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Chemometric and analytical tools directed to improve the selection of raw materials for long-filler cigars manufacture

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Introduction

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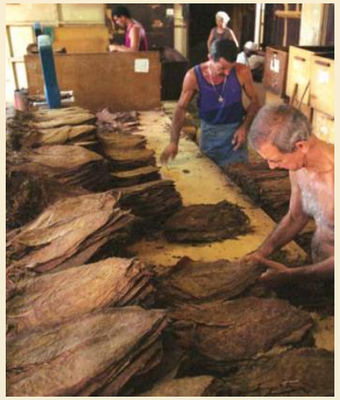
Different strategies to ensure consistency in the sensory properties of cigar blends



ılkaloids as ni	total nitrogen	org acids	total ash	ammonium	chloride	nitrate	sulfate	phosfate	lactate	acetic
35	36	37	38	39	40	41	42	43	44	45
2.1150e–02	4.2130e–02	6.2090e–02	0.2306	7.1400e–03	9.8100e–03	1.1440e–02	1.2880e–02	7.0300e–03	4.1900e–03	1.4550e–(
2.2530e–02	4.2470e–02	6.0520e–02	0.2264	7.6100e–03	1.0510e–02	1.1740e–02	1.2360e–02	7.4000e–03	3.8100e–03	1.4375e–(
2.3350e–02	4.2520e–02	6.2230e–02	0.2254	7.6200e–03	1.1050e–02	1.0910e–02	1.1820e–02	6.4600e–03	4.1875e–03	1.7600e–(
2.1960e–02	4.2840e–02	6.0620e–02	0.2291	7.0700e–03	1.0510e–02	1.2380e–02	1.2510e–02	6.8300e–03	3.4150e–03	1.2175e–(
2.3190e–02	4.1500e–02	6.4670e–02	0.2287	7.3500e–03	1.1330e–02	1.1530e–02	1.0490e–02	7.0100e–03	3.2700e–03	1.4900e–(
2.4270e–02	4.1950e–02	6.0740e–02	0.2268	7.2400e–03	1.1460e–02	1.0470e–02	1.1270e–02	7.4500e–03	3.3025e–03	1.3200e–(
2.2320e–02	4.1050e–02	5.8670e–02	0.2269	6.8800e–03	1.0560e–02	1.1440e–02	1.2510e–02	6.7600e–03	3.8000e–03	1.4150e–(
2.3960e–02	4.2500e–02	6.5080e–02	0.2290	7.4400e–03	1.0790e–02	1.0880e–02	1.1760e–02	7.7700e–03	4.2450e–03	1.1875e–(
2.5350e–02	4.3220e–02	7.0850e–02	0.2263	8.0800e–03	1.0980e–02	1.1170e–02	1.1540e–02	7.3100e–03	4.0125e–03	1.4400e–(
2.0260e–02	4.0590e–02	6.7870e–02	0.2301	6.6500e–03	9.4400e–03	1.3040e–02	1.2520e–02	8.2100e–03	2.8100e–03	1.3800e–(
2.2830e–02	4.1600e–02	6.8970e–02	0.2298	7.0700e–03	1.0160e–02	1.3370e–02	1.3400e–02	8.0500e–03	3.1075e–03	1.4025e–(
2.2090e–02	4.1160e–02	6.9690e–02	0.2301	6.7700e–03	1.0210e–02	1.2330e–02	1.1760e–02	8.2000e–03	3.3100e–03	1.4125e–(
2.1760e–02	4.0290e–02	6.7440e–02	0.2312	7.1800e–03	1.0640e–02	1.2130e–02	1.1870e–02	7.0000e–03	4.1325e–03	1.1825e–(
2.2600e–02	4.2100e–02	6.6050e–02	0.2301	7.5400e–03	1.0590e–02	1.2340e–02	1.2730e–02	7.3100e–03	3.5850e–03	1.6200e–(
2.3460e–02	4.2580e–02	6.8580e–02	0.2271	7.2400e–03	1.1120e–02	1.1150e–02	1.1700e–02	6.9100e–03	3.1100e–03	1.4375e–(
2.1830e–02	4.1130e–02	6.6840e–02	0.2281	6.8800e–03	1.0280e–02	1.1900e–02	1.2650e–02	7.3100e–03	3.4100e–03	1.3425e–(
2.3430e–02	4.2560e–02	7.1860e–02	0.2293	7.6600e–03	1.0820e–02	1.1380e–02	1.2910e–02	7.7400e–03	3.8700e–03	1.1950e–(
2.2590e–02	4.1580e–02	6.9550e–02	0.2308	6.9800e–03	1.0540e–02	1.1750e–02	1.2580e–02	7.1300e–03	3.5725e–03	1.4125e–(
1.9380e–02	3.9820e–02	7.2980e–02	0.2359	6.5000e–03	9.4800e–03	1.2800e–02	1.2610e–02	6.9800e–03	3.3225e–03	1.2200e–(
2.0580e–02	4.0370e–02	6.7900e–02	0.2303	6.8100e–03	9.5500e–03	1.2620e–02	1.2090e–02	7.5400e–03	2.9575e–03	1.2675e–(
2.1860e–02	4.1720e–02	7.0680e–02	0.2306	7.4300e–03	9.9600e–03	1.2150e–02	1.2030e–02	7.3500e–03	3.6450e–03	1.0675e–(

Quality indexes

$$\text{Strenght index} = \frac{[A]}{[B] + [C] + [D]}$$

IMPROVEMENTS ?

