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Research Paper

Using chemometrics to characterise and unravel the near infra-red spectral changes induced in aubergine fruit by chilling injury as influenced by storage time and temperature

Farahmand Babellahi [a], *Maria L. Amodio* [a,*], *Federico Marini* [b], *Muhammad M.A. Chaudhry* [a], *Maria L.V. de Chiara* [a], *Leonarda Mastrandrea* [a], *Giancarlo Colelli* [a]

[a] *Dip. to di Scienze Agrarie, degli Alimenti e dell'Ambiente, Università di Foggia, Via Napoli 25, 71122 Foggia Italy*
[b] *Department of Chemistry, University of Rome "La Sapienza", P.le Aldo Moro 5, I-00185 Rome, Italy*

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The early non-destructive detection of chilling injury (CI) in aubergine fruit was investigated using spectroscopy. CI is a physiological disorder that occurs when the fruit is subjected to temperatures lower than 12 °C. Reference measurements of CI were acquired by visual appearance analysis, measuring electrolyte leakage (EL), mass loss and firmness evaluations which demonstrated that even before three days of storage at 2 °C, the CI process was initiated. An ANOVA-simultaneous component analysis (ASCA) was used to investigate the effect of temperature and storage time on the Fourier transform – near infra-red (FT-NIR) spectral fingerprints. The ASCA model demonstrated that temperature, duration of storage, and their interaction had a significant effect on the spectra. In addition, it was possible to highlight the main variations in the experimental results with reference to the effects of the main factors, and with respect to storage time, to discover any major monotonic trends with time. Partial least squares-discriminant analysis (PLS-DA) was used as a supervised classification method to discriminate between fruit based on chilling and safe temperatures. In this case, only significant spectral wavebands which were significantly influenced by the effect of temperature based on ASCA were utilised. PLS-DA prediction accuracy was 87.4 ± 2.7% as estimated by a repeated double-cross-validation procedure (50 runs) and the significance of the observed discrimination was verified by means of permutation tests. The outcomes of this study indicate a promising potential for near infra-red spectroscopy (NIRS) to provide non-invasive, rapid and reliable detection of CI in aubergine fruit.

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* *Corresponding author.*
E-mail address: marialuisa.amodio@unifg.it (M.L. Amodio).
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Nomenclature

CI	chilling injury
ANOVA-SCA or ASCA	analysis of variance-simultaneous component analysis
SCA	simultaneous component analysis
FT-NIR	Fourier transform – near infra-red
PLS-DA	partial least squares-discriminant analysis
NIRS	near infra-red spectroscopy
VIS/SWIR	visible/short wave infra-red
EL	electrolyte leakage
PLSR	partial least squares regression
rDCV	repeated double cross-validation
DCV	double cross-validation
CV	cross-validation
SNV	standard normal variate
C1	conductivity of the solution at time zero
C2	conductivity of the solution after 2 h of incubation
C3	conductivity of the solution after defrosting after being kept at −20 °C for 24 h
Xc	centred matrix
m^T	transposed mean spectrum of the samples
X_θ	matrices accounting for the effect of temperature (θ)
X_t	matrices accounting for the effect of time (t)
X_{res}	residual matrix
p_θ	loading vector of the SC model for the effect of the factor "temperature"

1. Introduction

Various factors influence the quality of the stored fruits and vegetables among which temperature and time of storage holds particular importance in terms of the occurrence of chilling injury (CI) (Kader, 2013). Conventionally, most fruits and vegetables are recommended to be stored at low temperature to enhance shelf life and improve the retention of nutritional quality. However, when exposed to low temperatures sensitive produce is prone to CI (Fallik, Temkin-Gorodeiski, Grinberg, & Davidson, 1995). Basically, CI induces damage to the cell membranes resulting in the solute diffusion and increased tissue permeability (Concellón, Añón, & Chaves, 2005).

The aubergine, or eggplant (*Solanum melongena* L.) is a non-climacteric fruit which is popular and economically important worldwide but it suffers from severe CI when stored at temperatures below 12 °C (Concellón et al., 2005). CI in eggplants is a physiological disorder which leads to pitting in peel, flesh browning, blackening of the seeds and increased decay, particularly in the calyx. This can be more severe when the fruit is relocated to market temperatures after being exposed to chilling temperatures (Fallik et al., 1995; Shi et al., 2018). Therefore, the early detection of CI is crucial for the correct re-conditioning of the fruit preventing postharvest losses.

Various conventional analytical essays may help to detect CI before symptoms become visible, such as phenolic content, anthocyanin content, malondialdehyde content, polyphenol oxidase, peroxidase, catalase activity, and electrolyte leakage (Concellón et al., 2005; Fan et al., 2016; Shi et al., 2018). However, all the aforementioned techniques are destructive, time consuming, and comparatively expensive. The possibility of having non-invasive, accurate, and rapid techniques for evaluation and detection of the CI would be worthwhile.

A variety of non-destructive optical techniques have been successfully applied for quality assessment of agricultural and horticultural commodities in the past few years (Amodio, Ceglie, Chaudhry, Piazzolla, & Colelli, 2017; Chaudhry et al., 2018; Cortés et al., 2017; Erkinbaev, Henderson, & Paliwal, 2017; Munera et al., 2018). In this regard, near infra-red spectroscopy (NIRS) has proved to be effective method for the estimation of compounds comprising polar functional groups such as −OH, C−O, and N−H (Blanco & Villarroya, 2002) and it can serve as a substitute for predicting the presence of specific chemical constituents in fruit and vegetables without prior sample preparation.

