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ST1 Yang / Alkaloid analysis

Pretreat spl by spaying with $NaOH_{aq}$ (≤ 1hr)
Solvent : MeOH / $MeCl_2$ 1:3 v/v → recovery ≈ 95%
optimized from study of MeOH, acetone, CH_2Cl_2 and hexane

The N of the pyrollidine ring of nor-nic can react with the carbonyl of acetone

Prepn of stds

Nic. is usually contiminated with 5-6% of myosmine
Anatabine not commercially available

use NPD response factor
determine N(alcaloid)/N anabasine at 3 levels

R = 0,91 hornic
1,47 myosmine   1,00 anabasine
1,07 anatabine

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Carry over in the injection liner
inj. port 220-230 °C
silanization of liner surface
use of Carbowax/KOH packing to minimize interaction with liner walls

ST2 ELLIS

/ No ingredients

5.6 – 5,8 ← Commercial TL

filler pH whole range 5,6 – 5,8

5,33 – 5,33 smoke pH 5,25 – 5,35

0,82 – 0,80 FTC nicotine 0,75 – 0,82

Tar	Nic
1	0,2
5	0,4
16	1,1

- More TPM on the pad ⇒ less nic breaktrough
  nic trapping > 99.3 %
  breakthrough ≃ 1 μg / cig
- humectants appear to contribute to nic retention
- amonia filler and "smoke pH" are not contributors to nic retention on CF pad

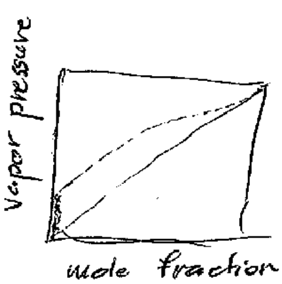
ST 3 BANYASZ

aqueous amines

tendency to reside in cavities

" " aggregation

micro heterogeneity → phase sepn



positive deviation to ideality

tremendous impact at very low concentration

activity coefficient ~ 150

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High diln : isolated nic molecules in hydration shells

higher conc : aggregation

beyond minimum : aggregated cages dispersed in nic

ST4 Paine

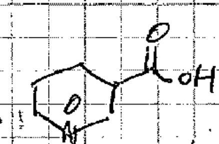
Nic more susceptible to oxydation than reduction

nicotinic acid, a major product

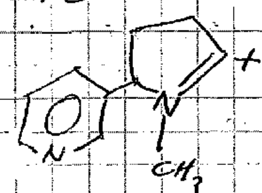
pyridine ring relatively resistant

most prone to oxidn [3C directly bonded to the pyrrolidine N atom
each of which leads to a different family of products

principal site being the 5' site



nic. acid



ST5 Boswell (Spielberg)

STG PEELE / Townsend

FC : NNK - dominant
BU : NNN - dominant
OR : low or non-detectable

TSNA in smoke: generally accepted to be transferred
not pyrosynthesized

Microbial mechanism
accepted for BU

Curing
wet bulb temp does not exceed 45°C, even in stem drying

150-300 gallons LPG per cure (570 - 1140)

14 lbs of NO_x per 1000 gallons combusted
2-4 lbs of NO_x per cure 1.0 - 1.9 kg

Barn	Burner	TSNA [ppm]	NO_x	
R&D electric	Heat exchange	0	n/a	n/a
" LPG	dir-fired	2	0.4	0.18
Comm LPG	"	13	2.5	1.14 *

* estimated on fuel usage

differences in air flow

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R&D burn	Treatment	TSNA [ppm]
electric	w/o NO_x	1
LPG	w/o NO_x	5
electric	with "	> 100
LPG	with	> 100

Dose response

1 lbs/cure added → 8-9 ppm
4 → 100 - 110 ppm

dramatic increase during the yellowing after infection

Fuel	Burner	Samples	TSNA [ppm]	
Stick (wood)	flues	6	0.3	
Diesel	heat ex	27	1.0	(RJR)
LPG	" "	23	0.0	(Turkey)
Stick LPG	direct	1	5.9	
LPG	direct	43	11.0	

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27 barns converted to heat exchangers
Eastern belt tobacco
Field data through 30.8.99

both types of barns available in the farms so that the tobacco (green) effect can be controlled

heat exchange	1.3 ppm
direct fired	7.1 ppm

upper-stalk leaves seem to increase the difference between the 2 types

No significant accumulation during storage after curing

- lab experiments to identify other reaction mechanisms/products
- work on Burley
- plan an expansion of the heat exch. barns

