69th Tobacco Science Research Conference

The Scientific Basis of Harm Reduction and the Risk Continuum

Application of near infrared spectroscopy to detect mold contamination in tobacco

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Outline

1 Backgrounds

2 Experimental

3 Results and Discussion

4 Conclusions

Backgrounds

Mold infection is a significant postharvest issue for processors of tobacco.

- **direct product loss**
- **value reduction of product**
- **serious economic losses**
- **food safety issue, mycotoxin**

Identification of moldy tobacco is very important.

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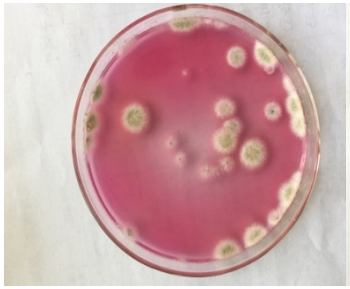
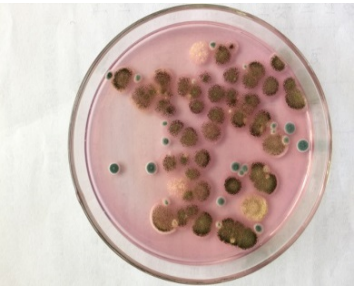
Why to do?

Traditional techniques:

- Visual inspection
- Microbiological detection : 6 day
- Chemical composition detection: complex sample preparation

However, mold mostly is undetectable at early stages by traditional detection techniques.

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Why to do?

NIR spectroscopy has many advantages

- **Rapid**
- **Little or no sample preparation**
- **Spectra fingerprints**

However， the shortage is insufficient sensitivity.

In our work, NIR spectroscopy technique combine with GBA algorithm were used in detection of mold infection in flue-cured tobacco samples was studied. The result show it was effectively.

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How to do?

Experimental

2.1 Material and methods

Instrument and methods:

- Nicolet Antaris Ⅱ Filter-Near Infrared (FT-NIR) Analyzer (Thermo fisher Corp., USA)
- TQ Analyst 9.3 software (Thermo fisher Corp., USA)
- MATLAB (Matlab 6.X, The MathWorks, Inc)
- Gas Chromatography-Mass Spectrometry (GC/MS) (Agilent Corp., USA)
- Liquid Chromatograph-Mass Spectrometer (LC/MS/MS)(Agilent Corp., USA)
- Continous-Flow analysis (CFA) (Bran Luebbe Corp., Germany)

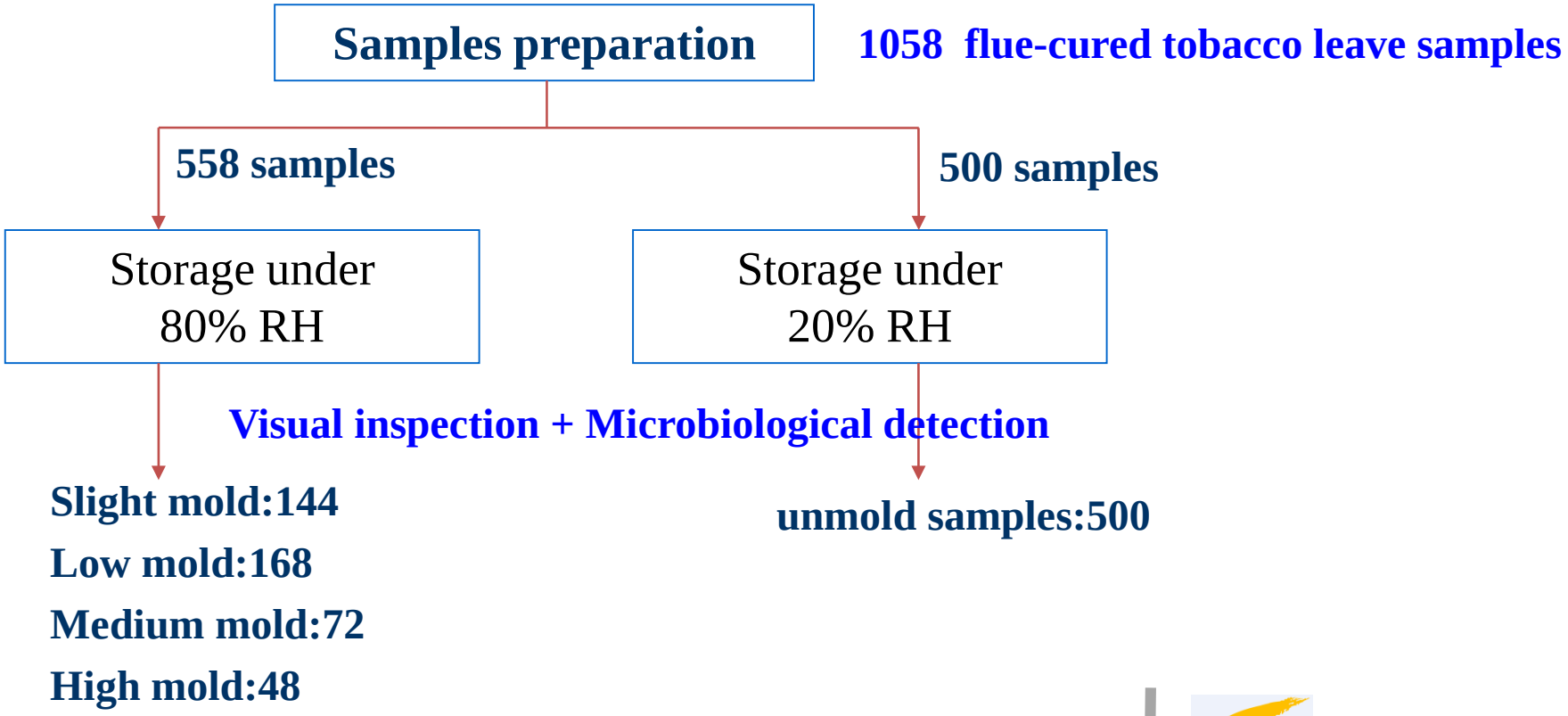
Statistical analysis:

- Monte Carlo sampling -partial least square (MCS-PLS)
- Good to bad (GBA) algorithm
- Principal Component Analysis (PCA)

How to do?

2.1 MATHERIAL AND METHODS

Samples:



How to do?

2.2 PROCESS

Samples

↓

FT-NIR Spectra Collection

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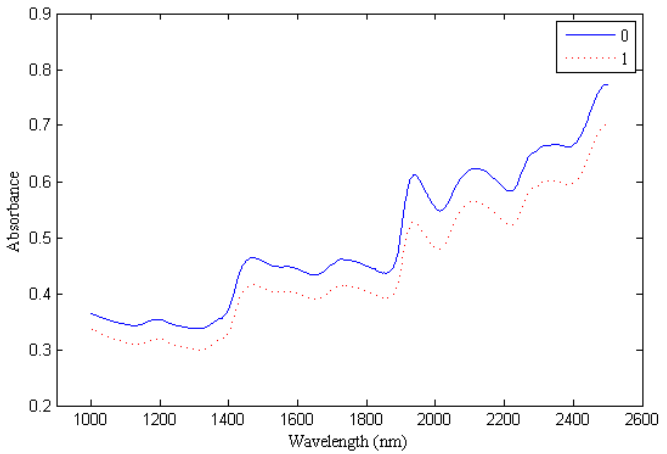
Informative features selection

↓

Model establishment and validation

↓

Applied on the detection of mold contamination in tobacco



Unmold Mold

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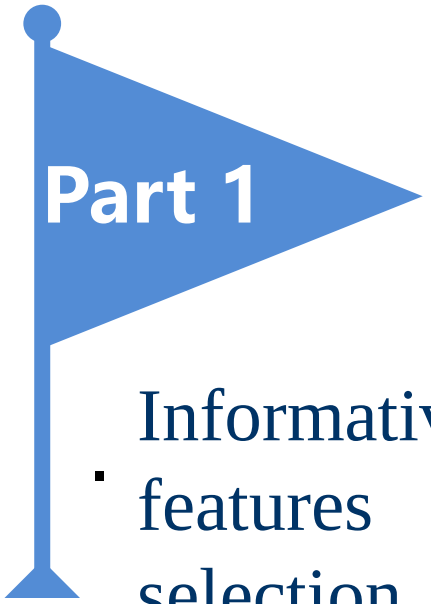
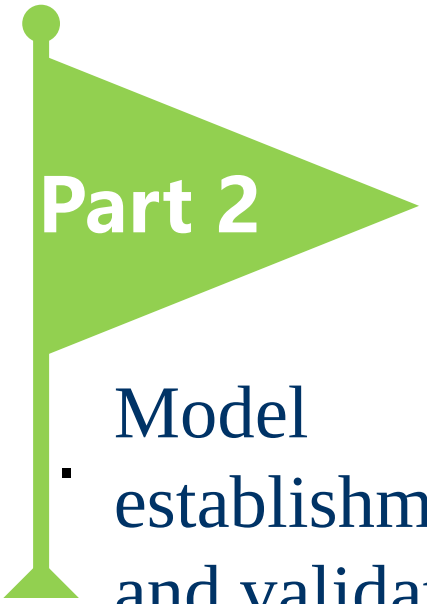
3 Results and Discussion



- Informative features selection.



