

Distinguishing Carrot's Characteristics by Near Infrared (NIR) Reflectance and Multivariate Data Analysis

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Abstract

This study presents an attempt to predict the sensory quality of carrots using Near Infrared (NIR) technology and multivariate data analysis (e.g. PCA, PLS-DA) to analyse some of factors modulating carrot sensory quality using classification system. The aim is to introduce a technology to develop a sensor that can non-destructively predict the sensory quality of different commodities. Such sensors could be used online in warehouses and public fruit markets. A NIR spectrometer with Photo Diode Array (PDA) detector, was able to classify different carrot samples according to their cultivar and production system (organic, conventional) with a high accuracy. Non-destructive classification and modelling was based on optical reflectance in NIR range (700-1100 nm). Different cultivars and different production system samples were correctly classified in rates of > 64% and > 82% respectively. The ability of NIR to classify samples with different cultivars and production systems indicates that NIR is able to predict the sensory quality of carrots, which are mainly dependant on cultivar, production system and year.

Keywords: NIR, carrots, production system, cultivar, Principal Component Analysis (PCA), Partial Least Square - Discrimination Analysis (PLS-DA).

Introduction

Non-destructive techniques for measuring quality parameters of different agricultural commodities are gaining attention. These techniques, e.g. X-ray, sonic and ultrasonic, nuclear magnetic resonance (NMR) and visible and near infrared (NIR) light, are based on the measurement of optical, chemical and / or physical properties of the product, which can be correlated with the quality characteristics of the targeted crop. These techniques have been evaluated for the assessment of the internal and / or external

quality traits of intact fruits and vegetables (Peiris et al., 1999). The advantages of these techniques include fast execution, ease of use in process control and grading systems and require limited sample pre-processing (Lammertyn et al., 2000).

NIR spectroscopy is one of these non-destructive techniques. It gained widespread acceptance for analysing agricultural products since its development by Norris and Hart in 1965 (Parnell and White, 1983). 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 with the NIR range of the spectrum. Hence, constituents such as starch and sugars (C-H), alcohols, moisture and acids (O-H), and protein (N-H) 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 %. The use of NIR technology provides for a faster and safer work environment and does not require chemicals.

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). Slaughter (1995) managed to measure non-destructively the internal quality of peaches and nectarines as characterised by their Soluble Solid Content (SSC), sorbitol and chlorophyll contents using visible and NIR spectroscopy. Abu-Khalaf and Bennedsen (2002) managed to predict plums' SSC using NIR reflectance. Kawano et al. (1992) used an optical fibre with interactance mode to study the sugar content in peaches.

Bellon et al. (1993) used the wavelength region between 800-1050 nm and developed a NIR instrument coupled with optical fibres to detect sugar at the speed of three apples per second with a standard error of prediction of 2.4 g/l of glucose. A relationship between NIR spectra and apple fruit quality parameters, such as pH, acidity, sugar content and texture parameters, was established by Lammertyn et al. (1998) and Moons et al. (2000). Hirano et al. (1998) managed to detect internal molds in nuts using NIR spectroscopy. Moreover, NIR has been used in the analysis of dairy foods (Rodriguez-Otero et al., 1997).

Optical measurements can be made 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 relative high intensity: Reflectance in the visible and infrared regions ranges up to 80 % of the incident energy, and
2. Reflectance measurement is not adversely affected by low-intensity background light.

Previous researches reported use of different wavebands. In this research, wavelengths between 700-1100 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 targeted crops was found to be deeper than for other ranges
2. Water absorbance peaks are less strong and broad than they are at other ranges, and the risk to mask spectral information correlated to low concentration constituents is low
3. The cost of instrumentation for this range is relatively low, it is portable and suitable for process control and for in situ field measurements
4. The bands are ascribed to the third and fourth overtones of O-H and C-H stretching modes and are expected to be separated due to anharmonicity, and
5. Lower absorbance at these wavelengths allows transmission optics.

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

Consumers are becoming increasingly concerned about the quality of agricultural commodities, especially regarding how, when and where the foods are produced. Information on origin and labelling such as 'organically grown' led to an increase in product preference (Alvensleben and Meier, 1990). One of the main differences between conventional and organically growing systems is the use of artificial fertilizer. Conventional growing is characterized by the use of easily soluble compounds of nutrient chemicals, whereas in organically growing, nutrient is supplied in a slowly soluble form from naturally occurring source (Oelhaf, 1978).

