content all increased than that of before storage. The total TSNA content decreased as the water content increased from 7.7% to 17.5%.

Results-Part 3

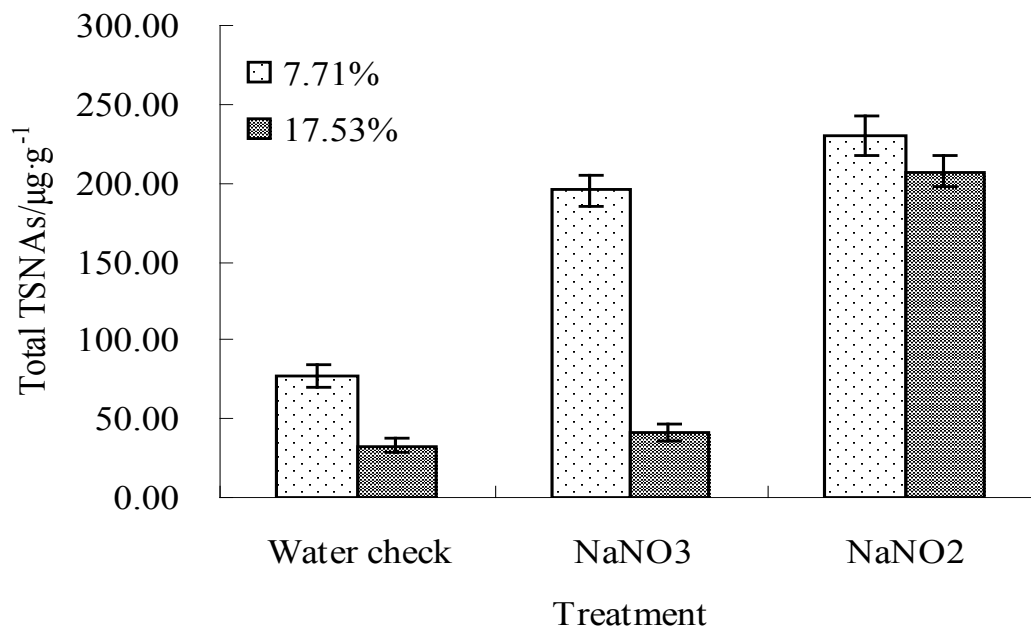
Effect of adding nitrate and nitrite to tobacco leaves with different moisture content on TSNA formation after 60°C storage for 15 d



TSNA content in burley tobacco leaves added with nitrate and nitrite with different moisture content after 60°C storage for 15 d



Total TSNA content in burley tobacco leaves added with nitrate and nitrite with different moisture content after 60°C storage for 15 d



From an initial TSNA level of 2.8 ppm, TSNAs in the water check increased after storage to 77 and 33 ppm in the low and high moisture tobacco, respectively.

Nitrite sprays caused a very marked increase in both the low and high moisture tobacco, to 230 ppm and 207 ppm, respectively.

The addition of **nitrate** caused a much smaller increase in the high moisture tobacco but a significant increase in the low moisture tobacco, to 195 ppm.

Conclusion

- ❖ The moisture content was closely related to the formation of TSNAs during high temperature storage. TSNAs increased most in the low moisture tobacco, and less in higher moisture treatments after 45°C storage.
- ❖ After 60°C storage, nitrite sprays caused a very marked increase in TSNA contents for both the low and high moisture tobaccos, nevertheless, nitrate sprays caused a much smaller increase in the high moisture tobacco but a significant increase in the low moisture tobacco, suggesting that nitrate may be more stable in the high moisture tobacco.

- ❖ Reduction of nitrate accumulation in harvested and cured tobacco and proper increase of the moisture content of cured tobacco would be effective pathways to reduce TSNA formation during high temperature storage.

Acknowledgement

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Thank You !