- Model establishment and validation

- Explanation of selected wavelength

3 Results and Discussion

3.1 Informative features selection

Steps:

- 1000 sub-models were built based on MCS
- Calculate the root mean square error (RMSE) of each sub-model
- Collecte 1% percentage of sub-models in SEM and BEM
- Calculate the GBA value :

$$GBA(i) = \frac{Frequency\ of\ feature\ i\ in\ 1\%\ SEM}{Frequency\ of\ feature\ i\ in\ 1\%\ BEM}$$

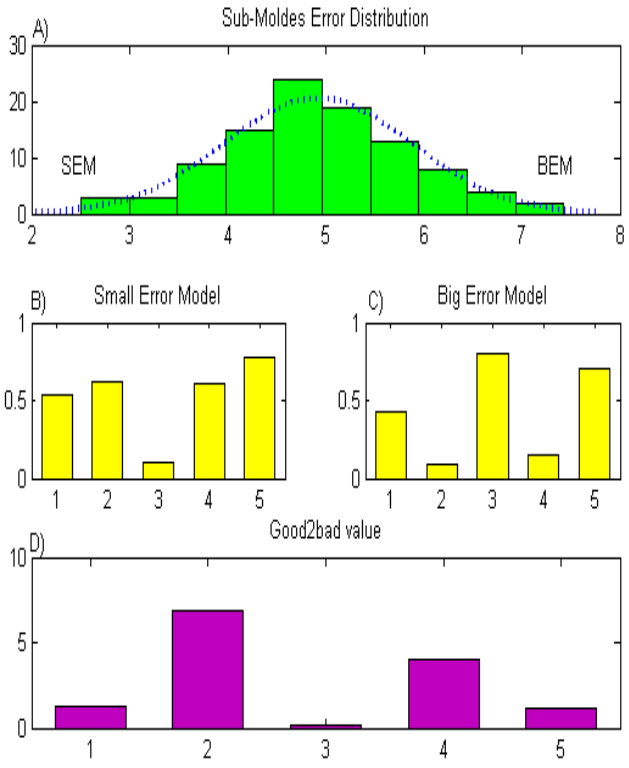


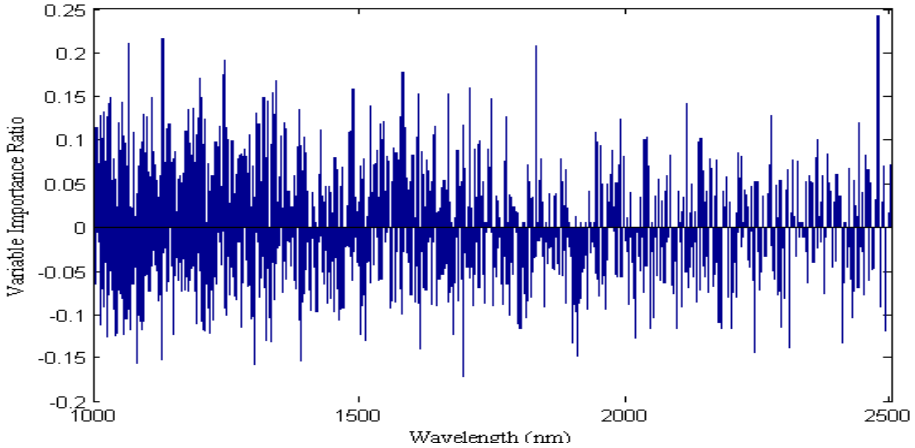
Figure 1 GBA Algorithm chart

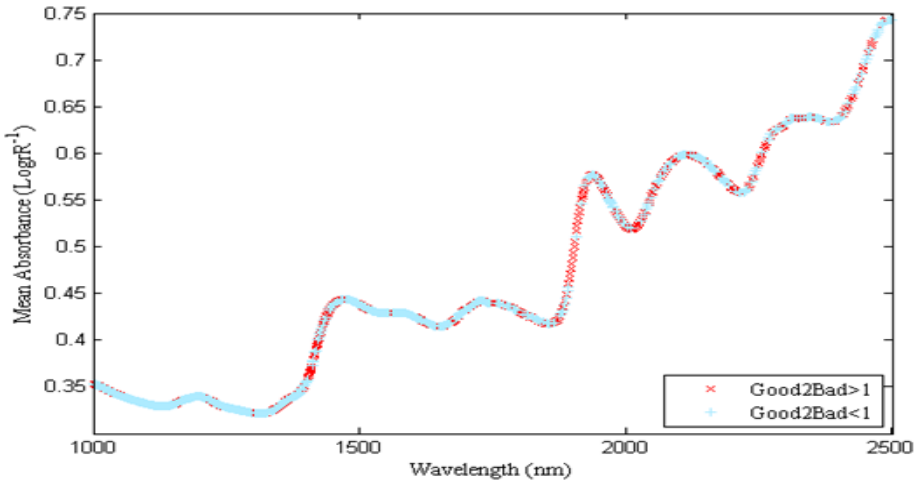
3 Results and Discussion

3.1 Informative features selection

Results:

- Calculate of the GBA value of each features
- Selecte the features which GBA value is more than 1, the bigger the better
- They are 1066 nm, 1130 nm, 1832 nm and 2474 nm



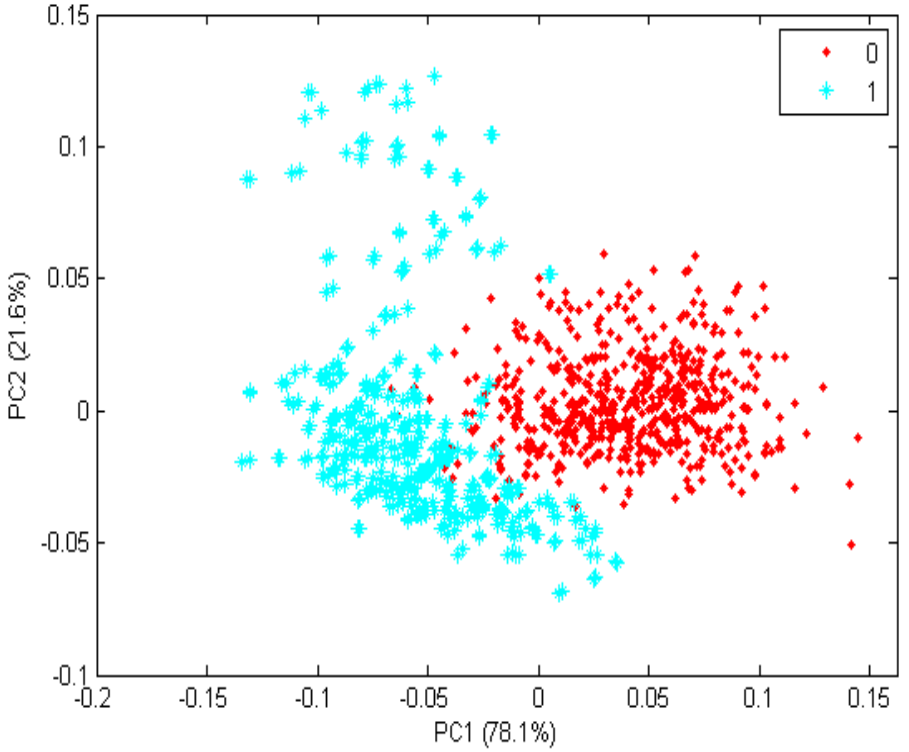


3 Results and Discussion

3.2 Model establishment and validation

Model establishment

- 1058 samples
- Optimal features of NIRs including Abs [1066 nm], Abs [1130 nm],Abs [1832 nm] and Abs [2474 nm]
- PCA-DA analysis
- Achieved low classification error rate as 2.92% with a Wilk’ s λ 0.216 ($P < 0.001$)

