Food Chemistry **27** (1988) 61–75

Near-infrared Reflectance Prediction of Quality, Theaflavin Content and Moisture Content of Black Tea

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(Received 27 November 1986; revised version received 2 April 1987;
accepted 23 May 1987)

ABSTRACT

Studies on a total of 134 black teas of wide-ranging type, origin and quality have indicated that the moisture content, theaflavin content and tea tasters' assessment of overall quality can be successfully estimated by near-infrared reflectance (NIRS) spectroscopy. Using the Neotec Compscan 7000 system, prediction equations have been derived on unground tea leaves. Moisture content was predicted with SEP 0·39% over a range of 8·9% to 17·3% using three linearly summed terms. Total theaflavins, in the range 1·5 to 26·5 μmoles/g, was estimated (two-term equation) with a standard error of 3·3 μmoles/g. Overall quality scored by five tasters, over a range of 25 to 74, was predicted by a two-term equation with an accuracy of ±15·6. This accuracy is favourably comparable to that achieved by one taster predicting the mean score of four others.

INTRODUCTION

There are many types of tea consumed in large quantities worldwide, all of which are fundamentally derived from the tea plant *Camellia sinensis*. Differences in black teas occur due to variation in green leaf composition, due to genetic characteristics of the clones and to growth conditions, and to the method of production of the finished material.

Many factors contribute to the overall quality of a tea, such as colour, appearance, flavour and mouthfeel, and these, in turn, are influenced by levels of certain chemical constituents produced during manufacture (Wood & Roberts, 1964). During enzymic fermentation and the subsequent firing stages, complex oxidation and condensation reactions lead to the formation of orthoquinones, bisflavanols, theaflavins and thearubigins from the catechin precursors (Roberts, 1958*a*,*b*).

The theaflavin and thearubigin fractions constitute up to 30% of the dry weight of black teas and significantly affect the colour and mouthfeel of the resultant tea liquors (Roberts, 1958*b*; Wood & Roberts, 1964). The thearubigins represent the bulk of the black tea pigments and consist of an unstable, poorly defined, heterogeneous group of compounds whose contribution to quality is far from fully understood. The theaflavins, however, are stable and chemically well characterised. They are highly coloured compounds with good solubility in water and are considered to contribute positively toward the appearance and mouthfeel of black tea infusions (Wood & Roberts, 1964; Hilton & Ellis, 1972; Cloughley, 1980). This has led to the suggestion that theaflavin content may be included in ISO standards to provide an indication of quality or authenticity of UK imported teas. Methods for the determination of theaflavin are relatively lengthy and subject to considerable interlaboratory variation. For this reason near-infrared spectroscopy (NIRS) has been assessed not only for the determination of theaflavin but also the direct measurement of overall quality.

The use of NIRS for the determination of moisture in agricultural products is both well understood and documented (Polesello & Giangiacomo, 1983). Its specific application to moisture measurement in tea is important with regard to the economics of tea trading; just 2% difference in moisture content can represent nearly £50 per tonne of tea solids. The keeping quality of tea also depends upon its water content; very high levels allow the growth of micro-organisms and mould spoilage.

MATERIALS AND METHODS

Materials

A total of 134 commercial tea samples were collected, representing the produce of 24 countries, numerous tea gardens and covering a complete range of leaf sizes. The greatest number of teas came from Kenya (22), Sri Lanks (20), Assam (16) and Malawi (15), but teas from sources such as Russia and Taiwan were also represented (Table 1).