Sensory quality prediction using NIR spectroscopy was investigated on peas (Marten and Martens, 1986), meat sausages (Ellekjaer et al., 1994) and black and green tea (Yan et al., 1990).

Carrot (*Daucus carota*) is one of the most commonly used vegetables for human nutrition. It is regarded as a desirable healthy food because of its high vitamin and fibre content (Negi and Roy, 2000). NIR spectroscopy has been successfully used to determine the total carotenoids and sugars in different carrot varieties (Schulz et al., 1998). Haglund et al. (1999) stated that the year, growing system and cultivar had an impact on carrots' sensory quality, and as taste is an important internal quality parameter, they found that the conventional growing system produced carrots with high carrot-taste, while the organic growing system produced carrots with high bitter-taste.

This research aims to extend the knowledge of using NIR for sensory quality. The idea is that, if NIR reflectance is able to classify carrots according to their production systems and cultivars, this will give an indication that NIR has a potential to sense the

carrots sensory quality, which mainly depend on production system and cultivar (Haglund et al., 1999). In this research, this investigation was done by:

1. Examining the possibility of NIR reflectance to classify carrot samples according to their production systems (conventional / organic), and
2. Examining the possibility of NIR reflectance to classify different cultivars of carrots.

Materials and methods

Carrot samples

In this research, three experiments have been carried out in a sequential order (Table 1):

1. The first experiment was basically a feasibility study. The goal was to screen whether the NIR spectroscopy is able to distinguish between different samples of carrots, and to identify the most important position along the carrot (tip, mid-section or crown) where spectral information can be measured. For this experiment, two samples of carrots were purchased from a local supermarket. After being in refrigerator, carrots were kept at room temperature (20 °C) for 24 hours for equilibration before the experiment. Apart from the fact, that one sample was organically produced, and the other conventionally grown, little information was available about the samples.
2. For the second experiment, three cultivars were used, 160 samples from each cultivar, giving a total of 480 carrot samples. Half of the sample in each cultivar was organic and the other half was conventional. For each day of experiments, just 160 samples, 80 samples organic / conventional of one cultivar, and 80 samples organic / conventional of the other cultivar, were measured by NIR spectrometer. These cultivars were:
 - 1) Cultivar 1: Bolero F1 (maintainer is Vilmorin). It is a Nantes type
 - 2) Cultivar 2: Rodelika OP (open pollinated) (maintainer is Allerleirauh). It is not a Nantes type. It is grown from organic seed, and
 - 3) Cultivar 3: Nerac F1 (maintainer is Bejo Zaden). It is a Nantes type.
3. The third experiment involved two cultivars, Bolero F1 and Rodelika OP. There were 160 samples from each cultivar, giving a total of 320 carrot samples. Half of samples in each cultivar were organic and the other half was conventional. For each day of experiment, just 160 samples, 80 samples organic / conventional of one cultivar, and 80 samples organic / conventional of the other cultivar, were measured

by NIR spectrometer. In addition to these samples, sixteen samples with a deviating taste or aftertaste were also measured.

Samples of carrots in the second and third experiments were obtained from the Danish Institute of Agricultural Sciences' (DIAS) station, Aarslev, Denmark. The carrots were ready to be marketed and consumed according to the DIAS's regulations. After being in a cold store, carrots were removed and kept at room temperature (20 °C) for 24 hours for equilibration before the experiment.

The goal of the second and third experiments was to examine the possibility of NIR reflectance to classify carrots according to their cultivars and production systems using NIR reflectance. The third experiment had the same goal as the second experiment, but it had 16 samples with a deviating and relatively bad taste, or aftertaste (mouldy, earth like taste) deviating, due to inappropriate storage, and we would like to investigate the ability of NIR to detect these samples that had this bad taste.

Table 1. The three experiments that have been carried out

Experiment no.	Duration (days)	No. of samples measured by NIR spectroscopy	Samples characteristics
1	One day	25	—
	Total no. of samples	25	
2	First day	160	C1, E1 ^a
	Second day	160	E2, E3 ^a
	Third day	160	C2, C3 ^a
	Total no. of samples	480	
3	First day	160 + 8 ^b	C2, E2, O ^b
	Second day	160 + 8 ^b	C3, E3, O ^b
	Total no. of samples	320+16 ^b	

^a C: conventional, E: ecological (organic). The number followed representing the cultivar.

^b O: The deviating and relatively bad taste samples.