Introduction

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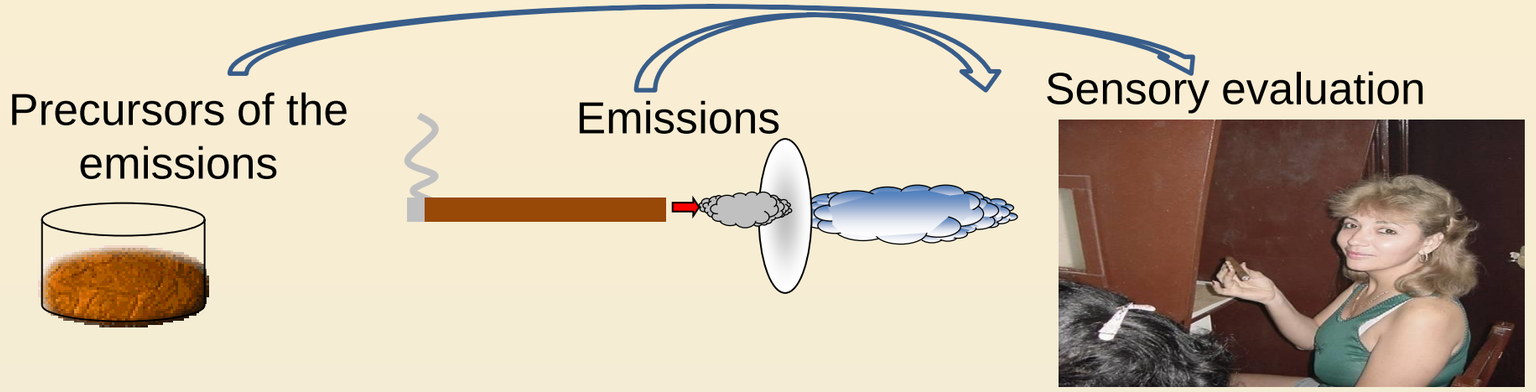
Modern approach: Using objective chemical information of the available raw materials



Multiple Reg./Corr. Techniques

Mixture-amount response surface models

Research project directed to warrant the availability of chemical information through a monitoring system

1. **Defined important chemical components to measure** and its specifications
2. **Analytical methodologies adapted to the industrial environment and to the conditions of the country**
3. Defined the best sampling time and the minimal sample size
4. Estimated the evolution of the chemical content during the pre-processing and industrial processing

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Objectives:

1. To define which of the chemical - physical properties of the cigars and the mainstream smoke are necessary to explain variations in the sensory strength.

2. To evaluate the fitness for purpose of analytical processes by NIR spectroscopy applied to the cigars and raw materials.

3. Developing a procedure to evaluate the fitness for purpose of traditional analytical methods in an industrial context where certified reference materials are unavailable.

Materials and Methods

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Two lots of tobacco raw materials and cigars

322 tobacco dust samples

- Growing seasons
- Locations
- Raw material type
- Processing phase

1890 cigars

27 cigar products, 70 units

Diameter (mm): 19.8 (50)
(ring gauge) 16.7 (42)
15.0 (38)

Length (mm): 192
165
124

Blend type: Light
Medium
Full

96L
Diameter: 1**9**.8
Length: 1**6**5
Blend: **L**ight

Product Codes		
99L	69L	59L
99M	69M	59M
99F	69F	59F
96L	66L	56L
96M	66M	56M
96F	66F	56F
92L	62L	52L
92M	62M	52M
92F	62F	52F

Three data sets

Cigars: 4 replications x 19 variables
Smoke: 8 replications x 14 variables
Sensory: 40 replications x 6 variables

Univariate and multivariate correlation and regression techniques

Materials and Methods

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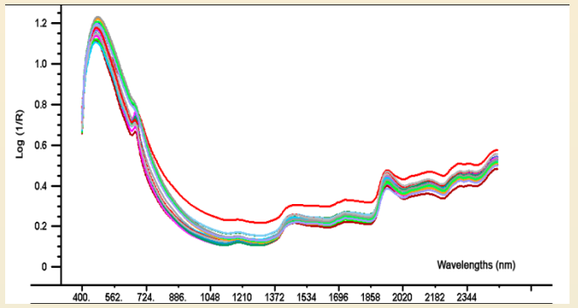
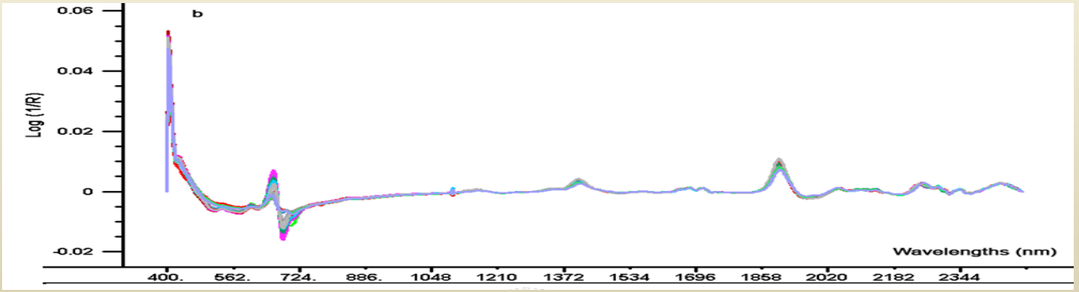
Borges, A.; Pérez, C.; Jiménez J y Álvarez, M: Near infrared analysis of total alkaloids as nicotine, total nitrogen and total ash in Cuban cigar tobacco. **Journal of Near-Infrared Spectroscopy. 27 (2) 123–133, DOI: 10.1177/0967033518825345, 2019.**