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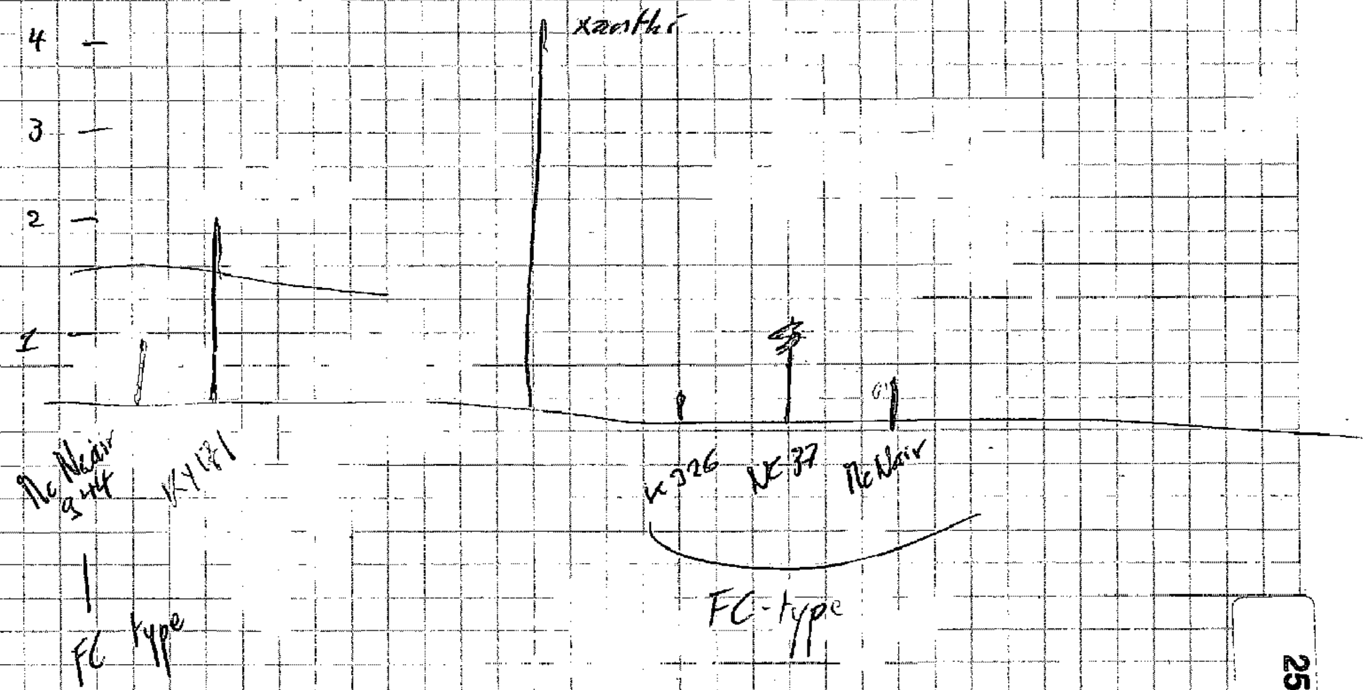
ST7 Wahlberg I

Objective: replace fire cured with new tobaccos
: low PAH & low TSNA

initial strategy: screening of genotypes under heavy fertilization

be suspicious about air curing conditions if NNK is present in very signif conditions

experiment: stalk cut on



Strong correlation Nic - TSNA in alkaloid lines
(as observed on previous slide between different varieties/genotypes

Hiparent
Hi intermediate
Lo interm
Lo

FC-lines lower than Burley-lines after air-curing

The level of secondary alcaloids had a significant impact on tot TSNA levels particularly in ...

Fertilization
150 or 250 lbs/acre nitrogen

Nicotine responded to the treatment
TSNA " as well
nitrate response was somewhat less clear depending on the variety
tested on
e.g. KY 171 had higher NO_3 aft lower fertilization

KY 174
TR Madole
MD

i.e. FC, BU, MD

What happens to the excess nitrite formed during curing from NO_3 as compared with what is "used" to form TSNAs?

→ goes to NO / NO_x gas?
→ reacts with other tobacco chemicals, i.e. "scavengers"?

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Scavengers compete with

antioxidants
ascorbic, tocoph., caffeic acid

amino acids & peptides
cysteine
glutathion } in particular the S-containing ones

lignin

Relative rate of moisture loss from the tobacco leaf during curing

- body
- lamina/midrib ratios
-
- amount of cuticular waxes

Take away on mechanism

- concentration of curing during the critical time period
- possibly breed genotypes with
  - higher levels of scavengers
  - leaf properties

(10)

ST 8 <u>WAHLBERG</u>

prof. Ray Long will present same/similar paper in China

Strategy for air curing

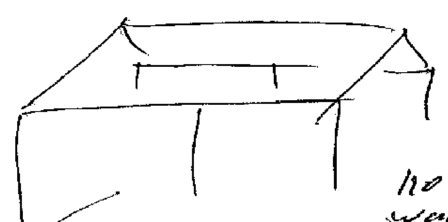
- effect of curing conditions and baling of tobacco
- effect of post curing drying

on TSNA formation

↓

design improve

- Curing structures
- ascorb. misting
- curing environment
- post curing practices
-



no walls

Experiments in FC growing regions

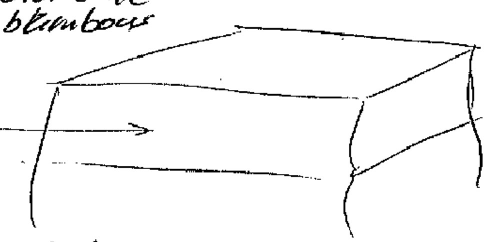
→ barn = Tractor shed + "snow fence"

used as wind protection ⇒ much higher ventilation than conventional barns

claie de bambous

or

Frame



black plastic →

probably only 1 layer of leaves

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The tractor shed and frames led to subjectively acceptable leaf with comparable or lower TSNA levels