Recently, Cen, Lu, Zhu, and Mendoza (2016) used hyperspectral imaging for detection of CI in cucumbers utilising supervised classifiers and feature selection techniques and similar research has been successfully been pursued using hyperspectral imaging on apples and peaches (ElMasry, Wang, & Vigneault, 2009; Pan et al., 2016). Moreover, Moomkesh, Mireei, Sadeghi, and Nazeri (2017) investigated the detection of freeze-damaged sweet lemons using reflectance, half-transmittance, and full-transmittance and visible/short wave infra-red (VIS/SWIR) spectroscopy combined with various machine learning techniques.

Tsouvaltzis, Babellahi, Amodio, & Colelli, (2020) successfully reported the possibility of classifying aubergine fruit based on temperature of storage (2 °C and 12 °C) using different optical-based techniques, and showed that Fourier-transform-near infra-red (FT-NIR) spectroscopy was the most efficient technique for this aim. Nonetheless, the effects caused by temperature and the duration of storage on the spectral response were not investigated. Therefore, objective of this study was to characterise the effect of storage time and temperature (together with their possible interaction) on FT-NIR spectra of aubergine stored at chilling and above chilling temperatures, applying ANOVA-simultaneous component analysis (ASCA), in order to evaluate whether these effects could be considered statistically significant and, if so, to associate the changes of the instrumental signals to the progress of the CI. Moreover, a secondary objective was to use these results to discriminate fruit stored at the different temperatures.

2. Materials and methods

2.1. Sampling

Aubergine fruit (cv. Fantasy) were hand-harvested from a commercial farm located in Molfetta, Italy (41° 12′ 0″ North, 16° 36′ 0″ East) in July 2019. After inspection for absence of any defects and uniformity in terms of size, 87 fruits were transported to Postharvest Laboratory of the University of Foggia within 2 h of harvest. Upon arrival at the laboratory, the fruit were placed at room temperature for temperature regulation, after which they were divided into three groups. The first group including 15 fruit was categorised as fresh eggplants for initial measurements, the second group comprised of 36 eggplants was stored at chilling temperature and the third group of 36 fruit were kept at safe temperature (i.e., 2 °C and 12 °C, respectively). Fruits were then removed from cold storage after 3, 6 and 10 d and were left at ambient temperature for 5 h for temperature regulation prior to acquisition of spectra.

2.2. *Electrolyte leakage measurement*

Electrolyte leakage (EL) was used as a standard technique. Measurements were carried out according to the method described by Fuchs, Zauberman, Rot, and Weksler (1989), based on six randomly selected fruit from each group. Seven discs (5 g) of each aubergine pulp with a thickness of 10 mm each were removed from the equatorial region of every sample using a 10-mm diameter cork-borer. The discs were incubated in 25 ml solution 0.3 M of mannitol at 20 °C. The conductivity of the solution was measured using conductivity meter (CM35, Crison, Carpi, Italy) at time zero (C1) and after 2 h (C2) of incubation with orbital shaking (DAS12500, Intercontinental equipment, Roma, Italy) at a speed of 60 cycles min^{-1}. In case of the last measurement (C3), the tube including the sample and the solution was frozen and then defrosted after being kept at −20 °C for 24 h. Results were stated as a percentage of total electrolytes leaking out of the tissue as shown in Eq (1). Determinations were performed in duplicate and the results were averaged.

$$\text{Electrolytic leakage}(\%) = \frac{C2 - C1}{C3} \times 100 \tag{1}$$

2.3. *Chilling injury evaluation*

CI symptoms in aubergine fruit appear both internally and externally. Thus, evaluation was based on a checklist of four external (i.e. calyx browning, peel discoloration, pitting, and firmness) and two internal indicators (i.e. pulp browning and seek blackening) carried out by four trained panellists. For each fruit, each CI symptom had a score based on the severity (i.e., 0 = no chilling symptoms (0% of indices), 1 = moderate chilling symptoms (<50% of indices), and 2 = severe chilling symptoms (>50% of the indices)).

2.4. *Firmness and mass loss evaluation*

The firmness of each fruit was measured using a Texture Analyzer (TA.XT2, Stable Micro Systems Ltd., England, UK) equipped with a 5-mm diameter probe which was used to penetrate the eggplant pulp with a loading speed of 50 mm min^{-1} in three positions on the equator, and subsequently averaged. The maximum force (N) obtained from the force–deformation curve was used as an indication of the fruit firmness. The average maximum force was used as the firmness index of the aubergines. Mass loss was measured for each fruit using an electronic balance (EU-C 7500 DR, Gibertini, Italy) as % loss between the day 0 and the end of each cold storage period.

2.5. *FT-NIR spectroscopy*

After fruit removal from each cold storage, fruit were kept at room temperature for 5 h for temperature regulation, prior to FT-NIR spectra acquisition. A multi-purpose FT-NIR analyser (MPA, Bruker Optics, Ettlingen, Germany) was used to acquire three scans per sample taken along the longitudinal direction of the fruit and averaged to formulate a representative spectrum for that particular sample. Reflectance mode was utilised during spectral acquisition over the absorbance range of 3600–12,500 cm^{-1} at an interval of 3.8 cm^{-1} (scanner velocity 10 kHz, sample scan time 64 scans, background scan time 64 scans). The instrument was equipped with a high-energy air-cooled NIR source (20 W tungsten-halogen lamp) and a permanently aligned and the highly stable ROCKSOLID interferometer (Bruker Optik GmbH, Ettlingen, Germany).