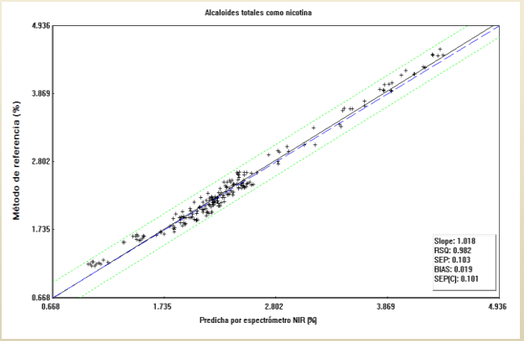
NIR spectroscopy models

- **Spectrometer:** NIR XDS Rapid content analyzer (FOSS, Denmark) 400-2500 nm with 2 nm interval (1 050 λ)



- **Calibration:** 75 % of the samples, PLS 1, PCR and MLR pre-processing MSC and 1st derivative (Norris-Williams) with 4 points smoothing window and 4 points for derivation





- Validation: 25 % of the samples (various statistics computed) SEP, Range/SEP, Std. dev./SEP.

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Borges, A.; Marquez, I;. Jiménez, J., Pérez, C. y Álvarez, M., Overcoming constraints of the control charting approach for measurement uncertainty assessments. **Accreditation and Quality Assurance, 20, 473-482, 2015, DOI: 10.1007/s00769-015-1161-0.**

Assessing the fitness for purpose of traditional and NIR spectroscopy analytical methodologies

Uncertainty of traditional analytical methods through control charting approach: total alkaloids as nicotine and total nitrogen.

$$u_c = \sqrt{s_{ta}^2 + u^2(\delta)}$$ random effect (spiking experiment) and systematic effect (method comparison study)

Three mass fraction levels, 54 test portions for each non-CRM

Estimation *on the dot* using 200 non-CRM
IIT lab vs. Reemtsma-ITG lab (ISO 17025 certified)

Applying the law of uncertainty propagation and partial derivation to the equation for estimating bias:

$$u(\delta i) = \sqrt{u^2(a) + (b-1)^2 \frac{1}{n}\, u^2(w_{\text{ref } i}) + \overline{w}_{refi}\, u^2(b) + cov(a,b)}$$

Coef. Estimated through IRLS Linear regression analysis (method comp)

Assessing fitness for purpose

Relative bias under intermediate precision ⟷ Relative bias under reproducibility conditions

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Univariate Pearson correlation coefficients between the sensory strength and the 33 variables in the cigars and smoke data sets.

Sensory property	Total alkaloids as nicotine	Total nitrogen	Total ash	Ammonium	Chloride	Nitrate	Potassium	Nicotine/ tar
Strength	0,640**	0,563**	-0,518**	0,434*	0,547**	-0,495**	-0,463*	0,431*

** Significant Pearson correlation coefficient, p-value < 0,01;
* Significant Pearson correlation coefficient, p-value < 0,05

7 out of 19 variables in the cigars data set

1 out of 14 variables in the smoke data set

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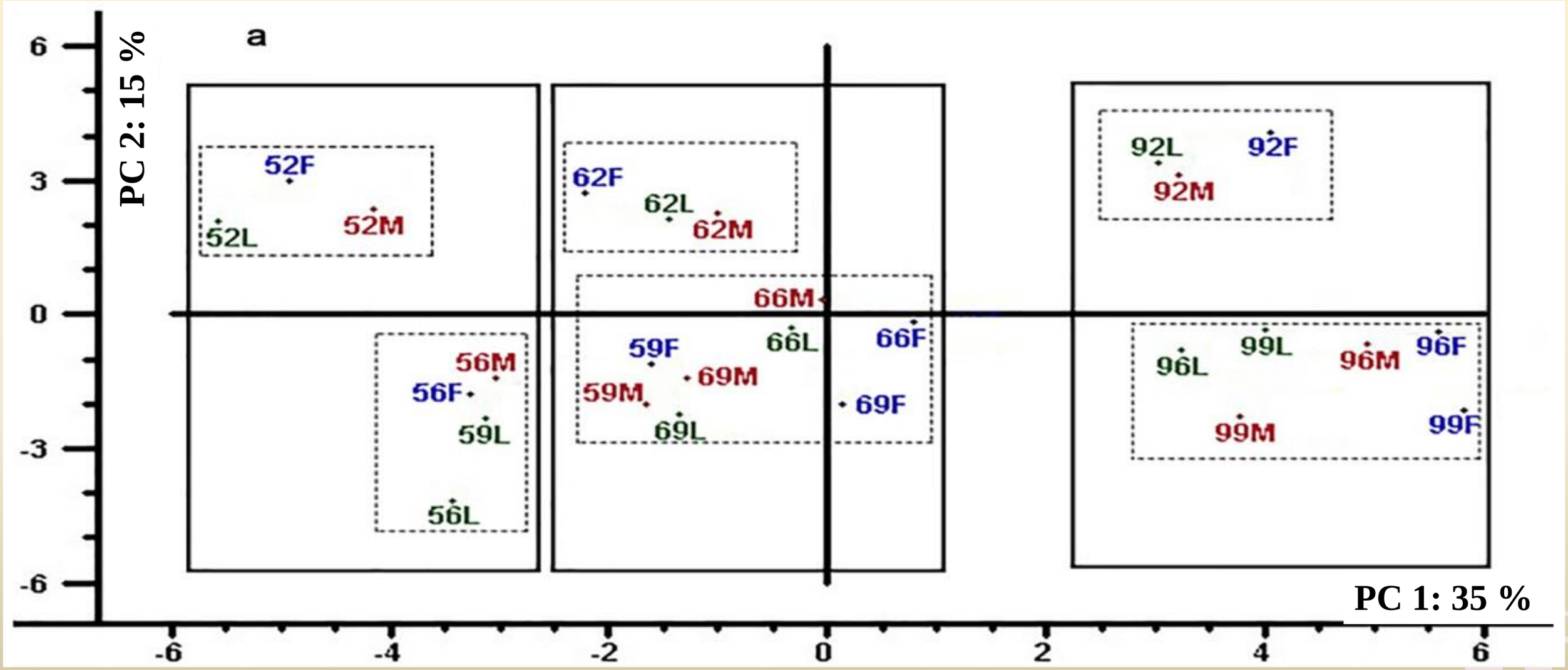
Score plot of the PCA analysis comprising the 33 variables of the cigars and smoke data sets