Reidsville, NC   dryer conditions than Burley region

TSNA ascorbic

- no misting → 16 ppm
- misting with water → 20 ppm
- " with 5% ascorb → 5 ppm

KY-171 fertilized @ 250 lbs nitrog/acre

Curing

open shed	5 plants/stick	10" apart on the rail
	= not very dense	
crowded shed	7-8	6-8"
log barn	7-8	6-8

something said about "moldy"

no difference

Crowded ~~[illegible]~~ vs open shed
stripped vs baled

but the log barn had high NO_2 levels

90 day TSNA

	1	S
	1,2	baled
crowded	0,8	S
	1.2	b
LB	6.8	S
	4.5	b

less TSNA than would expect from increased NO_2 levels observed

- curing in well-ventilated betterr than humid log barn
- high NO_2 levels does not necessarily lead to high TSNA
- heating after week 3 very signif. increases TSNA

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What can be done

- heat/μwave prior to critical period
- use well ventilated with rapid decrease of humidity during week 2 and 3
- use fully air-condition controlled barns
  ↳ 83-85 °F
  decrease at EOY to ~75°F linearly
  than cycle from 300 - 550 h to simulate day/night temperature
  75 - 83°F

STG Djordjevic

"lowering or eliminating WNK in tob and cigt smoke might in theory reduce the

objectives
~~reduce~~ monitor CO burden

13 smokers 21-55 y healthy
6 non-smokers ≥ 20 FF cigt/day (≥ 1mg/cig
≥ 7 Fägerstrom index ("nic dependence")

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split into 2 groups A & B

phase A — CIGRx / their brand • 7 d

phase B — switch • 7d ← • 3-day biomonitoring
3d

CO before & after smoking
plasma nic & cot
urinary cot NNAL + NNAL Glucuronide

CigRx — FC, no additives; KS; CA/charcoal filter; 1.1 mg nic/cigt

CTRL → blended commercial
KS
CA
1.1.

	CigRx	Comm
NNK	22	518
NO_3	1.8	186
NO_2	1.47	2.01
nic	22.2.	15.9
TPM	8.5	17.8
nic	1.1	1.1
puff	9.7	8.1
NNK	6.5	130
BaP	5.4	11.2
pH	7.1	6.2

— all from transfer
ng/cigt
"

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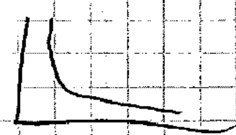
nic in plasma slightly higher for CigRx

CO 50% lower "

NNAL urinary 0,66 (CigRx) vs 1.42 (commercial)

See Hecht's paper on NNAL in 1999 cessation study

NNAL can be observed for weeks after cessation even if the reduction is dramatic in the early days
traces detected after 120 d



ST 10 OGDEN LC-115-115

NNK 0,1 µg/cig in 115 0,4 µg/cig in 55

NNAL rapidly and extensively converted from NNK
can alkylate DNA if metab. activated
extensive glucuronidation
considered detox - product

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ST 14/15 COLLAZO

Reliably capture CA fibers
Positively identify CA fibers
Accurately/consistently count them

- CF pad not ideal
- fiber collection could develop electrostatic charges

Replace CF pad with Dacron membrane
- flat surface
- collects fibers
- allows most of smoke particles through

CA fibers essentially unaffected by tar

Tested
- 3 brands
- counted between 0.4 and 3 fibers/cig

median length 375 - 437 µm } --- too long / wrong length/ϕ ratio
ϕ 20 - 50 µm } near zero probability of deep-lung deposition

second paper

2505627752

all fibers observed ~~were~~ had an aerodynamic dynameter ≥ 10 µm and confirmed to be ≥ 22 µm (and probably much larger)
They had 2 impactors (multistage) with respective cut off at 10 and 22 µm

Note: the higher the flow rate, the lower the cut off, if you consider the respiratory tract as an impactor. At higher flow rate, the trapping distance is shorter.

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$$CI = 1 - \frac{\text{\% Change in uptake of marker A}}{\text{\% Change in cig}^{t}\text{ smoke yield for marker A}}$$

Compensation index

- $CI = 0$ no compensation
- $0 < CI < 1$ partial compensation
- $CI = 1$ complete compensation (no change in uptake regardless of cig^t yield)
- $CI > 1$ over-compensation
- $CI < 1$ under-compensation

Biomarkers

Nicotine	Blood, urine	Nicotine	
Cotinine	", ", saliva	Nicotine	Inter-individual variation in nicotine metabolism
CO Hb	Blood	CO	
Exhaled CO		CO	

∄ biomarkers for tar

Cotinine ≠ marker for nicotine; = marker for exposure to smoke

2505627753

ST 17 Stotesbury BAT GC-Pyrolysis

Pyrolysis of cigarette ingredients labelled with stable isotopes

see presn in Brighton/1998

Question

Is pyrolysis meaningful

Validation

Range of temperature 200 - 800°C

- Rapid evaln of large number of substances
- Minimal sple prep
- Scan covers a large range of different components in 1 run

Pyrolysis vs smoke chemistry

Predicting rate of transfer intact into smoke based on pyrolysis compared with litterature (data on actual transfer into smoke/^{14}C data)

Stable vs radio isotopes

- sensitivity is much lower => need to overload cigt compared with regular applicn

The aim of the paper was to validate last years' results for vanillin and anisole which were different from published data. They repeated the pyrolysis study with ^{13}C- and ^{18}O-labelled products. These additives were also loaded (5mg/cig) on cigt and smoke transfer studies were performed.

