

Deproteinization Effects on Homogenized Leaf Cured (HLC) Products*

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SUMMARY

The HLC deproteinized product [depro] with 34 % of its original dry weight removed as heat precipitable protein fractions, shows a reduction in the levels of most of the cured product constituents and the major pyrolyzate constituents while the levels of polycyclic aromatic hydrocarbons and volatile nitrosamines in the pyrolyzates from the HLC deproteinized product were increased.

This indicates that the precursors of the polycyclic aromatic hydrocarbons in the pyrolyzate from the HLC deproteinized product were not removed with the protein precipitates. Another possibility is that some constituents in the protein precipitates that were removed from the HLC deproteinized product may have had an inhibitory effect toward the formation of the polycyclic aromatic hydrocarbons in the pyrolyzates of the HLC control and flue-cured reference during their pyrolysis, since the protein precipitates were not removed from these products.

Generally, the levels of polycyclic aromatic hydrocarbons from the pyrolyzates of the HLC products were higher than those from the pyrolyzates of the flue-cured reference. This could be a result of the tobacco maturity or curing methods. The level of the volatile nitrosamines in the pyrolyzates of the HLC control was lower than those levels in the pyrolyzates of the HLC deproteinized and flue-cured reference products. This could be due to pyrolytic interaction between some constituents in the sample and the protein precipitate which was not removed from the HLC control product. Additional work is needed to clarify these differences.

A reduction in the level of solanesol was evident in the HLC deproteinized product probably due to the association of solanesol with the chloroplastic protein precipitate that was removed from that product. In addition, the level of solanesol was highest in the flue-cured reference, which is in agreement with previous reports that solanesol concentration increases with tobacco maturity.

This report demonstrates that HLC can be used to manipulate the chemical composition of tobacco. The levels of some major constituents were decreased while the levels of polycyclic aromatic hydrocarbons were increased in the pyrolyzate from the same tobacco product.

* Contribution from the Tobacco Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Oxford, N. C., and the Department of Crop Science, North Carolina State University, Raleigh, N. C. Paper No. 8535 of the Journal Series of the N. C. Agricultural Research Service, Raleigh, N. C.

Received: 13th October 1982 — accepted: 5th December 1983.

ZUSAMMENFASSUNG

Deproteiniertes [depro] HLC*-Tabakmaterial, aus dem 34 % des ursprünglichen Trockengewichtes als hitzefällbares Protein entfernt wurden, zeigt gegenüber normalem HLC-Material und konventionell getrocknetem Tabak eine Verminderung in den meisten Bestandteilen des getrockneten Produktes und des Pyrolysates. Der Gehalt an polycyclischen aromatischen Kohlenwasserstoffen und an flüchtigen Nitrosaminen war im Pyrolysat des deproteinierten Materials hingegen höher.

Dies deutet darauf hin, daß die Vorläufer der polycyclischen aromatischen Kohlenwasserstoffe nicht mit dem ausgefällten Protein entfernt wurden. Eine andere Möglichkeit ist, daß einige Bestandteile der Eiweißfraktionen, die in den beiden Vergleichsproben (normales HLC-Material und „flue-cured“-Tabak) noch enthalten sind, die pyrolytische Bildung von polycyclischen aromatischen Kohlenwasserstoffen hemmen.

Im allgemeinen waren im Pyrolysat der HLC-Produkte mehr polycyclische aromatische Kohlenwasserstoffe enthalten als im Pyrolysat des auf herkömmliche Weise getrockneten Vergleichstabaks. Die Ursache dafür könnte in der Tabakreife oder auch im Trocknungsverfahren liegen. Im Pyrolysat der HLC-Kontrolle war eine geringere Menge an flüchtigen Nitrosaminen enthalten als im Pyrolysat des deproteinierten HLC-Materials und in dem der konventionell getrockneten Vergleichsprobe. Wechselwirkungen während der Pyrolyse zwischen einigen Probebestandteilen und den nicht ausgefällten Proteinen im HLC-Produkt könnten der Grund dafür sein. Zur Aufklärung dieser Unterschiede sind weitere Untersuchungen erforderlich.

