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Evaluation of shelf-life of fresh-cut pineapple using FT-NIR and FT-IR spectroscopy

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ABSTRACT

The aim of this work was to investigate the loss of freshness of fresh-cut pineapple samples stored at different temperatures using non-destructive spectroscopic methods. Three lots of fresh cut pineapples (*Ananas comosus* L. cv. *Golden Ripe*, from Costa Rica), packaged in PVC trays (250 g) were analyzed during storage at three different temperatures (5.3, 8.6 and 15.8 °C). Loss of quality of these fruit was evaluated by chemical and microbiological parameters and using NIR and MIR spectroscopy. The FT-NIR spectra were acquired in reflectance mode directly on the slice of fresh-cut pineapple, over the range 12,500–3900 cm^{-1}, while FT-IR spectra were collected over the range 4000–700 cm^{-1} using an horizontal ATR cell. Some chemical and microbiological parameters were also measured. Principal component analysis (PCA) was applied to the second derivative of the spectra to uncover molecular modifications occurring over the storage time. A clear discrimination between "fresh" and "old" samples was obtained and a stability time corresponding to the time of the initial loss of freshness was defined at each temperature. The stability times revealed by NIR spectroscopy were in good accordance with those evaluated by MIR. At each temperature the stability times (i.e. the initial loss of freshness times) defined by spectroscopic techniques (4–5 d at 5.3 °C, 3–4 d at 8.6 °C and 1 d at 15.8 °C) were associated with a mesophilic bacteria count ranging between 10^5 and 10^6 CFU g^{-1} and lower than the maximum limit for mesophilic bacteria ($<5 \times 10^7$ CFU g^{-1}) given by French hygienic regulations at consumption.

These results show that NIR and MIR spectroscopy could support conventional techniques (chemical and microbiological analysis) in studying shelf-life of fresh-cut fruit. In particular these techniques define the initial loss of freshness time, indicating a product which rapidly will be no longer acceptable if stored beyond that time. The main advantage of using IR spectroscopic techniques is to rapidly draw a profile of the product related to its change in quality.

1. Introduction

Market sales of ready-to-use fresh fruit have grown rapidly in the recent years as a result of changes in consumer attitudes. The international Fresh-cut Produce Association (IFPA) defines fresh-cut products as "any fruit or vegetable or combination thereof that has been physically altered from its original form, but remains in a fresh state". Quality of fresh-cut fruit products determines their value to consumers and is a combination of attributes, properties or characteristics including appearance, texture, flavour and nutritional value.

Because the tissue integrity of these products has been altered during processing, fresh-cut fruit are more perishable than the original raw materials (Shewfelt, 1994). Fresh-cut processing causes wounding, increases metabolic activities and delocalization of enzymes and substrates. This may lead to deterioration such as browning, softening, decay and off-flavour development. These manipulations result also in increased rates of respiration and ethylene production and may reduce the shelf-life of fresh-cut fruit commodities (Varoquaux and Wiley, 1997). On the whole fresh-cut products have a short shelf-life, up to some days, because they have to preserve sensory and nutritional characteristics of fresh products and pathogenic microbial species must not develop (Shah and Nath, 2006).

Several studies have been carried out to monitor the shelf-life of fresh-cut fruit and vegetables by using chemical, physical and microbiological indices. In addition predictive models are useful tools both to understand degradation phenomena during shelf-life and to optimise storage conditions, especially when kinetic phenomena are modelled under dynamically varying conditions

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(Corbo et al., 2006). Mathematical models to predict microbial stability, chemical and sensory quality of different fresh-cut vegetables and fruit have been studied by several authors (Riva et al., 2001; Lauridsen and Knøchell, 2003; Rocha and Morais, 2003; Lavelli et al., 2006; Zanoni et al., 2007; Montero-Calderon et al., 2008).

