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NIR-HSI for the non-destructive monitoring of in-bag hazelnut oxidation☆

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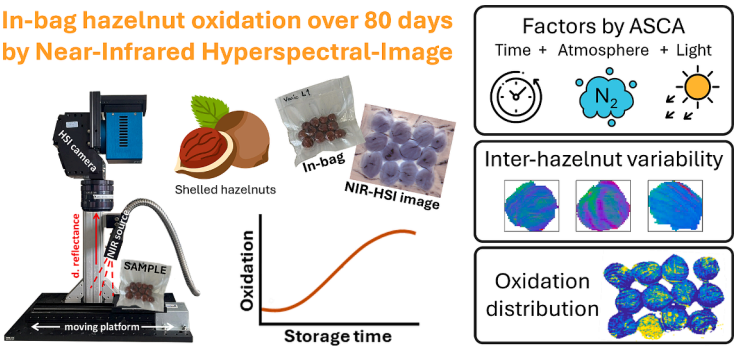
HIGHLIGHTS

- NIR-HSI enables non-destructive monitoring of in-bag hazelnut oxidation.
- Vacuum storage minimises oxidation, while light exposure accelerates it.
- Spectroscopic findings align with sensory analysis of rancidity.
- NIR-HSI effectively studies hazelnut variability and oxidation distribution.
- Promising methodology for real-time food quality and shelf-life assessment.

GRAPHICAL ABSTRACT



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ABSTRACT

This study explores the application of Near-Infrared Hyperspectral Imaging (NIR-HSI) as a non-destructive method for monitoring the oxidation of hazelnuts (*Corylus avellana)* stored under different atmospheric conditions (air, nitrogen, and vacuum) and light exposures, directly inside plastic bags, without the need for packaging removal nor sample preparation. Hazelnuts were imaged over a 78-day storage period, and data were analysed using ANOVA-Simultaneous Component Analysis (ASCA) to quantify the effects of time, atmosphere, and light on oxidation. Individual hazelnuts within each bag were segmented for analysis to study inter-hazelnut variability. Images were studied individually to detect oxidation patterns on the surface. Results indicate that time is the primary driver of oxidation, as expected, with atmosphere and light significantly impacting oxidation rates. Vacuum storage was most effective at reducing oxidation, while light exposure under ambient conditions accelerated oxidation via photooxidation. Sensory analysis confirmed that spectroscopic indicators of oxidation corresponded to perceptible sensory changes, validating NIR-HSI as a reliable tool for assessing hazelnut quality.

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This study underscores the potential of NIR-HSI and chemometrics for quality control and shelf-life assessment in hazelnuts and other oxidation-prone food products.

1. Introduction

The Mediterranean region provides the ideal climatic conditions for cultivating high-quality hazelnuts (*Corylus avellana)*. The combination of mild winters, warm summers, and well-drained soils creates an optimal environment for the growth of hazelnut trees, resulting in superior quality nuts with distinctive sensory properties [1]. Consequently, approximately 75 % of the global hazelnut production comes from this area, mainly Turkey, Italy, France and Spain [2,3]. The quality of Mediterranean hazelnuts is recognized by four Protected Designations of Origin (PDOs) and two Protected Geographical Indications (PGIs), underscoring the diversity and authenticity of hazelnut products from this area [4].

Hazelnuts can be consumed in various forms – natural, blanched, roasted, or further processed into products such as slices, chopped nuts, oil, or flour; each of these forms offers unique culinary properties for a wide range of applications [5]. The versatility of hazelnuts contributes to their demand in international markets, which continues to grow due to their high nutritional value [3]. Hazelnuts are rich in unsaturated fatty acids, particularly oleic acid, as well as vitamins E and B, magnesium, and dietary fibre, making them an excellent choice for a balanced diet [6].

All hazelnut producers must dry their harvests to facilitate storage, transportation, and commercialization since freshly harvested hazelnuts have a high moisture content, which makes them prone to spoilage due to microbial growth and enzymatic activity. The moisture content of freshly harvested hazelnuts typically ranges from 20 % to 30 %, which needs to be reduced to around 5–7 % to ensure long-term stability. However, the air-drying process used to reduce moisture content does not decrease the likelihood of rancidity and browning reactions, that lead to undesirable colours, off-flavours, and textural changes [7,8].

The primary cause of rancidity in dried hazelnuts is lipid peroxidation, which mainly affects unsaturated fatty acids such as oleic and linoleic acids. Lipid peroxidation is initiated by the action of oxygen, light, and sometimes metal ions, resulting in the formation of hydroperoxides and secondary oxidation products, which contribute to off-flavours and reduced consumer acceptability. This reaction is autocatalytic, meaning that its rate increases as the concentration of peroxidation products rises, accelerating the deterioration process. Once initiated, lipid peroxidation keeps the reaction going during its shelf-life. It is for this reason that controlling factors such as temperature, oxygen exposure, and light during storage is essential to mitigate this process [9,10]. In addition to lipid peroxidation, browning reactions, such as Maillard and enzymatic browning, can also occur during the drying and subsequent storage of hazelnuts, further impacting their colour and nutritional and sensory quality [11].

Several strategies have been developed to address these quality issues and improve the stability of dried hazelnuts. Modified atmosphere packaging (MAP) is an approach where the oxygen content within the packaging is reduced and replaced with an inert gas such as nitrogen or carbon dioxide to minimise oxidative reactions, or vacuum packaging, that also limits oxygen exposure [12–14]. Additionally, storing hazelnuts in a cool, dark environment helps slow down the rate of lipid oxidation and browning reactions [10].

To evaluate the extent of these oxidative reactions, rancidity has been the historically used indicator, assessed using destructive methods. The main method is sensory evaluation, a widely used reference method that assesses rancid flavours and odours, but is subjective and dependent on the expertise and perception of panellists, and it is inherently time-consuming, labour-intensive, and requires the destruction of samples. More advanced instrumental techniques have also been proposed, these techniques typically involve extracting lipids and performing chromatography analysis or measuring oxidation markers such as the peroxide value, the ratio of oleic to linoleic and the iodine value (IV) or using tailored analysis methods such as the Rancimat. While these methods provide more accurate and reliable results, they are still time-consuming and require preparation and destruction of samples. While these traditional techniques provide valuable insights into lipid oxidation and off-flavour development, they are not suitable for large-scale or real-time monitoring and non-invasive quality control during packaging, storage and distribution [15,16].