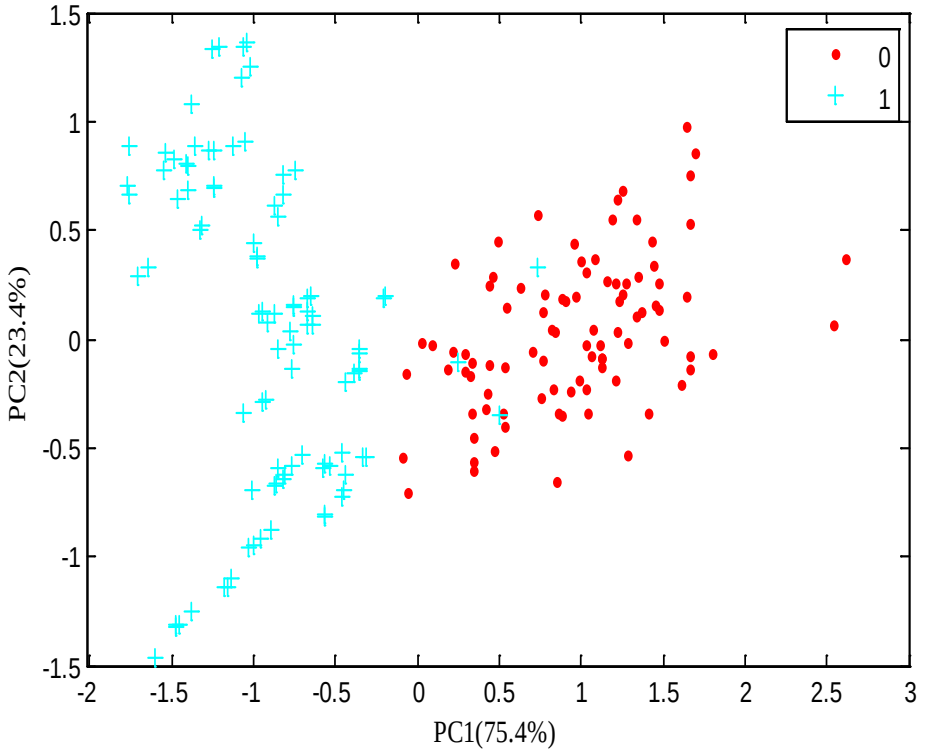


3 Results and Discussion

3.2 Model establishment and validation

Model validation

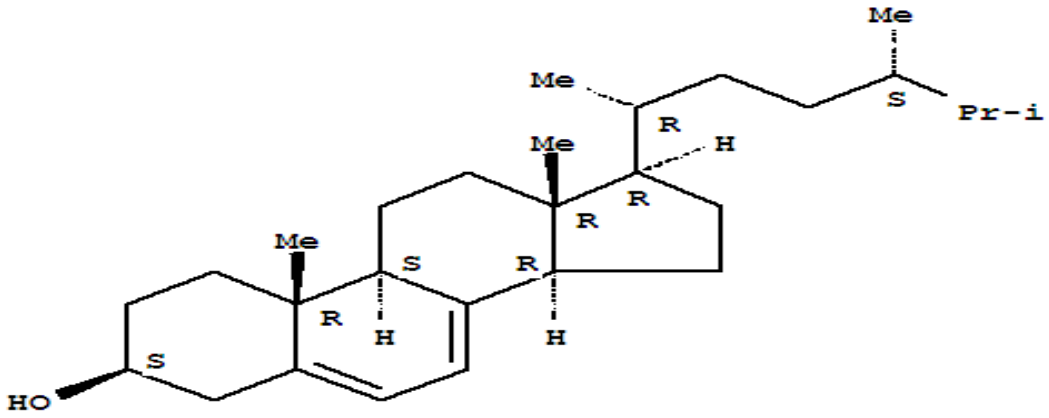
- Another 190 samples used for validation
- Optimal features of NIRs
- Use the Classification models
- The classification accuracies of unmold and mold were 94.57% and 96.94% respectively (100.00% slight mold, 100.00% low mold, 95.00% medium mold and 92.86% high mold).



3 Results and Discussion

3.3 Explanation of selected wavelength

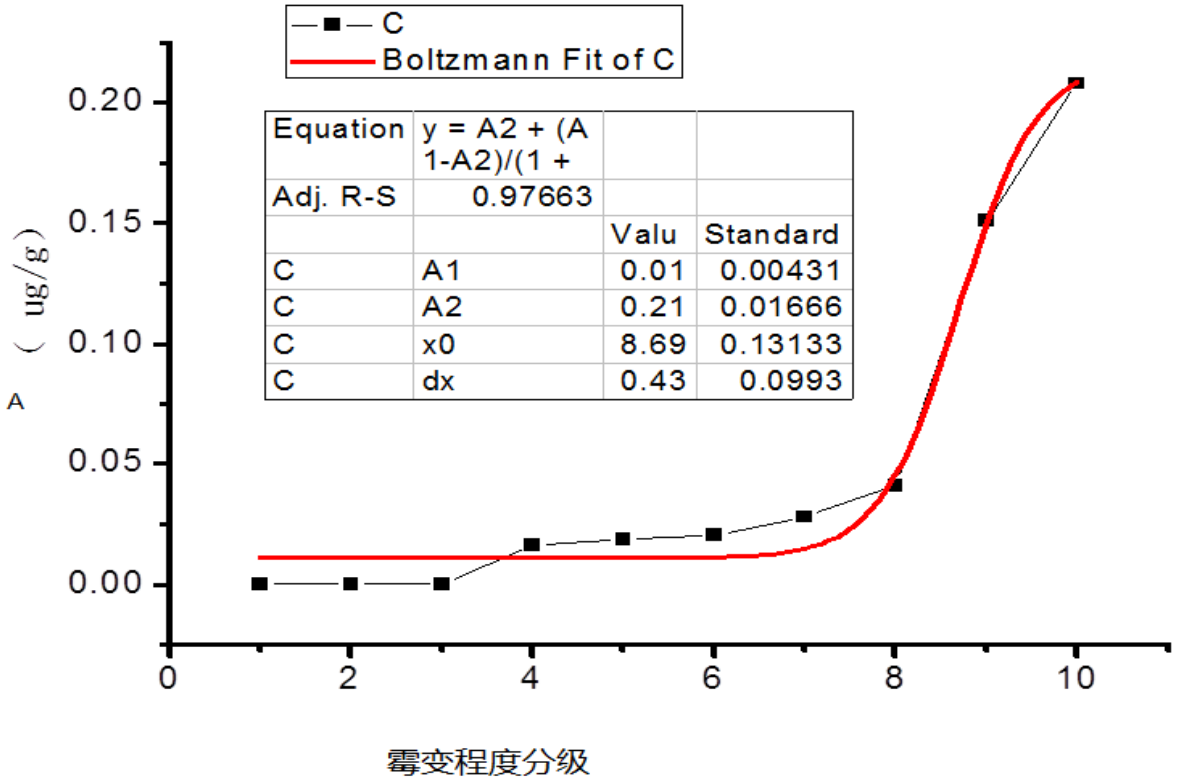
- Ergosterol, which are primary metabolites of micro-fungi .