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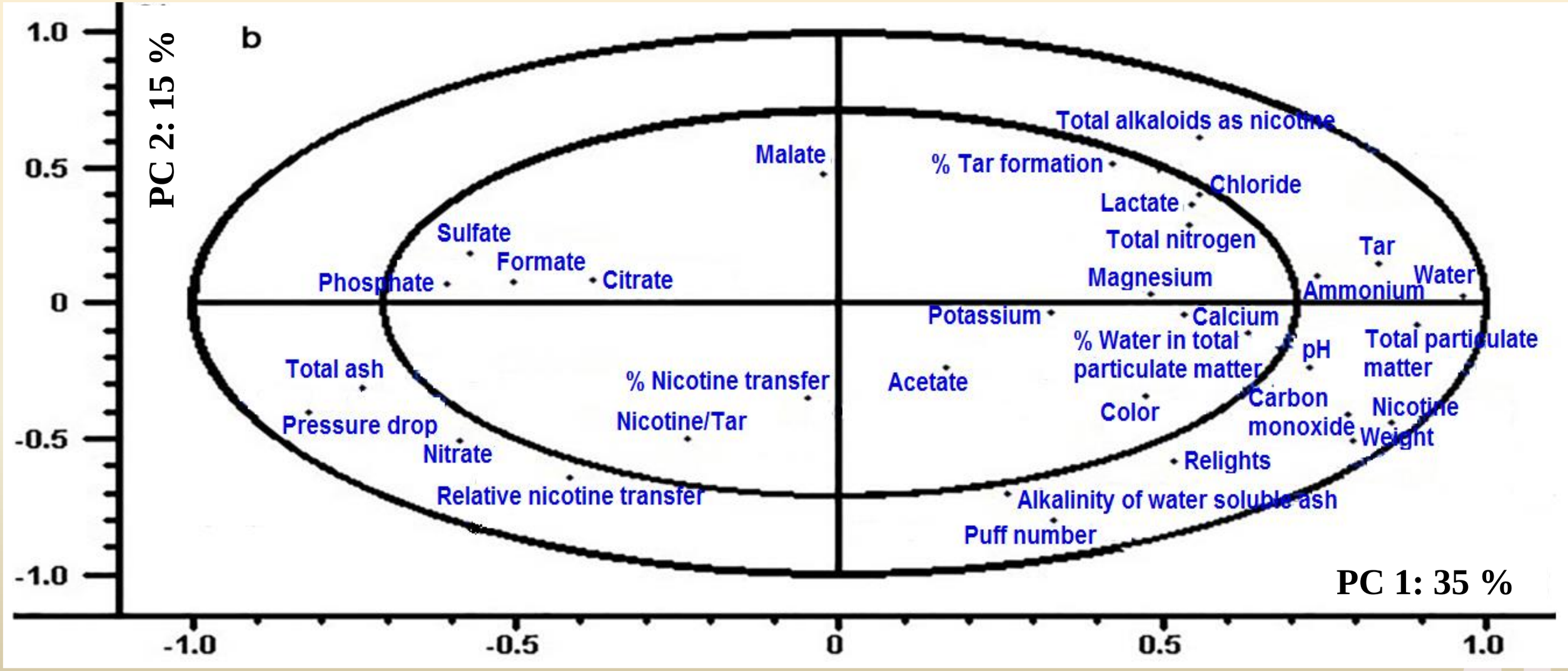
Loading plot of the PCA analysis comprising the 33 variables of the cigars and smoke data sets



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Standard and weighed coefficients of the PLS 1 prediction model of the sensory strength. Auto scaling as data pre-treatment.