TABLE 1
Origin of Teas Examined

Origin	*Number of samples*	*Origin*	*Number of samples*	*Origin*	*Number of samples*
Darjeeling	2	Sri Lanka	20	Mauritius	3
China	7	Tanzania	4	Zaire	1
Assam	16	Zimbabwe	3	Indonesia	1
South India	4	Papua New Guinea	2	Brazil	1
North India	10	Russia	1	Cameroons	1
Kenya	22	Java	4	Rwanda	1
Sumatra	2	Malawi	15	Taiwan	2
Bangladesh	2	Mozambique	7	Unknown	3

METHODS

Moisture determination

Moisture determination of the tea samples was carried out in accordance with the ISO method (ISO 1573-1980). 5 g of well-mixed test sample were weighed into a pre-dried and pre-weighed weighing bottle and dried in an oven at 103 ± 2°C. The loss on drying was measured by weight difference and was expressed as a percentage (w/w) of the sample before analysis. All determinations were carried out immediately after NIRS analysis to minimise water uptake between tests.

Theaflavin determination

Theaflavin analysis was carried out on unground tea samples so that theaflavin content of the infusions could be directly related to the tasters' assessments on similar infusions. The method used was based on that of Hilton (1983) as modified by Robertson & Jewell (1986). Duplicate 9-g samples of unground tea were infused by shaking samples in 375 ml boiling water in thermosflasks for 10 min. Resultant liquors were then filtered through glass wool and cooled to room temperature. 10 ml of the filtrate were then pipetted into 10 ml of isobutylmethylketone (IBMK) and shaken for 10 min. After allowing the layers to separate, 2 ml of the upper layer were pipetted into a test tube followed by 4 ml ethanol and 2 ml Flavognost reagent (2 g diphenylboric acid-2-aminoethyl ester dissolved in 100 ml ethanol). The mixture was shaken for 2 min and incubated at room temperature for 15 min. The absorbance at 625 nm was measured against a blank containing (1:1) IBMK/ethanol. Theaflavin was calculated on a dry weight basis according to the formula

$$\text{Theaflavin } (\mu\text{moles/g}) = \text{OD}_{625} \times \frac{4790}{\text{DM}}$$

where OD_{625} = optical density at 625 nm and DM = dry matter (%).

Theaflavin synthesis

Epitheaflavic acid was synthesised by ferricyanide oxidation of gallic acid and (+) catechin. The method was based on that of Collier *et al.* (1973). Catechin (1·1612 g) and gallic acid (0·3402 g) were dissolved in a minimum of distilled water (approx. 400 ml) and cooled to 4°C in an ice bath. A solution of potassium ferricyanide (1·32 g) and sodium bicarbonate (0·8 g) in water (40 ml) was added dropwise with stirring to the gallic acid, catechin mixture and the pH of the solution adjusted to 6·5 by addition of sodium bicarbonate (0·1 M). After cooling the solution, by standing in an ice bath for 15 min, the pH was further adjusted to 4·5 with hydrochloric acid (0·5 N). The resultant solution was extracted with five volumes of ethyl acetate, the extract dried with magnesium sulphate and concentrated by rotary evaporation to about 30 ml.

The extract was then applied to a Whatman GF/A disc and, after desiccation with silica gel for 16 h, scanned in the NIR spectrophotometer (Meurens *et al.*, 1986).

Theaflavin and thearubigin extraction

The method of extraction of theaflavins from tea was adopted from Collier *et al.* (1973) with the addition of methods by Roberts & Smith (1961).

Theaflavin-rich tea (240 g) was extracted with water (2400 ml) at 85°C for 5 min and the decanted extract freeze-dried. The resultant powder was dissolved to give a 4% w/v solution in a methanol/water mixture (1:3). The solution was extracted with five volumes of chloroform to remove caffeine. The methanol and residual chloroform was distilled from the decaffeinated solution at 30°C under reduced pressure and the solution extracted with five volumes of ethyl acetate. The ethyl acetate extract was washed with five volumes of an aqueous solution of sodium bicarbonate (2·5%) to remove soluble acidic thearubigins. The ethyl acetate was then reduced to 30 ml by rotary evaporation and dried onto a Whatman GF/A disc for scanning in the NIR spectrophotometer.

The aqueous residue after the ethyl acetate extraction provided a thearubigin-rich source which was also rotary evaporated and dried on to a Whatman GF/A disc for scanning.