Even if traditional methods have been successfully used for studying shelf-life of fresh-cut fruit and vegetables, they are expensive, slow, require considerable analytical skill and are not suited to automation. Rapid and non-destructive methods to investigate the loss of freshness of the food product have recently increased in importance. Infrared spectroscopy in both the near (NIR) and mid (MIR) regions appears to be one of the most powerful and convenient analytical tools which can be used for studying the shelf-life of food, given that absorption in these spectral ranges can be related, to a greater or lesser degree, to the main chemical components of foods, such as proteins, carbohydrates, fats and waters. In particular, in the NIR region (between 750 and 2500 nm), vibration and combination overtones of the fundamental O–H, C–H and N–H bounds are the main recordable phenomena (Williams and Norris, 2001), while the MIR measurements provide information on fundamental frequencies of chemical bonds in functional groups such as C–C, C–H, O–H, C=O and N–H (Colthup et al., 1990; Coates, 2000).

In recent years, NIR and MIR spectroscopy has become a valid tool supporting chemical methods. Several studies have been reported on the use of NIR and MIR spectroscopy as a rapid and cost-effective analytical tool to determine the food structure and properties in fundamental research and as on-line sensors for monitoring process (Ozaki et al., 2006; Nicolaï et al., 2007). Nevertheless, only few studies have addressed the application of NIR and MIR spectroscopy in shelf-life studies; Cattaneo et al. (2005) used NIR and MIR spectroscopy to study shelf-life of Crescenza cheese stored at different temperatures, while Sinelli et al. (2005) applied NIR spectroscopy to monitor the shelf-life of packed industrial ricotta cheese.

The aim of this work was to monitor, using Fourier transform (FT)-NIR and (FT)-IR spectroscopy, the main changes occurring during the commercial life of fresh-cut pineapple stored at different temperatures. In conjunction with this, microbiological and chemical changes were monitored with traditional analytical methods in order to evaluate the feasibility of FT-NIR and FT-IR spectroscopy as a rapid non-invasive approach for monitoring the shelf-life of fresh-cut fruit.

2. Materials and methods

2.1. Samples

Three lots of fresh-cut pineapple (*Ananas comosus* L. cv. *Golden Ripe*, from Costa Rica), packaged in PVC trays (250 g), supplied by the manufacturer at the beginning of their commercial life, were analyzed during storage at three different temperatures (4, 8 and 16 °C). Storage temperature was continuously monitored using small time–temperature recording devices (TB Econorma S.a.s, Treviso, Italy; water proof, ±0.5 °C temperature precision, diameter = 10 mm, thickness = 4 mm). Three samples were monitored for each temperature, resulting in average temperatures during storage of 5.3, 8.6 and 15.8 °C, with a standard deviation of replicates of about 0.5 °C.

Samples stored at 5.3 and 8.6 °C were analyzed every day over a ten day period, and those stored at 15.8 °C over six days. For each sampling, three trays were used to carry out chemical and microbial analysis, FT-NIR and FT-IR spectroscopy.

2.2. Chemical methods

At each sampling, fresh-cut pineapple was analyzed for total soluble solids content, titratable acidity and pH. Fresh-cut fruit pieces (50 g) were homogenized using an Ultra Turrax T25 (IKA WERKE, Germany) and filtered (Whatman paper no. 1). Total soluble solids content was determined on filtered juice using a hand refractometer (Atago mod. N1, Tokyo, Japan) and expressed as percent. Filtered pulp (10–15 g) was titrated with 0.1N of NaOH to pH 8.1, using an automatic sample titrator (Crison, Titromatic 2S-3B, USA). Titratable acidity was expressed as grams of anhydrous citric acid in 100 g of fruit (fresh weight). Soluble solids content and acidity results are the average of two determinations. pH was directly measured on pineapple slices using a pH meter (XS Instruments 510, Opto-Lab, Italia). Nine measurements, three readings from each of three slices, were carried out for each sampling.

2.3. Microbial analysis

The samples (10 g of pineapple) were aseptically removed from each package, transferred into a sterile bag, diluted with 90 mL of 0.85% sterile tryptone salt solution and homogenized for 30 s at 230 rpm in a stomacher (Seeword 400 Circulator, England). Decimal progressive dilutions were prepared and the following microbial determinations were performed: mesophilic aerobic bacteria count or total bacteria count (TBC) by pouring plates in Plate Count Agar (PCA) (Merck, USA) and incubation at 30 ± 1 °C for 48–72 h (ISO 4833:2003); yeast and mould spread plates in Yeast Glucose Chloramphenicol Agar (YCG) (Merck, USA) and incubation at 25 °C for 2–5 d. All the microbiological determinations were carried out in triplicate and the results were expressed as the average colony forming units per grams (CFU g^{-1}).

