Analysis on TSNAs and their relationship with alkaloids in cigar wrapper and filler tobaccos from different regions and varieties

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Background

- Cigar is an unique tobacco product widely consumed in the world. In recent years, cigar consumation in China grows rapidly.
- High quality and low harm cigar production highly depends on cigar tobacco since it is manually manufactured without industry manupulations.
- TSNAs are important harmful components found in tobacco and smoke, and the levels are much higher in air-cured and sun-cured tobacco which have been extensively investigated.
- Cigar tobacco is also cured by air or sun, and requires fermentation, and TSNA levels are assumed and proved very high.

Background

- Nicotine to nornicotine conversion is an undesirable trait which may lead to enhanced NNN formation and proved to be an important factor contributing to higher TSNA accumulation in air-cured tobacco.
- The status of nicotine conversion in cigar wrapper and filler tobacco was seldom reported.
- Investigation into TSNA contents and nicotine conversion rate in cigar tobacco is essential for low harm and high quality cigar production.

Objective

- Collect cigar tobacco samples from different regions worldwide and from different cigar varieties.
- Analyze contents of individual TSNAs and their precursors.
- Investigate the relationship between TSNA and their precursors, and identify the most imminent approach for TSNA reduction in cigar tobacco.

Materials and Methods

Samples from different regions:

55 samples fermented samples were collected

regions: Central America 18, South America 7,
Southeast Asia 13, China 17

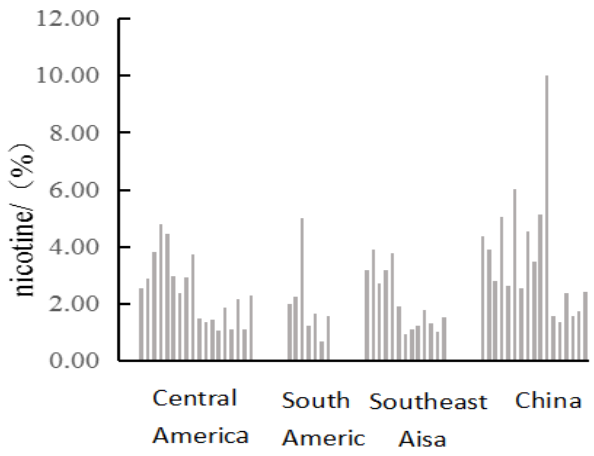
types: wrapper 13, binder 13, filler 29

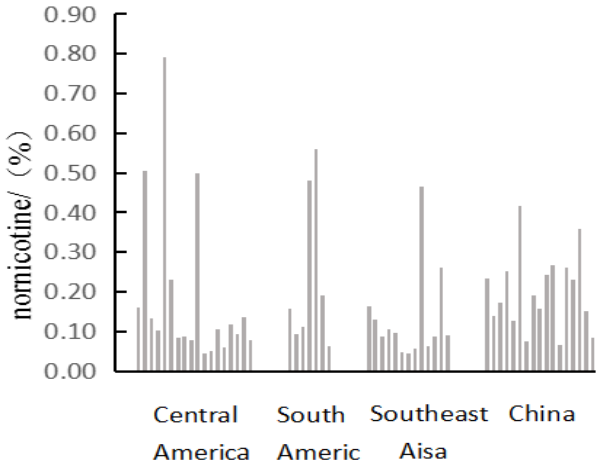
Samples from different varieties:

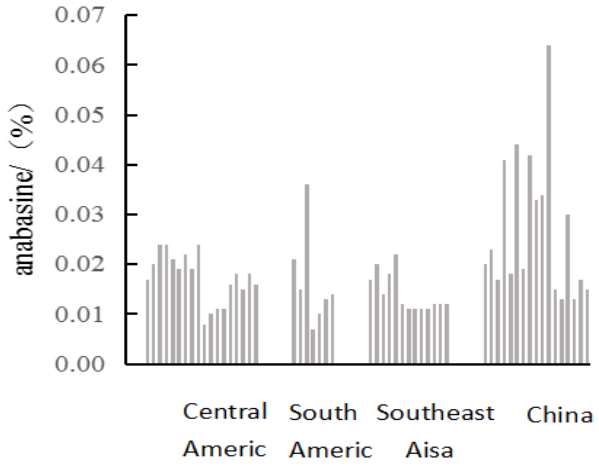
16 varieties originated from different countries were cultivated in 2018 in Sichuan, China. Air-cured and fermented tobacco leaves were sampled, freeze-dried and measured for alkaloids, TSNAs, nitrate and nitrite.

