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Near infrared (NIR) technology and multivariate data analysis for sensing taste attributes of apples

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A b s t r a c t. This paper concerns the feasibility of using near infrared technology and multivariate data analysis for detecting taste attributes of fruits. The aim is to introduce a non-destructive system related to development of taste components for detecting the characteristic taste parameters in different commodities. The method will have potentials for online fruit grading in warehouses and public fruit markets. A NIR spectrometer, with Photo Diode Array detector, was used to predict some of the internal quality parameters *ie* Soluble Solid Contents (SSC) and acidity; and to sense characteristic taste parameters of two different apple varieties (Aroma and Elstar). Non-destructive prediction and classification was based on optical reflectance in NIR range (700-1100 nm). A reasonable correlation between NIR and some quality parameters was achieved. Samples having the same ratio of SSC and acidity were correctly classified in 100 and 85% of the cases, respectively, at 10% significance level. Successful classification indicates that the NIR technique has a high potential for detecting taste characteristics of apples.

K e y w o r d s: NIR technique, taste, apples, multivariate data analysis

INTRODUCTION

In recent years, research has been focused on the development of non-destructive techniques for measuring quality parameters of different agricultural commodities. The aim is to develop new techniques which provide fast execution, can easily be used in process control and grading system, and require limited sample pre-processing (Lamertyn *et al.*, 2000). NIR spectroscopy is one of these non-destructive techniques. NIR methods have already been used to detect bruises on apples (Upchurch *et al.*, 1994) and to study dry matter content in onions (Birth *et al.*, 1985) and potatoes (Dull *et al.*, 1989). Abu-Khalaf and Bennedsen (2002) managed to predict plum Soluble Solid Content (SSC) using NIR reflectance. Kawano *et al.* (1992) used an optical fibre with interactance mode to study the sugar content in peaches. Slaughter (1995) managed to measure non-destructively the internal quality of peaches and nectarines as characterised by SSC, sorbitol and chlorophyll contents using visible and NIR spectroscopy. Bellon *et al.* (1993) used the wavelength region between 800 and 1050 nm and developed a NIR instrument coupled with optical fibres to detect sugar content at the speed of three apples per second with a standard error of prediction of 2.4 g l^{-1} of glucose. A relationship was established by Lammertyn *et al.* (1998), Moons *et al.* (2000) and Ozanich (1999) between NIR spectra and apple fruit quality parameters, such as pH, acidity, sugar content and texture parameters.

According to Ozanich (1999), NIR spectroscopy is uniquely qualified for analysis in food and related industries. NIR spectroscopy is particularly sensitive to molecules containing C-H, O-H, and N-H groups. These bonds interact in a measurable way in the NIR portion of the spectrum. Hence, constituents such as starch and sugars, alcohols, moisture and acids, and protein can be quantified in solids, liquids and slurries. In addition, the analysis of gases is possible. NIR is not a trace analysis technique and is generally used for measuring components that are present at concentrations greater than 0.1% (Ozanich, 1999). Kim *et al.* (2000) stated that 'spectral data of a fruit can be treated as

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a signature allowing fruits to be grouped on the basis of their similarities'. The use of NIR technology provides for a faster, safer work environment and does not require chemicals (Ozanich, 1999).

Previous researches reported use of different wavebands. In this research, wavelengths between 700 and 1100 nm were used. NIR in this range is promising and more useful for intact foods due to the following (Carlini, *et al*., 2000; McGlone and Kawano, 1998; Walsh *et al*., 2000):

1. Penetration of the radiation into the fruits was found to be deeper than for other wavelength ranges.

2. Water absorbance peaks are less strong and broad than they are at other ranges, and the risk of masking spectral information correlated to low concentration constituents is low.

3. The instrumentation that can be used at this range is low cost, suitable for process control and is portable enough for in situ field measurements.

4. The bands are ascribed to the third and forth overtones of O-H and C-H stretching modes and are expected to be separated due to anharmonicity.

5. Lower absorbance at these wavelengths allows for transmission optics.

Moreover, there is strong evidence that the range from 700 to 900 nm constitutes a 'diagnostic window' in which chemical compositions of samples can be investigated (Osborne *et al*., 1993).