Im deproteinierten HLC-Tabakgut war ein verminderter Gehalt an Solanesol zu beobachten, was wahrscheinlich auf die Mitfällung zusammen mit dem Chloroplasten-Protein zurückzuführen ist. Darüber hinaus zeigte sich der höchste Solanesolgehalt — in Übereinstimmung mit früheren Arbeiten, in denen über ein Ansteigen der Solanesolkonzentration mit der Reifung des Tabaks berichtet wurde — in den auf herkömmliche Weise getrockneten Vergleichsproben.

Die Versuche zeigen, daß die chemische Zusammensetzung des Tabaks mit Hilfe des HLC-Verfahrens gezielt verändert werden kann. Im Pyrolysat gleichen Tabakgutes verringerte sich der Gehalt an einigen wichtigen Inhaltsstoffen, während sich der an polycyclischen aromatischen Kohlenwasserstoffen erhöhte.

RÉSUMÉ

Un tabac reconstitué HLC* déprotéiné [depro], dont on a enlevé 34 % du poids à sec d'origine en tant que protéine précipitable à la chaleur, accuse une réduction

des principaux composants du produit séché et du pyrolysat par rapport au tabac HLC normal ainsi qu'au tabac séché de manière conventionnelle. Par contre, la teneur en hydrocarbures polycycliques aromatiques et en nitrosamines volatiles était plus élevée dans le pyrolysat du produit déprotéiné.

Cela indique que les précurseurs des hydrocarbures polycycliques aromatiques n'ont pas été précipités avec la protéine. Il se peut également que certains constituants des fractions de protéines encore contenues dans les deux échantillons-témoins (tabac HLC normal et tabac «flue-cured») inhibent la constitution par pyrolyse d'hydrocarbures polycycliques aromatiques.

En général, le pyrolysat des produits HLC contenait davantage d'hydrocarbures polycycliques aromatiques que le pyrolysat de l'échantillon séché de manière traditionnelle. Cela pourrait s'expliquer par la maturité du tabac ou par le processus de séchage utilisé. Le pyrolysat de l'échantillon-témoin HLC contenait une plus faible quantité de nitrosamines volatiles que celui du produit HLC déprotéiné et celui de l'échantillon séché de manière conventionnelle. Cela pourrait s'expliquer par des interactions survenues dans le cadre de la pyrolyse entre certains composants des échantillons et les protéines non précipitées du produit HLC. Il sera nécessaire de procéder à des études supplémentaires pour éclaircir ces différences.

On peut constater dans le tabac HLC déprotéiné une réduction de la teneur en solanesol, ce qui s'explique vraisemblablement par sa précipitation ayant accompagné celle de la protéine chloroplastique. De plus, la teneur la plus élevée en solanesol apparut dans le tabac-témoin séché de manière traditionnelle, ce qui confirme les travaux antérieurs ayant constaté que la concentration en solanesol augmente avec la maturité du tabac.

Ces études montrent que l'on peut recourir au procédé HLC pour modifier la composition chimique du tabac: pour des matières premières identiques, la teneur en certains composants essentiels diminue dans le pyrolysat tandis que la teneur en hydrocarbures polycycliques aromatiques augmente.

INTRODUCTION

Homogenized leaf curing (HLC) is an experimental system for curing tobacco (3, 4, 15). Previous reports indicate that HLC has potential for the production of a less hazardous tobacco product (3) because the macerated tobacco leaf is more amenable to chemical treatment and/or fractionation.

Thus far, attempts to change leaf chemistry and, ultimately, smoke chemistry have been limited to the treatment of the HLC slurry with ozone. Ozonolysis disrupts carbon-carbon double bonds of some constituents in the tobacco (10). However, efforts at this laboratory are currently being directed toward fractionation of the HLC slurry in order to modify leaf chemistry (3).

