

CORRELATION BETWEEN POST-CURING TSNA INCREASE AND ALKALOID AND NITRITE CONTENTS IN CURED LEAVES

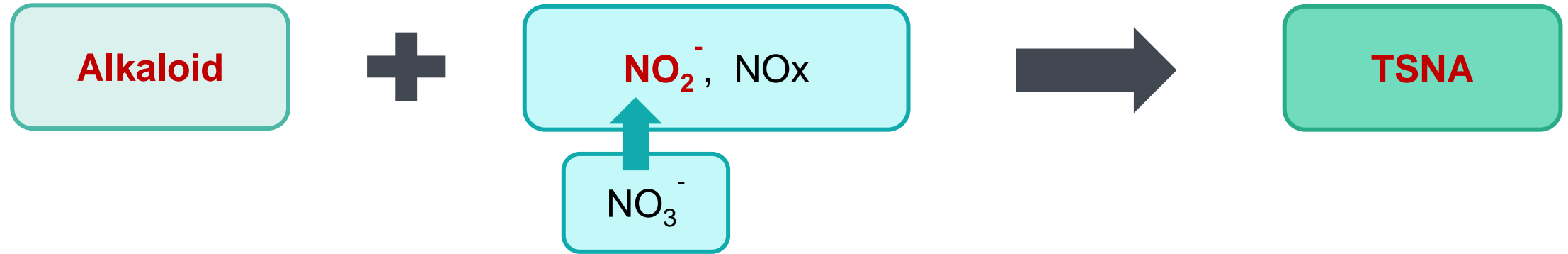
KAWANA M., MASUDA S., SATO N.

Japan Tobacco Inc., Leaf Tobacco Research Center (LTRC)

Background



- ◆ Alkaloid and nitrite in tobacco leaves are known to be involved in TSNA formation.



- ◆ Earlier reports have described that TSNA formation is also affected greatly by environmental conditions during curing and post-curing stages such as storage.
- ◆ Increasing of TSNA contents of cured leaves during storage is occasionally observed. However, the mechanisms of the increase have not been fully clarified yet.
- ◆ To clarify mechanisms of TSNA formation during storage, we intentionally produced cured leaves with different concentrations of alkaloid and nitrite contents and evaluated TSNA increase.

- Elucidation of correlation between post-curing TSNA increase and alkaloid and nitrite contents in cured leaves of burley tobacco

Step 1. Production of six cured leaf samples with different concentrations of alkaloid and nitrite contents

Sample	Content level		Conditions for sample production		
	Alkaloid	Nitrite	Variety	Cultivation method	Curing method
A	Low	Low	Variety 1	JT-LTRC STD	Curing 1
B	Low	High	Variety 1	JT-LTRC STD	Curing 2
C	Middle	Low	Variety 2	JT-LTRC STD	Curing 1
D	Middle	High	Variety 2	JT-LTRC STD	Curing 2
E	High	Low	Variety 3	Modified cultivation	Curing 1
F	High	High	Variety 3	Modified cultivation	Curing 2

Step 2. Treatment of the six cured leaf samples using high-temperature condition to promote TSNA formation