Quality assessment

The quality of the teas was assessed by five professional tea tasters using the scoring system with which they were familiar and which they would normally use within their own companies. In general, it was impossible to test all teas in one session. Accordingly, they were separated into workable batches of 25 samples, each batch including five anchor samples to provide interbatch control of tasters' performance. Because of the interbatch variability of tasters' scores and differences between scoring procedures of individual tasters, some treatment was necessary before data analysis could be performed. This treatment was in three parts:

(i) Batch correction: to achieve consistency between batches for each taster the scores for the standards present in each batch were aligned to each other.
(ii) Taster correction: to bring all scoring methods on to a common scale each taster's score was adjusted to cover a range of 0 to 100.
(iii) Consensus formation: the mean of the tasters' scores was used as quality data for NIRS analysis.

NIRS analysis

NIRS analysis of unground tea was carried out on a Neotec Compscan 7000 system (Pacific Scientific, Marlow, Buckinghamshire).

The granular sample cell was filled with tea and scanned over three-quarters of its length to give a scanned area of 45 cm^2. The movement of the sample cell across the scanning windows allows a better presentation of non-homogeneous and granular material to the instrument. NIR spectra were recorded over a range of 100 to 799 pulse points, in the reflectance mode, in the wavelength range of 2380 to 1380 nm. A computer program was used to randomly select 68 samples to form a calibration set, leaving 66 teas on which the prediction equations could be tested. For all three applications wavelength selection was carried out by a simple stepwise forward regression analysis program with the absorbance data in its first derivative form (segment size 7, gap size 12). Additional terms in the equations were included by linear summation. Software to perform these computational tasks is supplied by Pacific Scientific Ltd.

RESULTS AND DISCUSSION

Moisture prediction

The range of moisture content of teas in the calibration set ranged from 7·9% to 13·1%. Table 2 shows the calibration statistics for up to four additive terms in the equation. These equations were then tested on a separate set of 68 samples with a range of 8·5% to 17·3% moisture. Results are given in Table 3. The one-, two- and three-term equations produced acceptable predictions. The addition of the fourth terms appeared only to over-fit the calibration data, resulting in a slight increase in standard error. From Fig. 1 it can be seen that one point in particular lies well away from the fitted line and accordingly contributes largely to the standard error. The sample responsible for this point was a tea from Taiwan which had very poor colour and quality and contained large quantities of fibrous stalk material which clearly affected its prediction. The overall reliability of the equation is,

TABLE 2
Wavelengths, Constants and Calibration Statistics for Moisture in 66 Samples of Black Tea (range 7·9% to 13·1%)

Wavelengths (nm) (pulse points)				*Constants*				
λ_1	λ_2	λ_3	λ_4	k_0	k_1	k_2	k_3	k_4
2 050 (364)	—	—	—	11·0	146·6	—	—	—
2 050 (364)	1 625 (620)	—	—	7·1	133·4	315·9	—	—
2 050 (364)	1 625 (620)	1 942 (423)	—	7·8	125·1	314·7	40·8	—
2 050 (364)	1 625 (620)	1 942 (423)	2 175 (305)	7·9	120·4	369·2	53·2	−44·1

Number of terms in equation	*Calibration coefficient (r)*	*Standard error of calibration (s)*
1	0·93	0·51
2	0·98	0·43
3	0·96	0·39
4	0·96	0·40

TABLE 3
Prediction Statistics of Moisture in 68 Samples of Black Tea (range 8·5% to 17·3%)

Number of terms in equation	*Linear regression of reported versus calculated*			
	Slope	*Intercept*	*Standard error (SEP)*	*Correlation coefficient (r)*
1	1·11	−1·16	0·51	0·934
2	0·98	0·26	0·43	0·953
3	1·01	−0·11	0·39	0·962
4	1·01	−0·16	0·40	0·960