* homogenized leaf curing

In a previous report, *DeJong* and *Lam* reported on the application of HLC to alter leaf chemistry and produce protein as a by-product (3). They determined that the removal of the soluble (white) protein fraction from the tobacco would alter leaf and smoke chemistry. In their study, the levels of some major pyrolytic constituents from the pyrolyzate of their HLC deproteinized product were lower than those from the pyrolyzate of their HLC control product. Since pyrolytic studies generally indicate a qualitative relationship to cigarette smoke studies, *DeJong* and *Lam* concluded that the removal of the white protein fraction decreased some of the toxic components found in the smoke. However, they did not determine the levels of polycyclic aromatic hydrocarbons (PAH) in the pyrolyzates of their products. In preparing their products, they used a mature (not ripe) tobacco and concentrated on removing the white protein fraction which contained Fraction I and Fraction II proteins (6, 17).

For the current study, an immature tobacco was used because it contained more extractable protein (17). Efforts were directed toward the removal of both the insoluble (green) chloroplastic and soluble (white) protein fractions from the tobacco. Our objective was to examine leaf and smoke chemistry differences between the HLC control and HLC deproteinized [depro] samples. Both the HLC control and HLC deproteinized products from immature tobacco were further compared to a standard flue-cured reference from mature tobacco.

MATERIALS AND METHODS

During the 1980 season, 2 ha of flue-cured tobacco (*Nicotiana tabacum* L.), cv. SC-58, were grown at Oxford, North Carolina, utilizing normal cultural practices for flue-cured tobacco production. About 0.4 ha was grown to produce a conventional flue-cured product (reference) which was harvested in 3 primings with a Roanoke* multipass mechanical harvester and cured in bulk curing barns. After all harvests were processed, the cured weight of each priming was determined and random samples of each priming collected. These were combined proportionately to the cured weight of each priming to obtain a "flue-cured reference" representative of all harvests.

The transplanting of the remaining 1.6 ha was staggered 2–4 weeks to offset harvest dates and match expected pilot plant processing rates. The immature tobacco used to produce the HLC control and HLC deproteinized samples was harvested by removing all the leaves from the plant, 10 days before to 7 days after the button stage (7). The lower two thirds of the plants' leaves was removed with a Roanoke multipass mechanical harvester and the remaining leaves were harvested by hand the

same day. Some of this tobacco was used to optimize feed rate, anti-oxidant (sodium metabisulfite) addition, and juice pH procedures in the pilot plant.

The equipment used in the Tobacco Processing Pilot Plant has been previously described (3). Figure 1 shows a schematic flow diagram with processing rates that were used to obtain the HLC control and HLC deproteinized products. Each harvest of immature tobacco was divided and processed separately to produce the HLC control and HLC deproteinized products.

After all the harvests were processed, the HLC control batches were weighed and random samples were collected and recombined proportionately to obtain an HLC control sample representative of all harvests. The HLC deproteinized batches were sampled in the same manner.

The HLC control and HLC deproteinized composite samples and a composite sample of the flue-cured reference were used for analyses. Analyses were performed at this laboratory and the Tobacco Laboratory of the Richard B. Russell Agricultural Research Center, Athens, Georgia, using established methods for the analysis of tobacco leaf constituents (1, 5, 8, 9, 12, 16), pyrolyzates (11), polycyclic aromatic hydrocarbons (PAH) (13) and nitrosamines (2).

RESULTS AND DISCUSSION

During the preparation of the HLC deproteinized product, problems relating to pilot plant operation were encountered. Changes in the pilot plant procedure were evaluated in terms of extractable total nitrogen.

Although the conditions used in the pilot plant seem optimal for our system, these conditions may not be optimal for other facilities. The timing of homogenization, fiber-juice separation, and processing of the juice through the deproteinization steps seems to have been particularly critical to the protein extraction and removal procedure. Higher yields of protein from the tobacco might be realized if the time span between homogenization and fiber-juice separation is reduced, which may reduce losses from protein denaturation.