2.4. FT-NIR spectroscopy

The analysis was performed by using a FT-NIR spectrometer (MPA, Bruker Optics, Ettlingen, Germany) equipped with an integrative sphere. The spectra were acquired in reflectance mode directly on the pineapple slice, over the range 12,500–3900 cm^{-1} (resolution: 32 cm^{-1}; scanner velocity: 10 kHz; background: 128 scans, sample: 128 scans). OPUS software (v. 6.5 Bruker Optics, Ettlingen, Germany) was used for spectral acquisition and instrumental control. For each sampling, seven pieces of pineapple were analyzed at room temperature and the average spectra were used for further evaluations.

2.5. FT-IR spectroscopy

FT-IR measurements were taken using a spectrometer (VERTEX 70, Bruker Optics, Ettlingen, Germany) equipped with a deuterated triglycine sulfate (DTGS) detector. Spectra were recorded using an in-compartment benchmark attenuated total reflectance (ATR) through a top plate by use of a 45° germanium crystal with 11 internal reflections (Pike Technologies, Inc., Madison, USA). For both background and sample readings, 32 scans were co-added at a nominal resolution of 8 cm^{-1}; single beam spectra of the samples were collected and ratioed against a background of air. Fresh-cut pineapple slices were squeezed and pressed on the ATR plate, in order to cover the crystal with juice. For each sample, spectral data were collected in the range 4000–700 cm^{-1} at room temperature (20 ± 0.5 °C). OPUS software (v. 6.5, Bruker Optics, Ettlingen, Germany) was used for spectral acquisition, instrument control and preliminary file manipulation.

2.6. NIR and MIR data processing

Both NIR and MIR spectra were analyzed by using The Unscrambler (v 9.7, Camo, Inondhcim, Norway) software. In order to minimize the effect of baseline shifts, the spectral data were preprocessed by several mathematical treatments. The best pre-treatment

Table 1
Average values and relative standard deviation for total soluble solids content, titratable acidity and pH during storage.

Temperature (°C)	Total soluble solid (%)		Titratable acidity (g 100 g^{-1})		pH	
	Average	SD	Media	SD	Media	SD
5.3	11.9	0.24	0.71	0.08	3.61	0.12
8.6	10.5	0.08	0.80	0.12	3.54	0.09
15.8	11.9	0.17	0.75	0.11	3.59	0.13

useful to give the most important information was the second derivative transformation (Savitzky–Golay method, gap size = 15 data points). Principal component analysis (PCA) was applied, as an exploratory tool, to spectral data to uncover molecular modifications during storage. PCA identifies orthogonal directions of maximum variance in the original dataset in decreasing order and projects the data onto a lower-dimensionality space formed by a subset of the highest-variance components. The orthogonal directions are linear combinations of the original variables and each component explains in turn a part of the total variance of the data; in particular, the first significant component explains the largest percentage of the total variance, the second one, the second largest percentage, and so forth (Beebe et al., 1998; Naes et al., 2000). All spectral data sets were mean-centered before performing PCA calculations. For NIR and MIR data, PCA was performed over the range 11.000–3.950 cm^{-1} and 1800–870 cm^{-1}, respectively.

The values of the PC scores obtained by PCA applied to the all samples stored at three temperatures (5.3, 8.6 and 15.8 °C) were modelled for each temperature as a function of time during storage to identify the stability times, i.e. the time of initial freshness decay (Table Curve Software, v. 4.0, Jandel Scientific, San Rafael, CA, USA). The second derivative maximum value was calculated on the curve models and corresponded to the maximum acceleration of shelf-life process.

3. Results and discussion

Soluble solids content (SS), titratable acidity (TA) and pH showed little change during storage. Average values and the relative standard deviation for these parameters, measured during storage at each temperatures, are shown in Table 1. Similar trends for these parameters were found by Gil et al. (2006) and Montero-Calderon et al. (2008). Data variability reported in these studies could be associated with the type of cultivar and the maturity stages of the fruit.

