Interaction effects of temperature, moisture content and storage environment on tobacco-specific nitrosamine formation during burley tobacco storage

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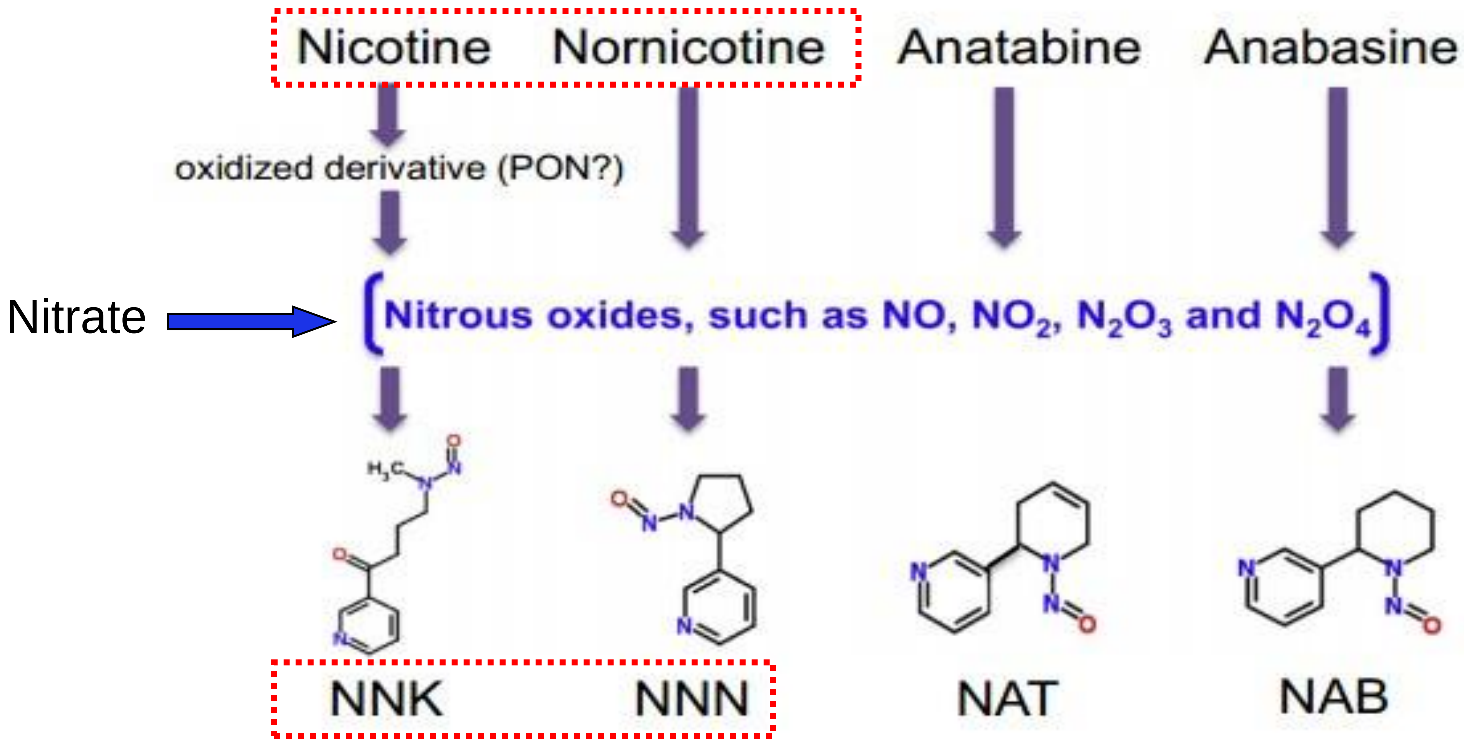
Introduction

- Tobacco specific nitrosamines (TSNAs) are important toxic components in tobacco and tobacco smoke, and NNN and NNK are reportedly strong animal carcinogens
- TSNA remains to be important research topics due to its specificity in tobacco and tobacco products.

Introduction

❖ **TSNAs are formed through the nitrosation of tobacco alkaloids**



Introduction

❖TSNAs are formed during both air-curing and leaf storage. A lot of research has been conducted on TSNA formation during air-curing of burley tobacco.