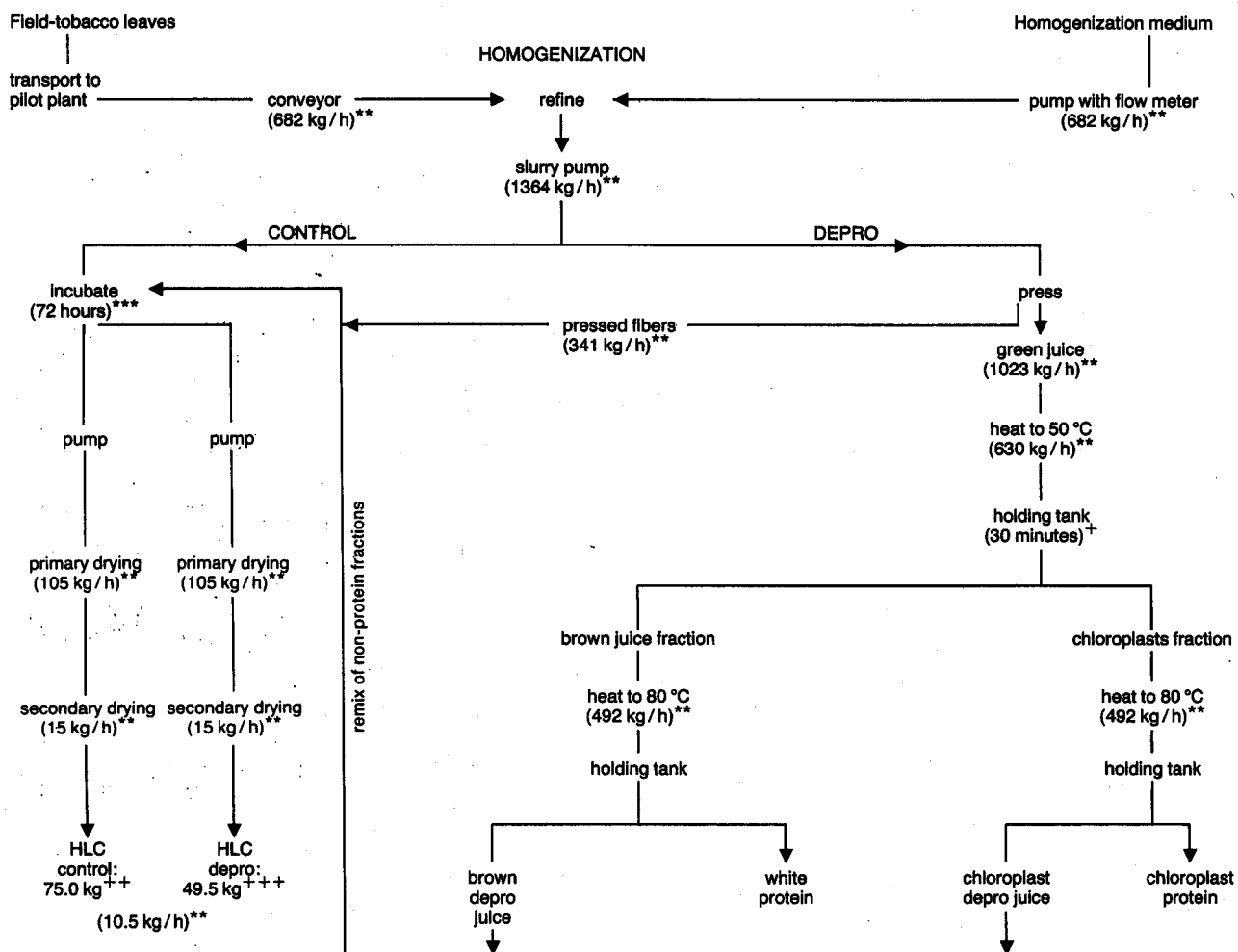
The average dry weight yield of the white and green protein precipitates was 6.9 % and 27.1 %, respectively (Table 1). About 40 % of the white and 15 % of the green protein precipitates were pure protein (total nitrogen $\times 6.25$). The impurities seem to be lipophilic substances.