Optical measurements can be done in different modes: transmittance, absorbance and reflectance. Chen (1978) stated that reflectance is generally easier to use for quality evaluation of agricultural products due to:

1. Its relatively high intensity: reflectance in the visible and infrared regions ranges up to 80% of the incident energy.

2. Reflectance measurement is not adversely affected by low-intensity background light.

Taste is an important internal quality parameter of a commodity. Fruit taste, as the major asset of fruit quality, is dominated by the sugar to acid ratio (Blanke, 1996). Apart from the sweet and sour taste, provided by sugar and acid, fruits have a distinctive taste which originates from their content of other chemicals. This taste is characteristic for the variety. Auerswald *et al*. (1999) quoted a number of authors indicating that the overall flavour of fruits is a combination of sugars, acids, aroma volatiles, amino acids and amines. This research aims at measuring these taste parameters, using NIR technology. The idea is that, if it is possible to distinguish two different varieties of the same commodity, having the same ratio of SSC and titratable acidity (this ratio dominates the fruit taste) (Blanke, 1996) then both components (sugar and acid) are eliminated, leaving only the characteristics taste (specific chemical compounds) parameters of each variety to be detected, which indicates that NIR technology can sense taste characteristics parameters of each variety. The ability of NIR spectroscopy to detect these parameters will open a possibility for applying NIR sensors for online grading of fruits according to their taste quality.

MATERIALS AND METHODS

Fruits

Two separate experiments on apples (*Malus domestica*) were carried out. The varieties Golden Delicious, Jonagold, Aroma and Elstar were used. These four apple varieties are among the most popular apples in Denmark.

- In the first experiment: One hundred apples of each Golden Delicious and Jonagold were used to predict SSC, acidity and firmness; classification of both varieties was carried out.
- In the second experiment: One hundred apples of each Aroma and Elstar were used to predict SSC and acidity; classification of samples having the same SSC/acidity values was carried out.

Apples were obtained from The Royal Veterinary and Agricultural University's (KVL) orchard research farm in September. After being in cold storage, apples were kept at room temperature (20°C) for 24 h for equilibration of a uniform surface temperature before the experiment.

Reflectance measurements

A Zeiss MMS1 (a fixed Si photo diode array) NIR enhanced spectrometer was used to collect reflectance readings over a wavelength range of 700-1100 nm in 2 nm increments, yielding 200 values per spectrum. For each variety (100 samples), the reflectance measurements were done in one day. For each apple, three reflection spectra were taken at three equidistant positions around the equator, in order to eliminate the spatial variability. The light source consisted of a 12V/100W tungsten halogen lamp. The lamp was calibrated before and after the reflectance measurement of each variety, using a standard reflectance plate made of barium sulphate ($BaSO_4$). Light from the lamp passed through a bundle of optical fibres to the fruit, and reflected light was transferred to a Photo Diode Array (PDA) detector through another bundle of optical fibres. A holder was designed to support apples and to direct the light, at a 45-degree angle, to the apples, to avoid specular reflectance and to maintain a distance of 1 cm, according to manu- facturer's recommendation, between the probe and the apples (Fig. 1). The integration time (time needed for a spectrum to be acquired) was 161 ms. The three spectra from each apple were averaged for further processing using multivariate analysis.

Taste attributes

After reflectance measurements, SSC and titratable acidity were measured on juice extracted from each apple.

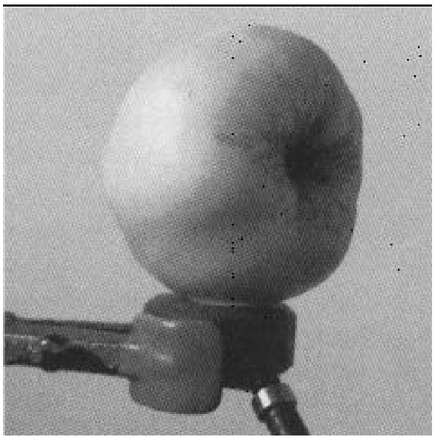

Fig. 1. The set up of the NIR probe, holder and apple. The holder directed the light at a 45° angle to the apples, and kept a constant distance of 1 cm between the probe and the apple.