Reflectance measurements

A scanning Zeiss MMS1 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. Three reflection spectra were taken at three equidistant positions around the mid equator (in the second and third experiments) of each carrot, in order to

average the spatial variability. The three spectra from each carrot were averaged for further treatment. The light source consisted of 12V/100W tungsten halogen lamp. The lamp was calibrated hourly, and before and after reflectance measurement of a batch of carrot samples, using a standard reflectance plate made of BaSO₄. Light from the lamp passed through a bundle of optical fibres to the vegetable, and reflected light was transferred to a Photo Diode Array (PDA) detector through another bundle of fibre optic. A holder was designed to support carrots and to direct the light in a 45 degree angle to the carrots (to avoid specular reflectance), and to maintain a distance of 1 cm, according to manufacture recommendation, between the probe and the measured carrot. The integration time (time needed for a spectrum to be acquired) was 150 milli second.

Multivariate analysis

The calculations were carried out using ‘Unscrambler’ v. 7.5 (Camo, ASA, Oslo, Norway), a statistical software package for multivariate 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 analysed.

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 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, in a decreasing order, the main structured information in the data. Plotting of the score vectors corresponds to plotting the objects in PC space (Esbensen et al., 2000).

NIR spectra can be used for modelling and / or classification with raw data or transformed data. The transformation used depends on many factors, mainly obtaining the lowest Root Mean Square Error of Prediction (RMSEP) and highest classification rate. The transformation techniques of NIR raw data are: Multiplicative Scatter Correction (MSC), first derivative and second derivative. MSC corrects the additive and multiplicative effects in the spectra and improves the predictive ability. The advantages of the second derivatives in fitting curves are:

1. Elimination of unnecessary parameters which reduces the likelihood of over fitting the data, and
2. Improves the extraction of chemical information from the raw data.

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 - Discrimination Analysis (PLS-DA), as a supervised classification method, was used for classifying carrot samples according to their cultivation method and cultivar using their NIR spectra. In PLS-DA, a dummy variable was used as dependent variable, with the values 1 for the targeted classified samples (e.g. conventional) and 0 for the other groups (e.g. organic). Then PLS-1 (which uses just one Y-variable) or PLS-2 (which uses more than one Y-variable) could be used for making the prediction model. Predicted values above 0.5 were assigned to the targeted group, and the values below 0.5 to the other group (Thyholt and Isaksson, 1997). The significant latent factors, i.e. the number of significant components needed for classification, were determined by random test set validation. This validation method was used since we have rather a high numbers of samples in our models.

Results

Analysing the spectra along the carrot length (tip, mid-section and crown) showed that the mid-section of the carrots is the only part in which the two production systems, i.e. organic and conventional, could be distinguished by PLS-DA. Using two dimensional plots of score vectors for PC1 and PC2, the production systems were separated along the first PC (Fig. 1). PC1 explained 94% of the variation between samples. For the first experiment, MSC pre-processing was used on NIR reflectance spectra. Three outliers were detected and deleted before handling PCA in Figure 1.