The dry weight of the flue-cured reference was 12.8 % of its fresh weight. Likewise, the HLC control and HLC depro dry weights were 11.0 % and 7.3 %, respectively. Removal of the proteinaceous precipitates resulted in a 34 % reduction in dry weight (Table 1).

About 50 % of the total nitrogen in the immature tobacco remained with the fibers, 45 % with the protein precipitates, and 5 % with the deproteinized juices. Recombining the fibers and deproteinized juices to produce the HLC deproteinized product resulted in a

* Reference to a company or product name does not imply approval or recommendation by the U. S. Department of Agriculture.

Figure 1.
General flow diagram for processing control and deproteinized [depro] HLC* tobacco.



* Equipment used has been described previously (3).

** Processing rates.

*** Incubation time.

+ Approximate time required for gravity separation.

++ Cured weight of HLC control obtained from processing 682 kg fresh leaf.

+++ Cured weight of HLC depro from processing 682 kg fresh leaf.

Table 1.

Fresh weights and dry weights obtained during processing of immature tobacco through the homogenized leaf curing system with percent distribution of dry weight in HLC deproteinization [depro] procedure.

	Fresh weight (kg)	Dry weight (kg)	Dry weight distribution (%)
Homogenization:			
tobacco leaves	1000	120	
sodium metabisulfite solution*	1000		
homogenate	2000	120	
Procedure:			
control**	1000	55	
depro**	1000	55	100.0
depro juice	678	9.8	17.8
green protein***	119	14.9	6.9
white protein*	66	3.8	27.1
fibers	137	26.5	48.2
Cured product:			
control**	1000	55 ^a	
depro***	815	36.3 ^a	

* Water cooled to 5 °C containing 4.4 g sodium metabisulfite per kg of water.

** Fresh weight of homogenate divided for the treatment.

*** 50 °C heat precipitate.

+ 80 °C heat precipitate.

** Cured HLC control: no protein precipitate removed.

*** Cured HLC deproteinized product comprises the deproteinized juice and fibers with white and green protein excluded.

^a From 500 kg fresh leaf weight.

Table 2.

Analyses (percent dry weight) on cured products — flue-cured reference (a), HLC control (b) and HLC deproteinized [depro] (c) samples, and percent change of HLC depro vs. HLC control.

Constituent analyzed	Flue-cured reference (%)	HLC control (%)	HLC depro (%)	Percent change HLC depro over HLC control
ash	17.30	17.00	17.30	+ 2
total reducing substances	12.60	5.20	1.04	— 80
nicotine	3.07	0.51	1.10	+ 116
total polyphenol	3.06	1.71	1.87	+ 10
α-amino nitrogen	0.27	1.70	0.28	— 84
fructose	2.78	0.30	0	— 100
solanesol	2.47	0.94	0.15	— 84
total nitrogen	2.06	2.64	2.22	— 16
sucrose	1.50	0	0	0
β-glucose	1.29	0.09	0.05	— 44
starch	1.25	7.90	5.10	— 35
α-glucose	1.12	0	0	0
chlorogenic acid	1.03	0.09	0.03	— 68
citric acid	0.73	0.55	0.16	— 71
malic acid	0.29	0.05	0	— 100
solanesenes	0.24	0.13	0	— 100

a: Prepared from conventional mature tobacco (cured weight: 64 kg / 500 kg fresh leaf weight).

b: Prepared from immature tobacco (cured weight: 55 kg / 500 kg fresh leaf weight).

c: Prepared from immature tobacco (cured weight: 36.3 kg / 500 kg fresh leaf weight).