A digital refractometer (RFM 90-Struers) was used to measure SSC. The SSC was expressed in Brix. Titratable acidity (citric acid) was measured, using a 719 S Titrino, by titrating the juice with 0.1 M NaOH to a pH 8.1 endpoint.

Firmness measurement was used in the first experiment on Golden Delicious and Jonagold apples. A Bosch penetrometer (model FT 327) was used to measure the firmness. Two measurements were taken on each fruit at opposite sides, at the middle point of each side, after removing 0.7-1.1 cm diameter disk of peel. The firmness was expressed in kg cm^{-2}.

Multivariate data analysis

The calculations were carried out using 'Unscrambler' v. 7.5 (Camo, ASA, Oslo, Norway), a statistical software package for multivariate data analysis. Matlab R.12 (The Math Works Inc., Natick, MA) was used as a bridge program between the spectrometer outputs and Unscrambler, to transfer the reflectance data to be analyzed.

Multivariate data analysis offers various methods for efficient simplification and interpretation of many different variables simultaneously. The methods reveal the main structures and relationships in large data tables, giving relatively simple output graphs and tables that have maximum information and minimal repetition and noise (Resurreccion and Shewfelt, 1985). Principal Component Analysis (PCA) is one of the multivariate techniques. PCA is a method that can be used to identify patterns in a data set derived from recording several characteristics at a time to eliminate redundancy in univariate analyses (Iezzoni and Pritts, 1991). The PCA is explained by Principal Components (PCs), which are composite variables, since they are linear functions of the original variables, estimated to contain the main structured information in the data. PCs are also called latent variables, and a PC is the same as a score vector (Esbensen *et al*., 2000).

NIR spectra, in general, can be used for modelling and/or classification with its raw or transformed (pre-processed) data. This transformation depends on many factors, mainly aiming at yielding: the lowest Root Mean Square Error of Prediction (RMSEP), the highest validation correlation (r^2), the lowest number of PCs, and the lower difference between the RMSEP and the Root Mean Square Error of Calibration (RMSEC) (Lammertyn *et al*., 2000). Obtaining the highest classification rate is the most important factor for classification process.

The transformation techniques for NIR raw data are: Multiplicative Scatter Correction (MSC), first derivative and second derivative techniques. MSC technique corrects the additive and multiplicative effects in the spectra. The general rule of thumb is that the first derivatives are useful in removing baseline offsets and that the second derivative corrects for baseline offsets and sloping baselines (Mobley *et al*., 1996).

Partial Least Square (PLS) technique was used for modelling apple quality parameters. PLS-1 (which uses just one Y-variable *eg* SSC or acidity) and test set validation were used for modelling.

Soft Independent Modelling of Class Analogies (SIMCA) classification technique and full cross validation were used for classification of apples according to their reflectance spectra. In SIMCA classification technique, the classification succeeds when there are very clearly distinguished groups of the targeted classes in the different SIMCA's pertinent graphs. Samples are considered wrongly classified when they are not belonging to any of the models of the targeted classes (Esbensen *et al*., 2000). SIMCA's classification tables (not shown in the results) were used for expressing classification percentage rate of different apple varieties.

RESULTS AND DISCUSSION

The stability of the light source in the spectrometer was quite acceptable, with a variation of less than 5% during the reflectance measurements.

Golden Delicious and Jonagold experiment

The chemical and physical reference values of 200 apples are tabulated in Table 1. The samples of both varieties were homogenous in terms of SSC, acidity and firmness, since the CV was small and about the same in both varieties. The SSC, acidity and firmness of each variety were significantly different between both varieties, at 5% significance level. The correlations between the three quality parameters were not high (<60%). The highest correlation was between SSC and acidity, with a correlation coefficient of 66.7%.