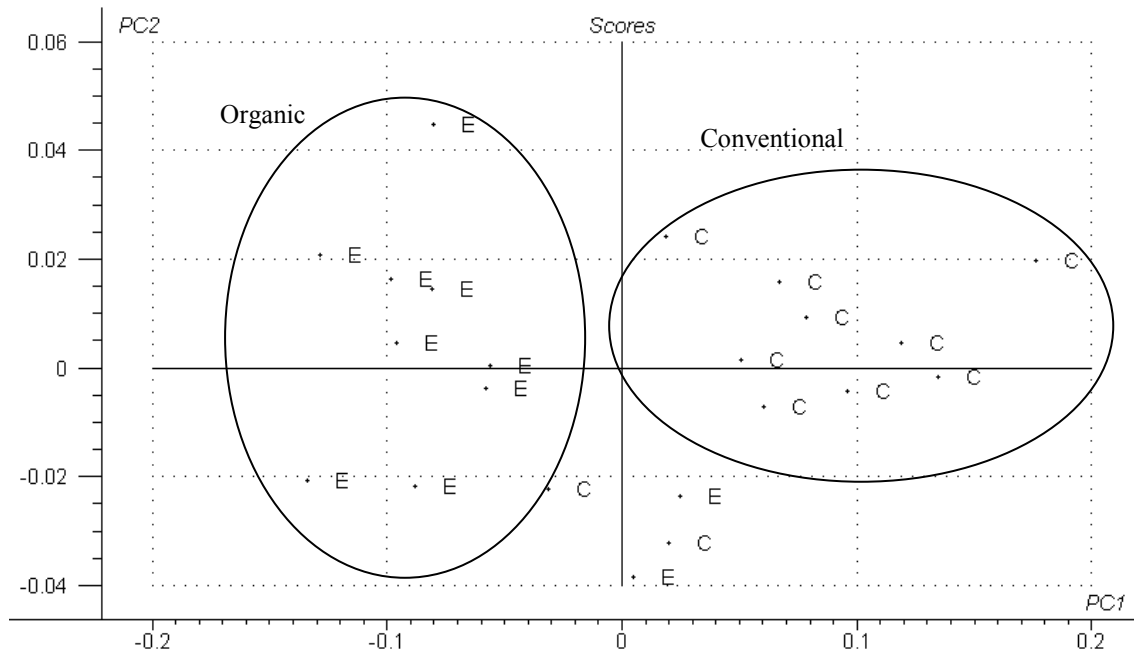


Figure 1. Score plot of NIR reflectance using MSC pre-processed technique at the mid-section part of the carrot samples in the first experiment. PC1 explained 94% of the variation between samples [Legend: C: conventional; E: ecological (organic)]

The average NIR reflectance of organic and conventional samples in the second experiment is shown in Figure 2. They look the same, but the reflectance of the conventional production system is higher than the organic production system, which indicates a significant difference between the samples investigated.

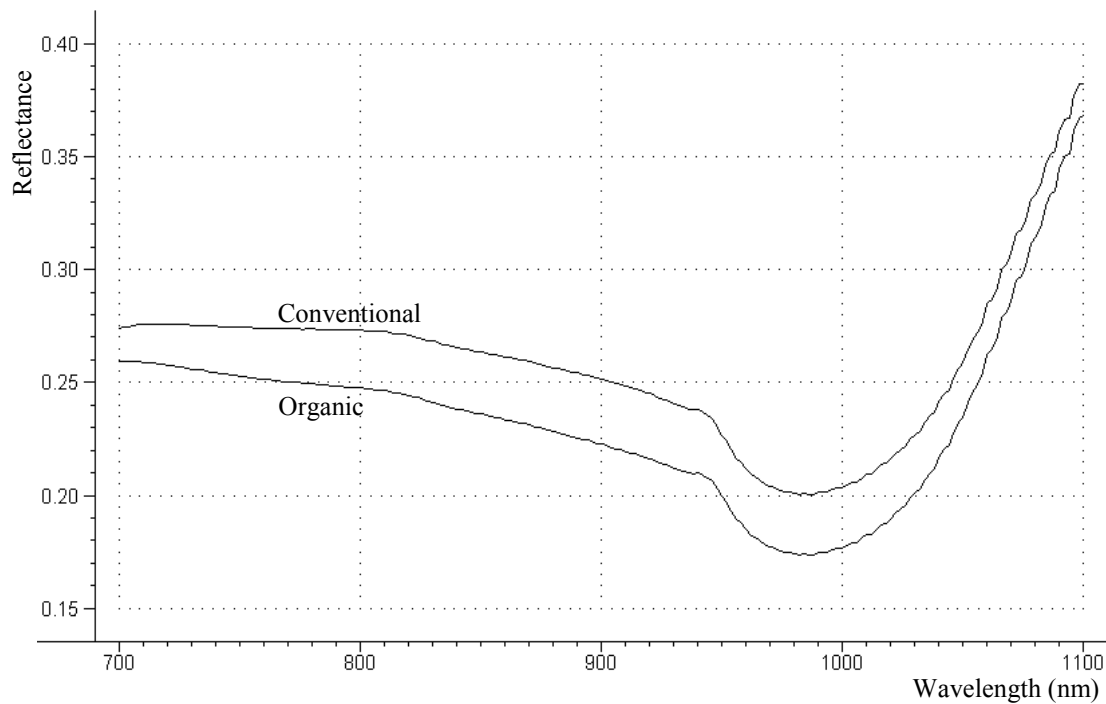


Figure 2. The average reflectance of organic and conventional samples in the second experiment