2.6. *Chemometrics*

2.6.1. *ANOVA-simultaneous component analysis (ASCA)*

To evaluate whether one or more controlled factors (and their interactions) have a significant effect on a multivariate signal, multivariate analysis of variance (MANOVA) is normally used as the generalisation of ANOVA; however, this approach is not effective when the number of variables/wavebands is greaterthan the number of measured samples and/or when the multivariate descriptors are highly correlated amongst each another and breaks down because it cannot handle singular covariance matrices (Stohle & Wold, 1990). Hence, ASCA was designed to be a multivariate exploratory technique to cope with data matrices resulting from an experimental design (Jansen et al., 2005; Smilde et al., 2005). In fact, ASCA combines a partitioning of the variability in the original data matrix X consistent with the scheme of the ANOVA, to the bilinear modelling of the effect sub-matrices attained utilising simultaneous component analysis; a method which, under the constraints of the ANOVA scheme, is mathematically identical to principal component analysis (Smilde et al., 2005). In particular, in the case of the present study, where two factors, namely “temperature” and “storage time”, were controlled and, hence, the effect of three terms (the two factors plus their binary interaction) has to be investigated, thus the first step of ASCA involves partitioning the centred matrix X_c according to:

$$X_c = X - 1m^T = X_\theta + X_t + X_{\theta \times t} + X_{res} \tag{2}$$

where 1 is a vector of ones, m^T is the transposed mean spectrum of the samples, X_θ and X_t are the matrices accounting for the effect of the main factors, $X_{\theta \times t}$ is the effect matrix for the interaction and X_{res} is the residual matrix, assembling the variability which has not been accounted for any of the previous factors Each of the effect matrices X_i is built as follows: all the rows corresponding to a level of the specific factor/interaction contain identical copies of the mean spectrum of all the observations collected at that level.

The significance of the observed effect was evaluated by successively permutation testing and interpretation of the design terms identified as significant carried out by SCA of the corresponding effect matrix.

2.6.2. *Partial least square- discriminant analysis (PLS-DA)*

PLS-DA was applied to the wavebands identified by the ASCA model to discriminate between fruit stored at the two different temperatures. PLS-DA, is a supervised classification technique which results from partial least squares regression (PLSR). In case of a PLS-DA, a regression model between the X (data acquired from instrument) and Y (dummy binary vector for coded samples) is developed. Classification of the samples is then accomplished based on the values of the predicted Y which, unlike those of the dummy matrix used for model building, are real-valued (Brereton & Lloyd, 2014).

In practice the reliability of the model is evaluated in prediction using an external dataset (i.e., samples neither used for modelling nor for model selection), and, simultaneously, to ensure that enough samples could be used for model development and validation, a repeated double cross-validation (rDCV) strategy was adopted. Double cross-validation (DCV) consists of two cross-validation loops (an inner and an outer loop) nested in one another. The inner cross-validation loop was used for model selection (i.e., for choosing the optimal pre-processing and the number of latent variables), whereas the outer loop contains the samples which are in turn treated as external validation sets. To avoid the estimate being biased by a specific division of samples into the different cancelation groups, the whole procedure was iterated for 50 times, hence the term repeated double cross-validation (Filzmoser, Liebmann, & Varmuza, 2009). In particular, different pre-processing methods were tested on the data, i.e., standard normal variate (SNV), derivatives calculated with different number of points and orders of the interpolating polynomial, and their combinations. As stated, for each cancelation group in the outer cross-validation loop, selection of the optimal model (in terms of optimal pre-treatment and number of latent variables) was used to predict the validation samples based on the minimum classification error in the inner CV loop. SNV + first derivative (i.e. a second order polynomial and 11 points interpolation window) served as the best pre-treatment while the most optimal model consisted of three latent variables.

3. Results and discussion

3.1. *Evaluation of chilling injury and quality losses*

The CI indices during storage are shown in Fig. 1. It can be clearly seen that the fruit stored at 12 °C almost did not exhibit chilling symptoms until the end of storage. However, fruits stored at 2 °C, started to show chilling symptoms after six days of storage but they were mostly internal. After six days, chilling indices continued to increase, showing severe CI symptoms, including browning, wrinkling, and scalds in the peel and pulp browning. Observed CI indices were in agreement those reported by Tsouvaltzis et al. (2020). In that study, the CI indices began to appear after four days. It is quite possible that this occurred in the current work, since at the second sampling after six days of storage the CI indices had almost reached 1.

The results of EL measurement of aubergines revealed that from an initial value of about 9%, there was a slight alteration in EL for fruit stored at 12 °C, whereas there was a constant increase of EL was observed for fruit stored at 2 °C, reaching 12% at the end of the storage period (Fig. 2). This confirmed a higher solute diffusion for cold stored fruit as a result to membrane damages and altered permeability (Concellón et al., 2005). The increase of EL observed in this study was less pronounced than in the findings of Concellón et al. (2005) where after 13 d of storage a 5 times increase was observed for fruit stored at 0 °C, but this can be due to the variety difference, since Japanese variety are known to be more CI sensitive than American (Concellón et al., 2007).

Mass loss increased during storage at both storage temperatures, particularly for fruit stored at 12 °C. This was expected since the metabolism is higher with the increase of the temperature. As Fig. 3A shows, at the end of storage period mass loss of fruit at safe temperature was 2.2 times higher than fruit stored at 2 °C. Regarding firmness of aubergine, the samples stored at 2 °C almost maintained their initial firmness. However, fruit at 12 °C lost 33% of the firmness (Fig. 3B). This could be directly related to firmness loss since higher enzymatic activity occurs at the higher temperature (Fan et al., 2016). Even if the recommended storage temperature is 12 °C, by storing fruit at a non-chilling temperature, quality degradation is unavoidably faster than at low temperatures.