However, the development of non-destructive techniques, such as near-infrared (NIR) spectroscopy provides an innovative approach to monitor the quality of hazelnuts during storage, allowing producers to assess oxidation levels in real-time. This technology can enhance quality control processes, ensuring that hazelnuts maintain their desired sensory and nutritional attributes throughout their shelf life [17–19]. Additionally, when Hyperspectral Imaging (HSI) is used, it also provides spatial information [20], as it can be used to analyse the distribution of rancidity within individual hazelnuts and the variability among hazelnuts in the same package without opening the packaging.

The aim of this study was to determine the suitability of NIR-HSI for monitoring hazelnut rancidity under different MAP conditions without removing the container bag. Furthermore, we evaluated the spatial distribution of rancidity both among hazelnuts within the same bag and within individual nuts. This research provides valuable insights into the effectiveness of MAP in mitigating oxidation and highlights the potential of NIR-HSI as a comprehensive tool for non-destructive quality evaluation.

2. Material and methods

2.1. Samples

The studied hazelnuts (*Corylus avellana*), of the 'Ribeta' variety, representative of the Mediterranean cultivars, were from a single batch (collected at the same time from a single orchard in 2023) to maintain consistency in the initial quality and composition of the nuts. The orchard is located in Alforja (Catalonia, Spain), with an altitude of 374 m of altitude and 18 km away from the Mediterranean Sea. The hazelnuts were shelled and placed in bags, with each bag containing 12 hazelnuts. Throughout the experiment, 18 bags were prepared: three atmosphere conditions (air, nitrogen and vacuum), two light exposure conditions (light and dark), and three replicates of each. Samples exposed to natural daylight were kept on a laboratory benchtop at ambient temperature (23 ± 3 °C). Samples stored in darkness were maintained at a constant 20 ± 1 °C and only exposed to light during spectral measurements. Two types of bags were used: one type for air and nitrogen atmospheres and another for vacuum. Both were made with the same polymer (low density polyethylene; LDPE); however, the vacuum bags were slightly thicker. This choice was necessary after preliminary tests, as the bags for air and nitrogen could not be vacuum sealed (due to them not being adaptable to the vacuum machine), while the vacuum bags could not retain sufficient internal gas volume for the air and nitrogen conditions.

2.2. Hyperspectral imaging

Hyperspectral image acquisition was performed using a NIR line-mapping hyperspectral camera (Fig. S2) covering a spectral range of 1000 to 1600 nm with 142 wavelengths and a typical spectral resolution of 4.2 nm (Headwall Photonics, Inc. Massachusetts. USA, kindly donated

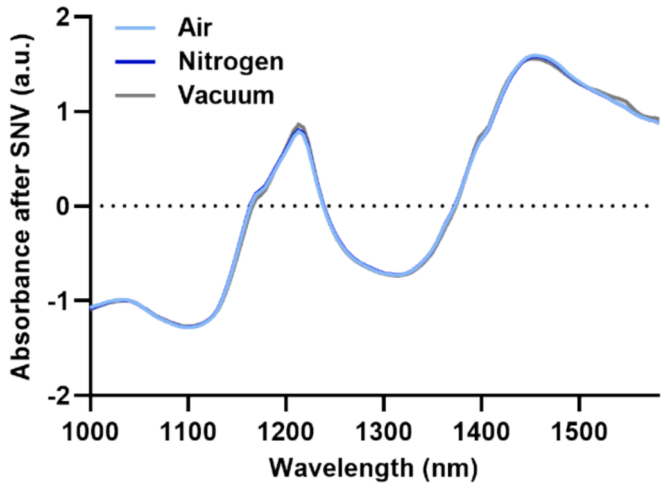

Fig. 1. Average NIR spectra (after SNV normalisation for scattering correction) of the hazelnut bags in the three packaging conditions.

by FOSS Analytics A/S, Denmark). The line mapping scanning system consisted of 320 sensors located at around 30 cm above the sample, measuring a 10 cm of field-of-view-line, and moving the sample underneath in steps of 300 μm, providing a spatial resolution of around 300 μm in both X and Y axes. The measurements were made in diffuse reflectance mode and converted to absorbance units by using blank and dark references used for calibration, measured at the beginning of each day [21]. Each hazelnut bag was imaged as a whole to capture a spectral profile representative of all nuts within the bag, obtaining images of approx. 5 × 10 cm. NIR-HSI images were acquired at progressively spaced intervals: every day at first, then every other day, twice a week and finally weekly; resulting in 23 points over a period of 78 days. This experimental design was designed to capture changes from early storage stages to advanced degradation, anticipating that oxidation might proceed faster in the first days after being shelled and stabilised at the end of the experiment.

After acquiring the images, the background of each of them was removed by applying a Principal Component Analysis (PCA) to each image and thresholding the scores (Fig. S3a), as the scores of the background are distinct from those of the pixels representing hazelnuts. This method also effectively removed reflections and shines on the plastic bags caused by wrinkles, which became more pronounced toward the end of the experiment due to increased handling. After that, the mean spectrum of each bag on each day was calculated. Additionally, to analyse the oxidation variability at the level of individual nuts, each hyperspectral image was further segmented into 12 distinct sub-images, with each sub-image representing a single hazelnut. This was done by developing a custom Matlab code that mostly automatised the process, based on the watershed transform (Fig. S3b) [22]. However, the final result for each image had to be checked, and some splits were corrected manually (Fig. S3c). The Matlab code is available at: https://www.chemosens.recerca.urv.cat/en/research/resources/.

2.3. Sensory analysis

A sensory analysis was conducted on the final day of the experiment to validate the spectroscopic findings with consumer-perceived sensory qualities. A panel of 18 non-trained consumers (nine men and nine women, aged 22–54, including two smokers) participated in the study. Due to limited sample availability, each panellist evaluated two hazelnuts from each storage condition (air, nitrogen, and vacuum packaging, under both light and dark conditions), resulting in a total of twelve samples per panellist. To ensure unbiased evaluation, the samples were presented in a randomised order (each condition per panellist at the same time) and coded.