The microbial growth on fresh-cut pineapple during storage at each temperature is shown in Fig. 1, and at each temperature shows a different trend. Also, the starting levels of microbial population were different, since the samples stored at the three temperatures belonged to three different lots.

The initial value ranged from 3 to 4 log CFU g^{-1} for mesophilic bacteria and reached about 7 log CFU g^{-1} after 8–10 d of storage at 5.3 °C, after 4–5 d at 8.6 °C and after about 2 d of storage at 15.8 °C (Fig. 1a). Similar increases were observed for yeasts at each temperature (Fig. 1b). As expected, the growth towards the final values of 7 log CFU g^{-1} was more rapid at the highest temperatures for mesophilic bacteria and yeasts. Similar values for mesophilic bacteria, moulds and yeast were obtained by Montero-Calderon et al. (2008), who found 7–7.5 log CFU g^{-1} of moulds and yeasts and 7 log CFU g^{-1} of mesophilic bacteria in fresh-cut 'Gold' pineapple packaged in different conditions and stored at 5 °C for 18 d.

The raw results of the spectroscopic data are shown in Fig. 2, including FT-NIR (a) and FT-IR (b) raw spectra of fresh-cut pineapple collected during storage. Both the near and the mid infrared spectra of all samples were quite homogeneous and did not show obvious differences and no outliers were identified *a priori* by visual inspection. In the FT-NIR spectra (Fig. 2a) the absorption bands at 10,244 and 6996 cm^{-1} are related to the second –O-H overtone and to the first –O-H overtone of water, respectively. The peaks at 8454 and 5623 cm^{-1} are associated with the absorption band of the second overtone and the first overtone of –C-H stretch, respectively. The absorbance at 5237 cm^{-1} is attributed to the –O-H combination band of water (Williams and Norris, 2001). The FT-IR spectra of pineapple (Fig. 2b) collected during storage are dominated by peaks attributed mostly to water (3356 cm^{-1} –O-H stretching and 1635 cm^{-1} –O-H bending) and to sugars and organic acids, in the range 1120–995 cm^{-1} (coupled C–O and C–C stretching vibration) (Coates, 2000; Workman, 2001). After a second derivative treatment, several spectral features became more apparent. In the NIR region, absorbance at 7274–7143 and 5408–5276 cm^{-1} has been attributed to water, while the absorbance at 5931–5631 and 4500 cm^{-1} are related to the first overtone –C-H stretch and combination band –C-H stretch of sugar and organic acids, respectively (Williams and Norris, 2001). MIR spectra are dominated by some peaks attributed to –O-H stretch (3691, 3394, 3232 cm^{-1}) and OH bending (1643, 1581 cm^{-1}) of water.

The absorption bands attributed to sugars and organic acids are more evident in the second derivative plot, which are dominated by peaks at 1500, 1434, 1380, 1290, 1180, 1141, 1107, 1056, 956 and 879 cm^{-1}. In particular, bands appearing between 1500 and 1290 cm^{-1} are associated with C–H_2 and C–H_3 deformation bands

