Supporting Information

Innovative Approaches for Estimating the Levels of Tobacco-Specific Nitrosamines in Cured Tobacco Samples

Samuel Kaiser, Frederico L. F. Soares, Jorge A. Ardila, Marcelo C. A. Marcelo, Jailson C. Dias, Liliane M. F. Porte, Carlos Gonçalves, Oscar F. S. Pontes and Guilherme P. Sabin.*

British American Tobacco (BAT), Cachoeirinha, Rio Grande do Sul 94930-000, Brazil.

*Corresponding Author: guilherme.sabin@bat.com; gpsabin@gmail.com

Table S1. MLR coefficient table for flue-cured Virginia Leaves. In highlight is the significant values

Coefficient Table				
Matrix-bound NNK (ng/g)	**Coeff. SC**	**Std. Err.**	**P-value**	**Conf. int(±)**
Constant	***544.64***	***35.52***	***$3.21x10^{-32}$***	***70.21***
Color		**Degrees of Freedom** = 1		
Color (Orange)	***-52.97***	***11.03***	***$3.84x10^{-6}$***	***21.80***
Color (Mahogany)	***52.97***	***11.03***	***$3.84x10^{-6}$***	***21.80***
Ripeness		**Degrees of Freedom** = 2		
Ripeness (Mature)	-28.50	28.07	0.316	55.47
Ripeness (Overmature)	-1.30	28.45	0.96	56.22
Ripeness (Imature)	29.80	53.33	0.58	105.40
Quality		**Degrees of Freedom** = 2		
Quality (Medium)	-1.80	37.53	0.96	74.17
Quality (High)	-35.85	39.00	0.36	77.07
Quality (Low)	37.64	74.85	0.62	147.93
Nicotine		**Degrees of Freedom** = 4		

Nicotine (Level 5)	***-59.84***	***20.93***	***0.01***	***41.36***
Nicotine (Level 1)	35.23	20.17	0.08	39.87
Nicotine (Level 4)	***-48.10***	***21.33***	***0.03***	***42.15***
Nicotine (Level 3)	13.95	23.60	0.56	46.65
Nicotine (Level 2)	58.75	69.67	0.40	137.68
Country	**Degrees of Freedom** = 5			
Country (Indonesia)	***70.45***	***21.42***	***$1.26x10^{-3}$***	***42.33***
Country (Bangladesh)	***-146.86***	***30.32***	***$3.22x10^{-6}$***	***59.92***
Country (United States)	***73.26***	***29.51***	***$1.42x10^{-2}$***	***58.32***
Country (Zimbabwe)	***53.89***	***18.71***	***$4.58x10^{-3}$***	***36.98***
Country (Brazil)	***99.15***	***17.95***	***$1.48x10^{-7}$***	***35.47***
Country (India)	***-149.89***	***68.45***	***$3.01x10^{-2}$***	***135.28***
Free NNK	***416.49***	***24.38***	***$1.22x10^{-36}$***	***48.18***
N = 162	**R^2 CV** = 0.85		**Condition number** = 33.54	
DF = 146	**R^2** = 0.87		**RSD** = 77.24	
	R^2 Adj. = 0.859		**Confidence level** = 0.95	

Table S2. MLR coefficient table for air-cured Burley leaves. In highlight is the significant values

Coefficient Table				
Matrix-bound NNK (ng/g)	**Coeff. SC**	**Std. Err.**	**P-value**	**Conf. int(±)**
Constant	***1241.70***	***40.66***	***0***	***81.04***
Color	**Degrees of Freedom** = 1			
Color (Brown)	15.72	25.77	0.54	51.37
Color (Light Brown)	-15.72	25.77	0.54	51.37
Ripeness	**Degrees of Freedom** = 2			
Ripeness (Mature)	85.13	44.37	0.06	88.42
Ripeness (Imature)	-85.13	44.37	0.06	88.42
Quality	**Degrees of Freedom** = 2			
Quality (Medium)	76.54	41.05	0.07	81.82
Quality (Low)	-14.77	80.37	0.85	160.18
Quality (High)	-61.78	56.46	0.28	112.52
Nicotine	**Degrees of Freedom** = 3			
Nicotine (Level 4)	***-149.30***	***61.76***	***0.02***	***123.09***

Nicotine (Level 3)	56.82	45.41	0.21	90.51
Nicotine (Level 5)	-85.69	64.35	0.19	128.26
Nicotine (Level 2)	***178.16***	***85.42***	***0.04***	***170.25***
Country	**Degrees of Freedom** = 5			
Country (Bangladesh)	***-893.70***	***89.55***	***$2.77x10^{-15}$***	***178.47***
Country (Mozambique)	-116.58	71.05	$1.05\ x10^{-1}$	141.60
Country (Brazil)	***919.24***	***56.52***	***$5.58x10^{-26}$***	***112.64***
Country (Uganda)	***91.04***	***42.97***	***$3.75x10^{-2}$***	***85.64***
Free NNK	***776.89***	***81.53***	***$1.91x10^{-14}$***	***162.49***
N = 85	**R^2 CV** = 0.91		**Condition number** = 11.59	
DF = 73	**R^2** = 0.94		**RSD** = 161.60	
	R^2 Adj. = 0.93		**Confidence level** = 0.95	