Sensory evaluations took place in a controlled sensory analysis room under standardized conditions, with uniform lighting and neutral background. Panellists were instructed to assess key sensory attributes related to oxidation using a 10 cm unmarked continuous scale [23,24]. The assessed attributes were: colour (light to dark), aromatic intensity, rancid aroma, hardness, crispness, oiliness, flavour intensity, rancidity, wood, sweetness and bitterness; based on Król *et al.* [25]. To minimize variability among non-trained panellists, all responses were normalized using Euclidean norm normalization before statistical analysis.

2.4. Statistical analysis

All the statistical analysis was performed in the Matlab (R2022b; MathWorks, Natick, MA) environment. The Hyper-Tools v4.0 toolbox (Hypertools, Bilbo, Spain) [26] was used for the image processing, and the PLS_toolbox 9.0 (Eigenvector Research Inc., Manson, WA) for building multivariate models, altogether with custom-made code for managing the data, automation of the data curation and fitting of sigmoid curves.

2.4.1. Anova-simultaneous component analysis

ANOVA-Simultaneous Component Analysis (ASCA) is a multivariate statistical method designed to separate and quantify the effects of different experimental factors on complex datasets [27]. In this study, ASCA was applied to determine how each factor (time, atmosphere and light) influenced the oxidation process in hazelnuts and to isolate the contributions of each factor and their interactions to the overall spectral variability. The same approach was used to study the data from sensory analysis.

The ASCA algorithm operates by combining the principles of Analysis of Variance (ANOVA) and Simultaneous Component Analysis (SCA), making it particularly useful for understanding NIR data, which is multicollinear. ANOVA is used to partition the data based on the factors of interest (e.g., time, atmosphere, and light), effectively separating the dataset into different matrices that represent to each factor or interaction. This step allows us to isolate how much of the total variability in the data can be attributed to each factor independently, which is one of the results of ASCA. This is validated by permutation test, permuting the DoE and recalculating the explained variance, in an analogous way to permutation test in univariate ANOVA; 1000 permutations were used [28,29].

Once the data is partitioned, SCA (analogous to PCA for ASCA models) is applied to each matrix to extract the main patterns or principal components that represent the variation within each factor or interaction. By applying SCA to each factor component isolated by ANOVA, ASCA allows us to visually assess the dominant trends associated with each factor, such as how oxidation changes over time, how it differs across atmospheric conditions, and how light exposure affects the process. ASCA interpretation is homologous to interpretation of scores and loadings obtained by PCA.

2.4.2. Partial leas squares regression

Partial Least Squares (PLS) regression was employed to model the relationship between the spectral data (independent variables) and the storage time (dependent variable). This technique was chosen due to its robustness in handling datasets with high collinearity among predictors and its ability to process data where the number of predictors exceeds the number of observations. PLS reduces the dimensionality of the predictor variables by projecting them onto a smaller set of latent variables that maximize the covariance with the dependent variable, ensuring both effective prediction and interpretability [30].

Prior to PLS modelling, the spectral data underwent pre-processing to enhance signal quality and reduce noise, which are critical for improving model performance. Pre-processing steps included Standard Normal Variate (SNV), Savitzky-Golay smoothing and derivative and

Table 1
Results of the ASCA model applied to the average NIR spectra of each bag (after SNV) considering all the experimental factors.

	Effect (%)	*p*-value
Storage time	22.65	0.001
Atmosphere	12.71	0.001
Light	9.08	0.001
S. Time × Atmosphere	9.67	0.001
S. Time × Light	5.42	0.001
Atmosphere × Light	2.12	0.001
Residuals	38.35	–

mean-centring. The PLS model was validated using a 5-fold cross-validation approach (with 5 iterations) to optimise the number of latent variables. Model performance was evaluated using the root mean square error of cross-validation ($RMSE_{CV}$) to quantify the predictive performance of the model.

3. Results and discussion

3.1. Assessment of factors affecting oxidation

3.1.1. Oxidation studied by NIR-HSI

As an initial exploration of the data, a global ASCA model was built to evaluate the effects of the considered factors (storage time, atmosphere, and light exposure) on hazelnut oxidation through the average NIR spectra of each bag at different points in time (summarised in Fig. 1). Structuring the data into a global model allowed for the identification of general trends in rancidity progression and the assessment significance of the considered experimental factors. This approach provided a comprehensive first analysis of the complex interactions that influence the properties of the hazelnuts.

The general ASCA model, summarised in Table 1, reveals the relative contributions of different factors to the oxidation process in hazelnuts. The results indicate that all the considered factors and their interactions are significant (p-value = 0.001). However, as expected, the 'Storage time' factor is the primary factor driving oxidative changes, accounting for around 23 % of the total variance. This significant effect reveals that the oxidation process progresses due to the autocatalytic nature of lipid peroxidation, where the accumulation of peroxidation products accelerates further reactions and degradation in the nuts.

Light exposure (Fig. 2a) emerges with a considerable impact, contributing 9 % to the variance of the NIR spectra. Light exposure, especially under ambient temperature conditions, can accelerate oxidation through a process known as photooxidation. Unlike autoxidation, which progresses slowly and requires an initial radical to trigger lipid peroxidation, photooxidation occurs in the presence of light and typically involves molecules that absorb photons and transfer energy to oxygen molecules [24,31]. Photooxidation in hazelnuts is particularly relevant for the lipids in the outer layers of the fruit, which are directly exposed to light. When hazelnuts are stored under ambient light and temperature conditions, the effect of photooxidation is accelerated [14,24]. The susceptibility of unsaturated fatty acids, particularly oleic and linoleic acids present in hazelnuts, to photooxidation means that light exposure can lead to rapid degradation and the formation of hydroperoxides and secondary products. These by-products contribute to rancid flavours and aroma, compromising sensory qualities and consumer acceptability. Additionally, the presence of light and variable ambient temperatures may exacerbate oxidative damage by creating an unstable environment that facilitates both thermal and photo-initiated oxidation pathways, accelerating the overall deterioration process. As expected, loadings of both factors (Fig. 2b and Fig. 2d) highlight different spectral regions, indicating that the compositional changes produced on the sample by the two types of oxidation are different and can be observed.