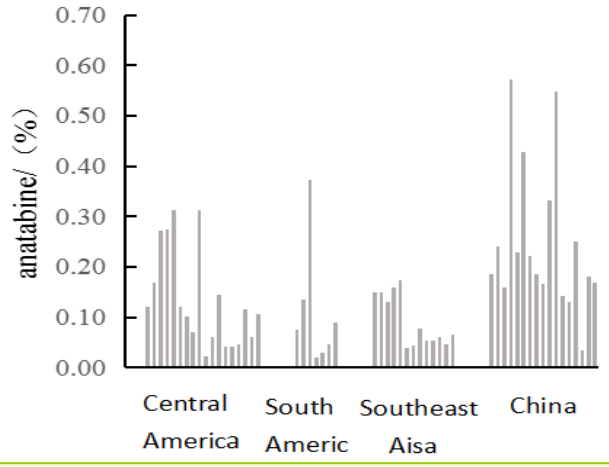
Percent nicotine conversion was calculated and expressed by the percentage of nornicotine in the total amount of nic + nornic

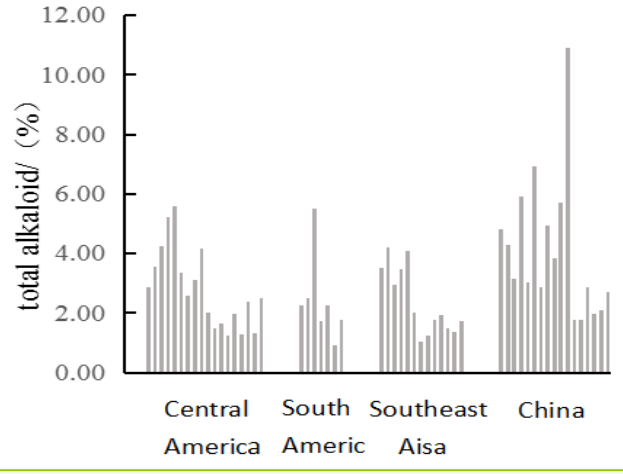
Contents of alkaloids in cigar tobacco from different producing areas