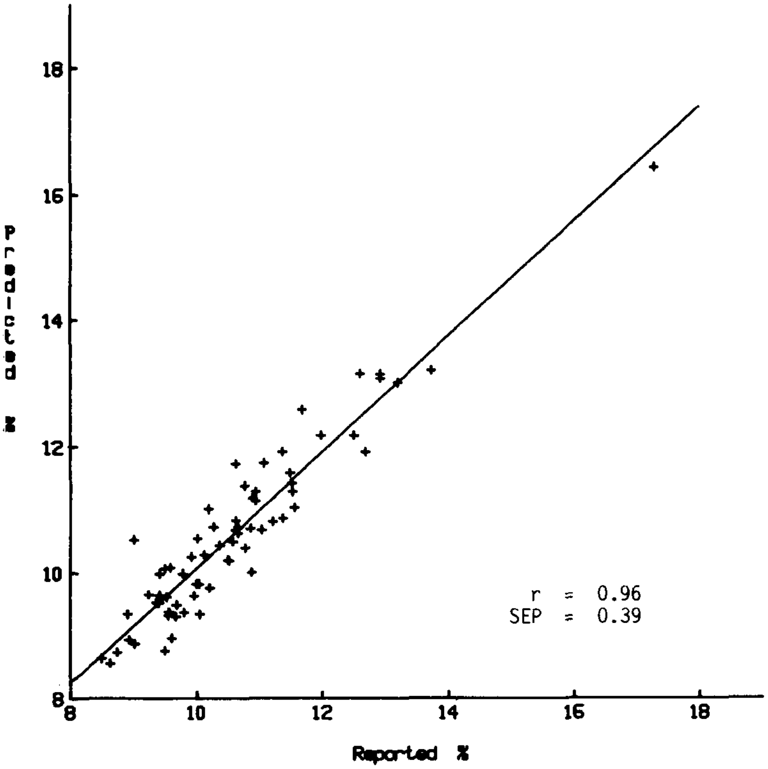


Fig. 1. The prediction by NIRS of moisture in black tea.

TABLE 4

Wavelengths, Constants and Calibration Statistics for Theaflavin in 66 Samples of Black Tea (range 1·5 to 26·5 μmoles/g)

Wavelengths (nm) (pulse points)				*Constants*				
λ_1	λ_2	λ_3	λ_4	k_0	k_1	k_2	k_3	k_4
2 320 (144)	—	—	—	31·6	638·5	—	—	—
2 320 (144)	1 755 (529)	—	—	34·5	451·7	−1 721·6	—	—
2 320 (144)	1 755 (529)	1 515 (708)	—	46·3	265·5	−2 843·4	−478·4	—
2 320 (144)	1 755 (529)	1 515 (708)	1 984 (395)	34·3	284·7	−2 694·7	−722·5	1 049·6

Number of terms in equation	*Calibration coefficient (r)*	*Standard error of calibration (s)*
1	0·73	3·8
2	0·80	3·4
3	0·84	3·1
4	0·86	3·0

TABLE 5

Prediction Statistics for Theaflavin in 68 Samples of Black Tea (range 1·5 to 26·5 μmoles/g)

Number of terms in equation	*Linear regression of reported versus calculated*			
	Slope	*Intercept*	*Standard error (SEP)*	*Correlation coefficient (r)*
1	1·06	−1·32	3·3	0·81
2	1·02	−1·16	3·3	0·80
3	0·97	−0·21	3·0	0·84
4	0·93	0·60	2·8	0·86

however, indicated by the result obtained for the sample containing 17·3% moisture, which was well outside the range of the initial calibration set.

Theaflavin prediction

The calibration was carried out on samples of theaflavin content ranging from 4·8 to 26·3 μmoles/g of dry matter. Table 4 shows the calibration statistics for up to four additive terms in the equations. These equations were evaluated on the test set comprising 68 teas containing from 1·5 to 26·5 μmoles/g theaflavin with the results as shown in Table 5. Prediction using an equation with just two terms gave a standard error of 3·5 μmoles/g and the addition of two more terms reduced this to 2·9 μmoles/g. This level of accuracy compares favourably to that achievable by the analytical procedures currently used, i.e. standard deviation approximately 5·5 μmoles/g (Reeves *et al.*, 1985), and is sufficient for screening purposes. The plot of analytical results against that predicted by NIR is given in Fig. 2.