- The NIR spectral bands related to ergosterol were observed at ~**1018** nm, **1190** nm, 1240 nm, 1360 nm, 1384 nm, 1436 nm, 1692 nm, 1730 nm, **1818** nm, 1910 nm, 2106 nm and **2382** nm et al**.
- Same of the wavelengths are closed to the selected wavelength. We can think of, is due to the spectral changes caused by ergosterol .

3 Results and Discussion

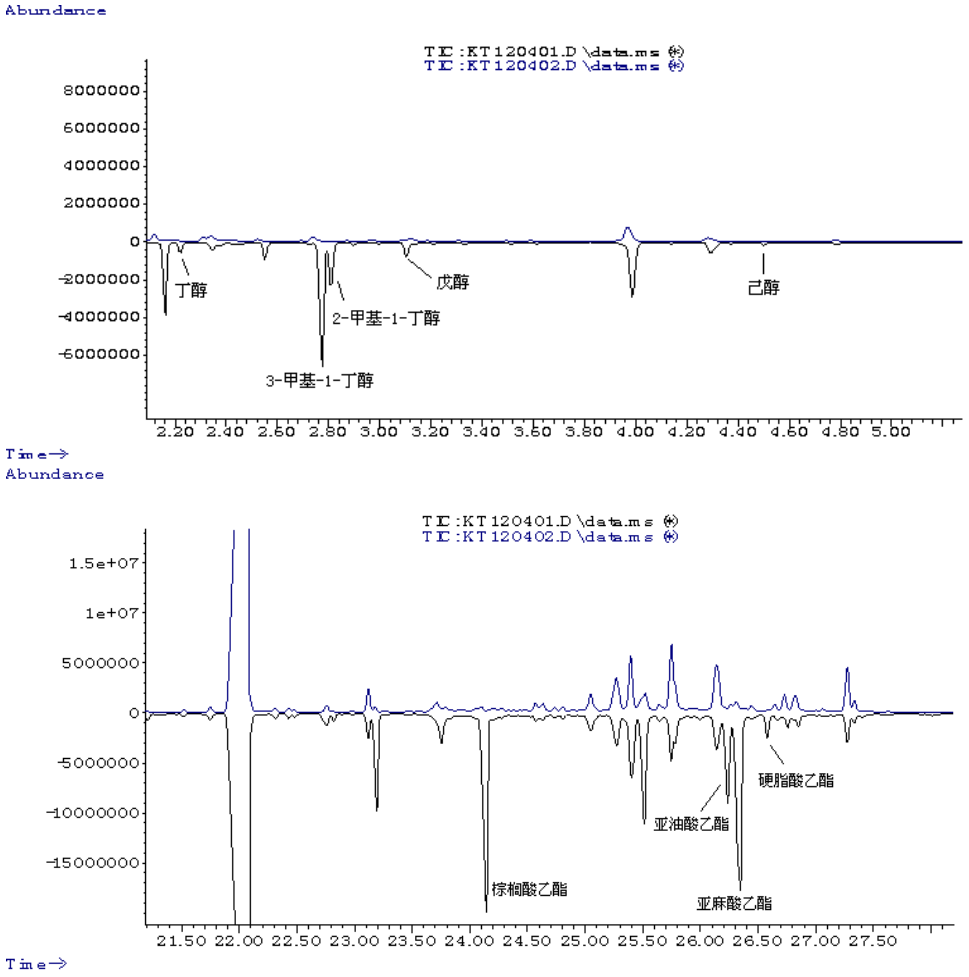
