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Multivariate accelerated shelf-life testing: a novel approach for understanding the shelf-life of edible mushrooms

Determinació de la vida útil de bolets comestibles amb proves accelerades i anàlisi multivariant.

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“Our virtues and our failings are inseparable, like force and matter. When they separate, man is no more.”

Nikola Tesla

Primerament, agrair als meus tutors i la seva infinita paciència per donar-me aquesta oportunitat i saber guiar-me i transmetre’m els coneixements tant necessaris per a la realització d’aquest treball.

Agrair també als meus amics per aguantar-me fins els pitjors moments i que han sigut un gran suport per mi.

Finalment, agrair a la meva família per poder comptar sempre amb ells i que, encara que no ho sàpiguen, m’han ajudat molt a portar el treball cap endavant.

REPORT

IDENTIFICATION AND REFLECTION ON THE SUSTAINABLE DEVELOPMENT GOALS (SDG)

This research investigates a typical edible mushroom consumed all around the globe, so it would align with the P for People, but ASLT and MASLT methodology can be applied later to other vastly different samples, so it could align with the P for Prosperity. The institution this research was conducted in, IDAEA-CSIC, works in environmental chemistry, so the most suitable P are Prosperity and Planet. As for concrete SDG within these categories, these are the main possibilities:

- SDG 2 (Zero Hunger): The development of accurate kinetic models leads to a deeper understanding of the shelf-life of edible products, effectively lowering the amount of wasted food due to inaccurate predictions.
- SDG 3 (Good Health and Well-Being): Some harmful additives are often mixed with edible products, but with the aforementioned deeper understanding of the products shelf-life and how storage conditions affect them, the additives would not be required, effectively subjecting consumers to less health risks.
- SDG 9 (Industries, innovation and infrastructure): The ASLT and MASLT methodology is still underrepresented in other sectors, such as pharmaceutical development, which, seeing the positive results of this research, they prove to be an interesting model to apply to other substances.
- SDG 13 (Climate action): Deeper understanding of mushroom kinetics can lead to saving costs and energy required by optimizing the storage conditions.

CONTENTS

1. SUMMARY

This project deals with the determination of the shelf-life of *Agaricus bisporus*, one of the most widely consumed mushrooms in the world. By storing these mushrooms at 5, 15 and 25 ºC, the effects of temperature were studied with 4 parameters: weight loss rate, ATR-FTIR spectrum PCA scores, green color change PCA scores and dark to white pixel ratio. These variables were measured over the span of 15 days to apply Accelerated Shelf-Life Testing (ASLT) and Multivariate Accelerated Shelf-Life Testing (MASLT) methodologies in order to estimate the kinetic parameters of the degradation reactions. ASLT yielded a shelf life of 10.88-12.09 days at 5 ºC, 4.13-8.53 days at 15 ºC and 4.51-4.52 days at 25 ºC and a global energy of activation of 30.6-34.6 kJ/mol. Univariate results were inconclusive and could not be calculated for the image related parameters, as they do not follow a discernible kinetic pattern. MASLT yielded a shelf life of 12.6 days at 5 ºC, 4.1 days at 15 ºC and 3.4 days at 25 ºC, with an energy of activation of 56.7 kJ/mol. This method based on PCA did allow the images' information to be used, thus proved a more effective tool to transform parameters with non-linear kinetic pattern into useful usable information. Further research is needed, but it is a promising line of investigation that can be applied to other substances.

Keywords: edible mushrooms, ASLT, MASLT, ATR-FTIR, colourgrams, RGB images, PCA.

2. RESUM

Aquest projecte tracta sobre la determinació de la vida útil de *Agaricus bisporus*, un dels bolets més consumits arreu del món. Sotmetent als bolets a tres temperatures diferents (5, 15 i 25 ºC), s'estudien els efectes que tenen en quatre paràmetres: la pèrdua de massa, la evolució de l'espectre ATR-FTIR amb PCA, la component verda del canvi de color amb PCA i una ràtio entre els píxels obscurs i clars de les imatges dels bolets. Aquestes variables s'han estudiat en un temps de 15 dies per poder aplicar els mètodes de Accelerated Shelf-Life Testing (ASLT) i Multivariate Accelerated Shelf-Life Testing (MASLT), que poden estimar els paràmetres cinètics de les reaccions de degradació. L'ASLT ha donat una vida útil de 10,88-12,09 dies per 5 ºC, 4,13-8,53 dies per 15 ºC i 4,51-4,52 dies per 25 ºC, així com una energia d'activació global de 30,6-34,6 kJ/mol. Els resultats univariants resulten poc conclusos i no s'ha pogut aplicar a les tècniques d'imatge, ja que no segueixen cap patró cinètic distingible. El MASLT ha donat una vida útil de 12.6 dies per 5 ºC, 4.1 dies per 15 ºC i 3.4 dies per 25 ºC, amb una energia d'activació de 56.7 kJ/mol. Aquest mètode basat en PCA ha aconseguit utilitzar la informació de les imatges i ha demostrat ser una eina molt útil per transformar paràmetres no lineals amb el temps en informació aprofitable. Encara es requereix una investigació més profunda i estudiar la possibilitat d'aplicar la metodologia per a altres substàncies.