The primary wavelength selected occurred at 2320 nm (pulse point 144) on

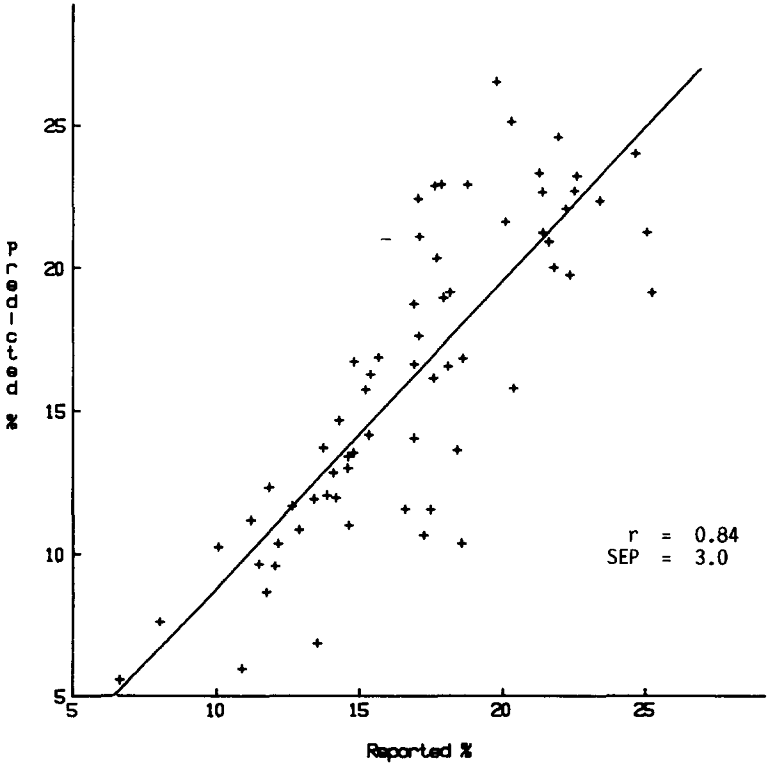


Fig. 2. The prediction by NIRS of theaflavin in black tea.

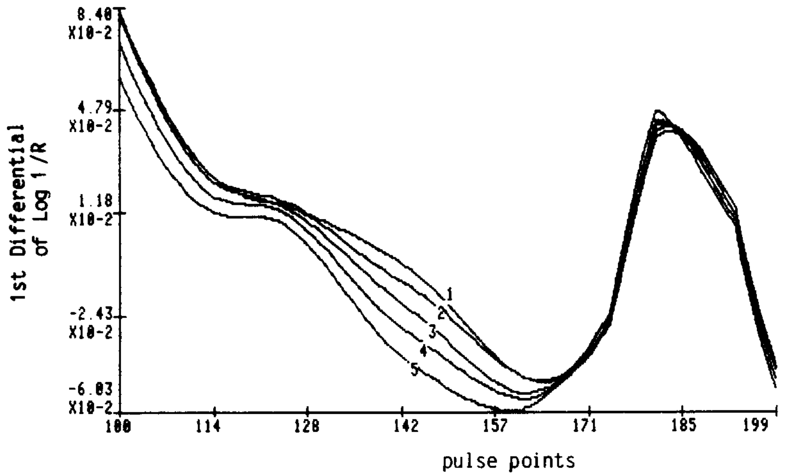
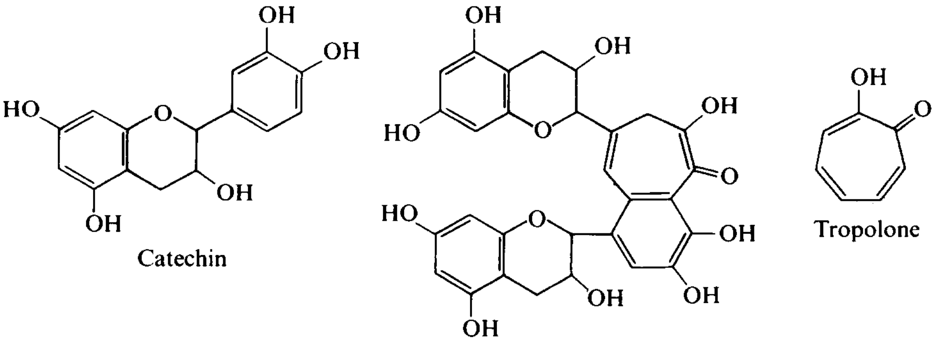