Materials and Methods 1



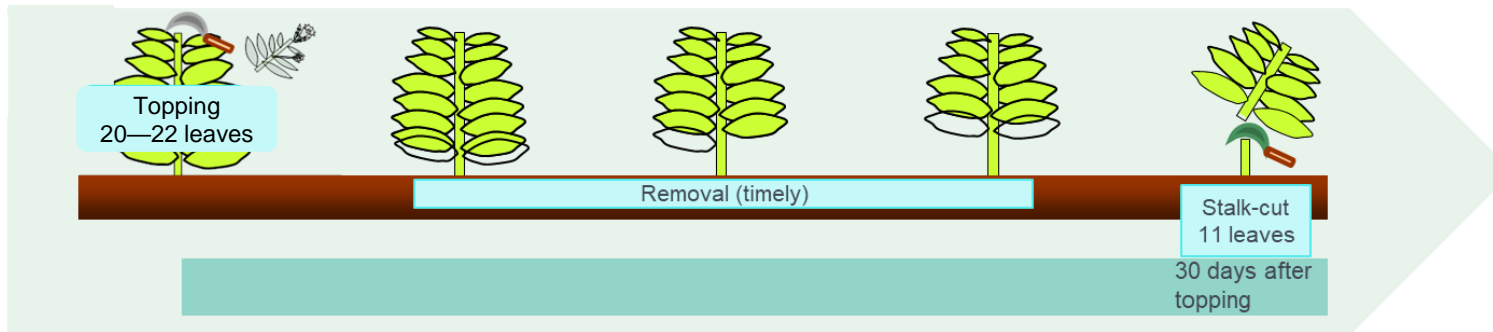
< For producing various Alkaloid content >

● Burley Variety

Variety 1: Low alkaloid variety Variety 2: JT-standard variety 1 Variety 3: JT-standard variety 2

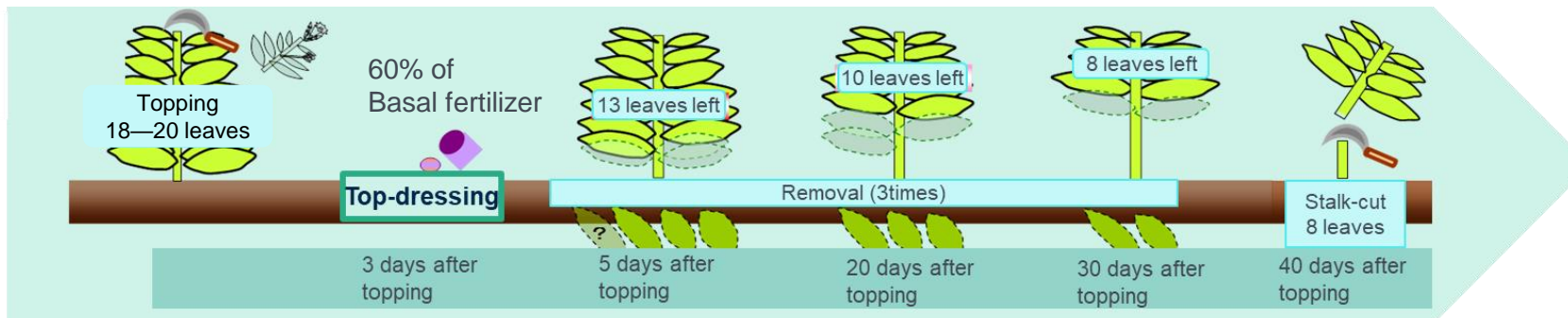
● Cultivation method

• JT-LTRC standard cultivation method



- ✓ Planting density: 1.2 m × 0.35 m
- ✓ Basal fertilizer: 120 N kg/ha
- ✓ Top dressing: none

• Modified cultivation method for alkaloid increase



- ✓ Planting density: 1.2 m × 0.35 m
- ✓ Basal fertilizer: 120 N kg/ha
- ✓ Top dressing: 72 N kg/ha



< For producing various Nitrite content >

● Curing method

- Curing 1 for low nitrite content: Low-humidity condition
Plastic greenhouse without shading
- Curing 2 for high nitrite content: Low-temperature and High-humidity condition
Plastic greenhouse with shading, insulation sheet
Roof watering during midsummer
Ultrasonic humidifier (10 a.m. to 3 p.m.)