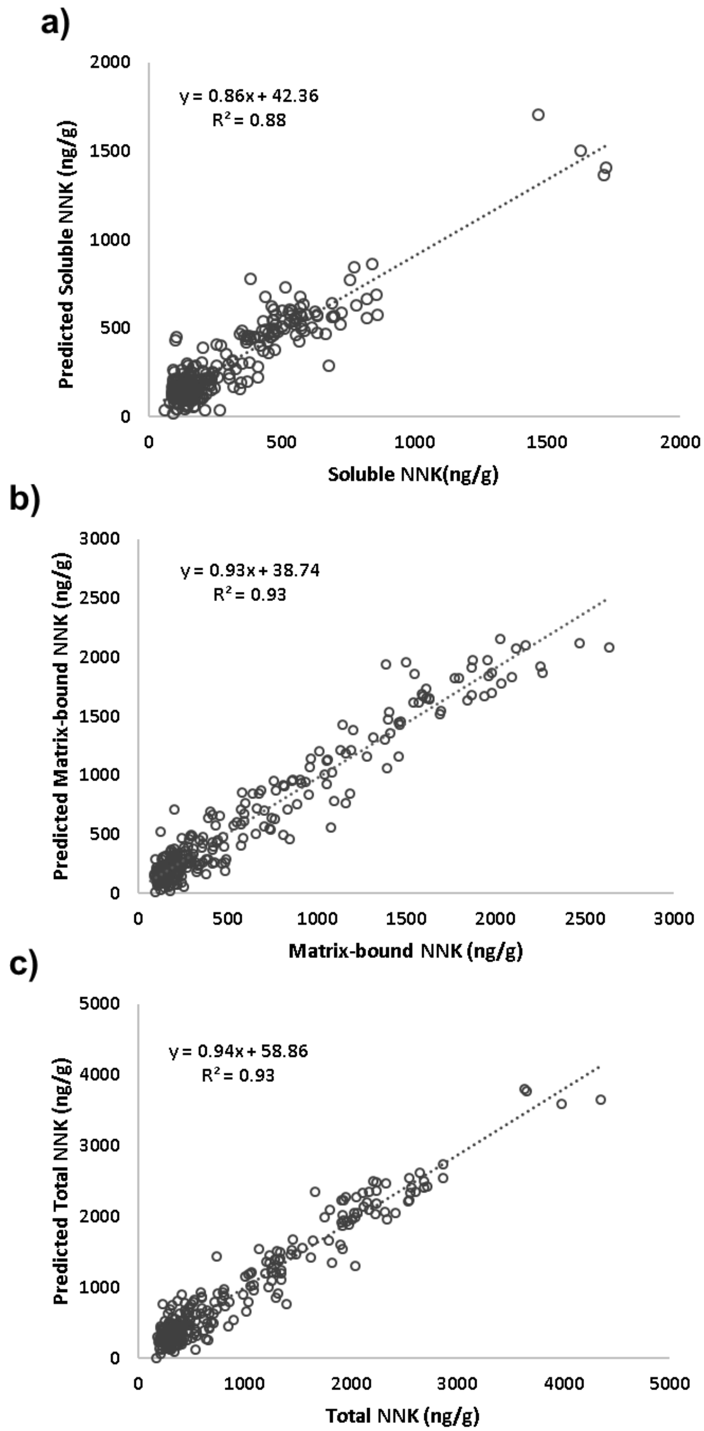

Figure S1. Real against the predicted for: a) Soluble NNK, b) Matrix-bound NNK and c) Total NNK by HTS-FIA-HRMS and O-PLS proposed method.