Even more importantly, atmospheric conditions (Fig. 2c) stand out as the second most influential factor (after time), explaining around 13 % of the variance. This finding suggests that MAP, due to the available oxygen levels, play a significant role in influencing lipid oxidation processes. The susceptibility of unsaturated fatty acids, such as oleic

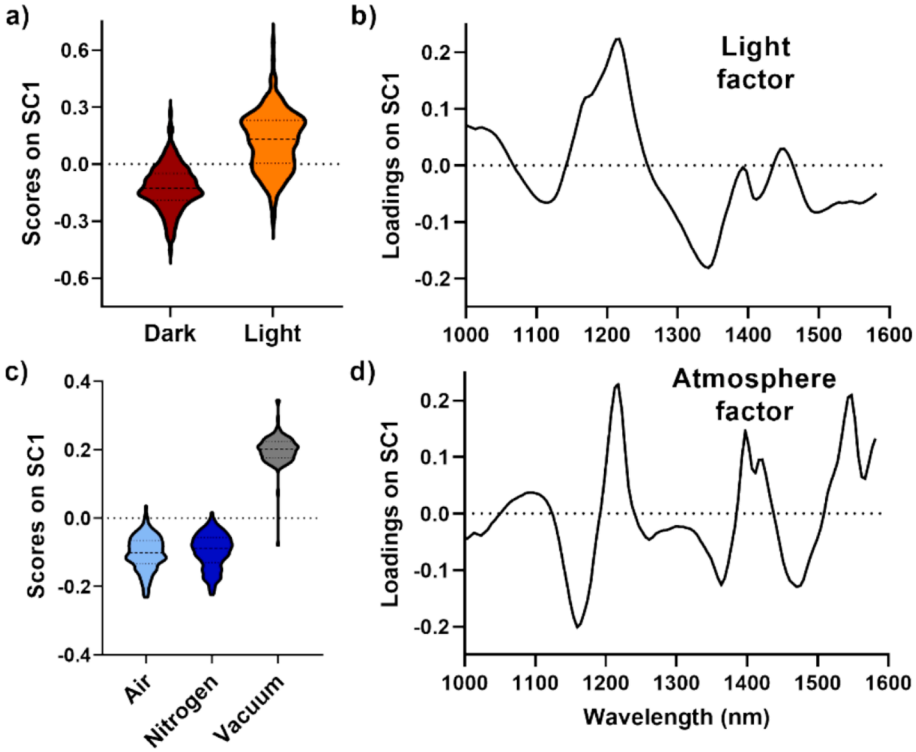

Fig. 2. Violin plots of the scores (a and c) and loadings plot (b and d) for the 'Atmosphere' and 'Light' factors, respectively, from the global ASCA model performed on the average NIR spectra of the hazelnut bags.

(C18:1) and linoleic (C18:2) acids, to oxidative reactions is well-documented, with their oxidation rates following the relative pattern of 1:10:20 for oleic, linoleic, and linolenic acids [31,32]. Reducing oxygen exposure appears to effectively limit the oxidation rate and the final state of oxidation, thereby better preserving the sensory and nutritional quality of hazelnuts during storage.

In addition, the interaction terms reveal complex dependencies between factors. The time-atmosphere interaction explains around 10 % of the variance, while the time-light interaction accounts for 5.42 % of the variance. This indicates that the effect of time on oxidation is influenced by the storage conditions, as expected. This is, oxidation accelerates more under specific storage conditions (exposure to light, ambient temperature and available oxygen, in this case), reinforcing the importance of MAP and light control to extend shelf life. Overall, these results demonstrate the effectiveness of HSI-NIR analysis for studying lipid oxidation in hazelnuts without the need to open the packaging, allowing for a non-invasive assessment.

Regarding the distribution of the scores of the atmosphere factor (Fig. 2c), they show that the main difference lies between the vacuum packaging and air and nitrogen packagings, the air and nitrogen packagings being similar to each other. When the ASCA analysis is repeated without the vacuum condition (submatrix without vacuum condition), the effect of atmospheres (difference between air and nitrogen) remains significant (p-value of 0.031) but very low (0.88 % of variance). Additionally, the spectra of the in-bag hazelnuts for the three packaging types (Fig. 1) show almost identical spectral properties. Therefore, it can be concluded that while spectroscopic differences may arise due to physical differences between vacuum and gas-filled bags (such as the distance between the hazelnut and the bag surface or the thickness of the plastic bag; Fig. S1), these are not the primary cause of the difference between gas-filled and vacuum bags. This is, instead, likely due to the permeability of the bag material used, which allowed oxygen to enter slowly over the 78-day study period, as observed by the gradual loss of gas volume in both nitrogen and air bags. This suggests that the polymer used in the gas-filled packaging is not entirely hermetic. As a result, oxygen diffusion occurred, impacting the internal atmosphere and leading to oxidation processes that influence both sensory and spectroscopic profiles, making air and nitrogen conditions more similar to each other and different to the vacuum one, which remained hermetically sealed. Consequently, to better study the rest of the factors, the dataset was divided to build three ASCA models, treating the three atmosphere conditions as separate experiments, as shown in Table 2.

The ASCA models for air and nitrogen atmospheres show consistent results: both time and light factors are significant. As expected, time emerges as the dominant factor influencing hazelnut oxidation, explaining 38.04 % and 42.59 % of the variance, respectively. This result emphasizes the role of time in driving oxidation processes when oxygen is present, even in reduced amounts as in nitrogen packaging. The total variance in the air and nitrogen atmospheres is similar (shown by the sum of squares, Table 2), and the variance explained by the storage time is also similar; this indicates that the oxidation state reached by the hazelnuts in both MAPs is similar. Light exposure also plays a significant role in both air and nitrogen conditions, accounting for around 11 % and 17 % of the variance, respectively. As already discussed, the presence of light likely induces photooxidation, a process that can occur even in low-oxygen conditions through reactions initiated by light-sensitive molecules present in the nut matrix such as polyunsaturated fatty acids or phenolic compounds.