For classification of carrot samples using their NIR reflectance, the first derivative pre-processed technique was used. This pre-processed technique was the only data that was able to separate reasonably the samples according to their cultivars and production systems. The loadings of the production systems and cultivars variables in PLS-DA are shown in Figure 3. This figure explains the relationship between different variables and how they are correlated. It is shown that some variables are positively correlated, e.g. cultivar 1 conventionally produced (C1) and cultivar 1 organically produced (E1), and others are negatively correlated, e.g. cultivar 1 (Var 1) and cultivar 2 (Var 2). Moreover, this figure indicates the possibility to classify samples with different cultivars and production systems, e.g. the conventional variable (Con) is in the upper right, while the organic variable (Eco) is in the lower left side. From 480 samples, 300 samples were used as calibration set, and 180 samples were used in test validation set in PLS-DA. The PLS-2 was used for making classification regression for the targeted group. Taking into consideration that the validation set contained samples from all targeted groups (cultivars and production systems). Table 2 shows the classification rate and the number of latent factors for different production system and cultivars of carrots. A high classification rate for production systems and relatively high classification rate for cultivars was obtained.

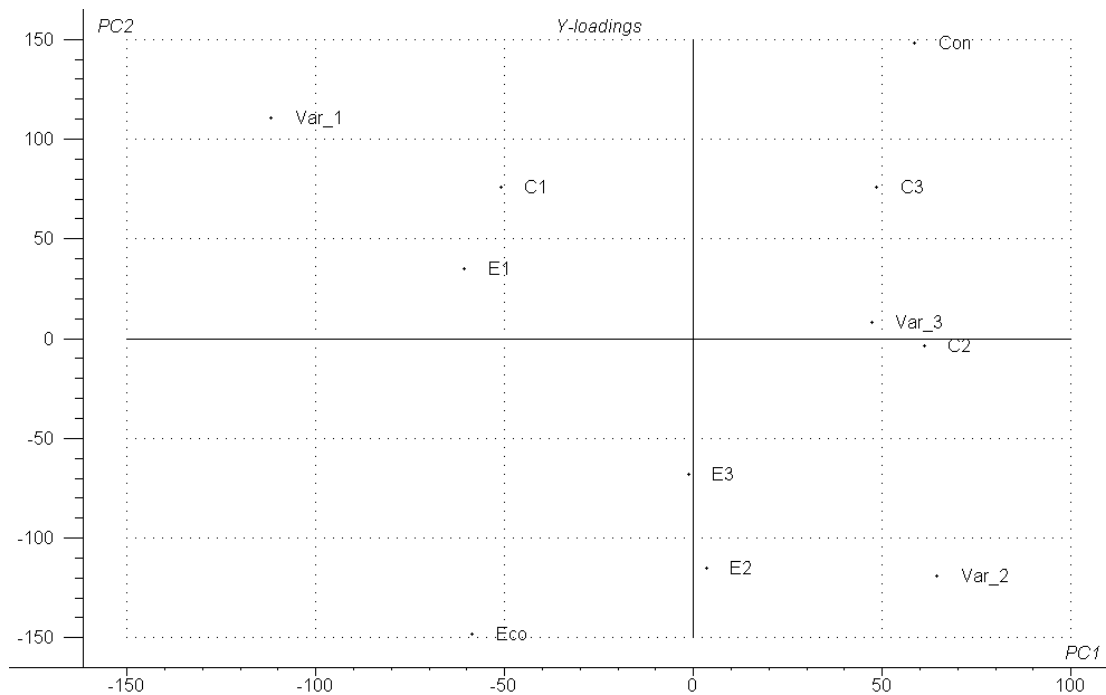


Figure 3. The loading of different cultivars and production systems (Y), using the first derivative pre-processed technique in the second experiment [Legend: Con: conventional; Eco: ecological (organic); E: ecological (organic); C: conventional; Var: cultivar, the no. followed is indicating the cultivar no.]

Table 2. Classification rate and number of latent factors of different samples in the second experiment, using their first derivative pre-processed NIR reflectance

Samples	Classification rate (%)	Numbers of latent factors (PCs)
Conventional	87.2	4
Organic	83.7	4
Cultivar 1	92.8	5
Cultivar 2	64.3	3
Cultivar 3	70.3	3

Figure 4 shows the average NIR reflectance of organic and conventional samples in the third experiment. They can be distinguished, which indicates a significant difference between the samples investigated.