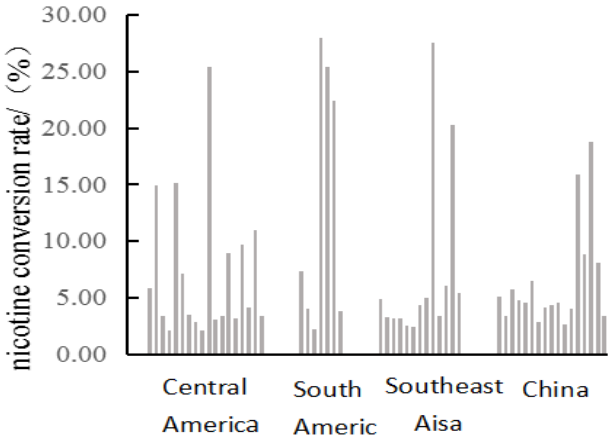






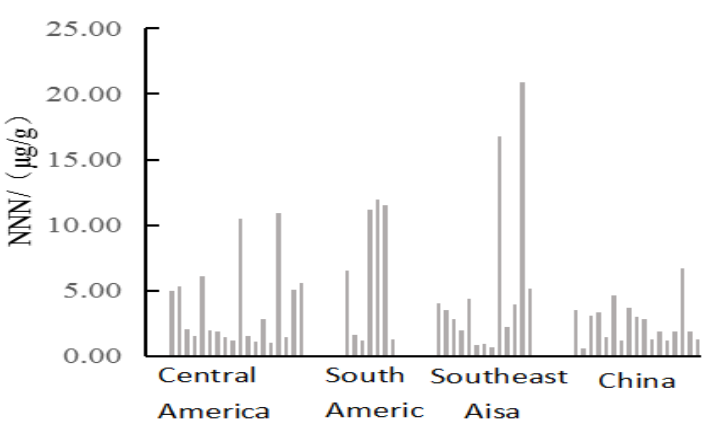


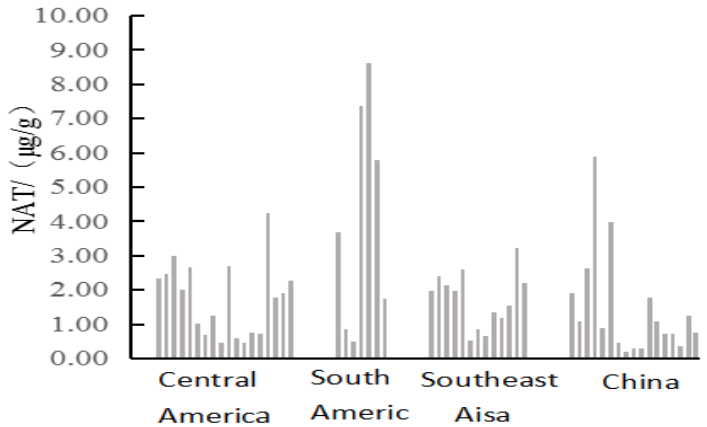


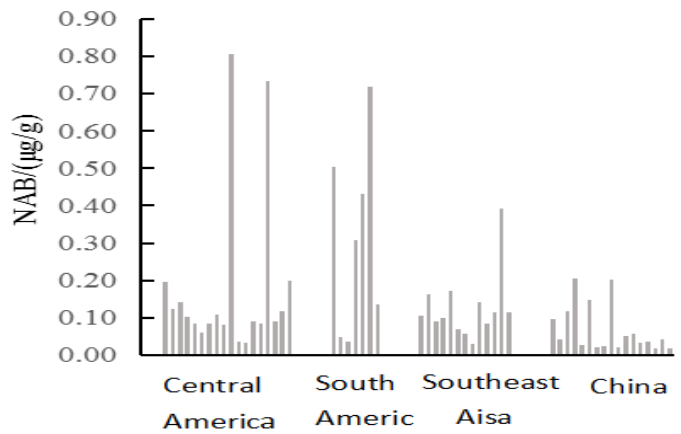


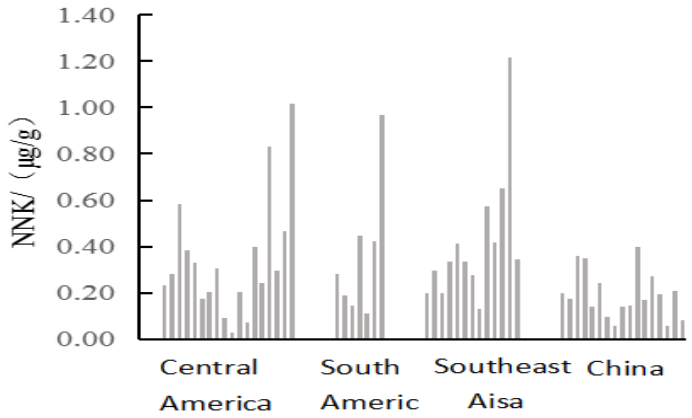
Nicotine and nornicotine contents varied greatly among different samples. There were no significant differences among different areas, meaning geographical location was not the determining factor affecting alkaloid level in cigar tobacco at the current stage. Comparing the percent nicotine conversion, we found the problem of nicotine conversion was extensively existed in cigar tobacco production.

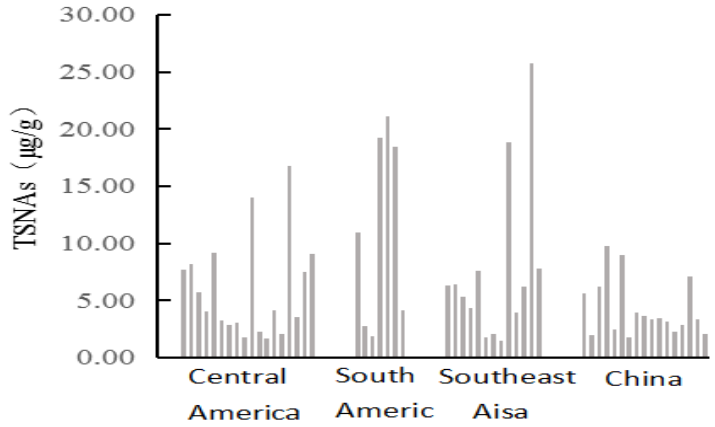
Contents of TSNAs in cigar tobacco from different producing areas