	Temperature and humidity during curing		Period (days)
	Average maximum temperature / day (°C)	Average minimum humidity / day (%)	
Curing 1	50	22	21-26
Curing 2	32	77	31-36



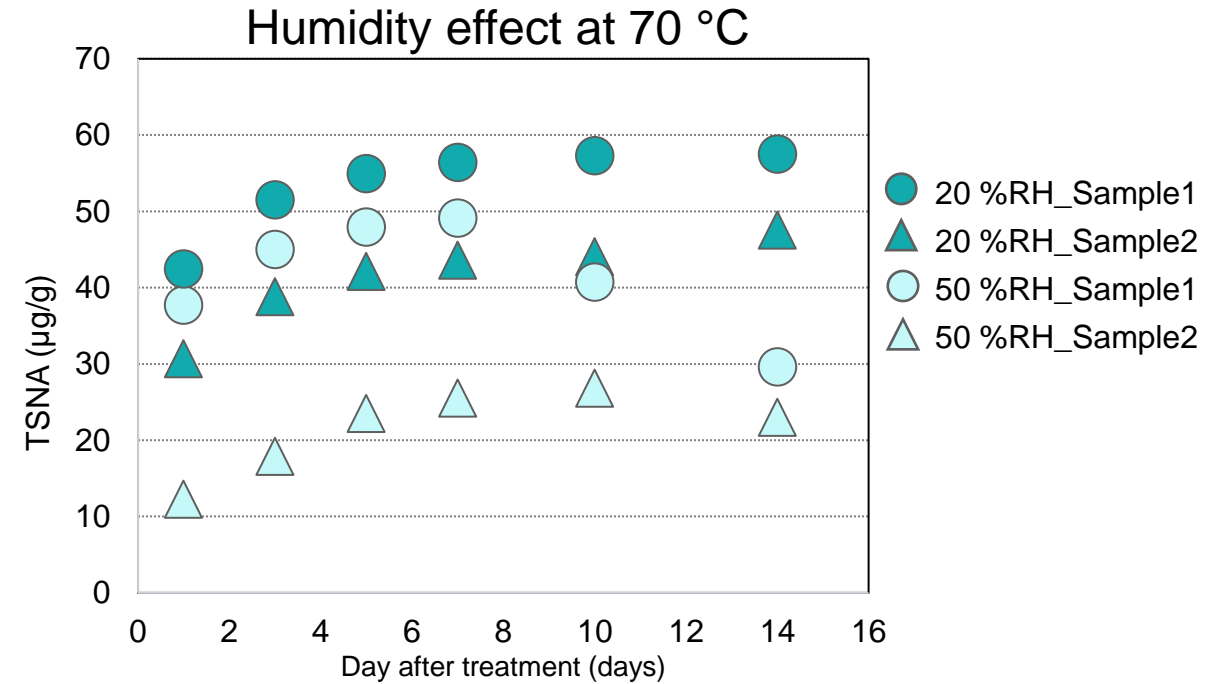
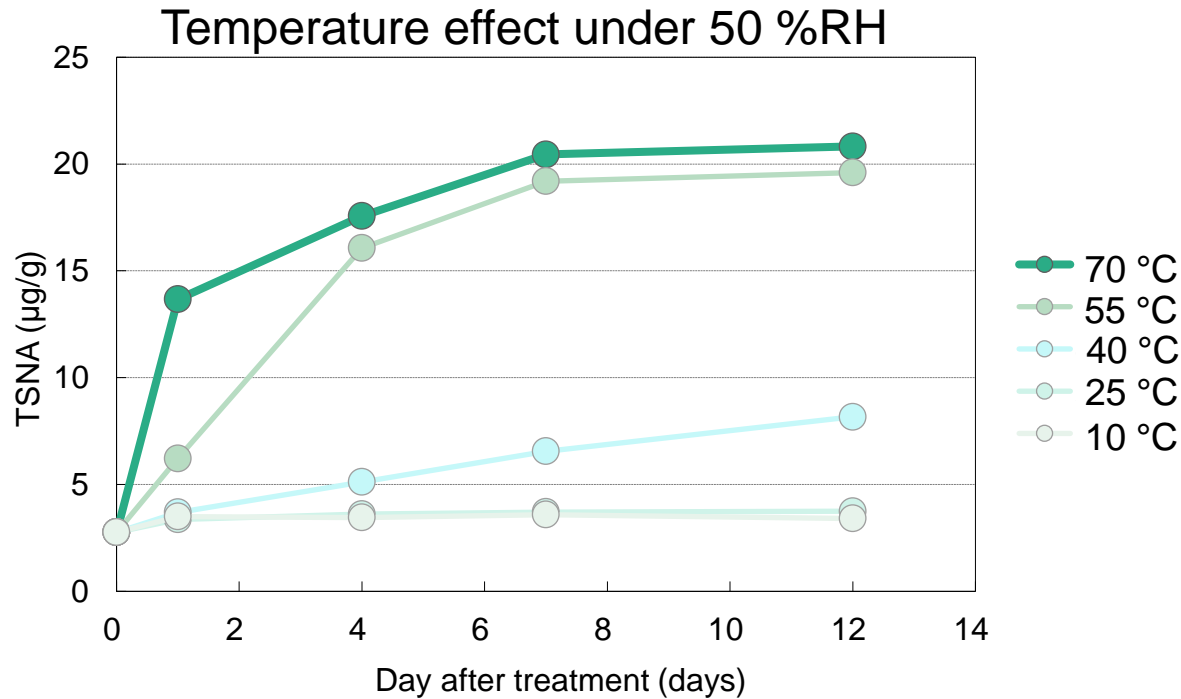
< Heat treatment (HT) >