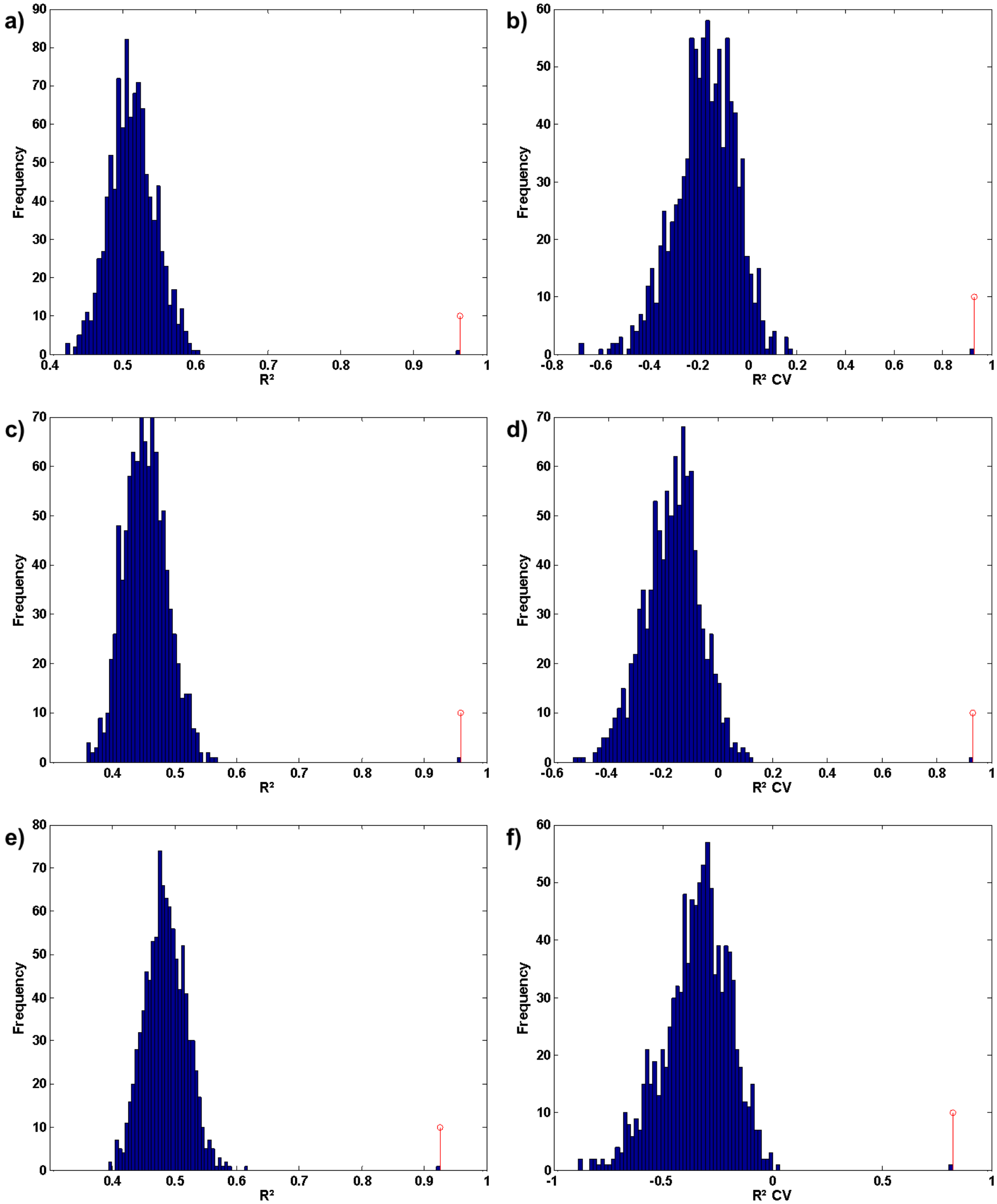

Figure S2. Histograms of permutation tests for R^2 (a, c and e) and R^2CV (b, d and f) for total NNK (a and b), matrix-bound NNK (c and d) and soluble NNK (e and f).

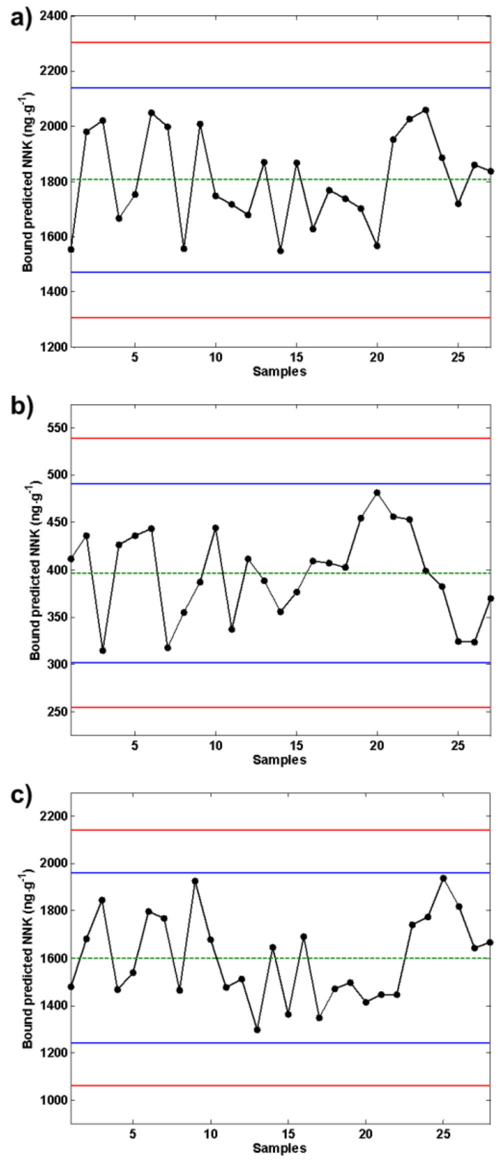

Figure S3. Control chart of predicted values of a) air-cured *Galpão Comum* tobacco, b) flue-cured Virginia tobacco and c) air-cured Burley tobacco for matrix-bound NNK in control samples.