❖Air-curing--microbial activity and curing-environment

❖Storage – high temperature, high nitrate level

Introduction

- As for the effect of humidity and leaf moisture on TSNA formation, different results were observed for curing tobacco and storing tobacco.
- During air-curing, the high humidity environment could promote TSNA formation.
- On the contrary, during leaf storage,TSNAs formed more for low moisture tobacco and under low humidity environment.
- Temperature and leaf moisture both have impact on TSNA formation, while no information is available on the interaction of these two factors.

In this research :

- Determine and mathematically describe the interaction effect of temperature and moisture content on TSNA formation by setting different levels of leaf moisture contents and storage temperatures;
- Evaluate the effects of changing storage environments on TSNA formation during long-term storage.

Part 1

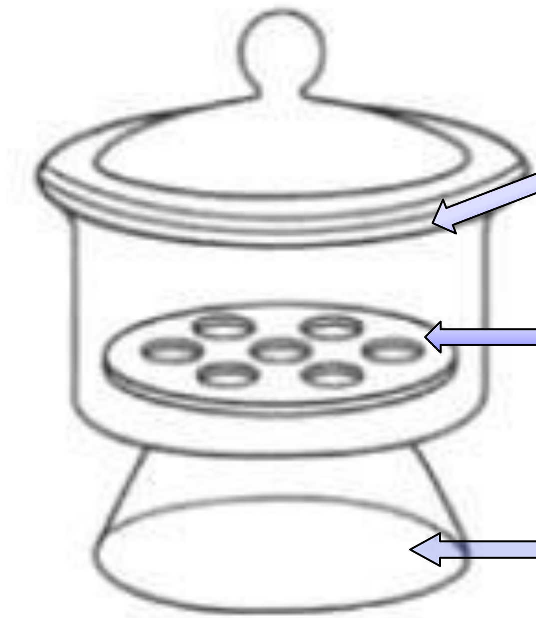
15-days laboratory controlled experiment

Interacting effect of storage temperature and moisture content on TSNA formation

Setting different levels of leaf moisture contents and storage temperatures.

Materials and Methods

- **Burley tobacco samples were TN86 from Yunnan, produced in 2014.**
- **The first step--Control moisture content of tobacco cuts by regulating air humidity of the storage environment.**



Seal with vaseline

Burley tobacco cuts 30g

Different saturation levels of potassium carbonate 300 mL

Glass desiccators stored at 24.5℃,RH 60% for 4 days.

Materials and Methods

Saturation level of K_2CO_3 solution	100%	60%	30%
Moisture content / %	7.71	15.30	22.10

Note: A saturated solution of potassium carbonate made by dissolving 110 g of K_2CO_3 in 100 mL of distilled water at 24.5℃.

❖**The second step--** tobacco cuts with known moisture contents were placed in reagent bottles and sealed tightly, stored in temperature-controlled chambers at 10℃, 20℃, 30℃, 40℃, and 50℃ for 15 days.

Chemical Measurements

- TSNA measurement:

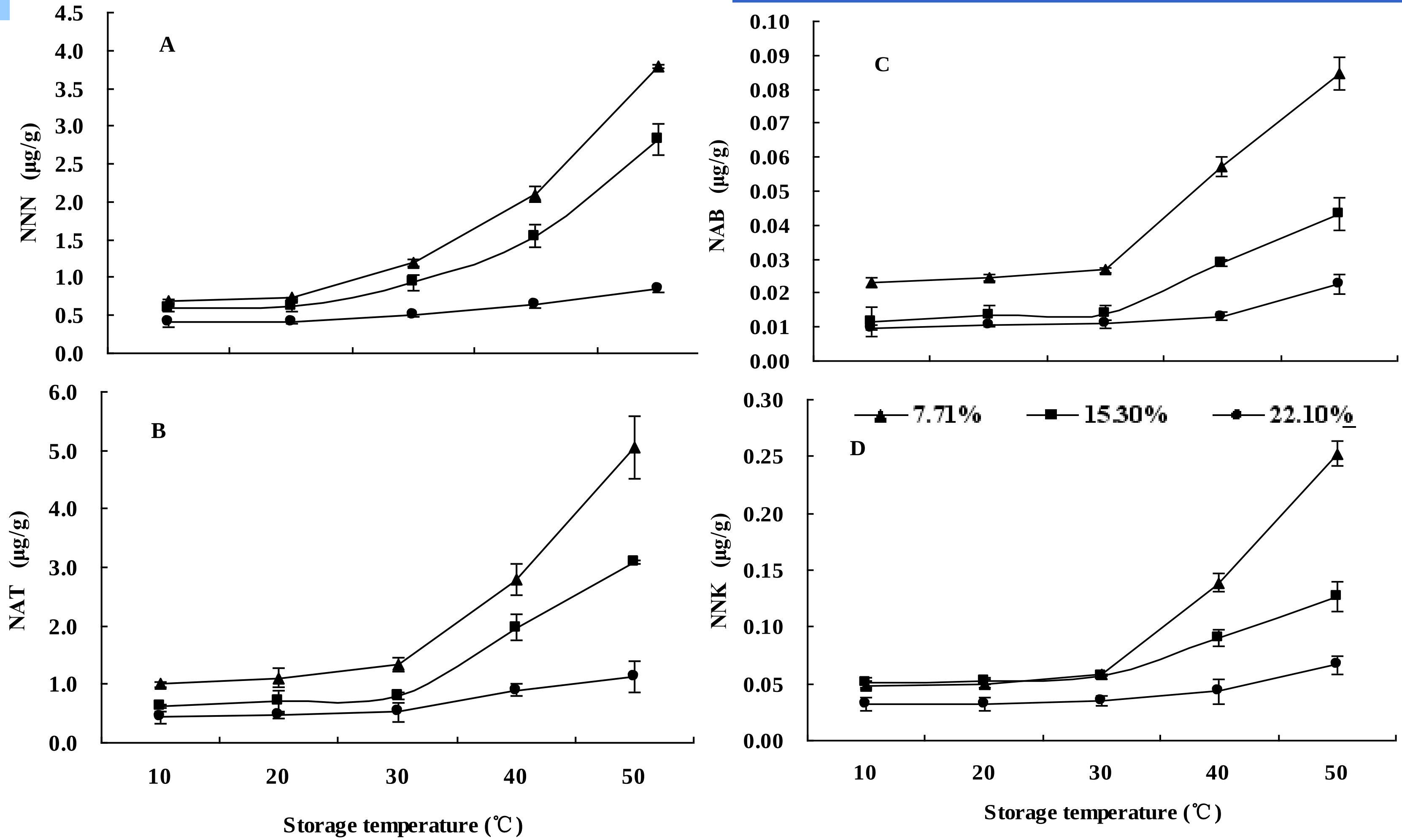
  Solid Phase Extraction–Liquid Chromatography/Mass Spectrometry (SPE-LC-MS/MS) method

- Individual alkaloids:

  Gas chromatograph (Agilent 7890A)

- NO_3-N and NO_2-N: **Colorimetric method**

Effects of storage temperature and tobacco moisture content on TSNA formation during storage



When storage temperature exceeded 30℃, significant differences ($P<0.01$) were observed both in individual and total TSNA contents among samples for tobaccos with different moisture contents.

Relationship between TSNA contents and storage temperature under different tobacco moisture contents

	Moisture (%)	$Y(m) = \alpha + \beta t + \theta t^2$					Moisture (%)	$Y(m) = \alpha + \beta t + \theta t^2$			
		α	β	θ	R^2			α	β	θ	R^2
NNN	7.71	1.295	-0.843	0.267	0.998	NAB	7.71	0.037	-0.019	0.006	0.984
	15.30	1.090	-0.660	0.200	0.995		15.30	0.018	-0.009	0.003	0.983
	22.10	0.447	0.076	0.031	0.999		22.10	0.015	-0.005	0.001	0.945
NAT	7.71	2.112	-1.412	0.398	0.993	NNK	7.71	0.109	-0.077	0.021	0.992
	15.30	1.150	-0.724	0.224	0.986		15.30	0.068	-0.023	0.007	0.991
	22.10	0.502	-0.123	0.051	0.971		22.10	0.044	-0.014	0.004	0.980
TSNAs	7.71	0.109	-0.077	0.021	0.992						
	15.30	0.068	-0.023	0.007	0.991						
	22.10	0.044	-0.014	0.004	0.980						

For each tobacco moisture content, TSNA contents followed a quadratic function relationship with storage temperature, showing that the effect of temperature on TSNA formation was affected by moisture contents. As the moisture increased, the effect of temperature became less prominant.