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ST 18 Jeff

Experiments

TG/DTA/MS relative rate of transfer of N-Me [illegible] in smoke

Pyrolysis studies

- Nic was not introduced in hot oven
  was very rapidly heated 400°C/min (better model of [illegible] in cgt)

	rate of transfer
nic	100
salt 1	95,3
2	98,4
3	95.3

Transfer occurs between 120 and 200°C

ST-21 COOK

Remarkable increase of transfer rate for superslims vs slim

Transfer rates highly dependent on the loading or concentration in the filler.

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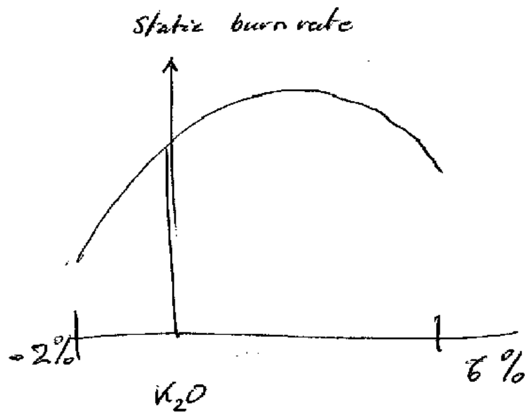
Organic Potassium = tot K − K bound to Cl^- and SO_4^{--}



ST 26 HWANG

CO_2, CO, TSP
Nic + 3-VP
UVPM, FPM, Solanesol
10-ℓ Tedlar gas sampling bag every 30 min
"SMPS"
"Anion" air cleaner led to higher-than-control CO levels!

not dipole oscillation (μw)
but ionic conductivity (RF)

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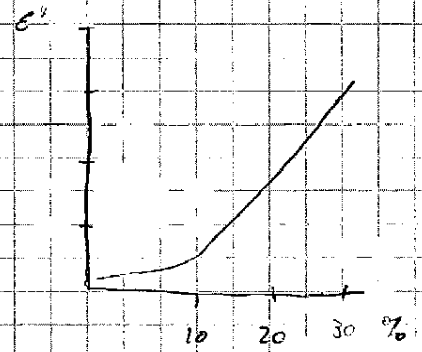
ST 29 Jones RF heating



∄ energy transfer if the material is totally dry

if different parts of the product to be dried are at different moisture content, the energy will be preferentially transferred to the zones at higher moisture.

RF much more easily incorporated into processes than μwave. Energy containment (viz operator safety + radio interferences) is a lot easier as frequencies are lower

Non-toxic destruction of beetles in all life-forms (eggs, larvae, bugs)
Tests in progress tend to indicate that volumetrically heating at 55°C + would quantitatively destroy all forms of the insect

ST 30

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ST 31 De Vos Lamina puffing

Capacitive heating is faster than convection heating since it does not depend on a temp gradient

Cooling by N_2 in closed loop to minimize aroma losses

Tobacco is preheated to 120°C / 35% OV by exposure to steam and than fed into the RF tube.

Δ specific power input (W/cm^3)
field strength
flash over

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ST 34 Hampl *

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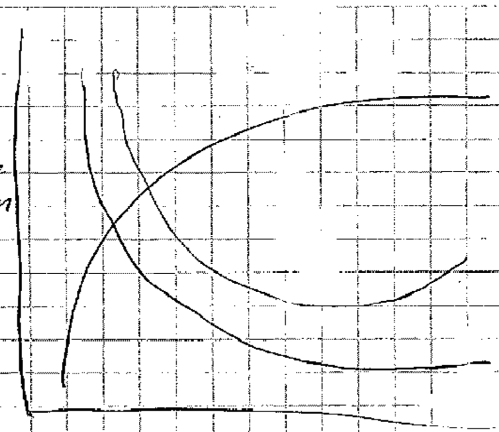
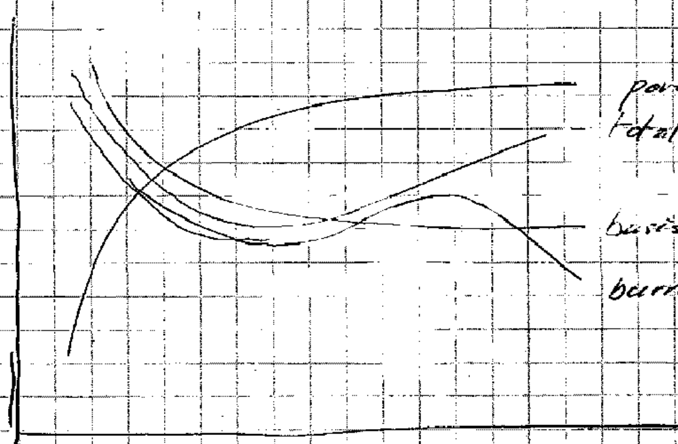


Un mécanisme important de rédn est le piégage des volatils sur le papier. Condensés sur la surface externe du papier, ils peuvent brûler complètement lorsque la braise progresse car ils sont maintenant exposés à une forte conc. d'oxygène.