Analytes	Standard coefficients.	Weighed coefficients.
Total alkaloids as nicotine	71.98	0.20
Total nitrogen	122.99	0.19
Total ash	- 29.40	- 0.13
Nicotine/tar	7.58	0.24
Relative nicotine transfer	0.02	0.11

Global model comprising all 27 products,

SECV= 0.36;
SEP= 0.42;
R^2 = 0.47;
R = 0.69

Narrow range of variation among the three blends that spanned the widest possible sensory space (mass fraction units):

Total alkaloids as nicotine: 0,00709
Total nitrogen: 0,00340
Total ash: 0,01053

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Friedman Test (Non-parametric equivalent to paired t-tests.

$\alpha = 0,05$

H_0: The paired means are equal

H_1: The paired means are different

Statistics	
n	27
χ^2 (Chi squared)	0,333
Degrees of freedom	1
p value	0,564 > α

The H_0 is acepted. For each product, the values predicted by the model are statistically equal to the observed (perceived) values by the tasters.

Conclusion

1) As a first approximation, the most important chemical and physical properties to predict the sensory strength of cigar's mainstream smoke are: the smoke analytes nicotine/tar ratio and relative nicotine transfer, together with the concentrations in the cigars of: total alkaloids as nicotine, total nitrogen and total ash. Raw materials specifications can be developed on these cigar related properties.

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Calibration statistics for NIR spectroscopy models

Analyte (mass fraction)	*n*	Model type	Factors/ variables	SECV (x 10^{-2})	SECV/ SEL	R^2 in calibration
Total alkaloids as nicotine	237	PLS 1	12	0.10	2.326	0.9909
		PCR	12	0.15	3.488	0.9692
		MLR	9	0.10	2.326	0.9861
Total nitrogen	237	PLS 1	11	0.11	2.535	0.9410
		PCR	11	0.18	4.147	0.8047
		MLR	9	0.13	2.995	0.9001
Total ash	219	PLS 1	10	0.48	6.977	0.9589
		PCR	10	0.65	9.448	0.8923
		MLR	9	0.43	6.250	0.9494

SECV/ SEL = 2 – 3 for most of the models

SECV lower than that reported in **Jingfeng *et al*,, 2007 y Poisson *et al*,, 2005** except that for total nitrogen

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Validation statistics for the NIR spectroscopy analytical methodologies

Analyte (mass fraction)	*n*	SEP	SEP/ SECV	Range/SEP	Std. dev./ SEP	r^2
Total alkaloids as nicotine	78	0.0011	1.10	33.72	8.36	0.99
Total nitrogen	78	0.0012	1.10	16.67	3.75	0.94
Total ash	69	0.0049	1.02	15.10	4.02	0.96

Performance parameters (AACC):
SEP/SECV < 1.2;
Range/SEP ≥ 15 good calibration for quantification
Std dev/SEP ≥ 8 good for applied research, ≥ 2,5 good for screening in breeding programs.

SEP lower than that reported in Jingfeng *et al*,, 2007 except for total nitrogen, Poisson *et al*, 2005 didn't report validation performance.