Analysis of variance for effects of tobacco moisture and storage temperature on TSNA formation

Source	df	NNN		NAT		NAB		NNK		TSNAs	
		F	P	*F*	P	*F*	P	*F*	P	*F*	P
Moisture content (*m*)	2	2490.5	<0.01 **	1581.1	<0.01 **	650.2	<0.01 **	483.8	<0.01 **	5293.1	<0.01 **
Temperature (*t*)	4	2899.1	<0.01 **	1643.6	<0.01 **	384.5	<0.01 **	511.9	<0.01 **	5782.1	<0.01 **
$m \times t$	8	480.5	<0.01 **	263.1	<0.01 **	69.3	<0.01 **	125.2	<0.01 **	939.5	<0.01 **

Storage temperature and moisture content of tobacco had significant effects (P< 0.01) on TSNA formation as well as significant interactive effects (P< 0.01)

Storage temperature-- the primary source of total TSNA variation -- 56.1% contribution rate, moisture content (25.6%), and interaction effect (18.2%)

The interaction model of moisture content and temperature on TSNAs formation

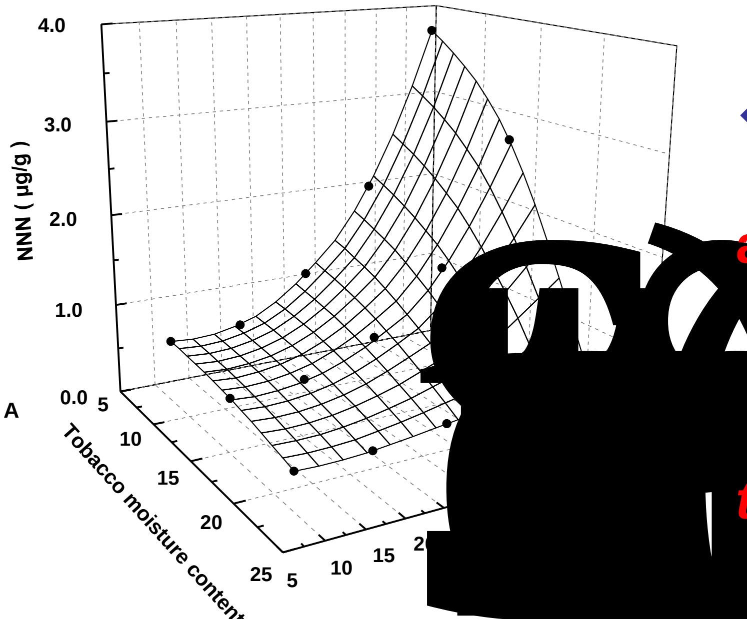
- **A two-dimensional equation was used to depict the response of TSNA formation to temperature and moisture content**

$$y = a + bt + cm + dt^2 + em^2 + fmt$$

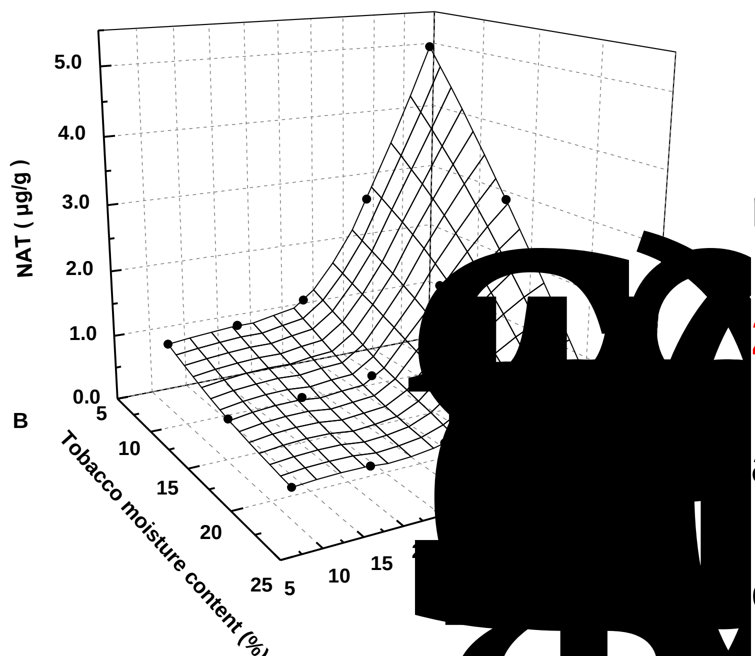
y **is TSNA content;** ***t*** **is storage temperature; and** ***m*** **is moisture content.**