Le papier voit sa porosité augmenter de plusieurs ordres de grandeur lorsqu'il commence à décomposer, se crevasser sous l'action de la chaleur.

L'addition de $Mg(OH)_2$. l'hydroxyde se convertit en MgO à ~350°C. Il en résulte

- une forte augmentation de la surface spécifique de la charge
- une réduction de porosité/formation de crevasse par un effet de frittage



paper parameters influencing SSS generation:
- porosity
- basis weight
- type & amount of filler additive
- " " of burn additive

In order to reduce sidestream

- reduce amount of tobacco burnt
- slow down the burn rate
- trap smoke on the paper
- achieve more complete tobacco combustion

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ST 36 Volgger

Purge & Trap @ 150°C / 120 ml/min He
trapping on tenax @ RT

desorb @ 250°C trap @ -150°C
flash heat @ 300°C

GC-MSD/sniff

Extraction efficiency is much more dependent on the polarity than on the boiling point of the compound. as the cellulosic matrix is highly polar.

furfural, furanon & acetol were the main three compounds driving the PCA analysis discriminating wood-pulp from annual-plant papers

Further discrimination could be made between papers with different burning additives such as none/citrate/phosphate/acetate

ST 37 Haselgruber

$CaCO_3$ calcite or aragonite
kaolin
TiO_2 (antase)
AlOH
talc (Mg hydroxide)
amorphous ...

Cellulose strongly interferes in IR with the individual fillers' spectra

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ST 37 ss

Removal of cellulose without degradation of the thermolabile filler is needed

this is carried out by oxidation

ashed and oxidized tipping base paper provides further ~~insight~~ material

ST 38 HAMPL

paper porosity is affected by

- fiber selection & refining
- pigment (filler) type & amount

|| 2 most important parameters

- basis weight
- wet pressing, calendering
- drying
- other manufacturing conditions

Refining & fiber bonding

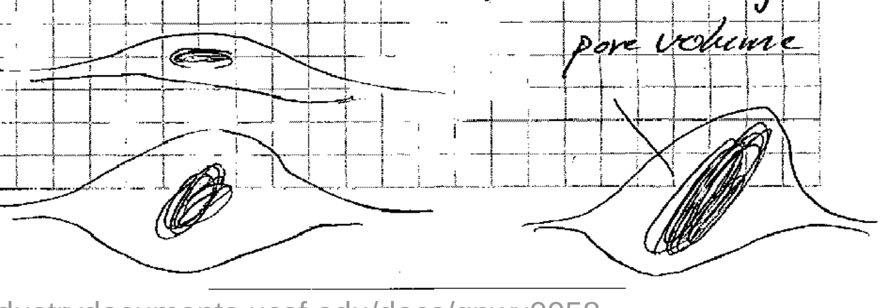
Cellulosic fibers form H-bonds

Regions of fiber-to-fiber bonding have zero porosity

Pigments interfere with f-to-f bonding by physically wedging themselves between fibers and thereby preventing bonding

As the particle size increases the pore volume and porosity increase

/ paper



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Porosity of paper can be controlled by using different ratios of particle-sizes in the filler

As the porosity drops with increasing refining, one can still achieve the same porosity from different pulps through the use of different ratios of small/large particle size filler

Pigment blending is not so obvious, plus, it affects other properties of the finished paper

STG

→ 8 TSNA

green: No or very low TSNA

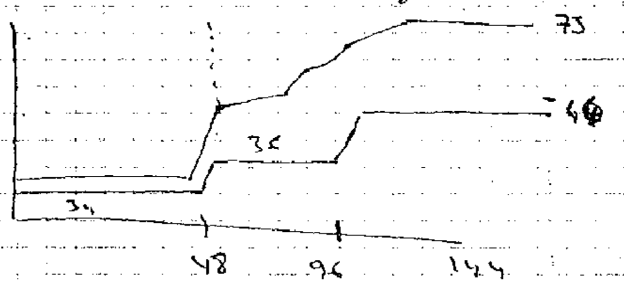
NNN prevalent in bu

NNK Fc transfer

Oriental: low Microb. mediated

Nitrate → NO_2 → NO_x → TSNA

NR drying curing Nitr.



courbe de curing

15 lbs of NO_x per 1.000 gallon combusted

Direct fireau: Oker

commercial: 150-700 gallon LPG

USEPA 14 lbs of NO_x per 1000 gallons combusted LPG

Barn Type	Burner	TSNA ppm	NO_x lbs / kg → kg
electr	Heat-exch		n/a / n/a
R&D LPG	direct fire	2	0.4 / 0.18
commercial	direct fire	13	2.5 / 1.13

) diff. in air flows

Barn type	Treatment	TSNA (ppm)
electric	—	1
LPG	—	5
Electric	with NO_x	7 100
LPG	with NO_x	7 100