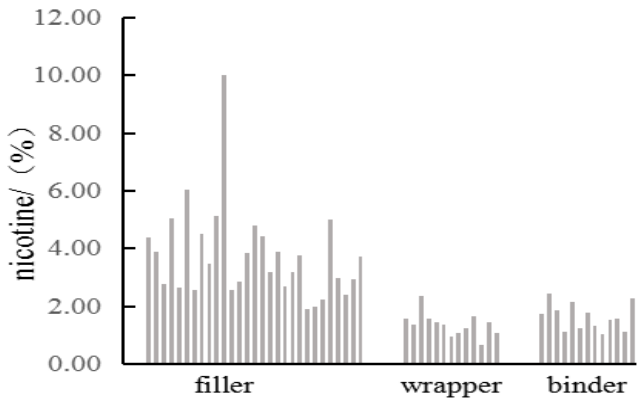


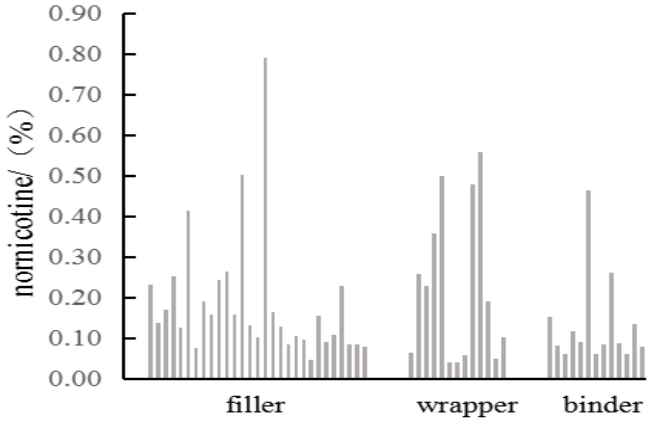


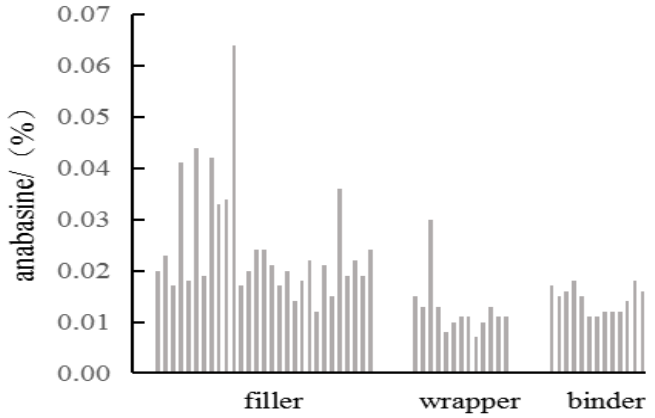


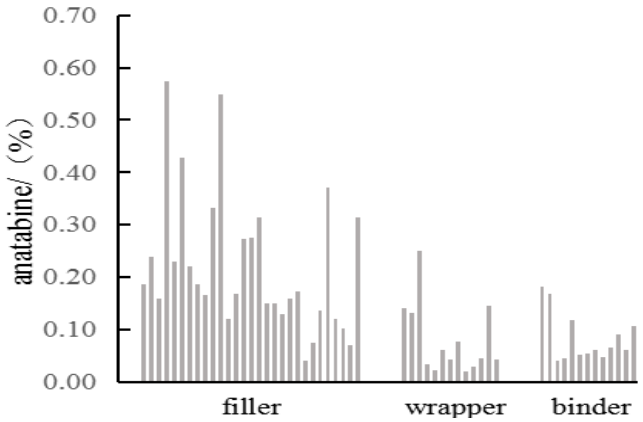
The variation of TSNA contents was great for all samples and for samples within the same producing areas, especially for NNN contents. Similarly, there was generally no significant difference among different areas when comparing the average data, indicating that the location was not decisive to TSNA levels, each area having a great potential for TSNA reduction.

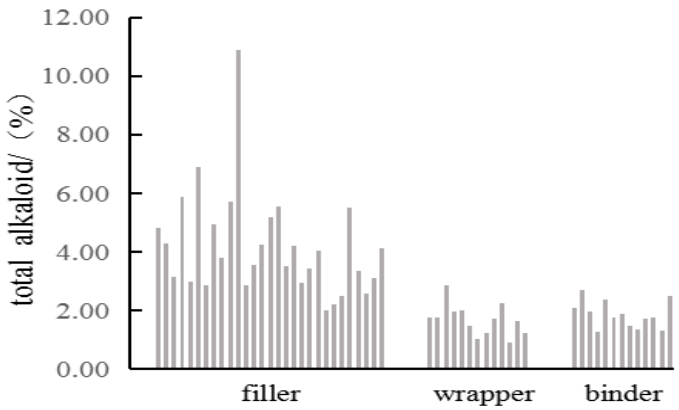
Alkaloids contents in different cigar tobacco types