T a b l e 1. Chemical and physical reference values of Golden Delicious and Jonagold apples

Variety	Golden Delicious				Jonagold			
Quality parameter	Mean	SDev*	CV* (%)	Range	Mean	SDev*	CV* (%)	Range
SSC (Brix)	12.13	0.90	7	9.20-13.90	13.60	0.72	5	12.12-15.65
Acidity (g/100 g)	0.57	0.07	12	0.41-0.78	0.83	0.08	9	0.67-1.08
Firmness (kg cm^{-2})	9.52	0.76	7	8.00-12.05	10.44	0.85	8	8.80-12.70

*SDev: Standard Deviation *CV: Coefficient of Variation = SDev / Mean 100.

For modelling (predicting) quality parameters by NIR reflectance, PLS-1 was used. For calibration, 130 samples were used. For validation, 70 samples were used. It was noticed that full MSC pre-processing technique yielded the best model, since it mainly yielded the lowest RMSEP. The result of modelling quality parameters by NIR is tabulated in Table 2. The predicted versus the measured SSC values is shown in Fig. 2.

The prediction of apple SSC, which has a correlation of 67.6%, six latent variables, 9.2% relative error (relative error = SEP / range) and 11% bias, was fairly acceptable. Bellon *et al*. (1993) built a SSC model with five latent variables between predicted and measured SSC values of 85%. Lammertyn *et al*. (2000) obtained correlation coefficients for SSC predictions between 80-90%, depending on the number of PCs and the pre-processing techniques. Moons *et al*. (2000) got a correlation greater than 80% for SSC prediction.

Using 130 apples for calibration is reasonable, since the maximum number of latent variables, used to construct the

T a b l e 2. Calibration results (130 samples for calibration and 70 samples for validation) of quality parameters for Jonagold and Golden Delicious apples

Quality parameter	RMSEP	r^2 (%)	PCs	RMSEC	Bias	SEP*
SSC	0.60	67.6	6	0.55	0.11	0.58
Acidity	0.08	71.1	4	0.07	-0.01	0.08
Firmness	0.78	<50	6	0.71	-0.05	0.78

*SEP: Standard error of prediction. $RMSEP^2 = SEP^2 + Bias^2$.

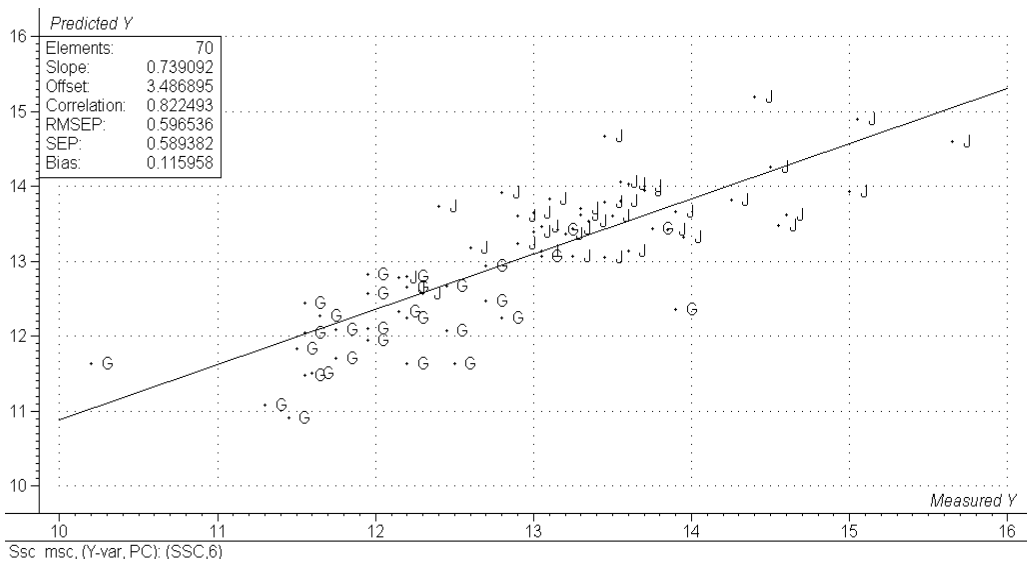

Fig. 2. The PLS model for SSC of Golden Delicious (G) and Jonagold (J) varieties.

calibration model, was six. This is in agreement with a statistical rule of thumb which states that the ratio of the number of samples to the numbers of variables should be equal to or larger than ten (Lammertyn *et al*., 2000). There are still about twenty times more samples than latent variables (principal components) in our model.