Results and discussion

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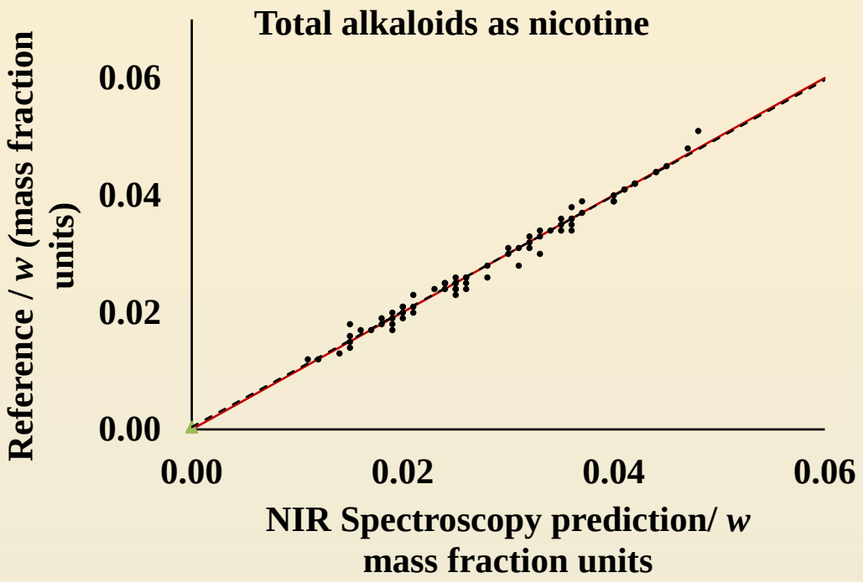
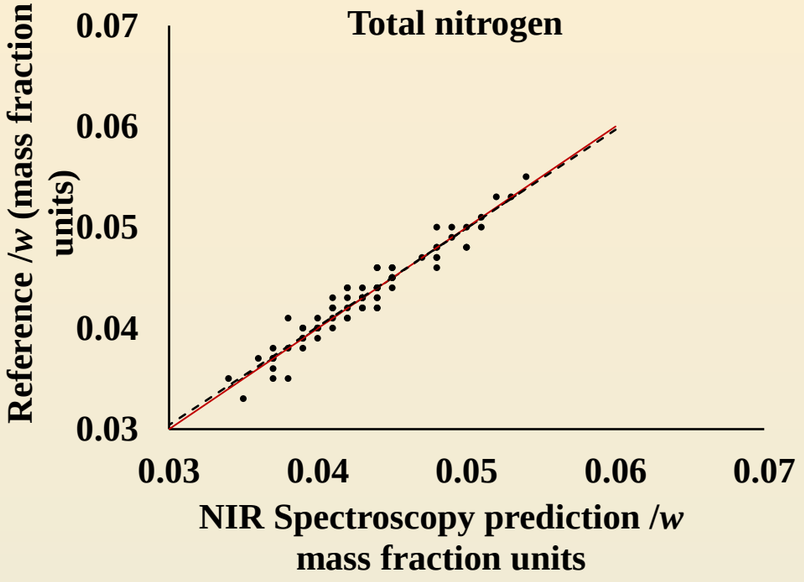
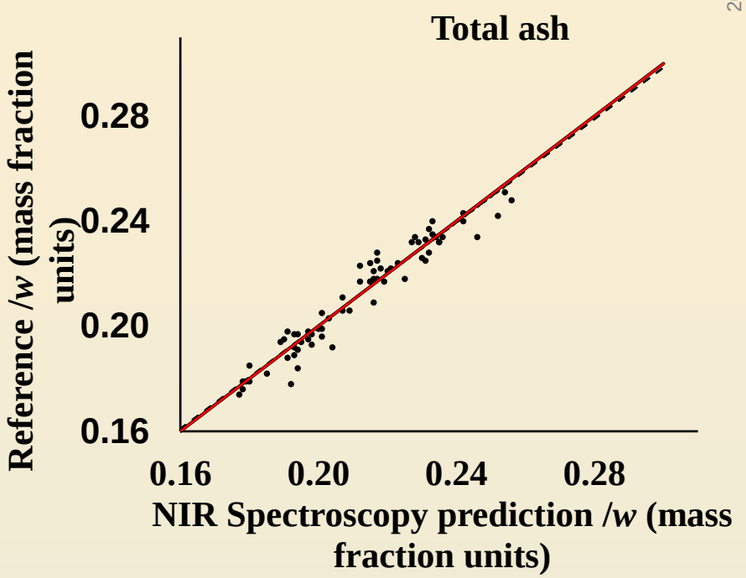
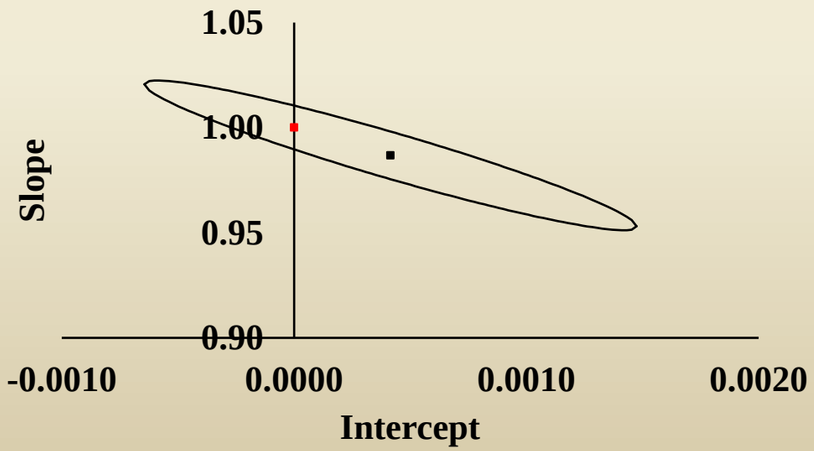
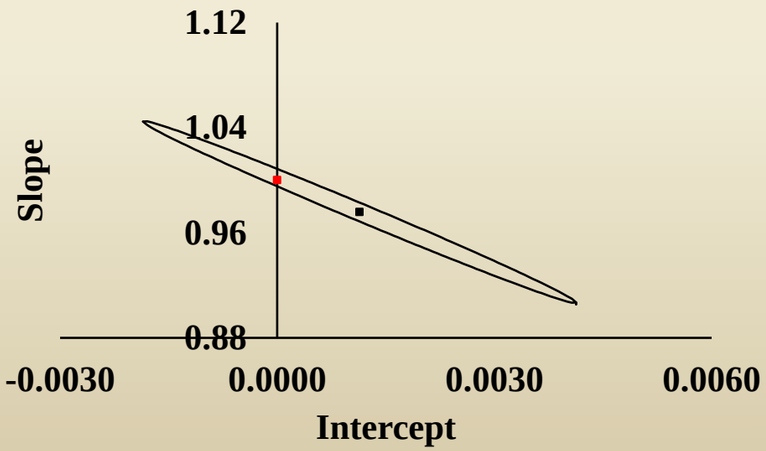
Non-significant systematic error through the mass fraction range assessed for the three analytes.