3.3 Explanation of selected wavelength

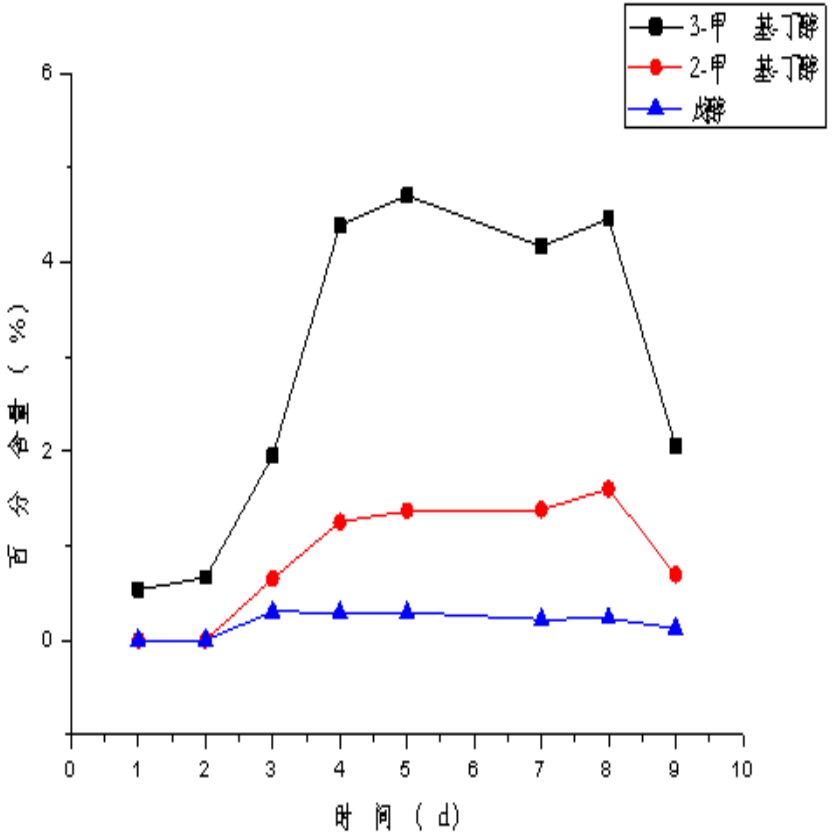


The content of ergosterol in diffrent moldy tobacco

3 Results and Discussion

3.3 Explanation of selected wavelength





3 Results and Discussion

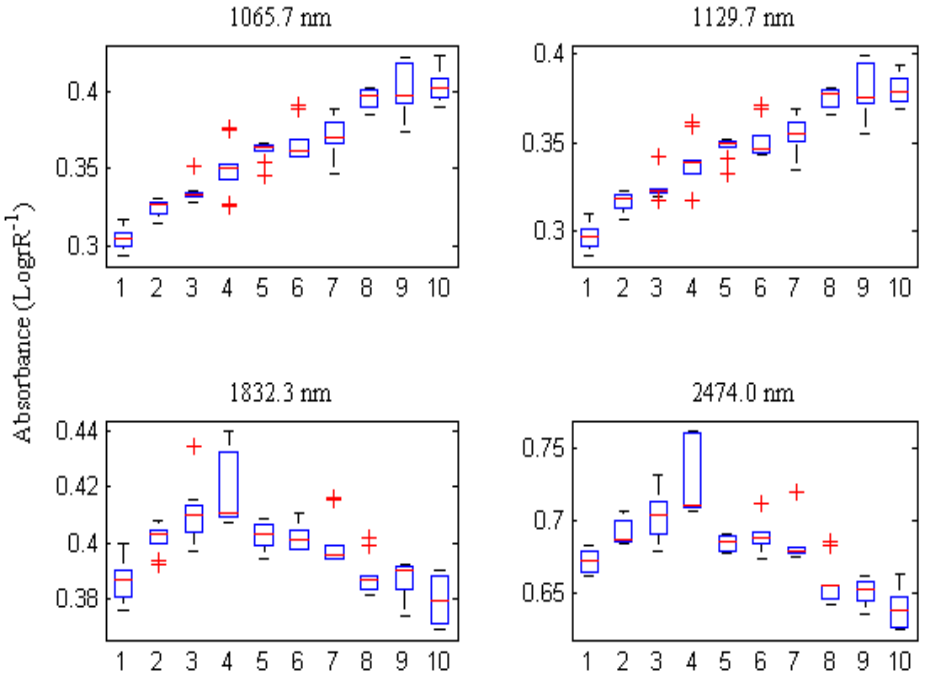
3.3 Explanation of selected wavelength

Chemical analysis of tobacco samples with different mold contamination severity（%）