● Treatment condition

- Temperature, 70 °C; Humidity, 20 %RH; Period, 14 days
- Treatment using high-temperature conditions to promote TSNA formation

Determining Heat treatment conditions to promote TSNA formation



Conducted in a 50 % humidity condition using cured leaves of burley tobacco

- TSNA started to increase over 40 °C
- Rapid TSNA increase was observed at 55 and 70 °C
- The rate of TSNA increase dropped after 7 days of HT at 55 °C and 70 °C

Conducted at a temperature of 70 °C using cured leaves of burley tobacco from two origins

- Both samples showed higher TSNA increase at low humidity (20 %RH)



Heat treatment at 70 °C and 20 % humidity was chosen to maximize TSNA increase rapidly





< Heat treatment (HT) >

● Treatment condition

- Temperature, 70 °C; Humidity, 20 %RH; Period, 14 days
- Treatment using high-temperature conditions to promote TSNA formation rapidly

< Chemical analysis >

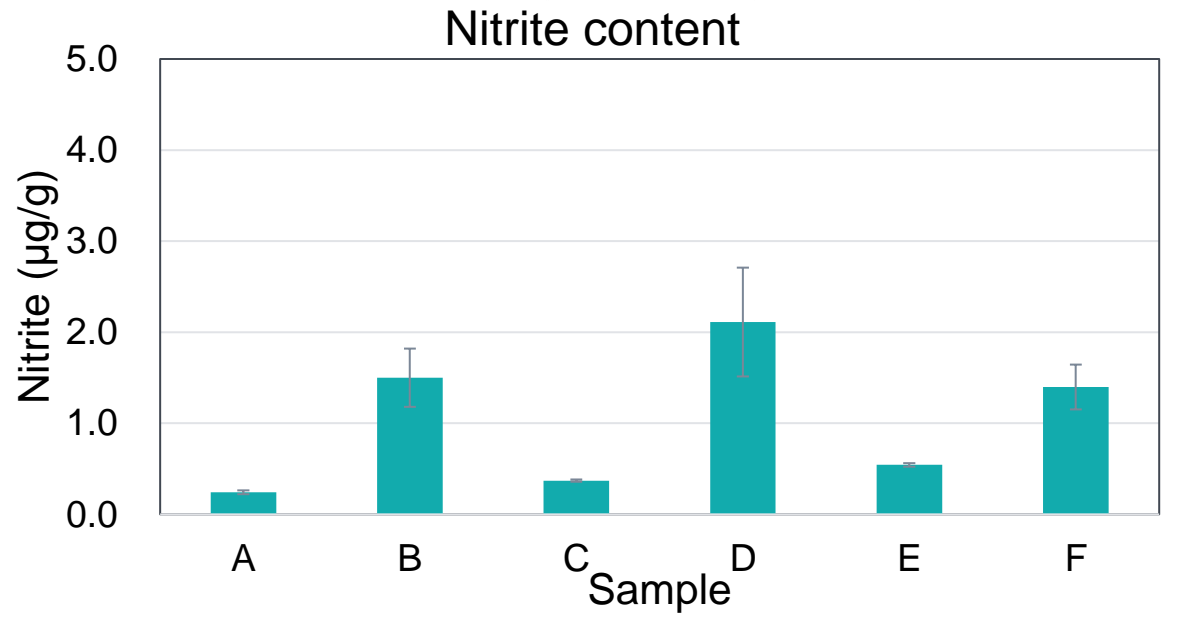
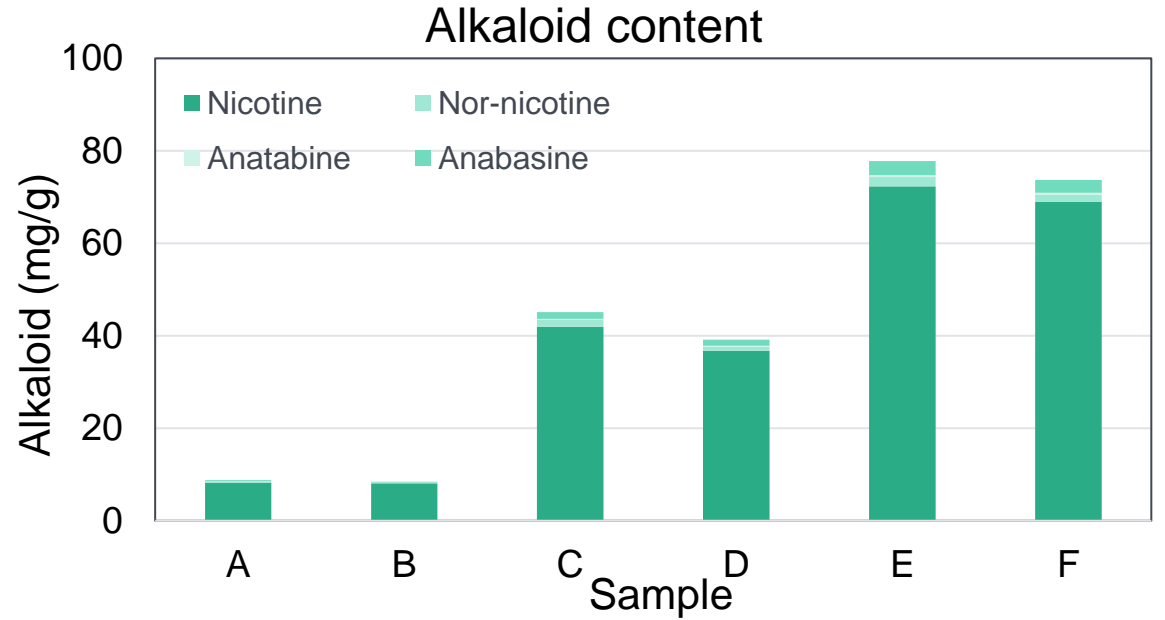
- Alkaloid (Nicotine, Nor-nicotine, Anatabine, Anabasine) -- JT standard method
- Nitrite -- JT standard method
- TSNA (NNN, NNK, NAT, NAB) -- CRM No. 72 (CORESTA Recommended Method)