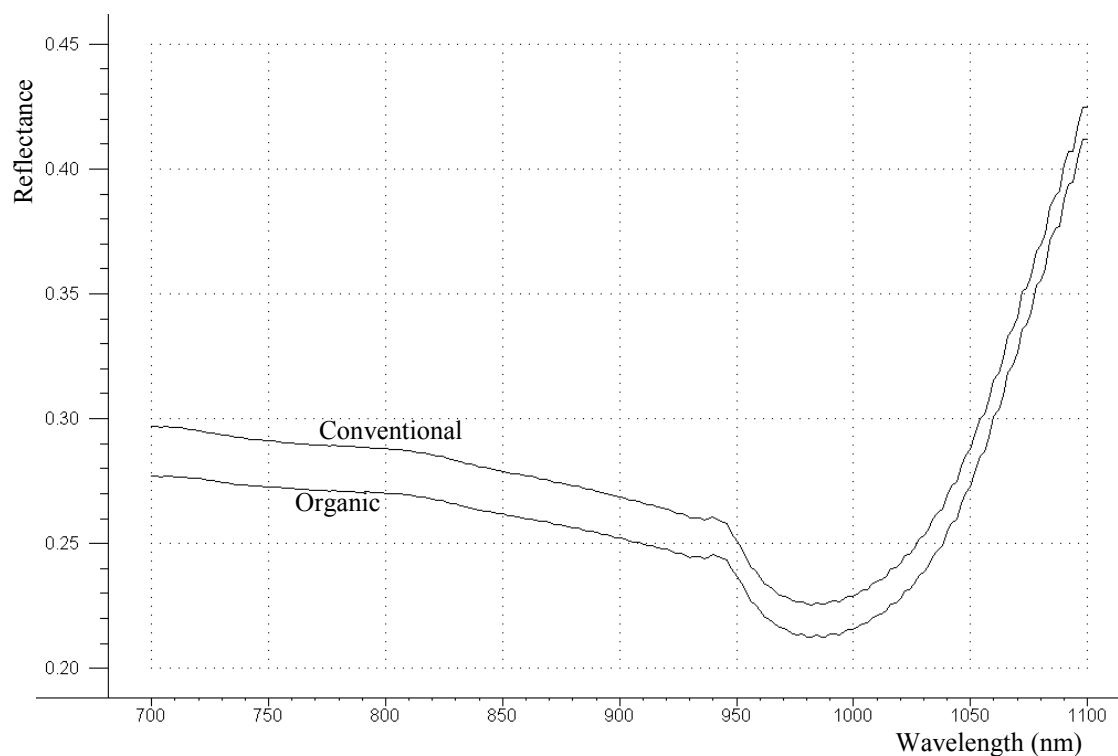


Figure 4. The average reflectance of organic and conventional samples in the third experiment

The first derivative pre-processed technique was also used for classification of carrot samples using their NIR reflectance in the third experiment. This pre-processed technique was the only data that was able to separate reasonably the samples according to their cultivars and production system.

The loading of the production systems and cultivars in PLS-DA is shown in Figure 5. The ability to classify samples of different cultivars and production system is also obvious, like in the second experiment. 120 samples (without the deviating-taste samples) out of 320 samples, were used as a test set for validating PLS-DA classification. Different classification rates are shown in Table 3. A high classification rate for production systems and cultivars using NIR reflectance was also obtained in this experiment, which supports the classification results in the second experiment.

Soft Independent Modelling of Class Analogies (SIMCA) was also tested for classification (Esbensen et al., 2000), but did not give better results than PLS-DA.