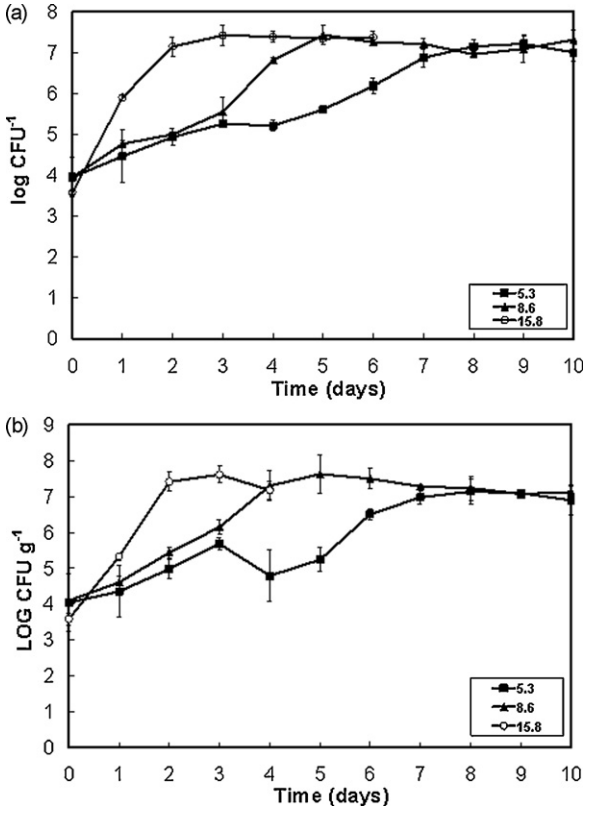

Fig. 1. Changes in mesophilic bacteria (a) and yeast (b) on fresh-cut pineapple during storage at each temperature.

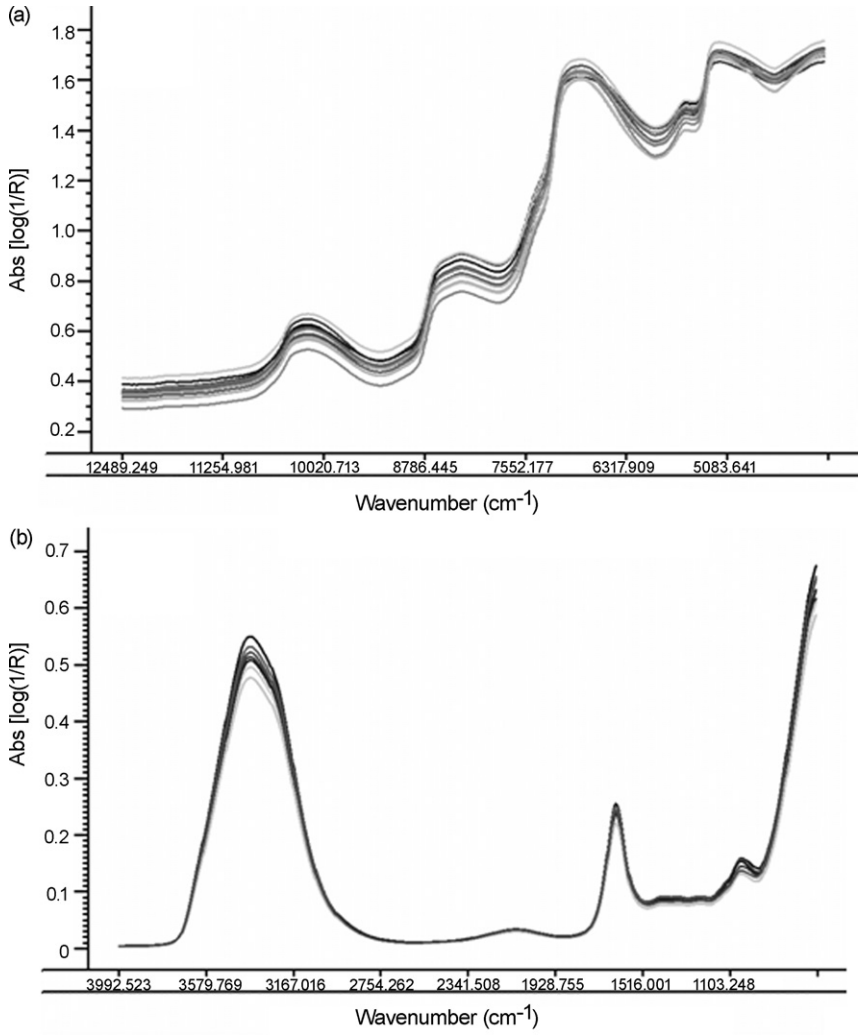

Fig. 2. FT-NIR (a) and FT-IR (b) raw spectra of fresh-cut pineapple during storage.

of sugars and organic acids, while the peaks in the region between 1180 and 1056 cm^{-1} arise mainly from C–O and C–C of sugars and organic acids (Coates, 2000; Workman, 2001).

Both for NIR and MIR spectra, PCA were performed on the second derivative spectra, and the sample scores calculated on PCs 1 and 2 were plotted, together with the loading plots of the first two principal components in order to uncover molecular modifications during storage.