Result 1



□ Six cured leaf samples varying in alkaloid and nitrite contents

alkaloid \ nitrite	Low	Middle	High
Low	A	C	E
High	B	D	F



● **Three degrees of alkaloid content**

- Low, < 10 mg/g
- Middle, 10-50 mg/g
- High, > 50 mg/g

● **Two degrees of nitrite content**

- Low, < 1.0 µg/g
- High, > 1.0 µg/g

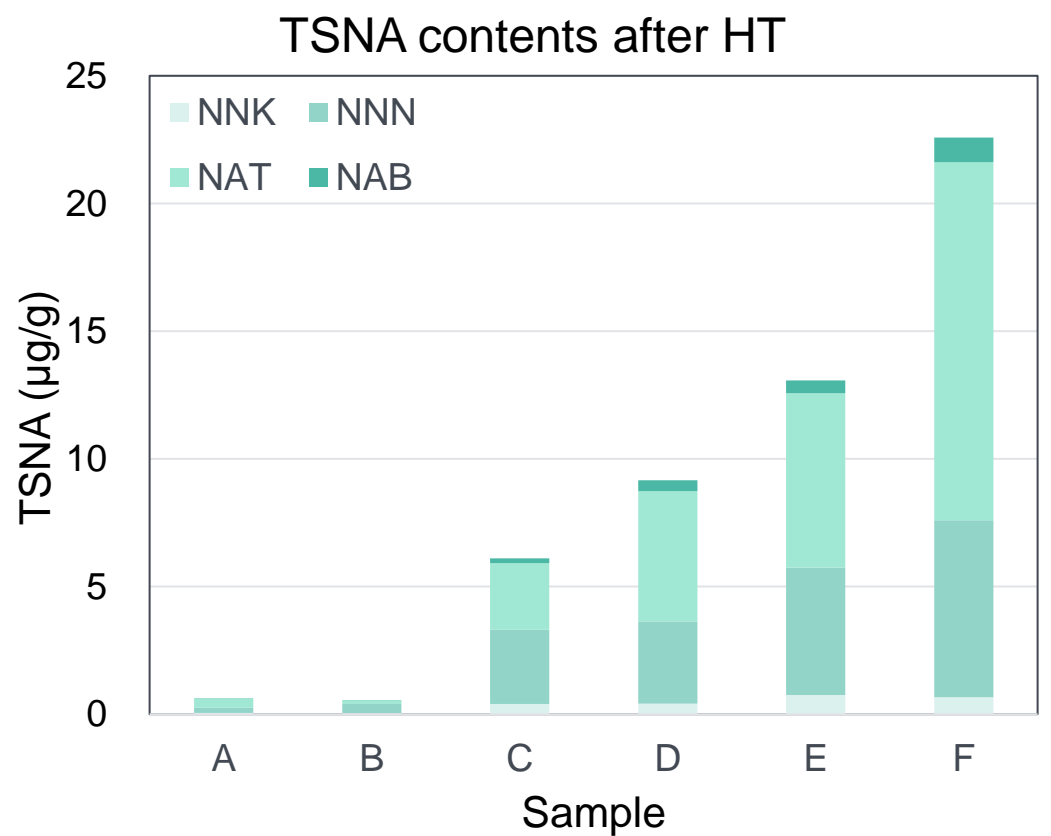
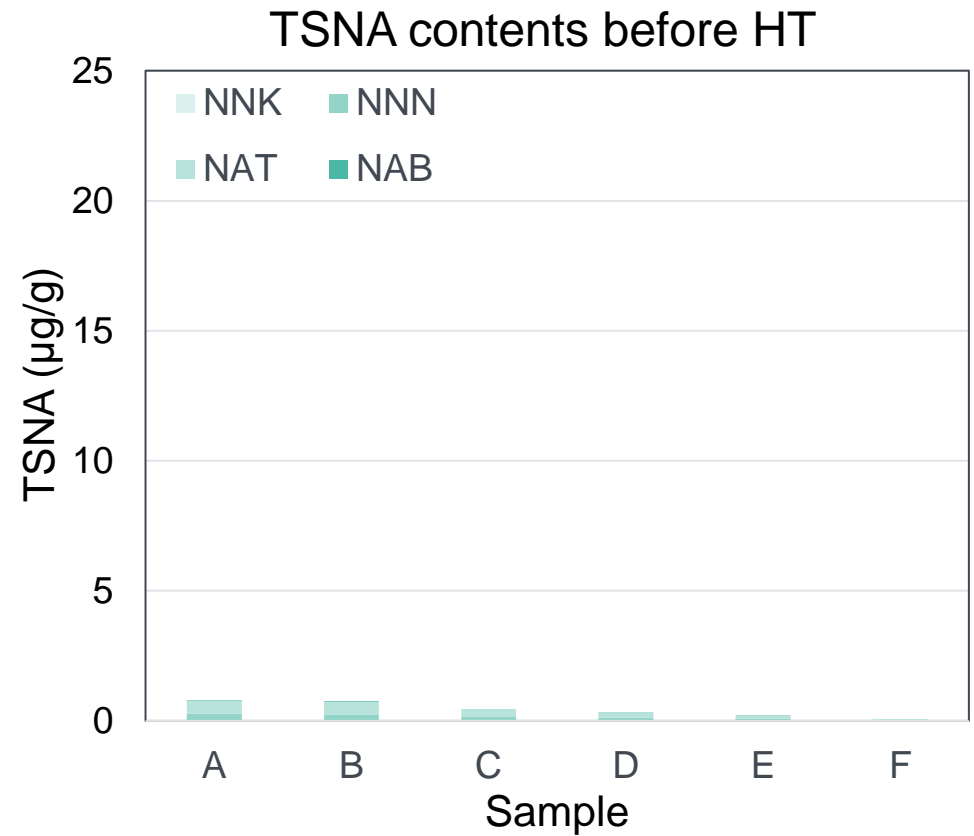
Six variations of cured leaf samples were prepared as a combination of three degrees of alkaloid and two degrees of nitrite.