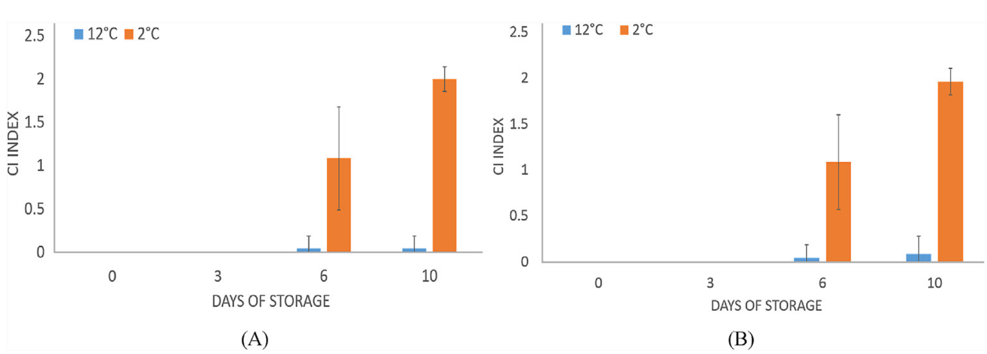


Fig. 1 – CI evaluation of aubergine fruit externally (A) and internally (B). The CI on fruit was scored as 0 = no chilling on fruit, 1 = slight CI symptoms and 2 = severe CI symptoms. Each point represents the mean of 12 fruit ± standard error (S.E.).

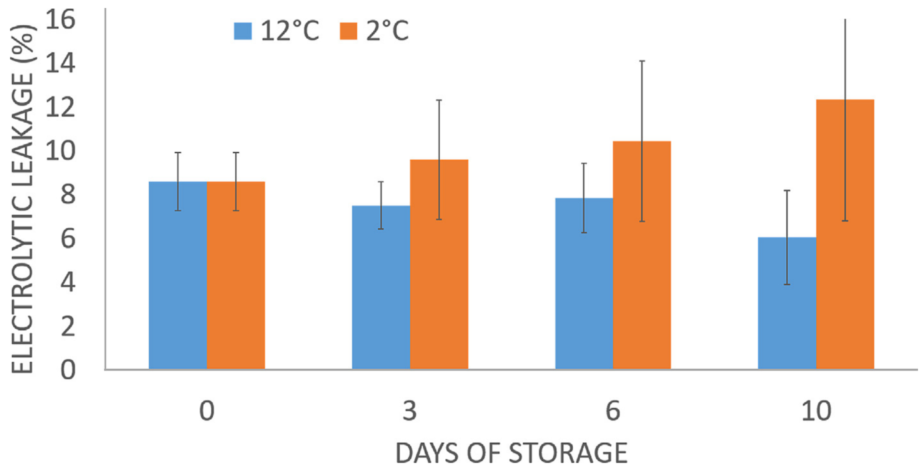


Fig. 2 – EL percentage from pulp tissue of aubergine during storage at 10 °C (blue line) and 2 °C (red line). Each value is the mean of six replicates.

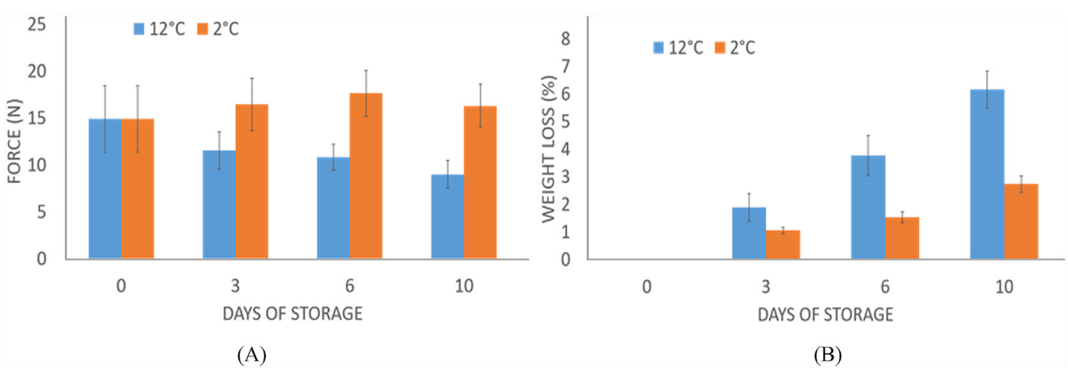


Fig. 3 – Firmness (A) and weight loss (B) of aubergine fruit stored at 12 °C (blue line) and 2 °C (red line). Data presented are the means ± SE of 12 replicate samples.

3.2. *ASCA on FT-NIR data*

This experiment was conducted based on a full factorial design, comprising the two main factors, temperature and storage time. The temperature included two levels (2 °C and 12 °C) and storage time had three levels (i.e. 3, 6, and 10 d). To investigate the effect of main factors and their interaction, a multivariate data analysis using ASCA was conducted on the data extracted from FT-NIR instrument as described at Section 2.6.1. The ASCA modelling was applied on the data after pre-processing to interpret the effects of any of main factors and their interaction on the data.

Subsequently, the mean-centred data matrix was partitioned according to the ANOVA scheme into the effect matrices for the three design terms and the residual matrix. The multivariate effect of each design term was then estimated by the sum of squares of the elements of the corresponding matrix. To evaluate whether the effect of each term could be considered as statistically significant, the value of the corresponding sum of squares was compared to its distribution under the null hypothesis, which was estimated non-parametrically by a permutation test (with 10,000 randomisations), as shown in Fig. 4. It can be clearly seen that all the effects were statistically significant, revealing that the spectral changes of aubergine fruits were affected by both temperature and storage time and that there was a non-negligible interaction between the two factors.

After showing that both the main factors and the interaction have a significant effect on the spectra, the next step regarding ASCA modelling was to interpret the observed variation using simultaneous component analysis (SCA) on the individual effect matrices. Initially, the effect of the temperature was explored, by computing a SCA model of the temperature effect matrix in which as briefly explained in Section 2.6.1. Half of the rows contained identical copies of the mean spectrum of the samples stored at low temperature and the other half were made up of identical copies of the mean spectrum of the fruit stored at high temperature, after centring.