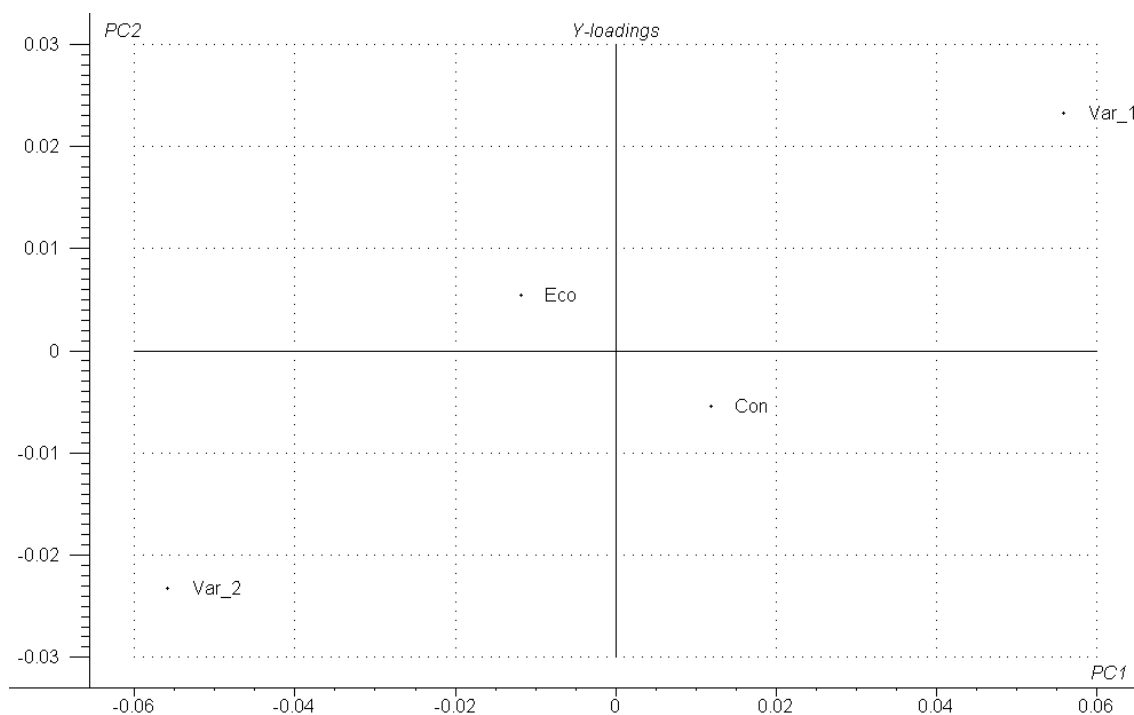


Figure 5. The loading of different cultivars and production systems (Y), using the first derivative pre-processed technique in the third experiment [same legend as in Figure 3]

Table 3. Classification rate and latent factors of different samples in the third experiment, using their first derivative pre-processed NIR reflectance

Samples	Classification rate (%)	Numbers of latent factors (PCs)
Conventional	88.7	5
Organic	82.7	5
Cultivar 1	98.3	6
Cultivar 2	96.6	6

To investigate the potential of NIR to detect the samples with relatively bad taste, or aftertaste due to inappropriate storage (16 samples), PCA was used. It was noticed that samples having bad taste (O) could be potentially detected in the score plot of the second derivative pre-processed NIR reflectance. Most of these bad tasting samples are located outside an imaginary circle (Figure 6).