Examining the score plot, obtained by applying PCA to NIR spectra over the range 11.000–3.950 cm^{-1}, in the area defined by the first two principal components, a satisfactory sample distribution was found according to the storage conditions (Fig. 3a). On the score plot, for each temperature, the number beside each point represents for each temperature the storage time in days. In particular, the first two principal components (100% of the total variance) were able to separate, for each storage temperature, the samples in two groups, named "fresh" and "old". In particular, "fresh" samples corresponded to fresh-cut pineapple stored up to 5 d, 4 d and less than 1 d at 5.3 °C, 8.6 °C and 15.8 °C, respectively. Conversely, "old" samples corresponded to samples stored for longer than 6 d, 5 d and 2 d at 5.3 °C, 8.6 °C and 15.8 °C, respectively. In an attempt to uncover the causes of these score patterns, the loadings of the first two principal components were studied and a number of significant features were identified (Fig. 3b). In particular, 8863 and 5631 cm^{-1} corresponds to the second and first overtones of C–H of sugar, respectively; 7251–7127 and 5415–5284 cm^{-1} corresponds to the first overtone and O–H bending and asymmetric stretching combination band of water and 4512 cm^{-1} corresponds to the combination band of the C–H.

In order to define a stability time, corresponding to the time of an initial freshness decay, the PC1 scores were plotted against time and modelled using a sigmoid function, expressed as

$$y = \frac{a + b}{[1 + \exp(c - x)/d]}$$

where a is the maximum shift (from initial to equilibrium value) of the considered index (PC1 scores), b is the transition center, x is the storage time, and c and d are two constants. The use of this type of sigmoidal function is justified by the nature (enzymatic or microbial-induced) of the transformation in progress (Benedetti et al., 2005; Sinelli et al., 2008). In Fig. 4 the modelling of these data is shown: the minimum of the second derivative of the empirical transition function, obtained interpolating PC1 scores at each temperature, allows the measurement of the related stability time. The stability time (5 d at 5.3 °C; 3.4 d at 8.6 °C and 1.1 d at 15.8 °C) was for each storage condition the time after which the product will be no longer acceptable as revealed by NIR spectroscopy.

The same multivariate approach was used to analyze the FT-IR spectra collected during storage (Fig. 5). The PCs score plot (Fig. 5a) showed that it was possible to obtain a good sample distribution along the PC1 axis according to the increase of storage time. As for NIR spectroscopy the first two principal components (91% of

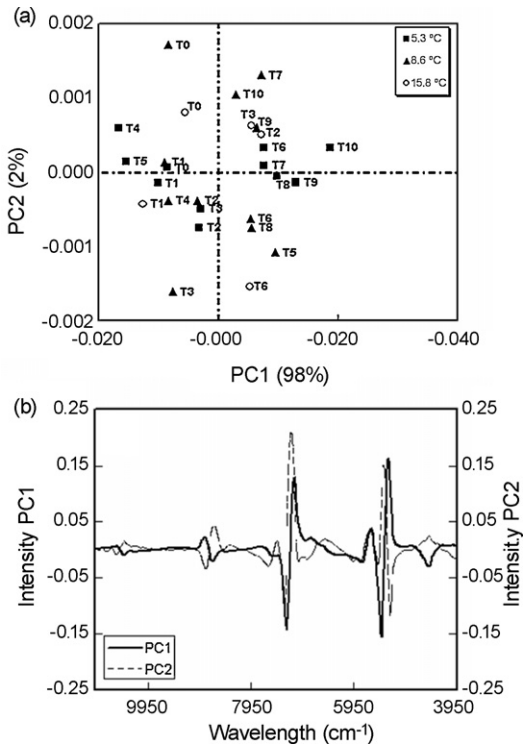

Fig. 3. Principal component scores (a) and line plots (b) on PC1 and PC2 of FT-NIR second derivative spectra of fresh-cut pineapple during storage.

the total variance) were able to separate the samples in two groups ("fresh" and "old"), at each storage temperature. The samples stored up to 5 d at 5.3 °C, up to 3 d at 8.6 °C and 1 d at 15.8 °C were considered "fresh", while all samples stored for longer times resulted "old". Loadings of principal components 1 and 2 (Fig. 5b) were characterized by contributions from water (1639–1500 cm^{-1}, OH stretching), sugar and organic acid (1500 cm^{-1} –C-H_3 scissoring vibration, 1184–950 cm^{-1}, C–O stretching vibration).