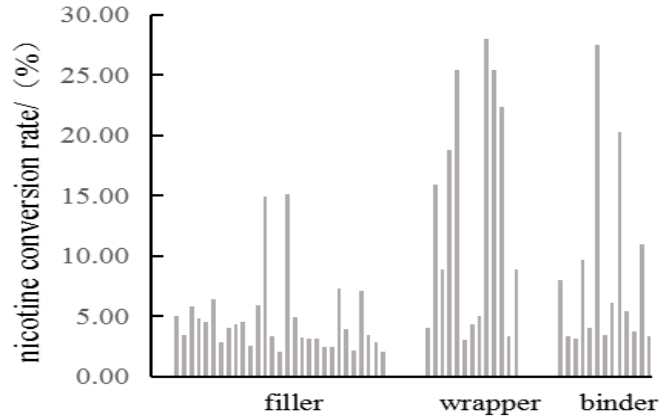






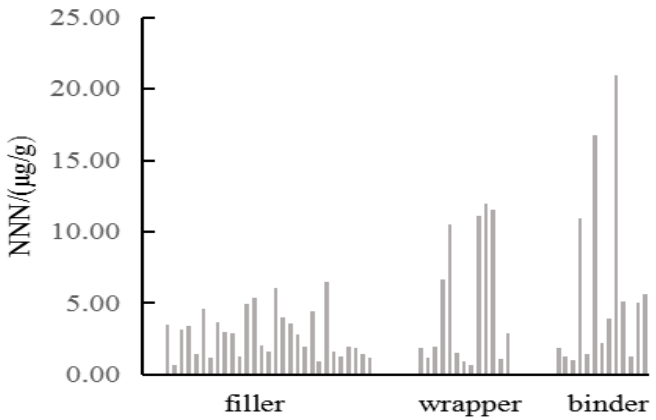


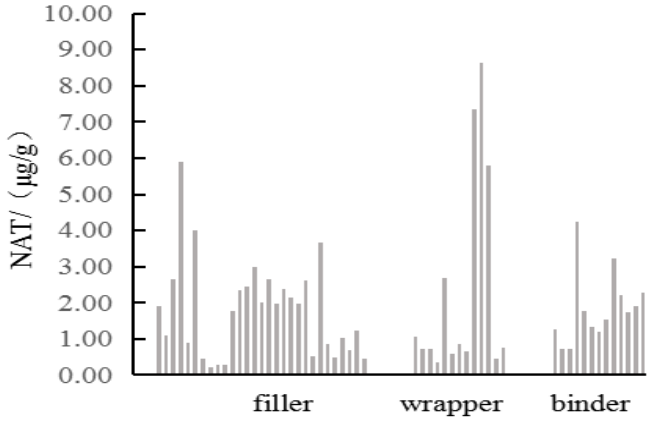


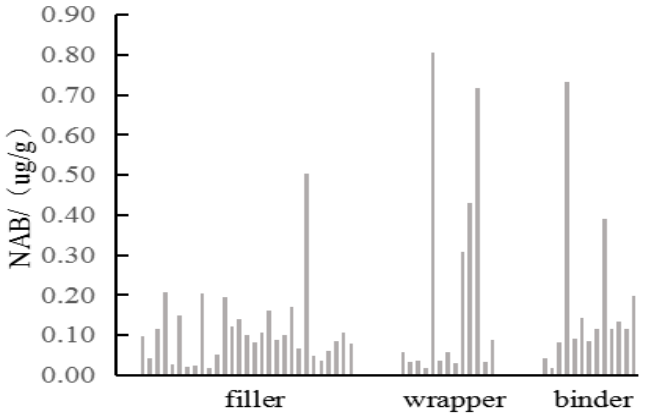


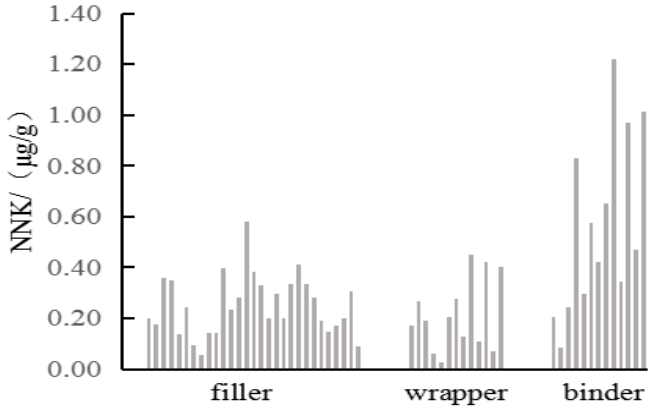
The samples were classified as fillers, binders and wrappers, and the alkaloid contents were compared among the three groups. Nicotine, anatabine, anabasine and total alkaloid contents in filler tobacco were generally higher, while the nornicotine contents and percent nicotine conversion didn't show much differences among cigar types, indicating that in all the three cigar types, there were problems of nicotine conversion.