- **Stepwise regression analysis were conducted to interpret the degree of influence of storage temperature and moisture content on TSNA formation.**

Moisture and temperature effects on TSNA formation in burley tobacco

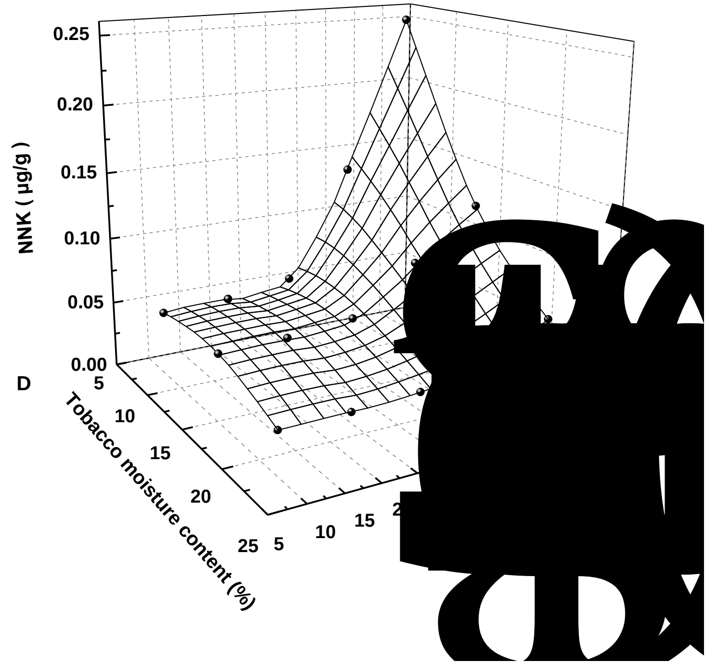


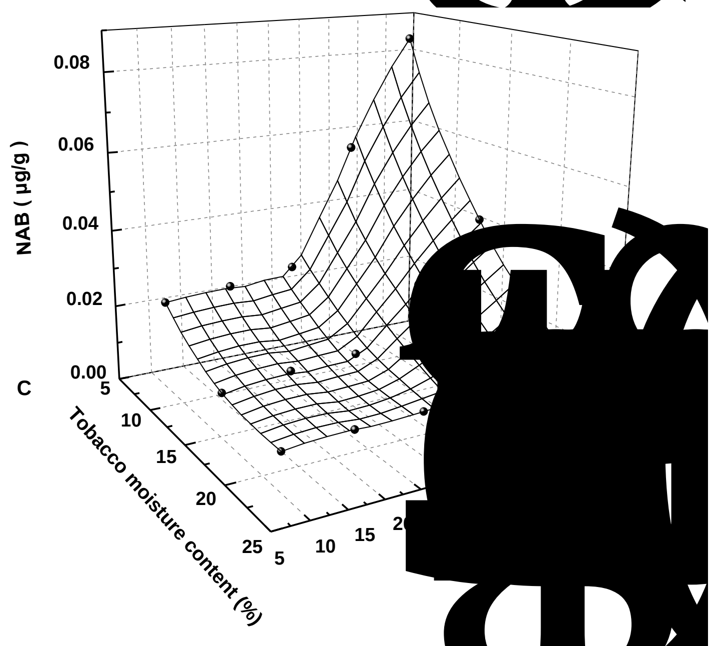
- NNN and NAT were **more closely associated with storage temperature**, and significantly affected by **the interaction of *t* and *m*.**



- According to the two-dimensional model when temperature exceeded **25.4℃** and **25.5 ℃**, the content of NNN and NAT increased significantly, especially for low moisture tobacco.