The most overall important ranges for modelling apples' SSC were: 1058-1066 and 1090-1100 nm, since these ranges have the highest regression coefficients (Fig. 3). The waveband between 940 and 970 nm is most likely related to water content.

Acidity modelling, with correlation of 71.1%, four PCs, 11.3% relative error and 1% bias, was also fairly acceptable. compared to any pre-processing techniques that the data was subjected to. The training set contained 70 samples and the testing set contained 30 samples for each variety. Full cross validation was used for building the PCs in SIMCA models for each variety. The classification percentage result depended on SIMCA's classification table and illustrations. Golden Delicious and Jonagold were correctly classified in 90 and 83.4% of the cases respectively at 5% significance level (Table 3). The overlapping is normal and expected, taking into consideration the inherent relationship between both varieties. Jonagold is a hybrid of Golden Delicious and Jonathan. These classification results took all taste attributes (SSC, acidity and characteristic taste parameters) into consideration, since we did not exclude any parameter when we classified the apples according to their spectra. This indicates that NIR technique has a high potential for detecting different tastes of fruits (taste is characteristic for the variety). It was found that all the NIR spectra range (700-1100 nm) had a high discrimination power. Unfortunately, there were not enough samples having the same SSC/acidity values to run classification on them.

Fig. 3. The regression coefficients of the wavelengths variables used for Golden Delicious and Jonagold apples' SSC modelling.

T a b l e 3. Classification results of Golden Delicious and Jonagold varieties at 5% significance level

Variety	Golden Delicious	Jonagold	Overlaping
		(%)	
Golden Delicious	90.0	0	10.0
Jonagold	3.3	83.4	13.3

No reliable relationship was found between firmness and NIR spectra. Kupferman (1997) stated that fruit firmness is generally very difficult to model, since many factors are involved. Theoretically, it is possible that scattering of light within the fruit may be related to firmness.

Classification based on the raw data of NIR reflectance of both varieties was carried out using SIMCA. The highest classification rate was obtained using the raw data,

T a b l e 4. Chemical reference values of Aroma and Elstar apples

	SSC (Brix)				Acidity (g/100 g)			
Variety	Mean	SDev	CV (%)	Range	Mean	SDev	CV (%)	Range
Elstar	12.21	0.61	5	13.9-10.8	0.89	0.08	9	0.72-1.09
Aroma	12.16	0.61	5	14-10.7	0.90	0.06	7	0.76-1.06

Aroma and Elstar experiment

The chemical reference values of 200 apples of both varieties are shown in Table 4. The samples of both varieties were homogenous in terms of SSC and acidity, since CV was small and about the same in both varieties. The SSC and acidity were insignificantly different between both varieties at 5% significance level. The correlation coefficient between SSC and acidity was very low (<50%). PLS-1 was used for modelling SSC and acidity using raw NIR reflectance for both varieties. For calibration, 140 samples were used. For validation, 60 samples were used. The modelling for SSC and acidity was not reliable.

It was noticed that some of the Aroma samples were overripe, which may cause failure of the SSC and acidity models. Over ripening of Aroma can be explained by the fact that Aroma is an earlier variety, while Elstar is picked later. Figure 4 shows the mean raw NIR reflectance spectra of Elstar and Aroma varieties. The reflectance spectrum of Aroma variety is higher than Elstar variety, which indicates that NIR can elucidate a significant difference between the samples investigated.

In the reflectance spectra scores plot of the second-derivative pre-processed data, groups (varieties) distinguished along the first PC, which explains 50% of the variation between samples, are quite clear (Fig. 5).