In contrast, the vacuum condition shows different results to the other MAP: the 'Storage time' factor is not significant, which reflects the substantial reduction in oxidation due to minimal exposure of hazelnuts to oxygen in this packaging. The 'Light' factor, even though significant, has a smaller effect (around 7 %) in vacuum than in the other atmospheres, suggesting that even in the absence of significant oxygen some photooxidative reactions may happen, but in a more limited way. Based on these results, the oxidation process will not be further investigated under vacuum conditions. Instead, air and nitrogen packaging conditions will be further studied, as they provide a more representative description of the oxidation process. The high residuals, particularly in vacuum (63 %), likely arise from natural biological variability among the hazelnut bags. Since hazelnuts are natural products, factors such as genetic differences, lipid composition, and microstructural variations contribute to differences in oxidation rates, even when stored under identical conditions.

Table 2
ASCA model results for each of the atmosphere conditions using the average NIR spectra (after SNV) of each hazelnut bag. *: significant factor (p-value < 0.05).

	Effect (%), Air	Effect (%), Nitrogen	Effect (%), Vacuum
Storage time	38.04*	42.59*	17.72
Light	10.52*	16.63*	6.88*
S. Time × Light	12.40	8.33	12.09
Residuals	39.04	32.44	63.32
Sum of Squares	73.79	73.32	85.31

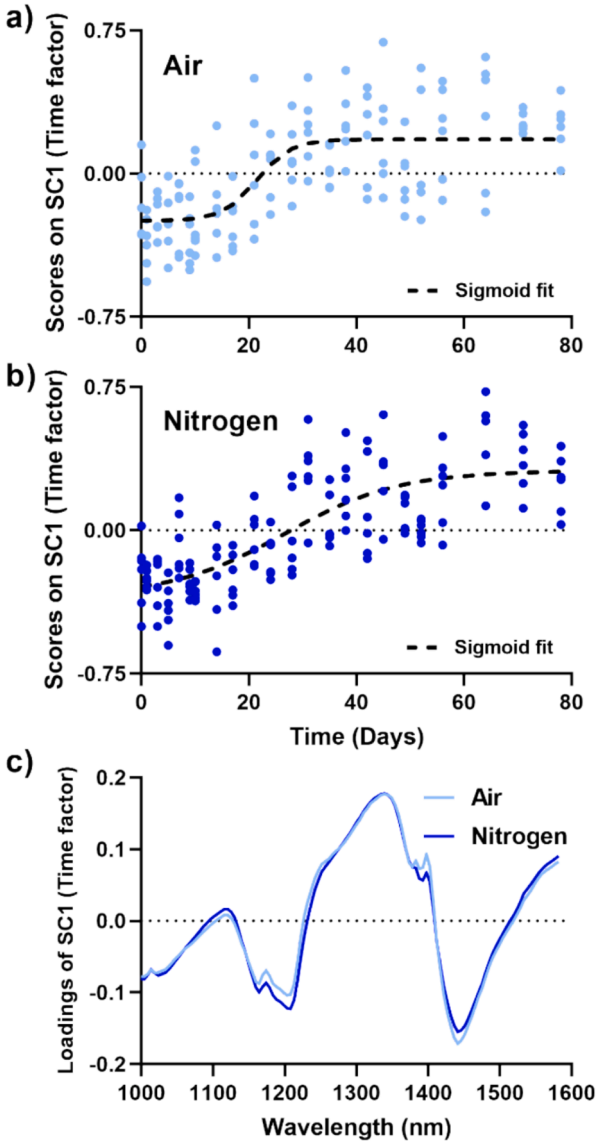

Fig. 3. Scores (a and b) and loadings (c) of the 'Time' factor of the ASCA models for the air and nitrogen atmospheres separately. A sigmoid curve is fitted to the average scores for each day, showing the evolution tendency over time; the R^2 values are a) 0.81 and b) 0.73.

For this, the PC1 scores for the 'Storage time' factor in the ASCA models for air and nitrogen atmospheres were compared (Fig. 3). This comparison reveals that, although both environments ultimately reach a similar oxidation state, the rate of oxidation differs between them. This difference is particularly significant, as it highlights the influence of oxygen availability in driving the speed of oxidative reactions. In the air atmosphere, where oxygen is readily available, the oxidation rate increases more rapidly, as indicated by the steeper slope of the PC1 scores over time. After reaching the highest oxidation rate, the scores seem to stabilise, though smaller changes may still occur. Oxygen accelerates lipid peroxidation by enabling the continuous formation of lipid radicals and hydroperoxides, which propagate oxidative degradation, especially of unsaturated fatty acids like oleic and linoleic acids. This readily available oxygen thus facilitates faster oxidation, potentially leading to noticeable rancidity earlier in the storage period [9,10].

Conversely, in the nitrogen atmosphere, where oxygen concentration is initially negligible and increases gradually over time, the oxidation rate progresses more slowly, as seen in the more gradual increase in PC1 scores over time. Although nitrogen packaging delays the early stages of oxidation by limiting oxygen exposure, the oxidative reactions do not cease entirely. Over time, oxidative changes still occur, likely due to residual oxygen or minimal oxygen diffusion through the packaging material. This slower, yet steady progression leads to an oxidation state eventually similar to that of the air condition.

Also, by looking at the loadings of this factor (Fig. 3c), specific spectral regions that contribute to the observed oxidative changes can be studied. In the NIR range, prominent loadings can be observed in regions associated with the second overtone of C–H stretching (around 1150 – 1250 nm) and first overtone of C–H stretching (near 1400 – 1500 nm). These bands are potentially linked to the aliphatic chains of unsaturated fatty acids such as oleic and linoleic acids, which are highly susceptible to oxidative degradation. As oxidation progresses, the intensity of these C–H related bands decrease, reflecting the breakdown of these fatty acids through lipid peroxidation. This breakdown leads to the formation of primary and secondary oxidation products, which ultimately contribute to off-flavours and the deterioration of sensory quality [31,33].

Additionally, the loadings in the region around 1300 nm suggest changes in O–H combination bands. These bands are likely associated with compounds formed during lipid oxidation processes, such as hydroperoxides. Although hydroperoxides are primary products of lipid oxidation, the spectral changes observed are more likely indicative of stable secondary oxidation products such as aldehydes, ketones and alcohols, which are responsible for rancid odours and tastes [34]. Another increase in loadings is observed from 1500 to 1600 nm, a region that may reflect C–H and O–H combination bands. Changes in this region could indicate the formation of oxidation by-products, as well as shifts in the moisture content due to oxidative reactions and degradation of the nut matrix over time.