Fig. 3. The first differential of the log 1/*R* spectrum in the 2380 nm (pulse point 100) to 2300 nm (pulse point 199) NIR region for whole leaf black teas with a range of theaflavin values (in μmoles/g theaflavin). Key: 1 = 24·02; 2 = 19·16; 3 = 15·73; 4 = 10·24; 5 = 4·79.

the derivative spectrum, which is in an area corresponding to C—H bond absorptions. Figure 3 shows the prediction of theaflavin around this area based on five teas covering the full range encountered. The general structure of the theaflavins and catechins is shown in Fig. 4 and the numerous C—H bond types can be seen. The structure of the pigmented polyphenolic compounds in tea due to fermentation is based on that of the catechins from which they are derived. The theaflavins, however, are set apart by the inclusion of the benztropolone ring structure. In order to study the assignment of specific wavelengths around the 2300 nm region, samples of (+) catechin and tropolone were scanned and their spectra compared with



Catechin

Theaflavin

Tropolone

Fig. 4. Chemical structures of (+)-catechin, theaflavin and tropolone.

that derived from synthesised epitheaflavin acid and extracted theaflavins. Figure 5 shows the basic similarities between the spectra of tropolone and the theaflavins and the differences with the spectra of catechin. Further information was provided by studies on a thearubigin extract of tea which gave a relatively featureless spectrum in the 100–200 pulse point region. This is probably due to the heterogeneous nature of this group of compounds. From these studies it would appear that an area around 2320 nm is specific to the theaflavins by virtue of their benztropolone grouping.

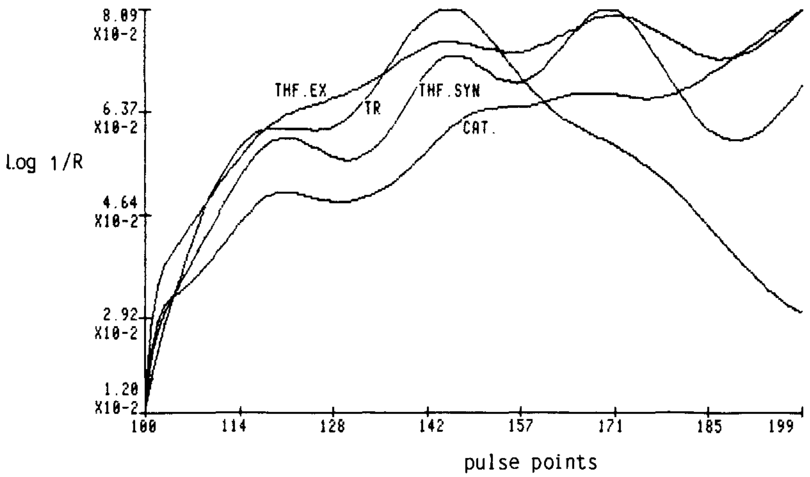


Fig. 5. The log 1/*R* spectrum in the 2380 nm (pulse point 100) to 2300 nm (pulse point 199) NIR region for extracted theaflavin (THF.EX), tropolone (TR), synthesised theaflavin (THF.SYN) and (±)-catechin (CAT).