Result 2-1



TSNA contents after HT

	alkaloid	Low	Middle	High
nitrite				
Low		A	C	E
High		B	D	F



TSNA contents increased extremely in samples with middle and high levels of alkaloid content

Result 2-2



Correlation between post-HT TSNA increase and alkaloid and nitrite contents

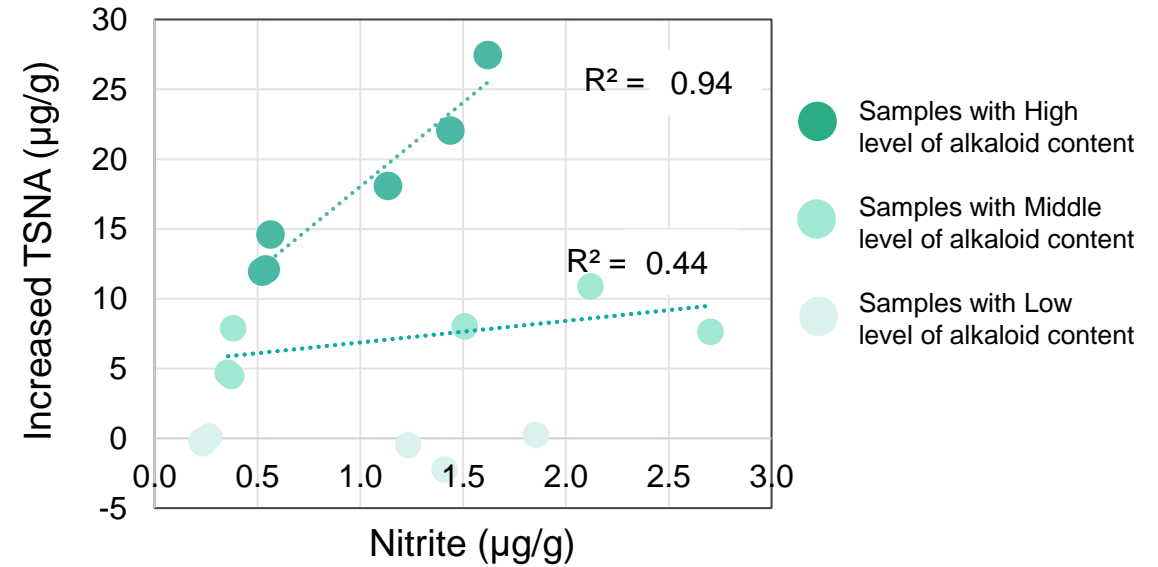
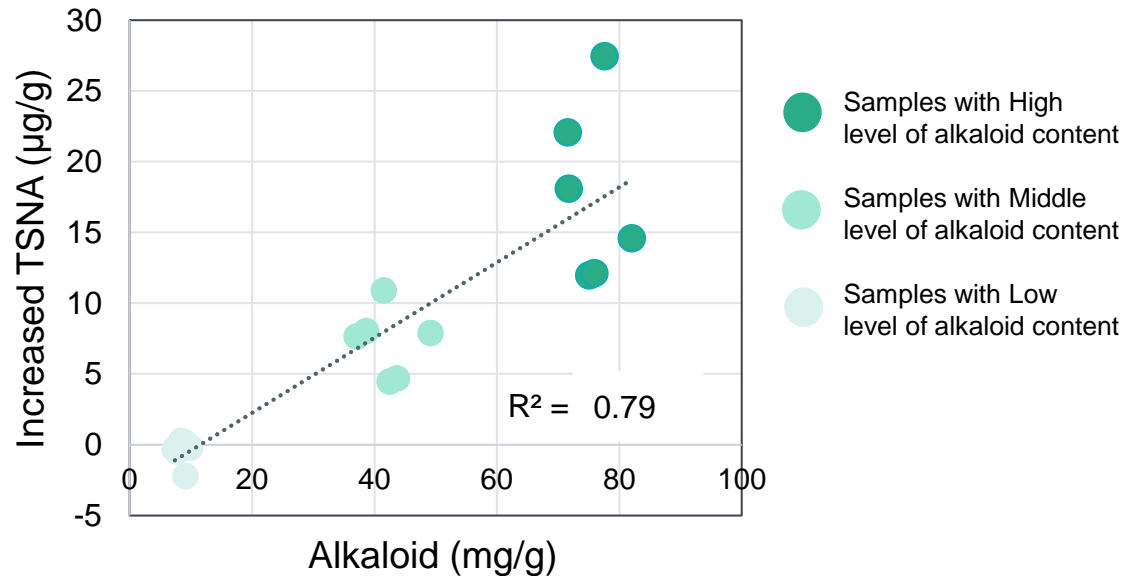


Fig. Correlation coefficient of increased TSNA and Alkaloid

- $R = 0.89$

Fig. Correlation coefficient of increased TSNA and Nitrite

- Middle level of alkaloid content, $R = 0.66$
- High level of alkaloid content, $R = 0.97$

Regarding samples with Middle and High levels of alkaloid content, there was correlation between the increased amount of TSNA contents after HT and alkaloid and nitrite content before HT.



- ✓ After HT, TSNA contents increased extremely in samples with middle and high levels of alkaloid content. For these samples, correlation between the increased amount of TSNA content after HT and alkaloid and nitrite contents before HT are indicated.
- ✓ Results of this study demonstrate that samples with middle and high levels of alkaloid content have a potential of TSNA increase depending on their post-curing conditions.
- ✓ However, among the samples with low level of alkaloid content, no increase was found in TSNA contents after HT even in the sample with high level of nitrite content.
- ✓ Results suggest that the mechanism underlying TSNA increase cannot be explained sufficiently by the alkaloid and nitrite contents alone. Further investigation must be undertaken to elucidate the TSNA formation mechanisms.

JT