In order to illustrate the variability related to the effects of a certain factor in ASCA model, residuals were projected on the simultaneous component (SC) space for that design factor. In the case of temperature, where a one component model explained 100% variance in the effect matrix, this was accomplished by calculating the score vector, as shown below (Zwanenburg, Hoefsloot, Westerhuis, Jansen, & Smilde, 2011):

$$t_{\theta+res} = (X_\theta + X_{res}) \times p_\theta \tag{3}$$

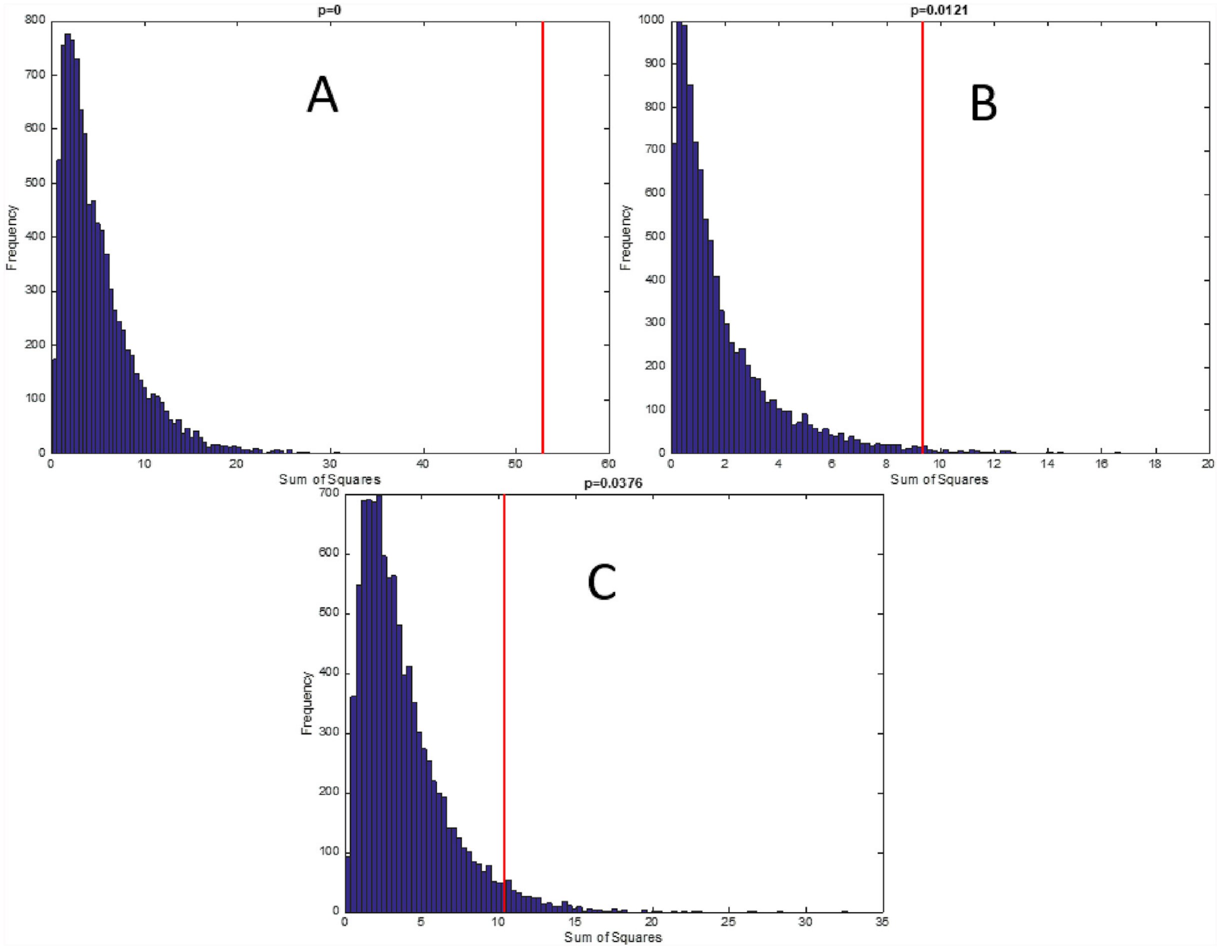


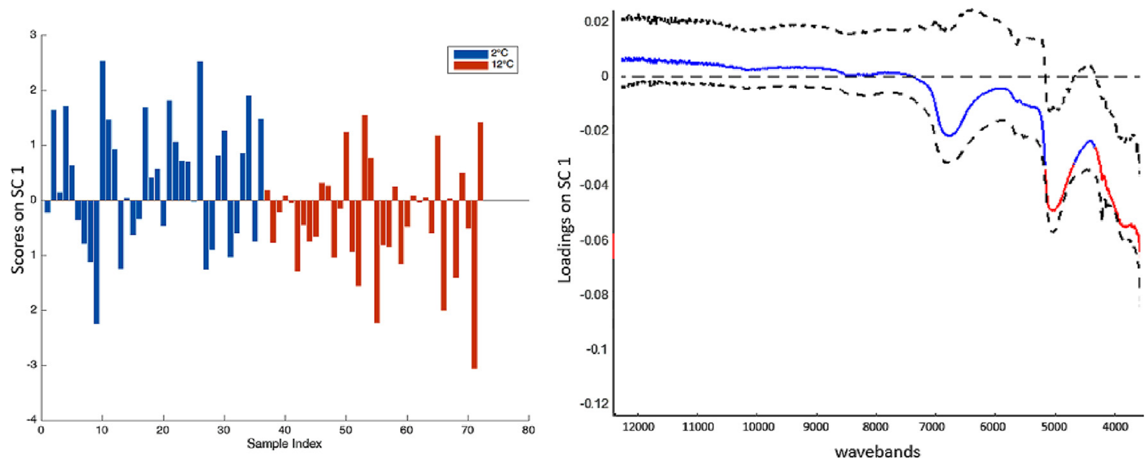
Fig. 4 – Assessment of the significance of the observed effects by comparing the experimental sum of squares (vertical red line) to its distribution under the null hypothesis, non-parametrically estimated via permutation tests (blue histogram). (A) Effect of temperature; (B) effect of storage time; (C) effect of temperature × storage time interaction.