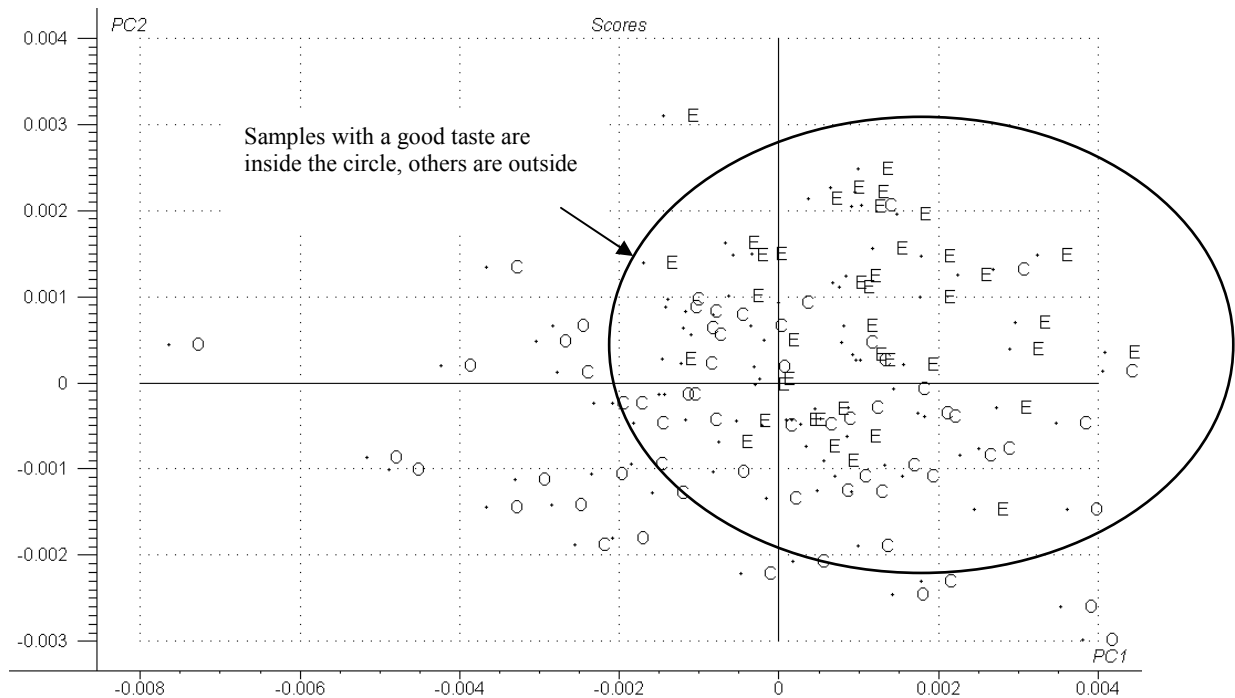


Figure 6. Score plot of samples (including the deviating taste samples) in the third experiment. The first and second PCs explained 39% and 13% respectively

Discussion

The results of this research indicate that there is a possibility of using NIR reflectance to classify carrots samples having different growing systems or different cultivars. Taking into consideration that the growing system and cultivar has an impact on carrots' sensory quality (Haglund et al., 1999). The method has potentials for evaluating the consumer satisfaction.

As NIR reflectance is influenced by a number of factors, care was taken to keep these as constant as possible during the experiments. The stability of the light source in the spectrometer, during all NIR reflectance measurements, was quite acceptable, with a variation of less than 5%. This indicates that any variation occur is not due to the light intensity difference.

The spectra were taken on whole parts of the carrots, and not the juice. Schulz et al. (1998) stated that the typical NIR spectra, obtained from the surface of the whole carrot, are the same as obtained from carrots juice. The organic and conventional carrots could be distinguished (Fig. 1), when the spectra were taken in the mid-section of carrots. This classification is mainly expected to be due to the difference in the internal characteristics of carrots, which NIR reflectance can sense. Osborne et al., (1993) stated that there are strong evidences, that the range from 700-900 nm constitutes a 'diagnostic window' in which chemical compositions of samples can be investigated.

In the second and third experiment, spectra were taken in the mid-section. This was based on the successful classification obtained in the first experiment, for carrots scanned in the mid-section (Fig. 1). The process of discarding pieces of 1 cm from the crown and from the tip agrees with this conclusion.

In Figure 2 and Figure 4, the average NIR reflectance spectra for the two types of production systems of carrots appear to have similar shapes, however, the average reflectance spectrum of carrots from a conventional production system is higher than the organic system, which indicates that there is a significant difference between both types of samples. This difference is due to the same reason that causes the classification in Figure 1 (i.e. diagnostic window). These figure support the possibility of classifying carrots according to their production system. It is expected that other varieties, different soil conditions, climate, irrigation etc., will produce different reflectance responses, requiring the system to be calibrated for the specific use.

The loading of the production system and cultivar variables in PLS-DA, using NIR reflectance, in Figure 3 and Figure 5, showed a very clear positively / negatively correlation between different variables, and showed the possibility of classification, since different targeted variables were located in different quarters of the loading plot. The classification of different samples with different production systems and cultivars had a high rate. In the second experiment (Table 2), with a classification rate > 64% for cultivars and > 82% for production systems, and in the third experiment (Table 3), with a classification rate > 96% for cultivars and > 82% for production systems. The reason

is suggested to be that spectral data of a commodity can be treated as a signature, allowing commodity to be grouped on the basis of their similarities (Kim et al., 2000) and that NIR range in 700-900 nm forms a 'diagnostic window' (Osborne et al., 1993). The NIR reflectance were measured randomly for carrots samples, and we got a high classification rate, which indicates that the classification was due to the different carrots characteristics, and not due to any other external factors (e.g. light stability, surface conditions etc.). Carrots' sensory quality depends on year, growing system and cultivar (Haglund et al., 1999), and since the NIR can predict the cultivar and growing system of carrots, this indicates that NIR is able to predict the sensory quality of carrots.

The NIR showed a potential to sense the deviating samples, which had a bad taste due to inappropriate storage. Figure 6 shows that these samples (O) are located outside an imaginary circle (or sphere, if it is in three dimension), which indicates the possibility of predicting samples having good or bad taste. Further researches are needed to find the threshold limit that predicts bad or good taste for different samples.

Conclusion

Carrots sensory quality depends on three factors: year, growing system (organic, conventional) and cultivar. NIR was able to predict cultivars and production systems with a high classification rate. Different cultivars and different production system samples have been correctly classified in rates of > 64% and > 82% respectively. This indicates that NIR is a reliable technique and has a high potential for sensing quality sensory of carrots. Further researches are needed for more investigations.

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