The use of the first differential transformation of the spectrum makes it difficult to explain why the particular wavelengths were selected. It would appear, however, that the prediction is achieved by use of an area of the log 1/*R* spectrum over the range of 2320 to 2294 nm (Fig. 5). The slope in this region of the log 1/*R* spectrum has a negative component by virtue of the downslope of the 'tropolone' peak and a positive one related to the upside of the C—H absorbance characteristic of catechins, etc. The nature of the prediction in first differential suggests that the positive slope component is the most significant and that the theaflavin content is actually predicted by the first term which is probably determining total polyphenolics in the tea (Fig. 6). Subsequent terms in the equation probably make the prediction more specific to the theaflavin. It is interesting to note that the tropolone peak was not taken specifically in the prediction and this may be due to large absorbances of other tea components in this area. Work is continuing to further elucidate the origin of the absorbances in this case.

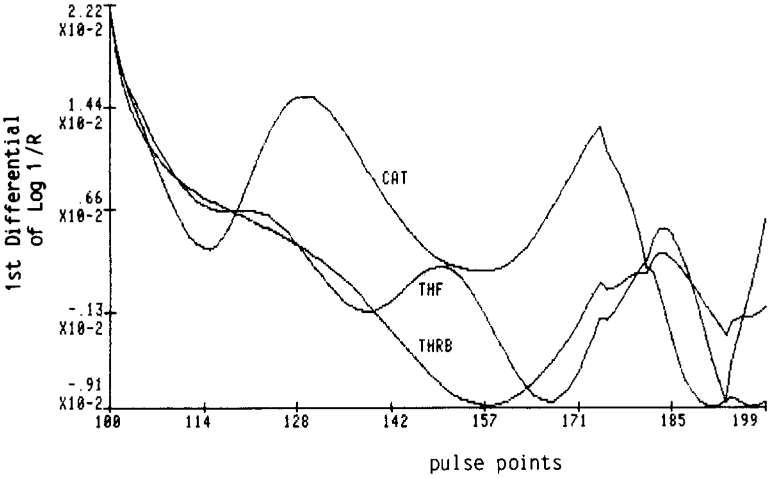


Fig. 6. The first differential of the log 1/*R* spectrum in the 2380 nm (pulse point 100) to 2308 nm (pulse point 200) NIR region for commercial (+)-catechin (CAT) and theaflavin (THF), and thearubigin (THR) extracted from black tea.

TABLE 6

Wavelengths, Constants and Calibration Statistics for Quality in 64 Samples of Black Tea (range 24·8 to 65·2)

Wavelengths (nm) (pulse points)				*Constants*				
λ_1	λ_2	λ_3	λ_4	k_0	k_1	k_2	k_3	k_4
2 130 (326)	—	—	—	−4·4	−1 496·1	—	—	—
2 130 (326)	2 306 (201)	—	—	76·0	−1 275·1	1 381·4	—	—
2 130 (326)	2 306 (201)	1 822 (485)	—	71·9	−1 306·6	1 072·8	1 078·2	—
2 130 (326)	2 306 (201)	1 822 (485)	1 768 (522)	46·8	−1 541·8	498·5	−1 172·4	−1 287·9

Number of terms in equation	*Calibration coefficient* (*r*)	*Standard error of calibration* (*s*)
1	−0·73	7·8
2	0·80	6·9
3	0·81	6·8
4	0·82	6·8

Quality prediction

The overall quality scores ranged from 24·8 to 72·7 in the calibration set. Table 6 shows the statistics obtained using up to four additive terms in the calibration equations. Prediction using the two-term equation gave a standard error of 7·8 (Table 7) and produced the plot as shown in Fig. 7. Although the standard error appears large it is, in fact, better than that obtained by any individual taster in the prediction of the consensus opinion of quality on the same teas. Some tasters predicted the consensus more closely than others, but in general the standard error was about 9. Using the theaflavin content as a predictor gave a standard error of approximately 10.