Where p_θ is the loading vector of the SC model for the effect of the factor "temperature". The corresponding scores plot, shown in Fig. 5A, shows how the difference between the scores from 2 °C to 12 °C can be considered statistically significant as evaluated by means of permutation tests. Figure 5B shows the loadings of all the variables on SC1, together with their 95% confidence interval, indicating the spectral regions mostly affected by the temperature (in red). As can be seen that only a reduced part of the spectral range was significantly affected by the temperature of storage (i.e. 5490–4740 cm^{-1} and 4555–3600 cm^{-1}). These regions are related to the stretching of O–H bonds, associated with sugars, and to the stretching of C–H bonds (Siedliska, Baranowski, Zubik, Mazurek, & Sosnowska, 2018). It is very possible that a different accumulation of sugars occurred at the different temperature as consequence of higher metabolism at the highest temperature of storage. Moreover, some authors have reported a reduction of sugars during aubergine storage at 5 °C but conversely observed an increase at 10 and 20 °C (Esteban, Molla, Villarroya, & Lopez-Andreu, 1989).

Because positive and negative scores were reported for SC1 samples stored at 2 °C and 12 °C, investigation of the loadings plot in Fig. 5B highlights that the significant bands (shown in red) are more intense, have higher pseudo-absorbance, when storage takes place at higher temperatures.

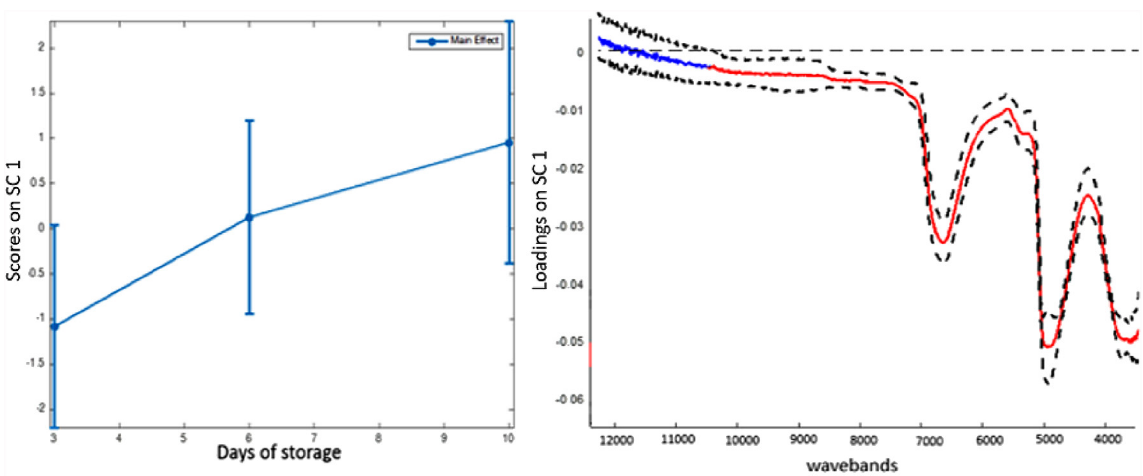
On the other hand, when considering the main effect of time, since this factor was examined at three levels, the first two SC jointly explain 100% of the spectral variance related to the design term. After projection of the residuals, scores and loadings for SC1 are shown in Fig. 6A and B. To account for the time trend, in Fig. 6A, for each level of the factor time the scores along SC1 for the corresponding observations are reported as means (i.e., the score which would result without projection of the residuals) ± standard deviation. As it can be seen that the scores increased with time, and almost all the range was significantly affected by the time of storage (10,000–3600 cm^{-1}). Nonetheless, since temperature and time of storage also showed a significant interaction, it may be better to investigate and interpret jointly the main effect of storage time and the storage time × temperature interaction, so as to highlight not only the average effect of storage time on the extracted spectra, but also how the two temperatures differently affected the temporal behaviour.

Thus, instead of analysing each factor separately, SCA was carried out on the matrix $X_{t+t\times\theta}$. Since the matrix $X_{t+t\times\theta}$ contained identical copies of six mean spectra only, corresponding



(A) (B)

Fig. 5 – ASCA analysis on FT-NIR data: SCA model of the temperature effect. (A) Scores plot for the effect with projected residuals; (B) variable loadings for SC1 (continuous line) together with their 95% confidence interval (black dashed lines): red and blue colours indicate whether the corresponding wavebands contributes significantly or not to the bilinear model, respectively.



(A) (B)

Fig. 6 – ASCA analysis on FT-NIR data: SCA model of the storage time effect. (A) Longitudinal plot of the scores along SC1 (expressed as mean ± standard deviation after projection of the residuals) vs time; (B); variable loadings for SC1 (continuous line) together with their 95% confidence interval (black dashed lines): red and blue colours indicate whether the corresponding wavebands contributes significantly or not to the bilinear model, respectively.

to the six different possibilities coming out from the combination of factor levels (3d at 2 °C, 6d at 2 °C, 10 d at 2 °C, 3d at 12 °C, 6d at 12 °C, 10 d at 12 °C), for each component there were only six different score values identifying the design cells. However, it is also possible in this case to project the residual matrix onto the SC subspace defined by the effect matrix for visualizing the variability associated with the factor + interaction levels and, consequently, to have a visualisation of the significance of the design terms.