} still TSNA microbial origin

4 pound NO_x / are added

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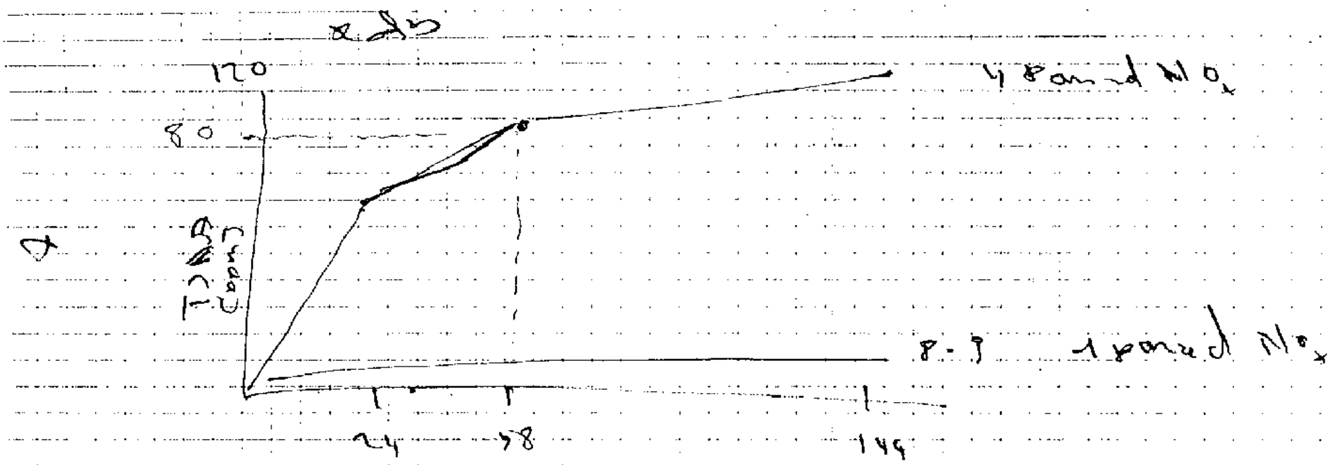

~~Lit NOx~~ Time post NO_x treatment (hours)

(NO_x introduced after 24 h)

Commercial

Fuel	Burner	Samples	TSNA
Stick (wood)	Elms	6	0.3
Diesel	~~Heater~~	27	1.0
LPG	Heat exch (Turkey)	23	0.0
Stick + LPG	Direct	1	5.9
LPG	Direct	43	11.0

Differences in the curing conditions, curing, quality of [illegible]

27 barns in East belt — more typical barns

Conventional barns conversion → 30.8.99 in East belt

Curing type	TSNA
Heat exch.	1.3
Direct fired	7.1

} comparison of heat exchange vs direct

→ No accumulation upon storage

Next: [illegible] complete lab. exp. on using artif. add. of NO_x
BM with Univ. Kentucky
Extend conversion of barns

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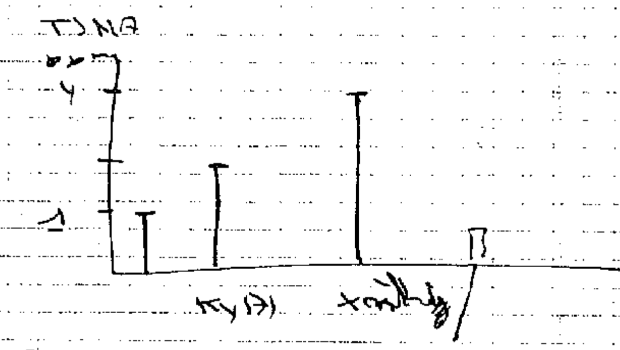
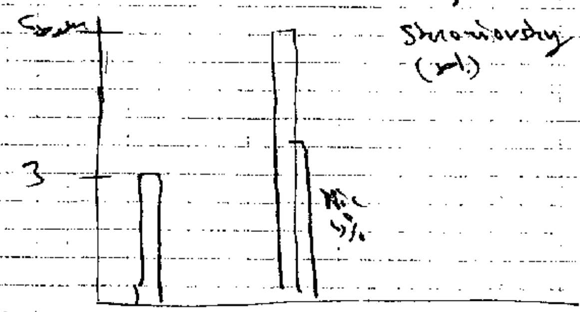
ST 7.

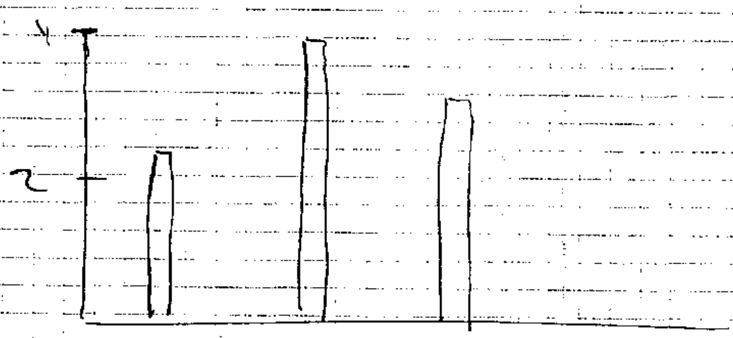
Strategy:

Screen tobacco grown under heavy N-fert rates

NNK present in high amounts ⇒ curing caused.