As was the case for NIR spectra, the PC1 FT-IR scores were plotted against time and the second derivative was calculated, in order to define the stability time, i.e. the initial freshness decay time. The stability times revealed by MIR spectroscopy (4 d at 5.3 °C; 3.4 d at 8.6 °C and 1.1 d at 15.8 °C) were in good accordance with those evaluated by NIR technique. At each temperature the stability times defined by spectroscopic techniques (4–5 d at 5.3 °C, 3–4 d at 8.6 °C and 1 d at 15.8 °C) are associated with the initial freshness decay times. In fact, at these stability times, the samples had a mesophilic bacteria count ranging between 10^5 and 10^6 CFU g^{-1} and lower than the maximum limit for mesophilic bacteria (<5 × 10^7 CFU g^{-1}) given by the French hygienic regulations at

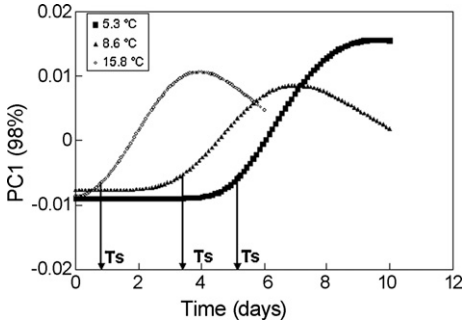

Fig. 4. Modelling of PC1 scores versus time for fresh-cut pineapple samples stored at 5.3, 8.6 and 15.8 °C.

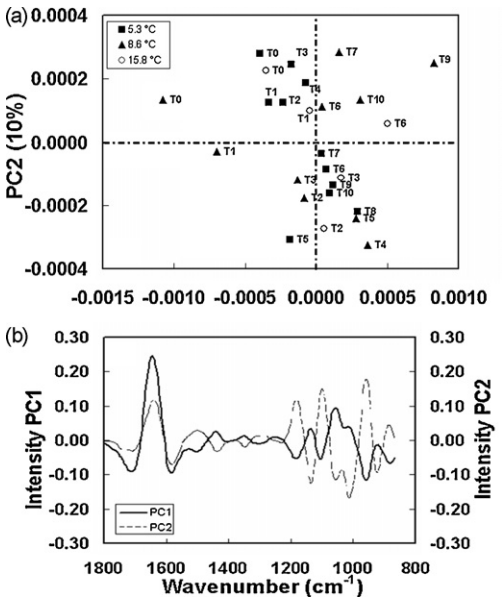

Fig. 5. Principal component scores (a) and line plots (b) on PC1 and PC2 of FT-IR second derivative spectra of fresh-cut pineapple during storage.

consumption (Ministère de l'Economie des Finances et du Budget, 1988).

Therefore we can assess that the spectroscopic methods are able to define, depending on temperature, the initial time for loss of freshness, indicating a product which rapidly will be no longer acceptable if stored beyond that time.

4. Conclusions

Results of this study show that non-destructive methods, such as NIR and MIR spectroscopy could support conventional techniques (chemical and microbiological analysis) in studying shelf-life of fresh-cut fruit. The main advantage of using the IR spectroscopic techniques is to rapidly draw a profile of the product related to its quality. In fact, FT-NIR and FT-IR spectroscopy monitor the molecular modification occurring in fresh products during storage and allow the definition of a stability time associated with them. The initial loss of freshness revealed by IR was confirmed by microbial decay, thus strengthening our results and rendering the use of fresh-cut fruit safe. In fact, the main changes occurring in the spectra during storage are associated with water loss and composition modification in the product, which might be also due to microbial development.

The approach used in this study can be applied to evaluate the loss of quality of other fresh-cut products, in which enzymatic and microbiological processes are responsible for freshness loss with a negative influence on the product quality and appearance during storage.

Acknowledgement

We dedicate this work to the memory of Prof. Marco Riva, a valued colleague and scientist, who has died unexpectedly.

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