To have a better perception of the storage time effects and the changes in temporal behaviour allied to the storage temperatures, the scores along SC1 and SC2 (together with their error bars representing the projected residuals) have been plotted as a function of time in Fig. 7A and B.

Examining Fig. 7A and B, it can be seen that the two main trends are linked to the effect of storage time. Component 1 accounted for a constant decrease of the score but an inversion was perceived at day 6 which might be related to chemical transformations caused by CI. Therefore, the spectral bands with negative loadings of this component will decrease their pseudo-absorbance with time which is contrary to that for bands having positive loadings. Instead, scores along component 2 showed a maximum corresponding to the sixth day of storage followed by a decrease. This alternative trend could be recognised as a

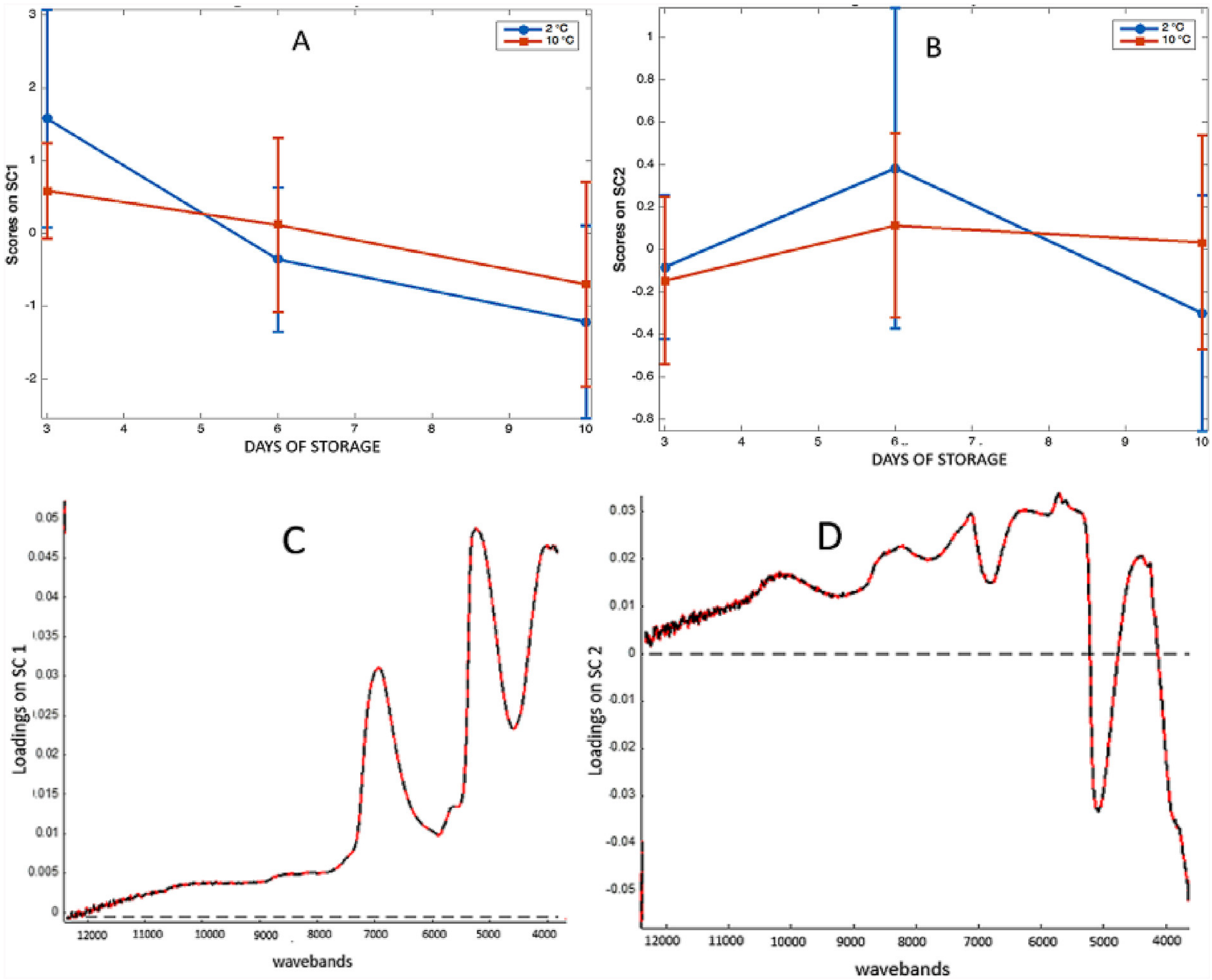


Fig. 7 – ASCA analysis on FT-NIR data: SCA model of the effect of storage time + temperatures × time interaction (A) Longitudinal plot of the scores along SC1 (expressed as mean ± standard deviation after projection of the residuals) vs storage time: aubergine fruit stored at 2 °C and 12 °C are indicated as blue circles and line or red circles and line, respectively; (B) longitudinal plot of the scores along SC2 (expressed as mean ± standard deviation after projection of the residuals) vs storage time: aubergine fruit stored at 2 °C and 12 °C are indicated as blue circles and line or red circles and line, respectively; (C) variable loadings for SC1 (continuous line) together with their 95% confidence interval (black dashed lines); (D) variable loadings for SC2 (continuous line) together with their 95% confidence interval (black dashed lines). In panels (C) and (D), red and blue colours indicate whether the corresponding wavebands contributes significantly or not to the bilinear model, respectively.

development of CI, since it was also described in the first component, which were then further transformed as time progressed. Thus, given the sign of the scores, it can be seen that the spectral regions having positive loadings along SC2 reached their minimum pseudo-absorbance on the sixth day of storage and then increased to their primary values at longer periods. The opposite occurred for variables with negative loadings.