Mold count	Total sugar	Reducing sugar	Nicotine	Total nitrogen	Potassium	Chloride
No mold	37.5	27.1	2.69	1.56	1.63	0.26
Slight mold	27.2	23.8	2.89	1.68	2.09	0.21
Low mold	21.1	18.9	2.83	1.57	2.10	0.20
Medium mold	8.36	7.83	2.87	1.93	2.39	0.33
High mold	2.80	2.39	2.60	2.25	3.01	0.50

3 Results and Discussion

3.3 Explanation of selected wavelength



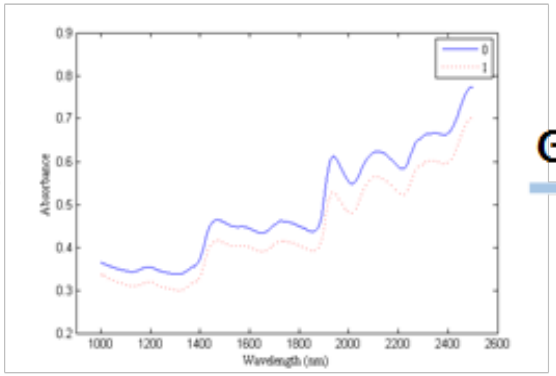
- Meanwhile, tobacco mildew is a complex chemical process.
- The four Optimal features of NIRs indicate that the process of the mildew

The absorbance of the selected wavelength

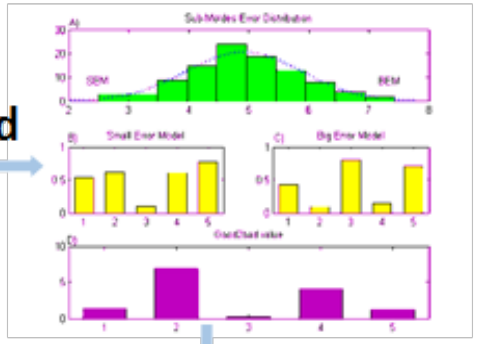
4 Conclusions

New method applied in prediction of tobacco at early stages of mold contamination.

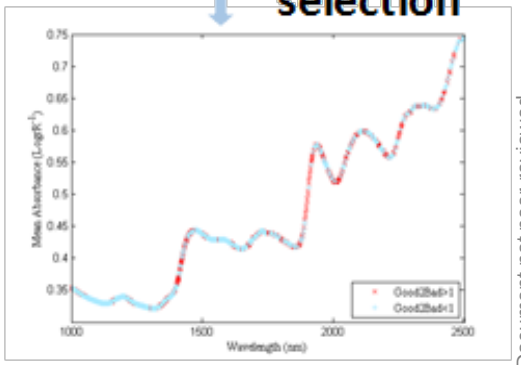
NIR



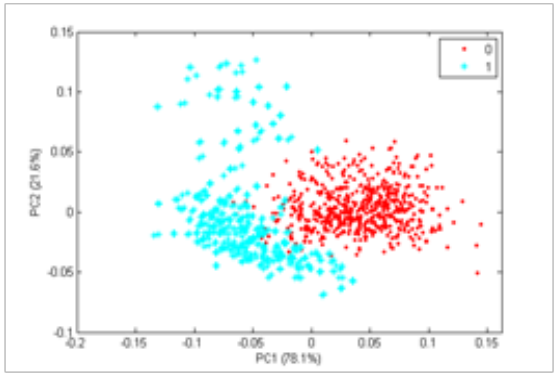
God2Bad



Informative features selection



Model calibration



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4 Conclusions

(1) A new algorithm that called GBA was set for informative features selection.

(2) A NIR Model for classify moldy and unmoldy tobacco samples was established and validated. The sensitivity is much higher than use sole of the spectra NIR model.

(3) Explanation of selected wavelength is connected with ergosterol, volatile and semi-volatile components, and some other chemical components in tobacco.The four Optimal features of NIRs could indicate the process of mildew in some way.

(4) Comparing with traditional technology such as Visual inspection + Microbiological detection, a new method of NIR combined with GBA and PCA-DA is applied in detecting mold contamination of tobacco, this method is rapid, accurate and effective.

Our team

Thanks!

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