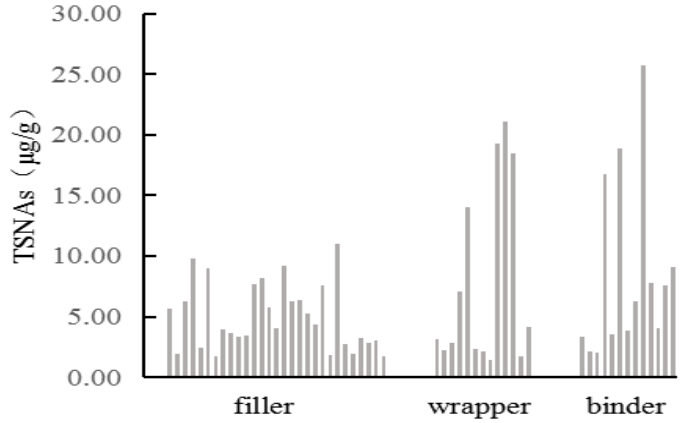
TSNAs content in three cigar tobacco types











The individual and total TSNA contents varied greatly among samples for all the three cigar tobacco types, especially the NNN contents having the greatest variation. All the cigar types had a great potential for TSNA reduction.

Correlation coefficient between TSNAs and alkaloids in cigar tobacco

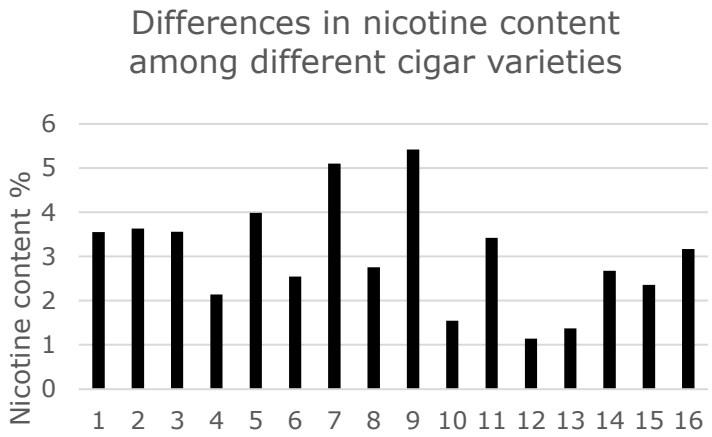
TSNA	Nicotine	Nornicotine	Anabasine	Anatabine	Total alkaloid	Nicotine conversion rate
NNN	-0.30*	0.55**	-0.25	-0.33*	-0.25	0.81**
NAT	-0.04	0.48**	-0.06	-0.01	0.01	0.54**
NAB	-0.27	0.29*	-0.19	0.30*	-0.24	0.55**
NNK	-0.25	-0.05	-0.14	-0.18	-0.18	0.15
TSNAs	-0.25	0.56**	-0.21	-0.20	0.20	0.79**

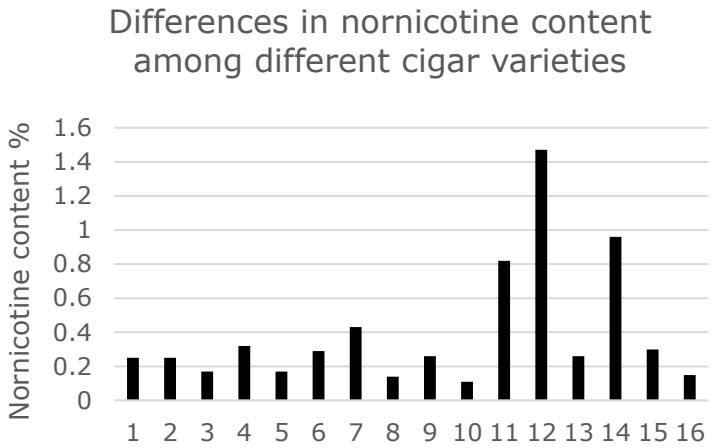
Nornicotine content and percent nicotine conversion had the most significant positive correlation with NNN and total TSNAs, indicating that nicotine to nornicotine conversion which resulted in increased nornicotine formation was the leading factor contributing to TSNA accumulation in cigar tobacco. Removal of nicotine converters from the population should be effective in lowering TSNA contents.