For the classification process, the second-derivative pre-processed data was used. Considering all apple samples, a training set of 70 samples and a testing set of 30 samples for each variety were used. Full cross validation was used for building the PCs in SIMCA models for each variety. The classification results are shown in Table 5. The Coomans plot (objects to model distance), (Esbensen *et al*., 2000) for all samples of Aroma and Elstar apples at 10% significant level, is shown in Fig. 6. It is clearly seen that SIMCA was able to classify Elstar and Aroma with a high performance at different significance levels. Clearly distinguished groups can be seen in the Coomans plot (Fig. 6). Aroma and Elstar were correctly classified in 94.6 and 81.1% of the cases, respectively, at 5% significance level. A relatively slight overlapping occurred, which is a normal phenomenon.

Searching for samples having the same SSC/acidity values, within 5%, 123 apples from both varieties were

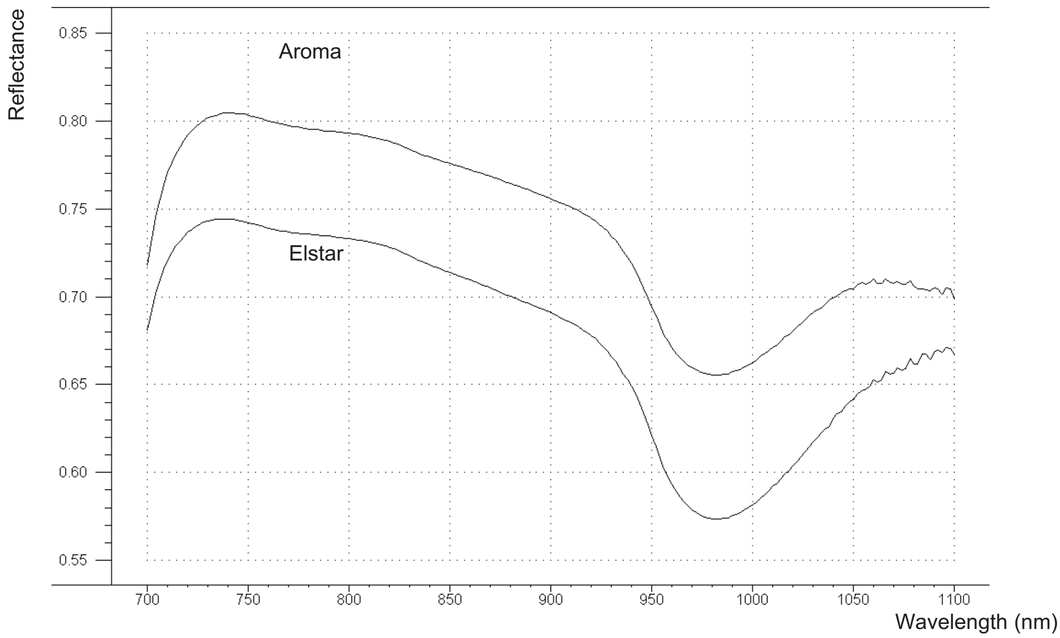

Fig. 4. Mean NIR reflectance spectra (raw data) of Aroma and Elstar varieties.

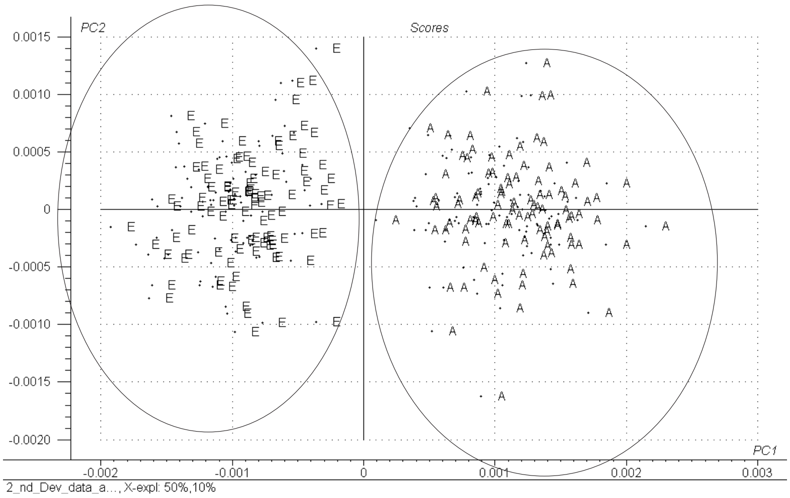

Fig. 5. Reflectance spectra scores plot of all Elstar (E) and Aroma (A) samples.