Moisture and temperature effects on TSNA formation in burley tobacco



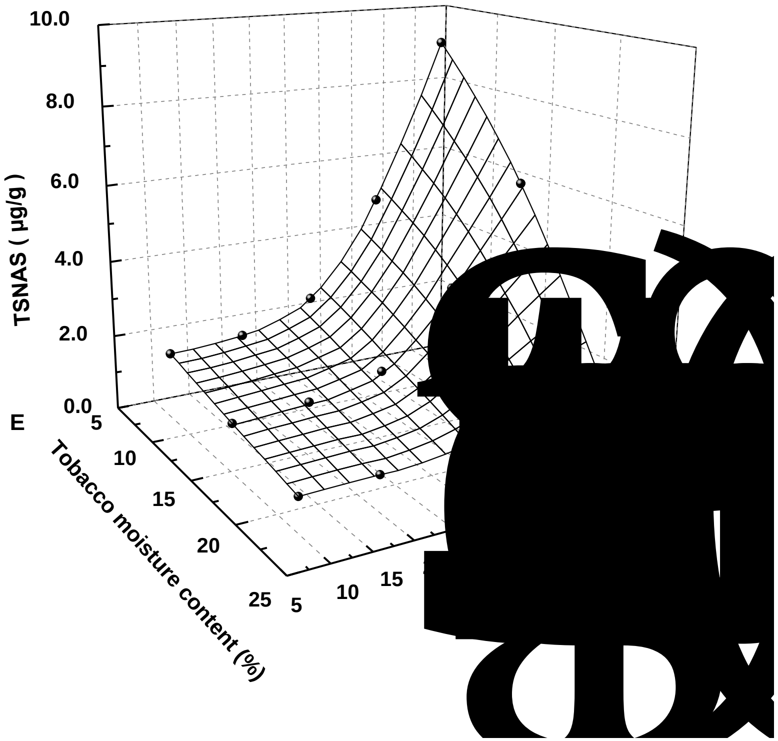


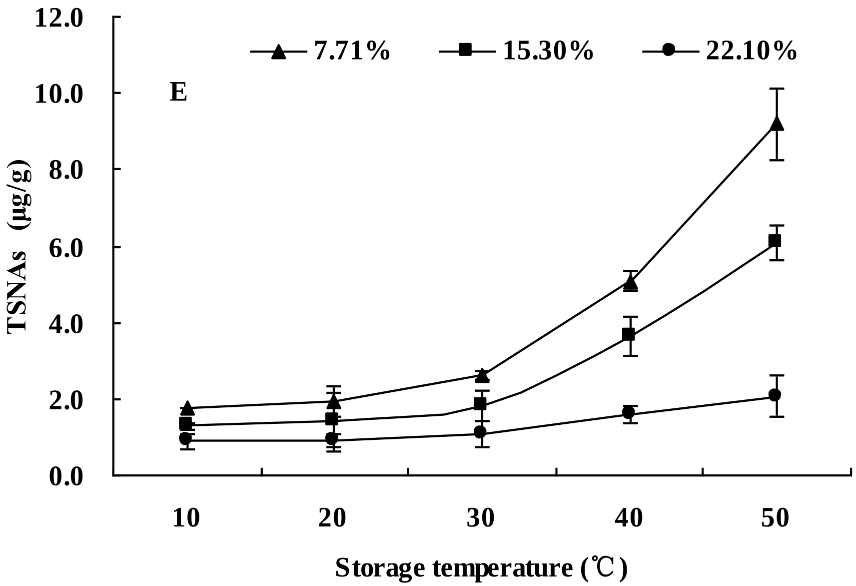
❖ $y_{NNK}=0.007+0.005m+5.748*10\text{-}4t-3.147*10\text{-}5m^2+1.061*10\text{-}4t^2-2.922*10\text{-}4mt$ （$R^2=0.932$）

❖ The model explained 93.2% of the variation in NNK formation.

❖ NNK was more closely associated with storage temperature and the interaction *of t* and *m.*

❖ when temperature exceeded 27.7℃, NNK content greatly increased.





- y_{TSNA}=-0.220+0.238m+0.023t-0.004m^2+0.004t^2-0.0103mt（R^2=0.961）
- **storage temperature--primary source of variation in TSNAs**
- **TSNAs content significantly affected by interaction *of t* and *m***
- **when temperature exceeded 25.5℃, TSNAs content greatly increased.**
- **Suggested Storing conditions:**

  temperature: less than 25 ℃

  moisture: higher range, but no greater than 18% to avoid going mouldy

Part 2

A long-term natural storage experiment

Storage environmental condition (temperature and humidity) on TSNA formation

Freshly cured tobacco stored separately in two geographic sites with distinctively different climatic conditions and in air-conditioned environments

Effect of environmental humidity and temperature on TSNA formation in burley tobacco during storage

- **Storage time: April 1, 2014 to October 15, 2014**
- **Temperature and relative humidity of storage sites were monitored by HOBO-U23 Pro-v2 data loggers that recorded data hourly.**