3.1.2. *Oxidation studied by sensory analysis*

To validate the results obtained by NIR-HSI spectroscopic analysis, a sensory analysis was conducted on the final day of the experiment. By comparing the sensory data with the spectroscopic findings, the goal was to confirm that the chemical indicators of oxidation corresponded with actual sensory changes perceptible to consumers.

The sensory data were also analysed using ASCA (results in Table S1), which revealed that both atmosphere and light factors significantly influence the sensory attributes, confirming the analysis by NIR-HSI. However, the residuals of this model were around 80 %. This high variability may come not only from the intrinsic variability of sensory analysis involving non-trained panellists, but also from substantial inter-hazelnut variability, as discussed in Section 3.3. Light exposure played the biggest role (Fig. 4a; 13 % of variance; *p*-value of

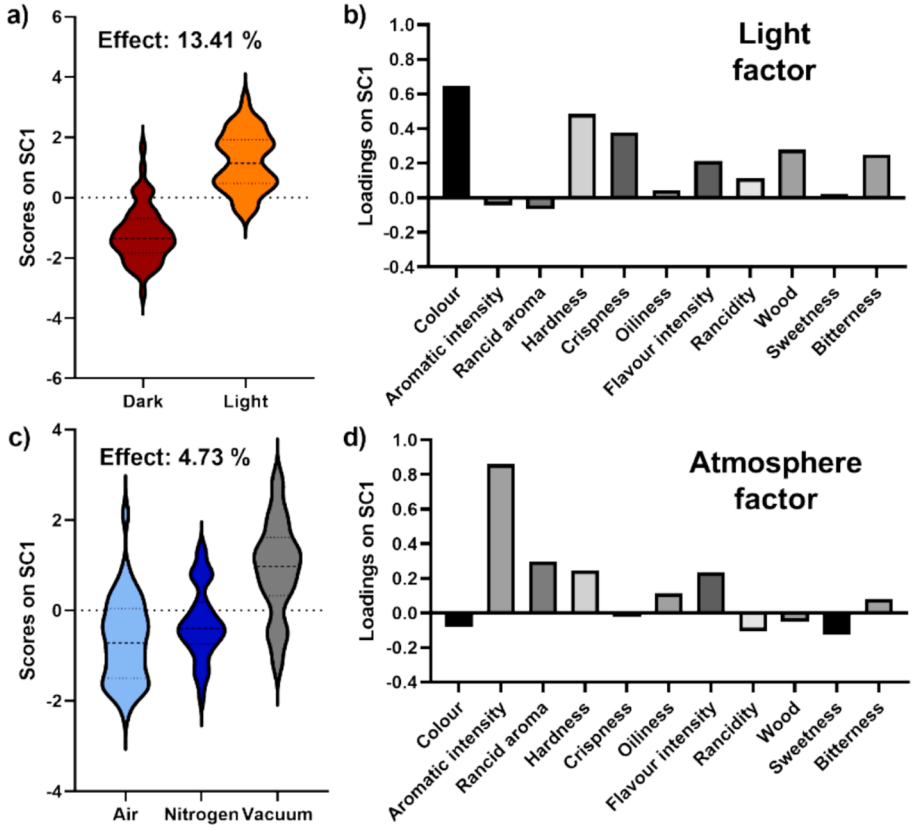

Fig. 4. Results extracted from the ASCA model applied to the sensory data of the hazelnut bags at 78 days of storage. a) and c) Violin plots of the scores of the first PC for the light and atmosphere factors, respectively. b) and d) Loadings plots of the first PC for the light and atmosphere factors, respectively.

0.001) as the panel observed notable differences in the organoleptic properties of the samples. Specifically, the samples exposed to light and ambient temperature were significantly darker, harder, and crispier, with more pronounced woody and bitter characteristics (Fig. 4b). These findings align with those of De Santis *et al.* [35] and Mexis and Kontominas [24], who also reported differences in several organoleptic properties, identifying crispiness (together with rancidity) as a consistent attribute between studies indicative of hazelnut degradation. The atmosphere effect was also significant but with a lower effect (Fig. 4a; 5 % of variance; *p*-value of 0.001). In this case, the panellists mostly noted a significant difference in the aromatic and flavour intensity, the hazelnuts conserved in vacuum being more aromatic. Here again, hazelnuts stored in air and nitrogen showed quite similar characteristics to each other but differed from those stored in vacuum. This confirms that the differences observed in the NIR spectra reflect the actual properties of the samples, rather than being attributed purely to spectroscopic effects.

3.2. Prediction of oxidation stage through NIR-HSI

Observing that there is a direct relation between the oxidation state of the hazelnuts and their NIR spectra, a PLS regression model was built using the average NIR spectrum of each hazelnut bag over time to predict the extent of oxidation as storage progresses, in equivalent days of storage, as an indicator. SNV, first derivative (Savitzky-Golay; 15-point window, second order polynomial) and mean-centring were used to preprocess the data, in this order. The model was cross-validated by a random 5-fold split, as there was no instrumental replicate involved.

The scatter plot (Fig. 5a) illustrates the predicted vs. actual values from the PLS model, exhibiting an overall good fit across most of the time points (10 to 50 days). However, non-linearity is observed at the extremes of the data, with the model losing predictive accuracy. This is, instead of having a linear response between spectra and time, the data exhibits a sigmoidal-looking relationship, as already described in Fig. 3. At the beginning of the storage period, oxidation appears to progress slowly, resulting in a less pronounced spectral response. This gradual onset of oxidation is typical, as fresh hazelnuts contain minimal oxidation products. As time advances, the rate of oxidation accelerates, leading to a stronger linear relationship between spectral changes and oxidation. In the later stages of storage, however, the rate of oxidation decreases, as indicated by the plateau effect in the predicted values. However, this does not necessarily mean that the oxidation stops, it may be that the compounds that describe the oxidation in the middle stages are not produced as intensely in this latter stage. This is, this stabilisation may occur because, as described in literature [10], the accumulation of oxidation products such as hydroperoxides and aldehydes reaches a point where the process slows due to substrate depletion or self-limiting reactions. Consequently, the spectral response also levels off, causing the model to lose linearity and the ability to properly predict oxidation levels at the beginning and end of the storage process.