TABLE 7
Prediction Statistics for Quality in 68 Samples of Black Tea (range 24·8 to 74·1)

Number of terms in equation	*Linear regression of reported versus calculated*			
	Slope	*Intercept*	*Standard error (SEP)*	*Correlation coefficient (r)*
1	0·84	8·8	8·9	0·69
2	0·89	4·3	7·8	0·77
3	0·88	5·1	8·3	0·73
4	0·88	5·1	8·1	0·75

The use of NIRS, therefore, is at least as good as the subjective methods presently available for the screening of quality of black teas used for the UK market. It must be noted, however, that the quality of a tea, although influenced by the many factors previously mentioned, is governed as far as the individual taster is concerned by the specific requirements that the tea has to meet. For example, a tea may have a poor flavour but still be valued highly for its colour contribution to a blend; conversely, a poorly coloured tea may have excellent flavour. Accordingly, the specific quality assigned by an individual taster is normally affected by the buying policies of his company. In this study, tasters were asked to base their judgement on the teas and not to take into account the specific use to which they might normally be put. The data produced, therefore, were, as far as possible, an expression of the absolute quality of the individual tea samples.

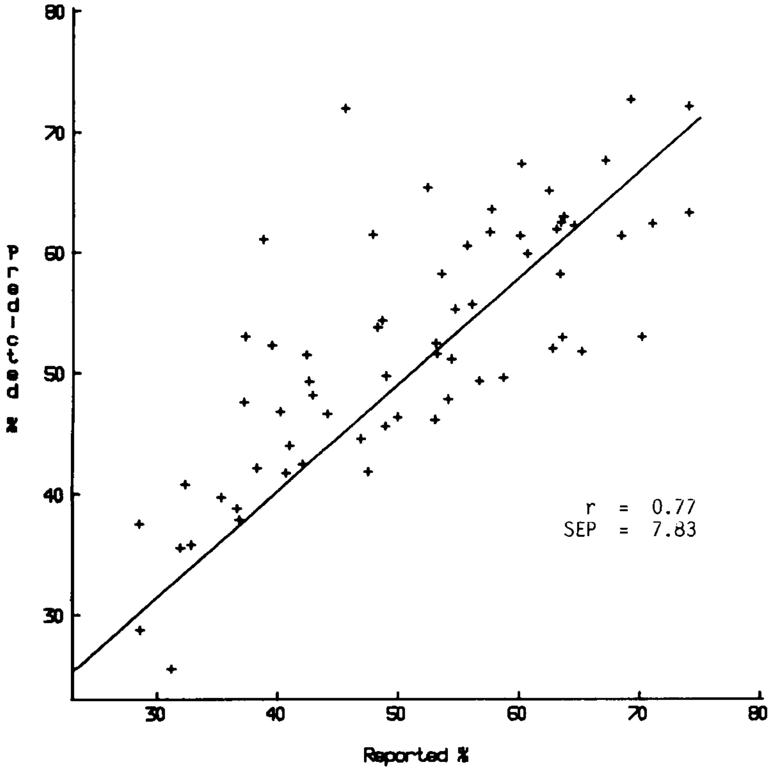


Fig. 7. The prediction by NIRS of quality in black tea.

CONCLUSIONS

Near-infrared spectroscopy provides a rapid method for the simultaneous estimation of the moisture content, theaflavin content and overall quality of black tea.

The ability to rapidly monitor these parameters in tea provides a further means of final product quality control at either the buying, importation or blending stages. Furthermore, the technique may well be appropriate for the continuous monitoring of the tea manufacturing process, thus enabling better control of the withering, fermentation and drying stages.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the assistance with funding from the Ministry of Agriculture, Fisheries and Food (MAFF), Food Science Division, and the following companies: Lyons Tetley, James Finlay, Liptons, Cadbury Typhoo and the Co-operative Wholesale Society, who also provided samples and the services of their tasters. The results of this work are the property of MAFF.

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