45 % reduction of total nitrogen from the starting material.

Constituents that were not removed by the deproteinization treatment would normally result in an increase of that constituent in the HLC deproteinized product. Constituents that are nearly equal in the protein and deproteinized juice fractions would remain constant in both the HLC control and HLC deproteinized products. Constituents that are concentrated in the heat-precipitable protein fraction would be reduced in the HLC deproteinized product.

The levels of most of the cured product constituents (Table 2) were reduced in the HLC deproteinized products. The most obvious ones were starch, sugars, α-amino nitrogen and solanesol, the levels of which were reduced by 35.4 %, 80.0 %, 83.5 %, and 84.0 %, respectively. The levels of some constituents remained nearly equal while those of others increased slightly. However, the level of nicotine increased by 115.7 % in the HLC deproteinized product.

The total nicotine weight was 280 g (55 kg × 0.51 %) in the HLC control and 399 g (36.3 kg × 1.1 %) in the

HLC depro representing a 42 % increase in total nicotine in the HLC deproteinized product. Although nicotine remained soluble in the deproteinized juices, accounting for part of the increased nicotine in the HLC depro, increased volatilization of nicotine during the drying of the HLC control (unpublished data) was responsible for inflating the percent increase of nicotine in the HLC deproteinized vs. HLC control sample.

The HLC control and HLC deproteinized samples were dried in a similar manner. However, deproteinization resulted in a 34 % decrease in dry weight and 25 % increase in water content of the HLC deproteinized slurry before drying. The HLC deproteinized sample dried at a slower rate due to its higher water content while the HLC control sample dried at a faster rate, attaining higher drying temperature which caused more nicotine to volatilize in the HLC control than in the HLC depro during drying.

Table 3.

Analyses* on pyrolyzates prepared from pyrolyzing cured products — flue-cured reference (a), HLC control (b) and HLC deproteinized [depro] (c) samples.

Constituent analyzed	Flue-cured reference	HLC control	HLC depro	Percent change HLC depro over HLC control
— mg / g pyrolyzed —				
nicotine	34.37	9.17	13.72	+ 50
phenol	1.94	1.92	1.12	— 42
<i>o</i> -cresol	0.45	0.48	0.30	— 38
<i>p</i> -cresol	0.44	0.55	0.34	— 38
<i>m</i> -cresol	0.41	— ⁺	0.34	—
catechol	0.68	0.87	0.66	— 24
hydroquinone	1.41	1.29	0.83	— 36
neophytadiene	0.98	0.65	0.36	— 45
palmitic acid	1.21	2.35	1.34	— 43
stearic/oleic acids	0.63	1.58	0.71	— 51
linoleic/linolenic acids	0.28	2.14	0.45	— 79

* mg constituent / g sample pyrolyzed.

⁺ No value due to co-eluting peak.

a: Prepared from mature tobacco (cured weight: 64 kg / 500 kg fresh leaf).

b: Prepared from immature tobacco (cured weight: 55 kg / 500 kg fresh leaf).

c: Prepared from immature tobacco (cured weight: 36.3 kg / 500 kg fresh leaf).

Except for α -amino nitrogen (83.5 % lower), starch (84.1 % lower), total nitrogen (21.9 % lower) and ash (about equal), all constituents were higher in the flue-cured reference than in the HLC control (Table 2).

The levels of the major constituents (Table 3) in the pyrolyzate from the HLC deproteinized sample were less than those in the pyrolyzate from the HLC control sample except for nicotine which was 49.6 % higher. The levels of nicotine and neophytadiene were highest in the pyrolyzate from the flue-cured reference. However, the levels of palmitic acid, catechol, stearic/oleic acids and linoleic/linolenic acids were lower in the pyrolyzates from the flue-cured reference and the HLC deproteinized sample than in the pyrolyzate from the HLC control sample. The levels of phenol, hydroquinone and the *o*-, *p*- and *m*-cresols were about the same in the pyrolyzates from the flue-cured reference and HLC control but their levels in the pyrolyzate from the HLC deproteinized sample were lower. Generally, the levels of the pyrolytic constituents from the HLC deproteinized sample were lower than those from the HLC control or the flue-cured reference.