The regression vector of the model (Fig. 5b) shows the wavelengths that contribute most to the PLS model, emphasizing spectral regions associated to oxidation-related compounds. These regression coefficients include bands related to C–H and O–H combination bands in unsaturated fatty acids, which are key markers of lipid peroxidation and drive the oxidation-related spectral changes used in the model, as described before.

3.3. Study of the variability among individual hazelnuts

The PLS regression model was used to study the variability among hazelnuts in each bag or the inter-hazelnut variability. For this analysis, each hyperspectral image of a bag containing 12 hazelnuts was segmented into 12 individual images, with each image containing a single hazelnut (Fig. S3). This segmentation enabled a focused approach, where the average spectrum of each individual hazelnut was analysed separately, rather than treating the bag as a single unit. For each hazelnut, its average spectrum was projected into the previously described (Section 3.2) PLS regression model. This methodology allowed to examine the inter-hazelnut variability in oxidation within each bag, this is, under the same conditions.

The prediction results, shown in Fig. 6, revealed high variability among hazelnuts within each bag, with an average relative standard deviation of 76 % in the bags. This may be attributed to each hazelnut being exposed to slightly different environmental factors, such as available oxygen and light. However, as this variability remains constant over the oxidation process, it suggests that the hazelnut already had high variability at the beginning of the process. This inherent biological variability among hazelnuts likely contributes to differences in their susceptibility to oxidation, influenced by factors such as lipid composition, moisture content, and surface characteristics.

Moreover, this segmentation and individualised analysis proved advantageous, providing a more accurate prediction (RMSE of 10 equivalent days of oxidation) of the overall oxidation state than the original PLS model (RMSE of 11 equivalent days of oxidation). Averaging the predicted storage days across individual hazelnuts yielded a more reliable estimate than predictions based on the average spectrum of the entire bag. This increased accuracy may be explained by two facts: firstly, using the mean spectra of each individual hazelnut and then averaging their values, the result is normalised by the number of pixels representing each hazelnut. This approach ensures equal weighting for each hazelnut rather than representing better the larger hazelnuts (that

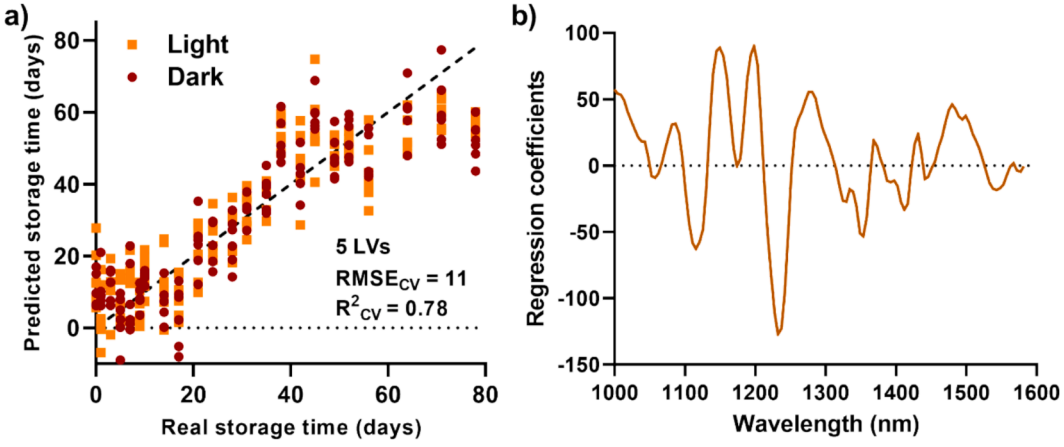

Fig. 5. PLS regression model for predicting equivalent days of storage using the mean spectra of hazelnut bags. a) Predicted vs. actual value plot for cross-validation data. b) Regression coefficients of the model in the left.

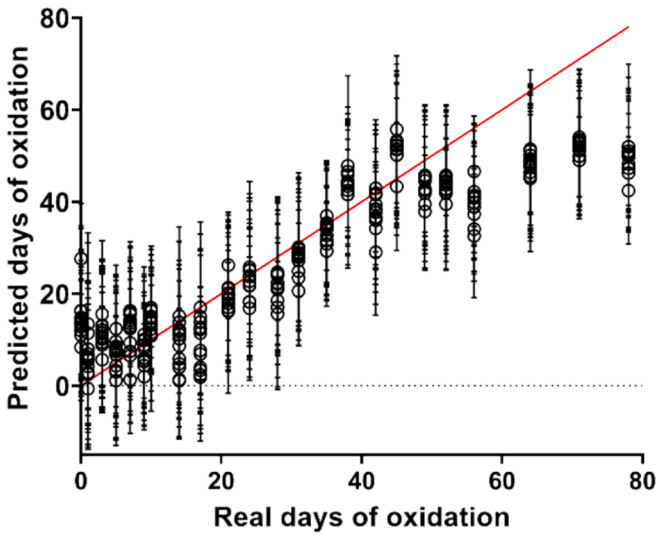

Fig. 6. Predicted equivalent days of storage for the average spectrum of each hazelnut against the actual values, shown as mean and standard deviation for each bag. Each dot represents the average prediction for a bag and the intervals represent the standard deviation of the predictions for the twelve hazelnuts in each bag.

may have more extreme values) as in the original model. And, the second fact may be that in the image-splitting process, the watershed transform also removes the pixels on the perimeter of the hazelnuts (Fig. S3b), which are usually the most extreme or inaccurate values as they are more affected by shadows and light scattering effects.

3.4. Study of the distribution of the oxidation

Realising that the spectra over the surface of a single hazelnut were not constant, the storage time PLS regression model was used to study the oxidation patterns on the surfaces of some example hazelnut bags over time. One bag is shown in Fig. 7, where the regression model is applied to the spectrum of each of the pixels of the bag (after removing background and reflections) over different stages of storage (approx. every 20 days).

As it can be seen in the image of day 0 (Fig. 7), there is already one hazelnut that is significantly different (marked with a cross), as it has a much higher predicted state of oxidation than the rest, as the yellow colour indicates. This is probably due to the hazelnut being already rancid when it was shelled. Also, it can be seen how this hazelnut does not oxidise much more, as the rest of the hazelnuts do, probably because it is already reaching a stabilisation as commented for the samples in the later stage in the PLS regression. It must be noted that the hazelnuts in the bags filled with air move freely inside the packaging, so in each acquisition they are randomly positioned in the image. However, it is still recognisable by its advanced state of oxidation compared to the rest of the hazelnuts in the same bag.