T a b l e 5. Classification results for all samples of Aroma and Elstar at different significance levels

Significance level (%)	Variety	Aroma	Elstar	Overlaping
			(%)	
5	Aroma	94.6	0	5.4
	Elstar	0	81.1	18.9
10	Aroma	100	0	0
	Elstar	0	97.3	2.7

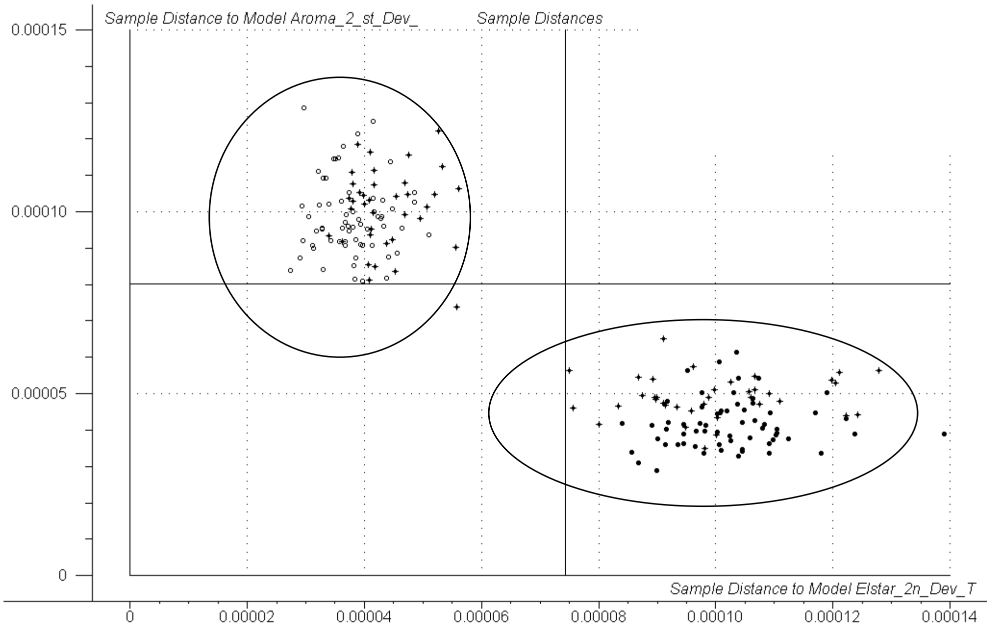

Fig. 6. Coomans plot for all Aroma and Elstar samples at 10% significance level.

selected. For classification of these samples, 40 and 20 samples were used for the training set and for the testing set, respectively, for each variety. Table 6 shows the results of SIMCA classification at 10% significance level. Figure 7 shows the Coomans plot for all Aroma and Elstar apple samples that have the same ratio of SSC and acidity. It can be noticed that classification result was reasonably good for samples having the same SSC/acidity values. Aroma and Elstar were correctly classified in 100 and 85% cases, respectively, at 10% significance level. The overlapping indicates that some of the characteristic taste parameters between the two varieties are common. The wavelengths with the highest modelling power, in classification models of both varieties, were: 700-750 and 1050-1100 nm. The discrimination power wavelengths between these varieties were: 730-734, 742-748, 768-790, 814, 822-826, 840, 860-862, 866-874, 922-924, 1036-1038 and 1076 nm. These different wavelengths should be investigated more, in order to find if they are related to the chemical composition (characteristic taste parameters) causing this discrimination between the samples having the same ratio of SSC and acidity, since in this classification, the SSC and acidity were kept out (by taking samples having the same values of SSC/acidity) and the classification was based only on the other characteristic taste parameters.