Three burley tobacco samples, each of 1kg,

Zhengzhou, Henan

Laboratory of Henan Agricultural University
113° 31’25”E,
34° 47’11”N,
103 m above sea level

Midu, Yunnan

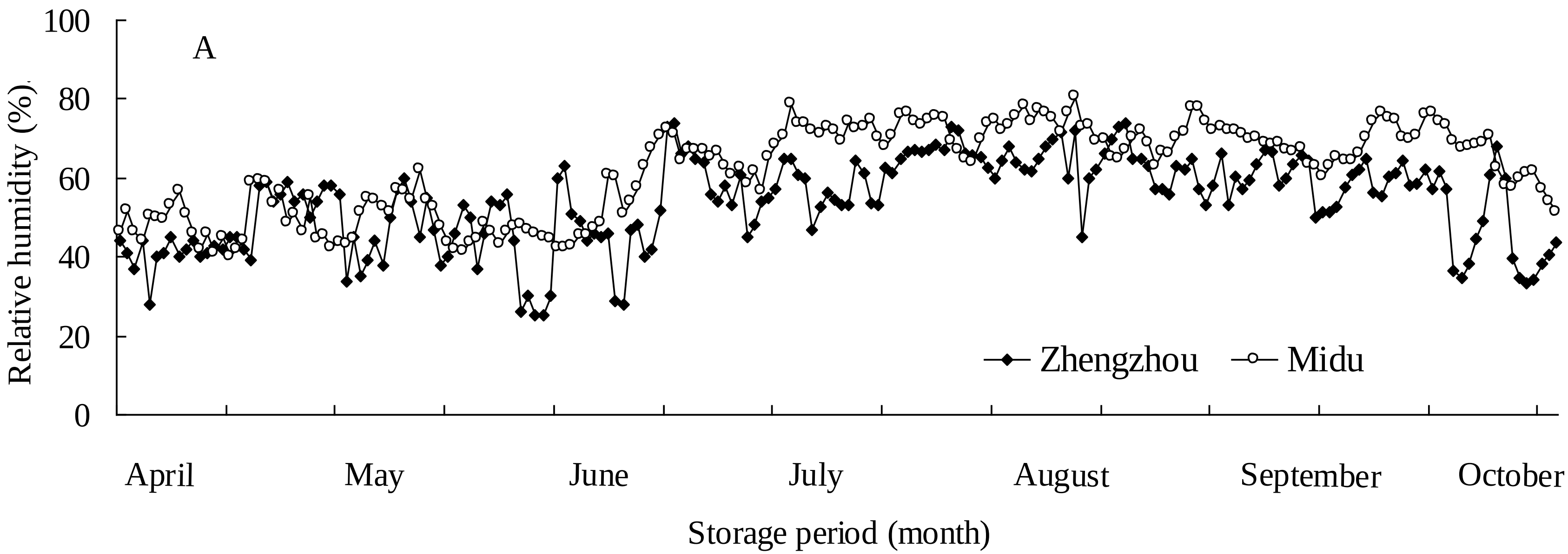
Tobacco station of Xinjie County
100° 25’35”E,
25° 23’30”N,
1680 m above sea level

Control (25℃)