It is noticeable (Fig. 7C and D) that storage temperature and time of storage (and their interaction) significantly affected all the FT-NIR spectra since all the loadings were statistically different to zero. Thus, according to the phenomena described previously for SC1, it could be concluded that by increasing the storage time, there is an increase in the pseudo-absorbance of all the bands possessing positive values in the loading on SC1, whilst the remaining variables decreased their signal.

3.3. *Classification model discriminating fruit stored at chilling temperature*

As the ASCA model showed that temperature had a significant effect on the FT-NIR spectra, a classification model using the PLS-DA algorithm was carried out on the data for classifying the fruit stored at chilling and safe temperatures. In order to check the validity of the ASCA model, as well as to reduce the complexity of the classification model, only those wavebands identified by ASCA were used to discriminate between fruit stored at different temperatures. In this regard, 36 samples stored at chilling temperature (3, 6, and 10 d of storage) and 36 samples stored at safe temperature (3, 6, and 10 d of storage) were labelled as chilled and healthy fruit, respectively. The final dataset resulted in a matrix including 72 samples × 369 effective

Table 1 – Results of classification model for discriminating fruit stored at chilling temperature.

Class	Number of LVs	Inner rDCV loop			Outer rDCV loop		
		Sensitivity	Specificity	Accuracy	Sensitivity	Specificity	Accuracy
Healthy	3 ± 0	91.1 ± 3.2%	84.7 ± 3.7%	87.9 ± 2.1%	90.2 ± 4.0%	84.7 ± 3.1%	87.4 ± 2.7%
Chilled		84.7 ± 3.7%	91.1 ± 3.2%		84.7 ± 3.1%	90.2 ± 4.0%	

wavebands. Those identified by ASCA are shown in Fig. 5B but the whole range of FT-NIR spectra comprised 2307 wavebands.

The use of rDCV allowed the external validation of the PLS-DA model to be repeated on the outer loop samples as many times as the number of DCV runs. This allowed not only a point estimate of the predictive ability of the model to be obtained, but also a corresponding confidence interval. Thus, the classification accuracy could be more robustly evaluated. In particular, when considering the outer DCV loop, i.e., the one mimicking an external validation set, it was found (Table 1) that 90.2 ± 4.0% of the healthy and 84.7 ± 3.1% of the chilled fruits were correctly classified, leading to an overall accuracy of 87.4 ± 2.7% (corresponding to a value of the area under the receiver operating characteristic (ROC) curve of 0.941 ± 0.016). Most of misclassified samples belonged to fruit after 10 d of storage, suggesting that by this time senescence may have had a higher impact on spectral changes than temperature and CI. This confirms the findings of the ASCA about the inversion of the score along SC2 when the effect of storage time + temperatures × time interaction was considered. These results, nonetheless, indicate good discrimination between the fruit according to the storage temperature, particularly considering that fruit are normally transported to market within a few days after harvest.

Moreover, to rule out the possibility that these outcomes resulting from chance correlation, the observed values of classification accuracy and of the area under the ROC curve were compared to their distributions under the null hypothesis, which were non-parametrically estimated by means of a permutation test with 1000 randomisations. For both the classification figures of merit, an empirical p-value <0.001 was obtained, thus confirming that the observed discrimination between the classes can be considered highly statistically significant. The validity of PLS-DA results based on the spectral region introduced by ASCA were endorsed by comparing with the results of work performed by Tsouvaltzis et al. (2020). However, in their study they used full range FT-NIR spectra (12,500–3600 cm^{-1}) of aubergine for discriminating fruit stored at chilling temperature, but the accuracy of the classifier (PLS-DA) was still less than this study.

The ASCA method is receiving attention for analysis of hyperspectral data, but few applications can be found for the postharvest handling of fruit and vegetable. For instance, Leisso et al. (2016) explored ASCA for gene expression and metabolism preceding for soft scald, a CI of 'Honeycrisp' apple fruit, but this method is time-consuming and compared to spectroscopy needs expertise even for sample preparation. For coffee analysis, De Luca et al. (2016) utilised this method for high-performance liquid chromatography-diode-array detector (HPLC-DAD), NIR data. However, after realising the effect of varieties and roasting time, the effective wavebands were not used as the input of classifier, unlike this study where the ability of ASCA was verified by setting its output as an input to a PLS-DA classifier.

Our study was designed at to use ASCA modelling as an additional investigation tool (Tsouvaltzis et al., 2020) to gather information on the effects of temperature and time of storage on the spectral data extracted from non-invasive instrument.

4. Conclusion

As chilling injury (CI) in subtropical fruits such as aubergine is a critical disorder, it is important to understand how the temperature and duration of storage may impact on fruit quality. To this aim non-destructive techniques such FT-NIR spectra can be used. For the first time, in this kind of study, the effects of temperature and storage time on fruit spectral response were investigated. By applying ANOVA-SCA, it was found that both the "temperature" and the "storage time" factors, as also their interaction, significantly affected the spectral profile of eggplant fruit over storage. For each factor the most significant wavebands were isolated. This information could be used to build a PLS-DA for discriminating eggplant fruit based on the temperature. The PLS-DA model was shown to classify fruit with high accuracy 87.4 ± 2.7% (90.2 ± 4.0% for healthy and 84.7 ± 3.1% for chilled fruit; evaluated on the outer loop of a repeated double cross-validation procedure). It is worth stressing that the proposed approach allowed the classification of chilled and healthy fruit by a rapid, comparatively cheap and non-invasive technique (FT-NIR), without requiring any sample pre-treatment and irrespective of the days of storage. From this viewpoint, the results achieved indicate this could be a promising tool for selecting and discarding unhealthy fruit and decreasing commercial food losses.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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