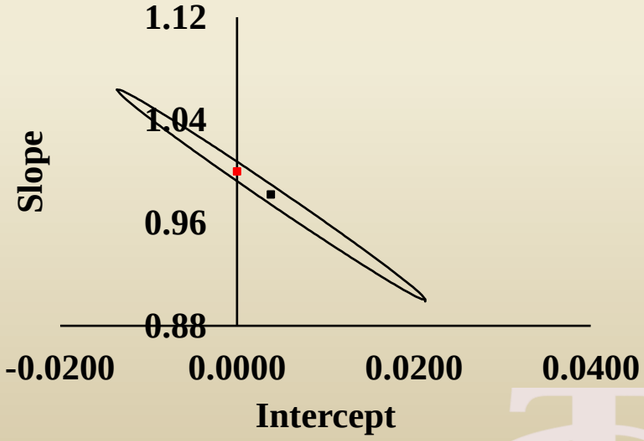












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Estimating the standard combined uncertainty of traditional analytical procedures and its components at three mass fraction levels

Analyte	w_{obs}	w_{ref}	δ	w_{obs}	$s_{(ta)}$
	Method comparison study			**Spiking experiment**	
Total alkaloids as nicotine	0.0272	0.0260	12.6×10^{-4}	0.0274	4.4×10^{-4}
	0.0376	0.0375	8.6×10^{-4}	0.0375	10.9×10^{-4}
	0.0478	0.0468	9.6×10^{-4}	0.0478	8.1×10^{-4}
Total nitrogen	0.0388	0.0409	-21.4×10^{-4}	0.0388	6.5×10^{-4}
	0.0515	0.0523	-8.8×10^{-4}	0.0510	10.6×10^{-4}
	0.0632	0.0665	-33.1×10^{-4}	0.0632	9.5×10^{-4}

Method comparison

Analyte	w_{ref}	$u(\delta)$	u_c	Relative u_c (%)
Total alkaloids as nicotine	0.0260	4.6×10^{-4}	0.0009	3.3
	0.0375	10.8×10^{-4}	0.0022	5.8
	0.0468	14.4×10^{-4}	0.0023	4.8
Total nitrogen	0.0409	0.1×10^{-5}	0.0007	1.8
	0.0523	33.4×10^{-4}	0.0043	8.3
	0.0665	48.3×10^{-4}	0.0059	9.3

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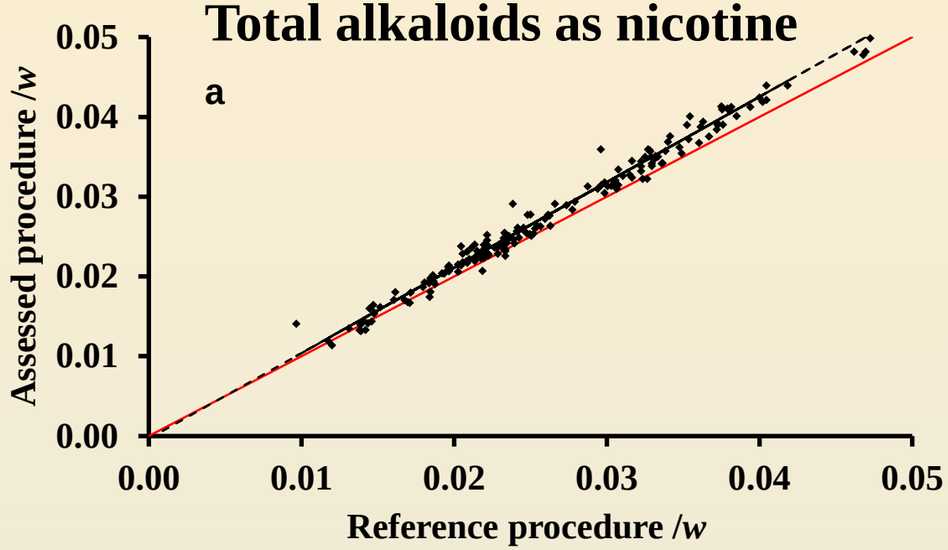
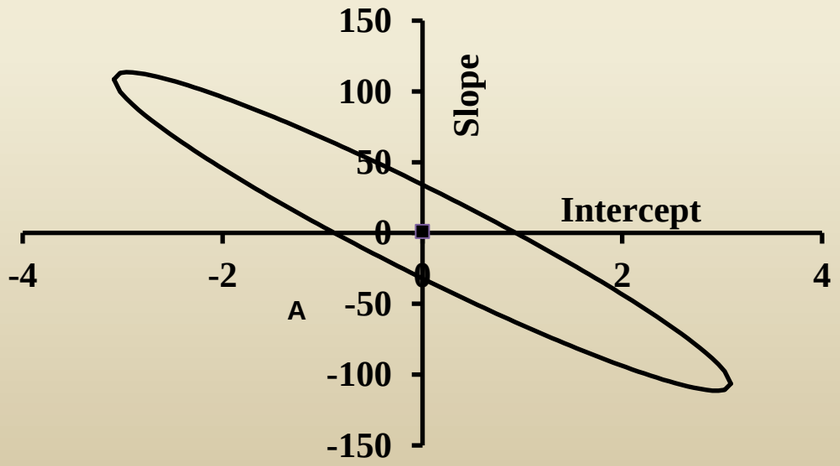
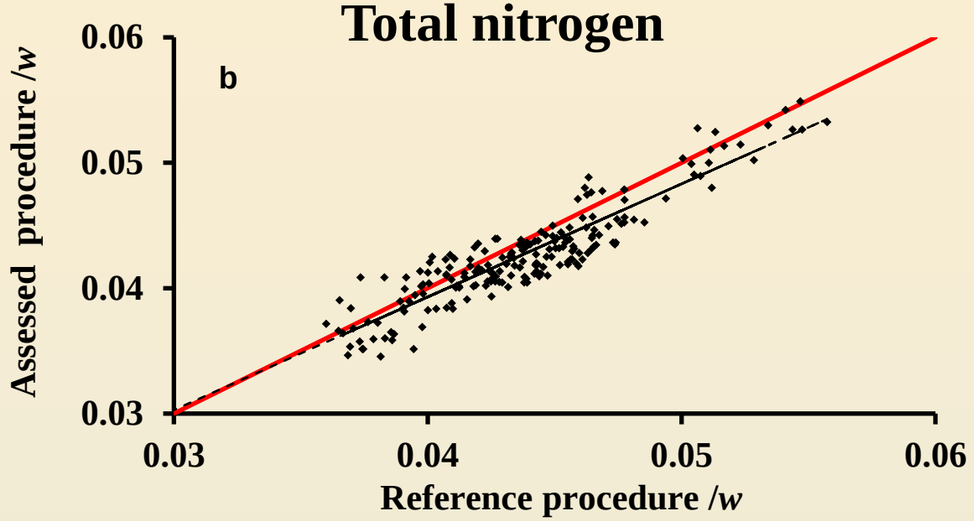
Non-significant systematic error through the mass fraction range