Laboratory of Henan Agricultural University

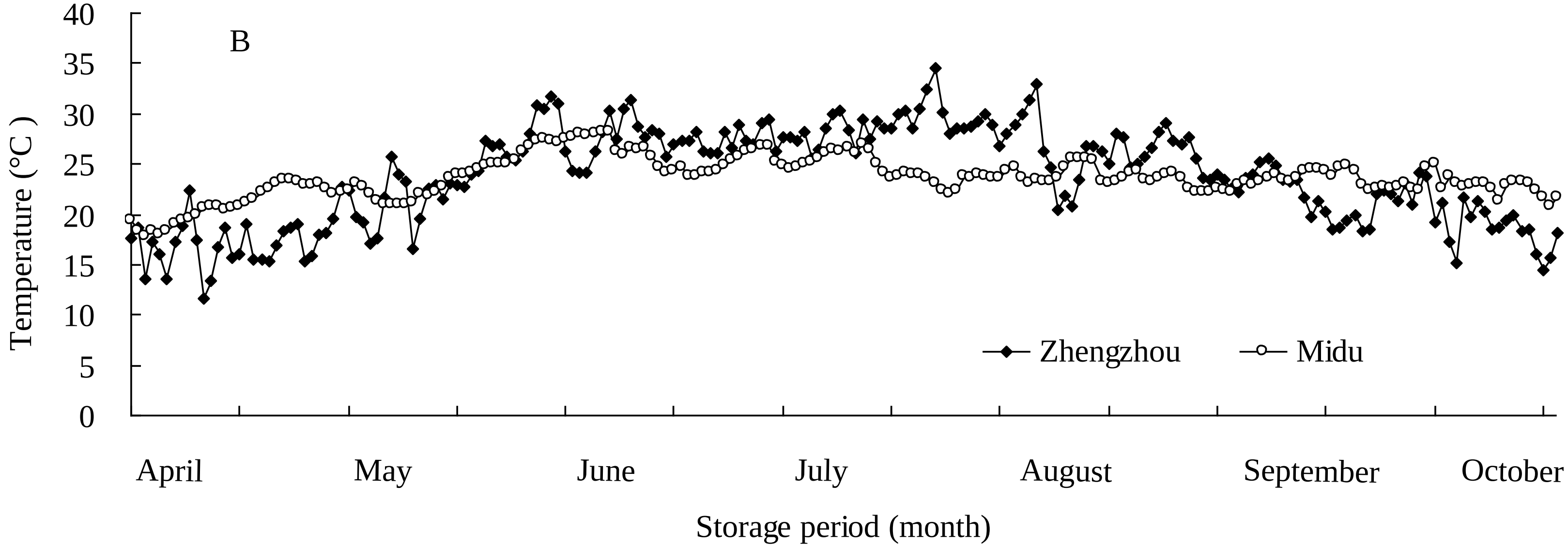
Daily humidity changes in two storage sites

- **Mean relative humidity in Midu, Yunnan was 61.9%, about 8% higher than that in Zhengzhou during entire storage period**
- **From early June, mean relative humidity in Zhengzhou was lower than in Midu by approximately 10.7%**



Daily temperature changes in two storage sites

- **Dali storage site—only 43 days of daily mean temperatures exceeding 25° C, the highest was 28.2° C**
- **Zhengzhou storage site— 93 days of daily mean temperatures above 25° C, the highest temperature (34.6° C) appearing in July, the monthly mean temperature 28.9° C.**



TSNA contents after storage at two different sites

	Storage site	NNN (μg/g)	NAT (μg/g)	NAB (μg/g)	NNK (μg/g)	TSNAs (μg/g)
Before storage		1.48±0.06	1.76±0.004	0.018±0.003	0.033±0.009	3.29±0.07
After storage	LT-HH (Midu)	3.57 ± 0.12	4.64 ± 0.07	0.11 ± 0.01	0.12 ± 0.01	8.45 ± 0.19
	HT-LH (Zhengzhou)	5.87 ± 0.05*	6.05 ± 0.22*	0.17 ± 0.02	0.3 ± 0.01*	12.39 ± 0.28*
	AC- 25℃	2.88±0.09	3.45±0.11	0.02±0.01	0.11±0.01	6.453±0.13
Net increase	LT-HH (Midu)	2.09	2.88	0.09	0.09	5.15
	HT-LH (Zhengzhou)	4.39	4.29	0.15	0.26	9.13

TSNAs content of tobacco stored in Zhengzhou was markedly higher than that in Midu.

After storage, TSNAs content of samples stored in Midu and Zhengzhou increased by 1.4-fold and 2.8-fold, respectively.

Conclusion and Discussion

- **Storage temperature was the main source for total variation of TSNAs and was the most critical factor contributing to TSNA formation during leaf storage.**
- **Temperature and moisture content have significant interactive effects on TSNA formation during burley tobacco storage.**
- **According to the two-dimensional model, when temperature exceeded 25.5℃, total TSNAs content increased significantly.**

Conclusion and Discussion

- The moisture content of storing tobacco should maintained at relatively higher level (but not exceeding 18%, the storage standard of burley tobacco, to avoid being mouldy)
- Burley tobacco stored at Midu, a region that did not have a high temperature season and with relatively higher air humidity, had lower levels of TSNAs.
- The control of temperature and humidity of the storage environment is an effective way to reduce TSNA formation during leaf storage.

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Thank You !