Additionally, Fig. 7 shows an uneven distribution of oxidation on the surfaces of hazelnuts, visible even from the day 0 image. The hazelnuts have some natural furrows on their surface, and they exhibit a lower predicted oxidation level at the bottom of the furrow than at the top. This variation could be purely caused by light scattering effects due to interaction with rugous surfaces. Alternatively, it is possible that this uneven distribution reflects a natural heterogeneity in the surface composition of the hazelnuts themselves. It can be seen that this difference in oxidation between the top and bottom of the furrows remains consistent throughout the oxidation process, suggesting that the variation is not only due to external factors like light scattering, but could also be attributed to intrinsic properties of the hazelnut surface. This highlight the potential of NIR-HSI to study the spatial distribution of

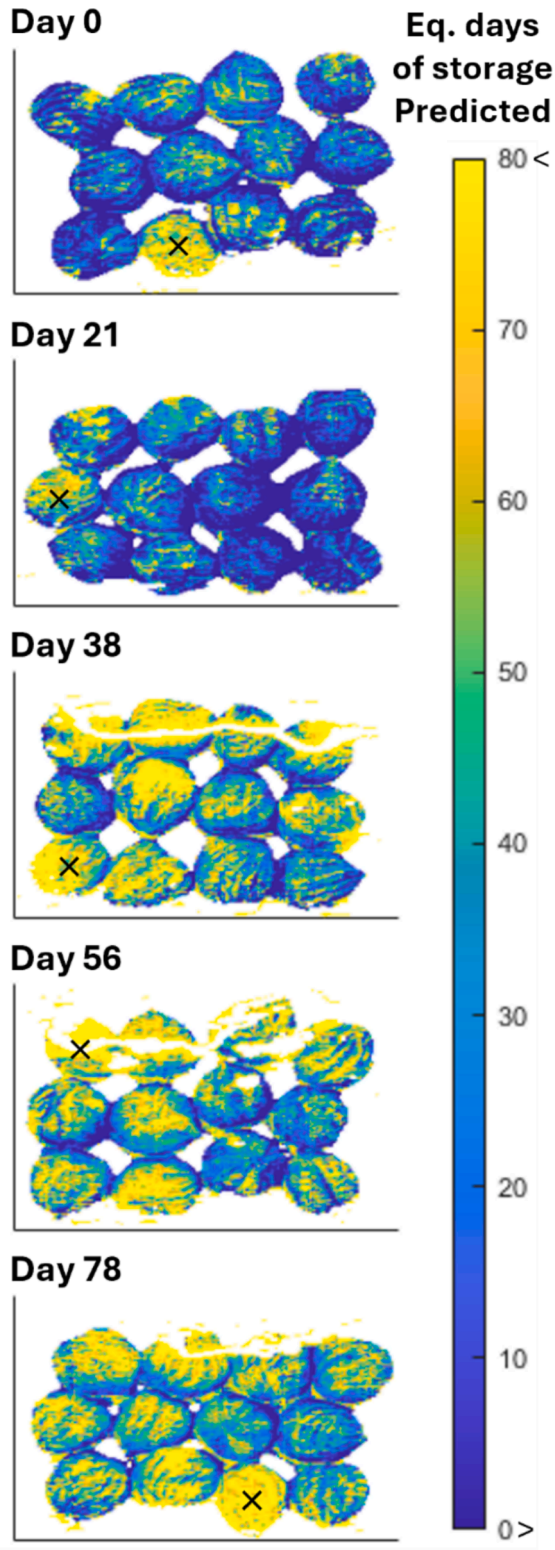

Fig. 7. Predicted oxidation state of each pixel of a single bag of hazelnuts over time. Example shown: bag filled with air, stored under ambient light and temperature, replicate 3.

oxidation and other changes caused by biological processes over time on the surfaces of hazelnuts and potentially other dry fruits, even inside their packaging, maintaining a non-invasive approach even when the product is still inside its packaging. However, challenges such as equipment accessibility, cost, and the feasibility of implementing NIR-

HSI at an industrial scale should be considered. Despite these constraints, advancements in HSI technology at a research scale and the decreasing cost of optical components are making NIR-HSI increasingly viable for large-scale applications at medium term.

4. Conclusions

This study demonstrates the effectiveness of NIR-HSI as a non-destructive and non-invasive method for monitoring the oxidation of hazelnuts stored under different atmospheric and lighting conditions, directly inside plastic bags. By using NIR-HSI, the oxidation process in real-time was assessed, detecting key changes in spectral regions associated with lipid degradation and oxidation. The segmentation of hyperspectral images of individual hazelnuts within each bag also allowed to explore the inter-hazelnut variability, confirming that oxidation rates and final oxidation states can differ even among nuts stored under the same conditions. This highlights the inherent biological variability in hazelnuts and the potential influence of storage positioning within the bag.

The results of the ASCA models confirmed that both MAP and light exposure factors trigger oxidative changes. Hazelnuts stored in air and nitrogen atmospheres showed similar oxidation levels, though the nitrogen atmosphere delayed oxidation onset slightly compared to air. In contrast, vacuum storage significantly reduced oxidation, emphasizing the importance of oxygen exclusion for extending hazelnut shelf life. The ASCA model also revealed that light exposure, especially under ambient conditions, accelerates oxidation through photooxidation, further compromising the sensory qualities of the hazelnuts. This was validated by the sensory analysis conducted at the end of the storage period, which confirmed that the chemical indicators of oxidation observed in the NIR spectra corresponded with detectable sensory degradation, such as increased rancidity, bitterness, lower aromatic intensity and changes in texture. This sensory confirmation validates NIR-HSI as a reliable method for tracking oxidation, showing that spectral changes are reflective of actual sensory impacts on the product.

This research underscores the potential of NIR-HSI as a non-invasive and rapid monitoring tool for hazelnut quality without package destruction or sample preparation. The methodology developed here could be applied to other nuts and food products prone to oxidation, providing a framework for non-destructive quality control and shelf-life assessment.

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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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2025.125906.

Data availability

Data will be made available on request.

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