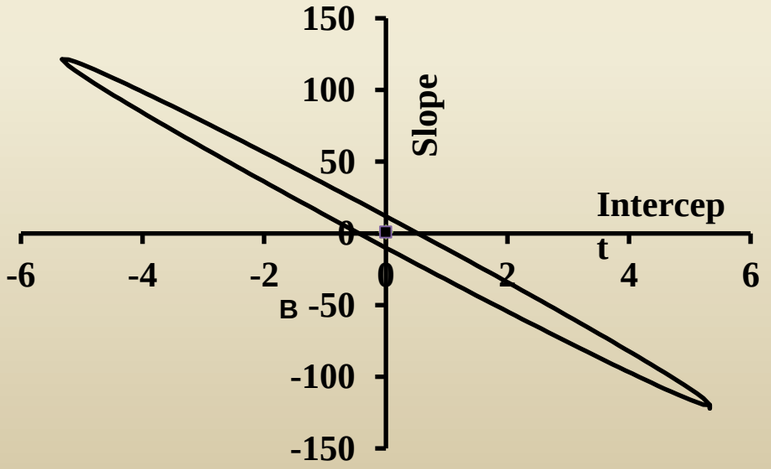
Total alkaloids as nicotine





Total nitrogen





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Assessing the fitness for purpose of NIR spectroscopy calibrations and traditional analytical procedures

Analyte	Relative SECV (%)	Relative SEP (%)	Method comparison study (%) (Intermediate precision)	Collaborative studies (%) (Reproducibility)
Total alkaloids as nicotine	3.55	**3.90**	**1.77 - 3.08**	**4.10[1]; 3.80[2]**
Total nitrogen	2.56	**2.79**	**0.0024 - 7.26**	**-**
Total ash	2.28	**2.32**	**2.47**	**-**

1. **Franke J.E.; Bennet, B.N.; Davis, R.E.; Thomsen, H.V.; y Johnston, K.S., Determination of nicotine in tobacco: Collaborative study. Beiträge zur. Tabakforschung International/ Contributions to tobacco research.. 19 (5): 259-265. 2001.**

2. **Crumpler, L.A. ; Zhang, W. ; Ma, Y., Routine Analytical Chemistry Sub-Group Technical Report . 2014 Collaborative study comparing crm35 for the determination of total alkaloids (as nicotine) in tobacco by continuous flow analysis to a new method with safer chemistry. Available online in: https://www.coresta.org/sites/default/files/technical_documents/main/RAC-052-1-CTR_SaferChemistry-CollStudy2014_March2017.pdf**

Conclusion

2) There were positive indications for evaluating the fitness for purpose of the NIR spectroscopy models. It allows moreover several advantages as follows if calculated in a year basis:

- It is needed the analysis of 2 100 additional samples, which represents 2.6 times more samples than the traditionally assessed. It can be achieved in a time period which is 3.5 times lower than the previously employed.
- Around 14 736 USD won't be expended for reagents acquisition and electricity utilization.
- It is avoided the utilization of 10 500 L of current water and 2 340 L of distilled water.
- It is expected that the Institute earn up to 136 000 USD through a scientifically based service

3) There was as well a positive indication for evaluating the fitness for purpose of the traditional analytical procedures used as reference for the NIR spectroscopy models.

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General scheme for applying the results obtained

Agricultural phase

Pre-processing phase
Sorting and classification workshop

Pre-processing phase
Stripping workshop

Pre-processing phase
Aging

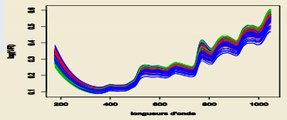
Industrial Phase
Cigar factory

Raw materials not suitable for long-filler cigars

Other product categories

Raw materials with high quality

Laboratory



Predicted with Deviation

Sample	Predicted	Deviation
"3"	3.642e-02	7.737e-04
"7"	1.641e-02	8.573e-04
"14"	2.849e-02	1.117e-03
"15"	3.347e-02	1.853e-03
"16"	1.994e-02	1.007e-03
"20"	2.475e-02	8.749e-04

Alkaloids and nitrogen	++++	+++		-	--
Ash	----	---		+	++

Concentration of the three components can be written in the labels of the traditional packages

Specifications

Thank you !

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