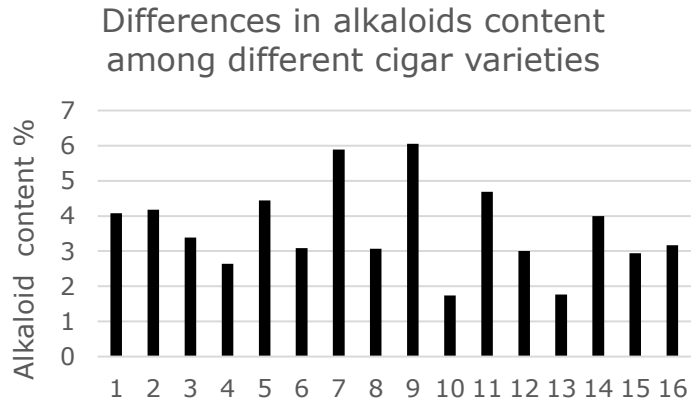
Alkaloid contents and nicotine conversion in bulk samples of different cigar varieties

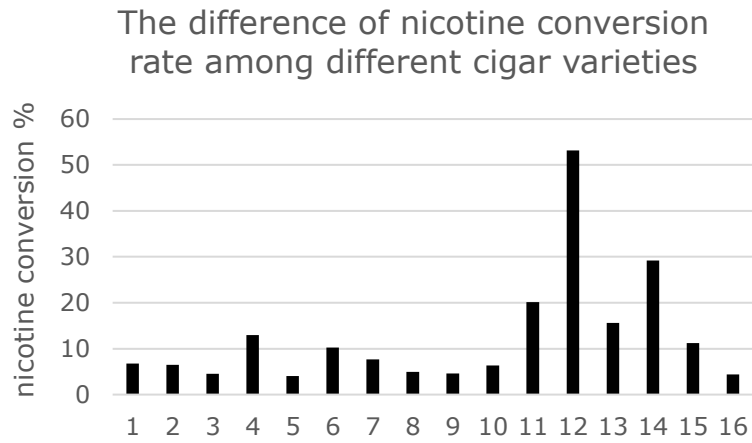
Number	Varieties	Number	Varieties
1	MNC	9	MKK
2	MSCA	10	CP
3	MFZS	11	CX2
4	PC	12	CX1
5	MFPP	13	GH-2
6	OLOR	14	BN
7	NJ	15	GH-1
8	DMD	16	C.B

- ✓ There were significant differences in alkaloid content among different cigar varieties.
- ✓ Percent nicotine conversion varied greatly among different varieties.
- ✓ The problem of nicotine conversion in cigar tobacco was extensively existed and very severe for some varieties, and the screening and improvement were much needed.