Table 4.

Analyses* of polycyclic aromatic hydrocarbons in pyrolyzates of cured products — flue-cured reference (a), HLC control (b) and HLC deproteinized [depro] (c) samples, and percent change of HLC depro vs. HLC control.

Constituent analyzed	Flue-cured reference	HLC control	HLC depro	Percent change HLC depro over HLC control
— μ g / g pyrolyzed —				
methylphenanthrene + anthracene	7.32	8.71	9.28	+ 7
methylpyrenes	2.60	2.55	3.45	+ 35
phenanthrene	2.00	5.26	7.99	+ 52
2-methylphenanthrene	1.96	1.69	1.76	+ 4
anthracene	1.45	2.38	3.30	+ 39
acephenanthrylene	1.16	1.13	1.99	+ 76
pyrene	0.76	1.48	2.75	+ 86
fluoranthene	0.64	1.56	3.08	+ 97
chrysene + triphenylene	0.51	0.63	0.97	+ 54
benz[a]-anthracene	0.30	0.69	0.76	+ 10
benzo[e]pyrene	0.032	0.09	0.24	+ 167
benzo[a]pyrene	0.060	0.17	0.52	+ 209

* μ g / g sample pyrolyzed.

a: Prepared from mature tobacco (cured weight: 64 kg / 500 kg fresh weight).

b: Prepared from immature tobacco (cured weight: 55 kg / 500 kg fresh weight).

c: Prepared from immature tobacco (cured weight: 36.3 kg / 500 kg fresh weight).

Table 4 lists the polycyclic aromatic hydrocarbons (PAH) determined in the pyrolyzates of the flue-cured reference, HLC control and HLC deproteinized products. The PAH levels are lowest in the flue-cured reference, intermediate in that from the HLC control and highest in that from the HLC deproteinized sample.

The nitrosamines determined in the pyrolyzates of the flue-cured reference, HLC control and HLC deproteinized products are listed in Table 5. The levels of nitrosonornicotine (NNN) in the pyrolyzates of the HLC control and HLC deproteinized products are about equal while the level of NNN in the flue-cured reference pyrolyzate was the highest. Generally, the levels of the volatile nitrosamines in all the pyrolyzates were lower than expected; however, the volatile nitrosamines levels determined in the pyrolyzate of the HLC control were lower than those levels determined in the pyrolyzates of the HLC deproteinized and the flue-cured reference products.

Table 5.

Analyses* of nitrosamines in pyrolyzates of cured products — flue-cured reference (a), HLC control (b) and HLC deproteolized [depro] (c) samples, and percent change of HLC depro vs. HLC control.

Constituent analyzed	Flue-cured reference	HLC control	HLC depro	Percent change HLC depro over HLC control
— ng / g pyrolyzed —				
nitrosonornicotine ⁺	1210	622	526	— 15.0
dimethylnitrosamine ⁺⁺	0.012	0.016	0.071	+ 344.0
diethylnitrosamine ⁺⁺	0.041	0.005	0.074	+ 1380.0
dipropylnitrosamine ⁺⁺	0.040	0.014	0.068	+ 364.0
dibutylnitrosamine ⁺⁺	0.152	0.045	0.119	+ 164.0
nitrosopyrrolidine ⁺⁺	0.119	0.011	0.021	+ 90.9

* ng / g sample pyrolyzed.

⁺ Non-volatile constituent.

⁺⁺ Volatile constituent.

a: Prepared from mature tobacco (cured weight: 64 kg / 500 kg fresh weight).

b: Prepared from immature tobacco (cured weight: 55 kg / 500 kg fresh weight).

c: Prepared from immature tobacco (cured weight: 36.3 kg / 500 kg fresh weight).

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