Determination of cocoa purine content by near infrared spectroscopy

Caffeine and theobromine are involved in cocoa flavour development. Measurement of their contents is still used to determine the cocoa content of chocolate. It also seems to be accepted that the contents of these two compounds are linked to genetic and geographical origin. This study, which was conducted on cocoa varieties of different geographical origins, showed that near infrared spectroscopy can be a rapid and non-destructive alternative for determining these compounds.

Experimental procedure

The study was carried out on 189 cocoa samples over 3 production years (1999 to 2002). The cocoas came from Ivory Coast, Venezuela and Trinidad, and were varieties of the Forastero, Criollo and Trinitario types. Sampling in this way ensured good representativeness of the spectral and chemical variability of fermented dried cocoas.

Method

- Wet chemical methods
  After reflux extraction in water, caffeine and theobromine contents were determined by HPLC (detection: 280 nm).

- Near infrared spectroscopy
  NIRS acquisitions were obtained on a Foss-Perstorp 6500 analyser using a spin cell. Spectral data were collected and processed with NIRS version 2 software (InfraSoft International). 3 g of cocoa taken from 100 g of shelled, ground and sieved beans (0.5 mm) were analysed by diffuse reflection from 400 nm to 2,500 nm in 2 nm steps.

Results

- Reference analyses
  Caffeine contents were between 0.08% and 0.74%, with an average content of 0.28%. The standard deviation for caffeine contents was 0.16. Theobromine contents were between 0.58% and 1.48%, with an average content of 0.94%. The standard deviation was 0.20.

- Calibration
  Partial Least Square models (PLS) were used to establish quantitative relations between NIR spectral bands and caffeine and theobromine content. The models developed (table1) fitted the data well, with coefficients of determination of 0.96 for caffeine contents and 0.86 for theobromine contents. The coefficients of determination for regressions (figures 1 and 2) between the reference values and the predicted values were 0.93 for caffeine and 0.81 for theobromine. The standard errors of prediction (SEP) were 0.04 for caffeine and 0.09 for theobromine.

Table 1. Calibration statistics for caffeine and theobromine.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>N</th>
<th>M</th>
<th>SD</th>
<th>SEC</th>
<th>R²</th>
<th>SECV</th>
<th>Number of PLS terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td>179</td>
<td>0.28</td>
<td>0.15</td>
<td>0.03</td>
<td>0.96</td>
<td>0.04</td>
<td>11</td>
</tr>
<tr>
<td>Theobromine</td>
<td>182</td>
<td>0.94</td>
<td>0.19</td>
<td>0.07</td>
<td>0.86</td>
<td>0.08</td>
<td>8</td>
</tr>
</tbody>
</table>

N: number of samples adopted by the model (t test)
M: mean
R²: coefficient of determination
SD: standard deviation of the calibration population
SEC: standard error of calibration
SECV: standard error of cross validation

Conclusion

Given the performance of the equations developed from representative sampling of spectral variability, NIRS can be considered as a routine purine analysis method. Whilst this study did not lead to any definitive conclusions on discriminating between genotypes according to their purine compositions, it did confirm earlier observations. With a tool enabling rapid determination of purine composition it will be possible to make progress in that field.