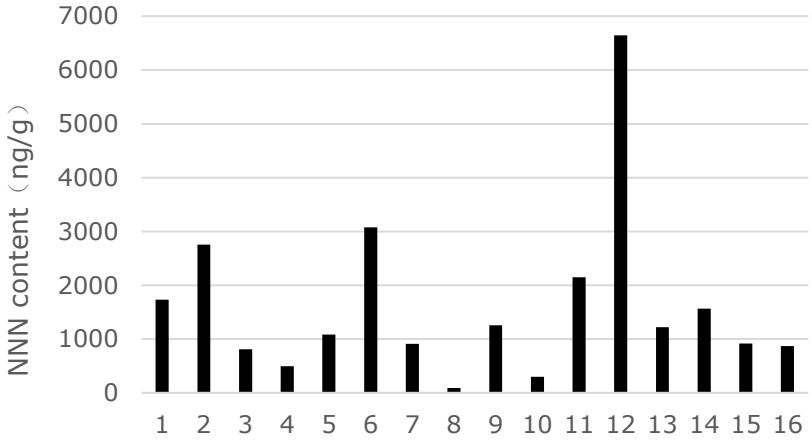


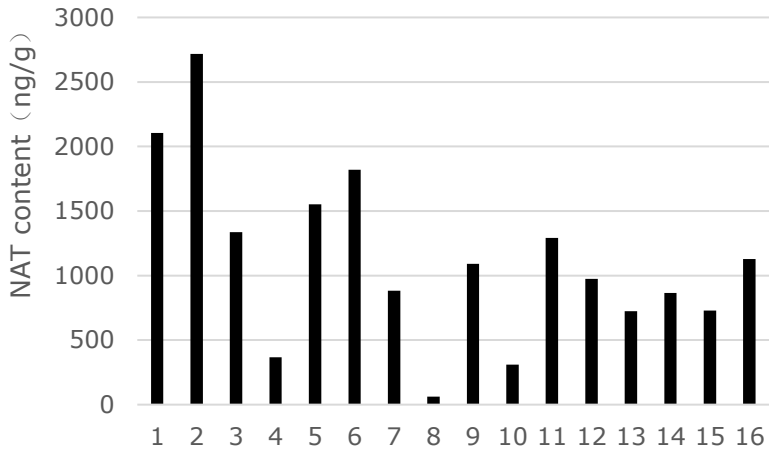


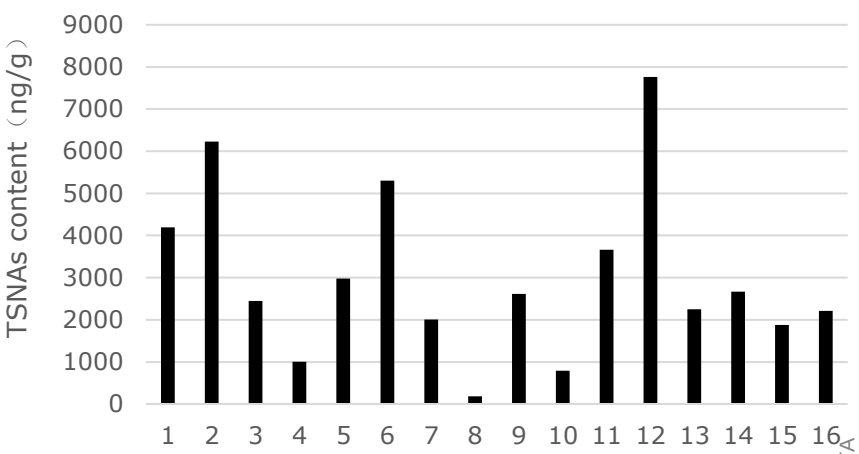




TSNAs content in bulk samples of different varieties



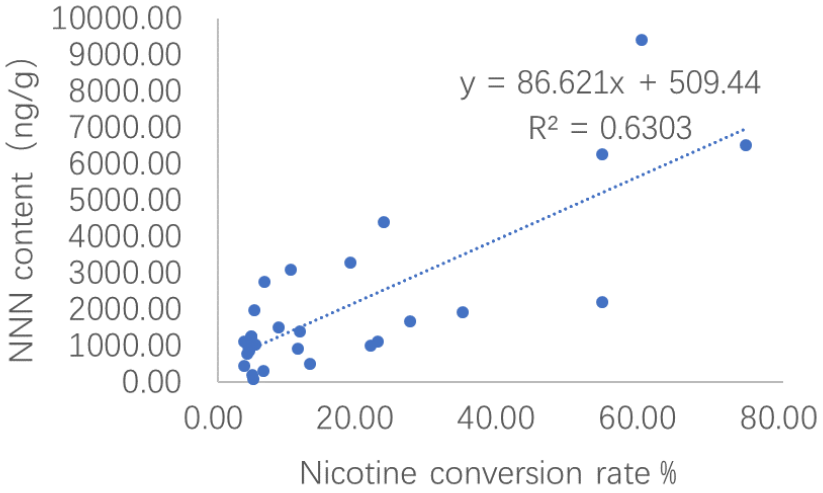




- Individual and total TSNAs contents varied significantly among different cigar varieties; total TSNA contents ranged from 181.7 to 7759.1 ng/g, with an average value of 3011 ng/g.
- NNN contents had the highest average value and greatest variation among all the four individual TSNAs.

Correlation analysis of TSNAs with alkaloids

	Nicotine	Nornicotine	Anabasine	Anatabine	Total alkaloid	Nicotine conversion rate
NNN	-0.368**	0.802**	-.232*	0.246*	0.016	0.794**
NAT	0.390**	-0.077	-0.024	0.426**	0.397**	-0.2
NAB	0.123	-0.057	-0.143	0.138	0.128	-0.035
NNK	0.294**	-0.390**	-0.161	0.022	0.121	-.404**
TSNAs	-0.199	0.676**	-.223*	0.349**	0.139	0.632**



- NNN contents were positively correlated with nornicotine contents and percent nicotine conversion.
- Total TSNA contents were also positively and significantly correlated with nornicotine contents and nicotine conversion.
- Nicotine to nornicotine conversion lead to increased levels of NNN formation, which contributed to high TSNA accumulation in some of the cigar varieties.

Conclusion

- Cigar tobaccos from different producing areas, different varietes and of different types all had the problem of nicotine to nornicotine conversion, which lead to abnormally higher levels of nornicotine contents.

- TSNA contents varied greatly among cigar tobacco samples. Nornicotine contents and percent nicotine conversion had positive and significant correlations with NNN and TSNA contents for cigar tobacco from different producing areas and different varieties.

- Currently, the existence of nicotine converters in the population was the direct cause of increased levels of NNN and TSNAs in cigar tobacco. The improvement of nicotine conversion trait in cigar varieties was the priority for TSNA reduction in less-harm cigar tobacco production.

THANK YOU !