T a b l e 6. SIMCA's classification results of all Aroma and Elstar samples having the same SSC/acidity values at 10% significance level

Variety	Aroma	Elstar	Overlaping
		(%)	
Aroma	100	0	0
Elstar	0	85	15

From these experiments, and keeping in mind that the same conditions (light stability, temperature, *etc*.) were equal as much as possible during the measurements, and taking into consideration that the SSC/acidity dominates the fruit's taste (Blanke, 1996) and that the fruit taste is characteristic for the variety, it can be noticed that:

- In the first experiment: NIR managed to classify Golden Delicious and Jonagold apples, in view of all the taste attributes (SSC, acidity and characteristics taste parameters), which indicates that NIR could sense the overall taste of apples.

- In the second experiment: NIR still managed to distinguish between the two varieties of apples, and also between samples having the same SSC/acidity values, which indicates that NIR could sense the characteristics taste parameters, since SSC and acidity were not considered in the classification process.

The high rate of classification in the first experiment was not due to significant differences between SSC and acidity, since in the second experiment, the SSC and acidity were not significantly different and still the classification had a high rate.

The reason for this successful classification is suggested to be that NIR range in 700-900 nm forms a 'diagnostic window' in which chemical compositions of samples can be investigated (Osborne *et al*., 1993), and also spectral data of a commodity can be treated as a signature, allowing commodities to be grouped on the basis of their similarities (Kim *et al*., 2000).

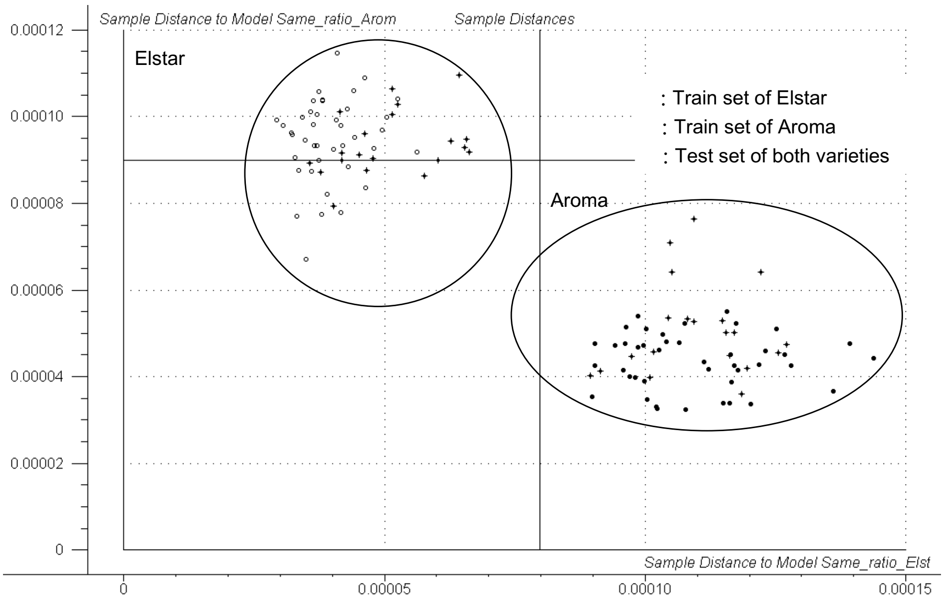

Fig. 7. Coomans plot for all Aroma and Elstar samples having the same ratio of SSC and acidity at 10% significance level.

CONCLUSIONS

1. NIR is able to predict some quality parameters; like SSC and acidity, when using very good spectrometer stability versus time.

2. NIR spectroscopy was able to classify the different varieties of apples with a reasonably high performance (>81%), and since the taste is characteristic for the variety, this indicates that NIR has a potential to sense the overall taste characteristics of fruit.

3. NIR spectroscopy was also able to detect different varieties of apples even when they had the same ratio of SSC and acidity, taking into consideration that the SSC/acidity dominates the fruit taste.

4. This classification indicates that NIR may have a potential to detect the components of the characteristic taste parameters of each variety, since SSC and acidity parameters were not taken into consideration in the classification process.

5. Further research is needed to build more robust quality parameter models, and to study the wavelengths that contribute to the discrimination between samples of two apple varieties having the same ratio of SSC and acidity.

6. NIR technique has a high potential for sensing the overall taste of apples.

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