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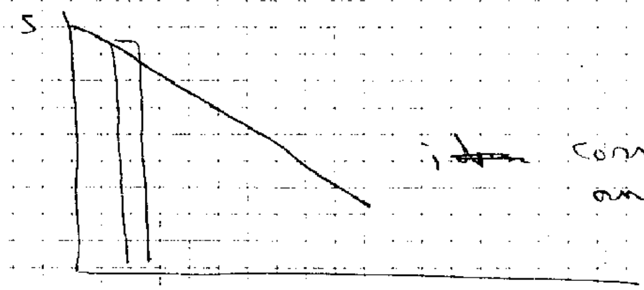
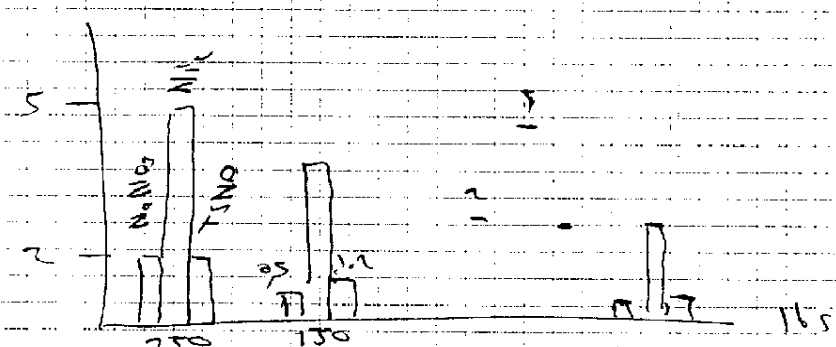


Fortification



sterilize tobacco (heat)

Modify curing

Nitrate green → Nitrite curing → NO_2 sav. TSNA

nitrate reducin bacteria

1000 ppm!

-excess lost react with NO_x scavengers

Facts of insulation

- Level of alkaloids
- Level of nitrosating agent
  - Lv. nitrite scav.
  - rel. rate of moisture loss

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antioxidants: - asc. acid
- Tocoph.
- caf. acid
. Amino acids
- Cyst.
- Glutath.
Lignin.

Rate of moisture loss Impacted by

. Body
. Laminal midrib ratio
. Leaf morphol.
. Amount & comp. of cuticular wax

Concluding comments

- TSNA decrease of precursors (NO_3) during curing period.
- Existing genotypes to be improved by breeding
- Lower TSNA levels by modif. A.C. procedures.

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ST 8

Obj.: optimize AC → ↓ TSNA + retain quality (easy dry out ⇒ no TSNA)

Stat: curing cond. + Barley
- effect of post curing drying
- new curing methods

"North Carolina" ⇒ FC ⇒ ↓ gorge. + some neige some protection

2) field structure

94	Nic	Sugar	NaNG	NaNO2	TSNA
Shed	4.7	4.7		10	1.5
Frame	4.2	3.4		10	<0.1
95					
Shed	4.4			<10	1.1
Frame	3.2			<10	1.6

Concl. Shed / frame comp. low TSNA level
Δ can also be attributed to climate

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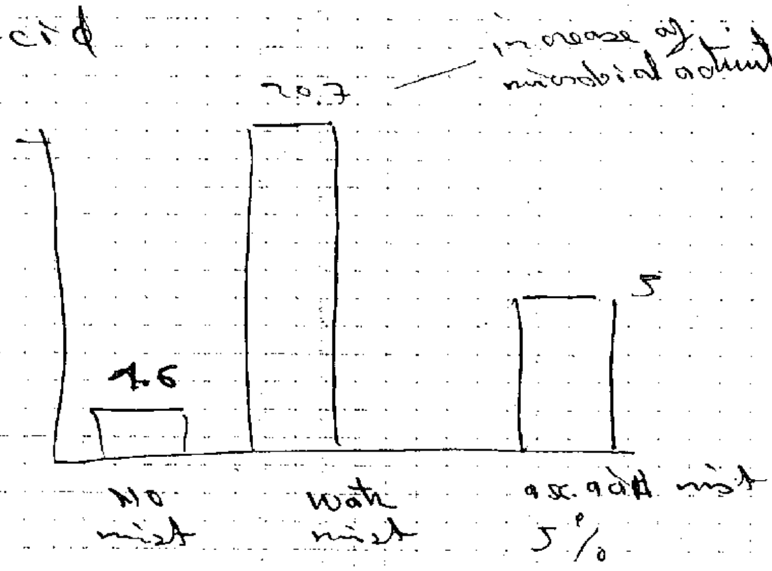
Asc. Acid



? Which stage mist

Curing environment:

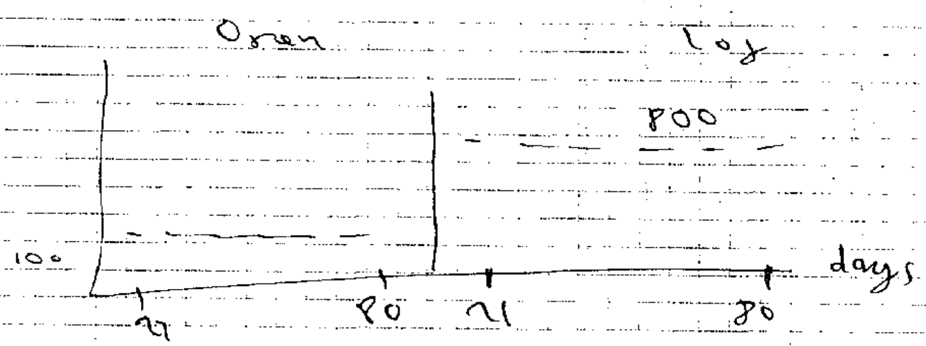
KY 171   1250 1611 N/A — shed   8 wt/stick

— crowded shed   7-8 wt/stick

— log. barn   7-8 wt/stick

Treatment: Freezedrying   oven dried 55°C or 85°C

Log barn ~~Stalks~~: high nic



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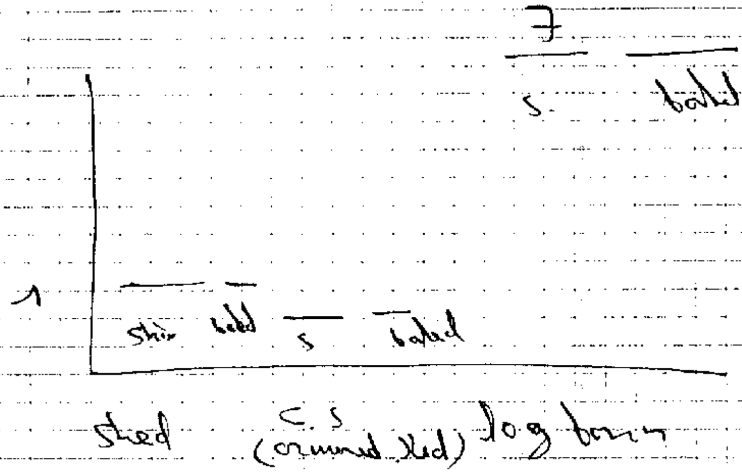

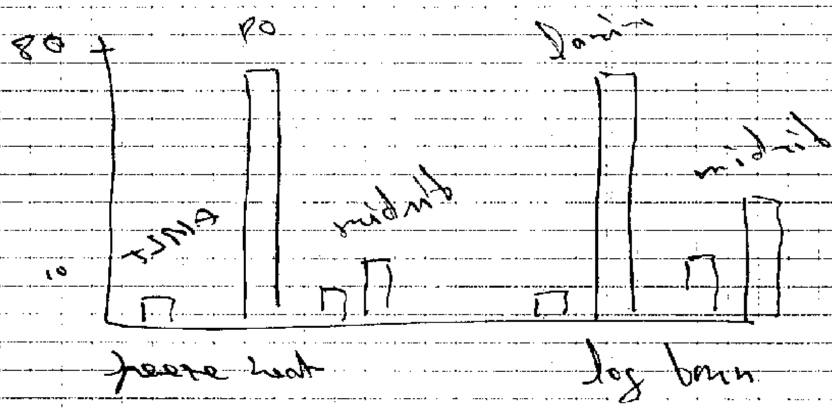

Heat drying leads to substantial TSNA formation
" Week 3

Heat before ⟹ ↓ TSNA formation

Measures:

- New TSNA-safe genotypes
- Modified AC curing well ventilated barn.
  rapid decrease of moist. content
  control

Control temp & humidity

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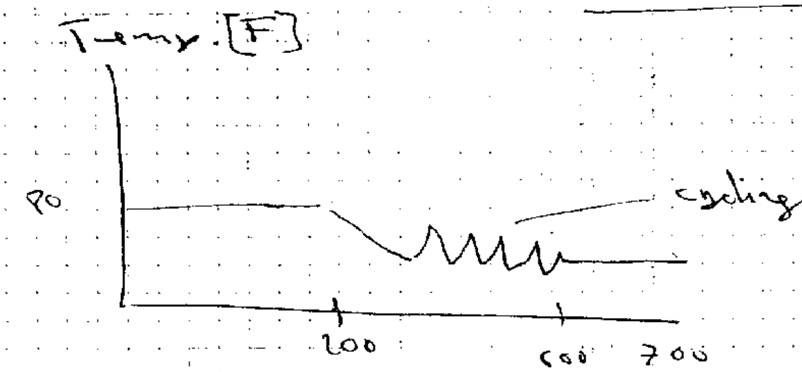
Controlled air drying ~~&~~ conditions.



ST 9

NNAL biomarker for NNK

Cig Rx™ : Tobacco NNK free

Test: 13 adults
6 Non smokers
21-55 men + women, 20 cig / day 1 mg cig.

Groups A: smoke Rx
B: " own brand.

Day 1-7 smoking.

Day 8-11 clinic measurements — CO ... ; Nic, cot blood ; urine NNK metab + NNAL ; NNAL Gluc.

Day 11 switch assignment

~~Tobacco constituents~~ ~~- TSNA~~