Paraules clau: bolets, ASLT, MASLT, ATR-FTIR, colorgrama, imatges RGB, PCA.

3. INTRODUCTION

Agaricus bisporus, more commonly known as the white button mushroom, is a very sought after fungus due to its widely beloved flavor, nutritional value and ease for cooking [1]. Being the most consumed mushroom, it is of upmost importance to accurately determine its shelf-life in order to prevent loses and to avoid health risks such as illegal additives[2]. Previous works have correlated its high respiration rate and humidity to an increased ripening rate, higher susceptibility to microbial attack and the undesirable enzymatic browning. These effects could be a consequence of the loss or damage of the cuticle, a protective membrane that covers the cap[3]. However, mushrooms are sensitive to outside stimuli as well, like temperature[4], relative humidity and atmospheric composition[5]. For that reason, there is quite a lengthy list of parameters that could be measured to follow its deterioration.

3.1. VARIABLES AND CUT-OFF CRITERIA

The quality of mushrooms has been quantified through a wide range of different variables. A few examples from Li et al.'s[7] study of shiitake mushrooms are organoleptic parameters, such as smell, color, pileus form, stipe shape or corruption degree. On the other hand, other instrumental parameters have been studied, for instance % of lost weight, color, firmness, springiness, chewiness, phenolic content, malondialdehyde content, total aerobic plate count and water status.

In this work, it was deemed unfeasible to assemble a team of experts to analyze the organoleptic properties, so, instead, some instrumental parameters were chosen. Namely, % of lost weight is very easy to replicate and is also non-destructive, meaning that it can be done even with limited samples. Next, the color was chosen as a quality parameter as most costumers would not trust an off-colored product. In previous works[6,7] it was studied via de Hunter L,a,b color space using a parameter known as ΔE, which requires specialized equipment. Due to the unavailability of such equipment and for simplicity's sake, it was opted to work with a scanned image of the samples in the RGB color space. Finally, the last variable would be the IR spectrum of the mushroom itself due to the availability of the instrument. Baskar et al.[1] studied the age of *A. bisporus* following the water IR peaks in the working interval, so it has some precedent.

The cut-off criteria are the conditions under which the sample, in this case the mushrooms, are considered to have deteriorated and cannot be sold. Unfortunately, cut-off criteria are normally selected on an experiment by experiment basis and there seems to be little consensus on what they should be[7]. Following the bibliography, where the criteria are based on the loss of organoleptic properties, it's been decided that the reference values, or cut-off criteria, will be the mean value of the variables when the mushrooms starts having an off odor and the membrane that covers the gills begins to open.

As the accelerated shelf life method requires, those variables will be tracked at 3 different temperatures, 5ºC, 15ºC and 25 ºC, which is what the available equipment makes possible. But before talking about this method, some background on simple kinetics is in order.

3.2.THE KINETIC THEORY

Normally, to study the shell-life of edible products, the general kinetic formula (Eq. 1) is used, where r is the rate of the reaction, P is a measurable property, k is the rate constant at that temperature and n is the order of reaction, which for food is generally either 0 or 1[6].

$$r = \frac{dP}{dt} = kP^n \quad (1)$$

In order to find the value of n of the reaction, the chosen property is studied overtime so that, eventually, the data can be fitted into the integrated forms of the kinetic formula (Eq. 2.1-3).

$$0^{th}\text{ Order: } P = P_0 - kt \quad (2.1)$$

$$1^{st}\text{ Order: } lnP = lnP_0 - kt \quad (2.2)$$

$$2^{nd}\text{ Order: } \frac{1}{P} = \frac{1}{P_0} + kt \quad (2.3)$$

Once the values of the constant rates at different temperatures are found, one may find more useful kinetic parameters such as the activation energy by use of Arrhenius' model or the activation enthalpy and entropy by use of Eyring's model.

For Arrhenius' model (Eq. 3.1)[6] a modified version of the formula is used when working at different temperatures (Eq. 3.2) that can find a rate constant k_{ref} at the reference temperature T_{ref}, in K, which would be the mean value of the studied ones. R is the ideal gas constant, whose value in this case would be 8,314 $J \cdot mol^{-1} \cdot K^{-1}$, E_a, in Joules, is the activation energy, the minimum amount required to start the reaction, A is Arrhenius' preexponential factor and T is the studied temperature in K.

$$k = Ae^{\frac{E_a}{RT}} \quad (3.1)$$

$$lnk = lnk_{ref} - \frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right) \quad (3.2)$$

For Eyring's model (Eq. 4.1), the activation Gibbs free energy ($\Delta G^{\ddagger}$) can be further developed and the equation is linearized into (Eq. 4.2)[7]. When calculating the value for the rate constant (k) at different temperatures (T) in degrees Kelvin, one can fit a linear regression to find the activation enthalpy variation ($\Delta H^{\ddagger}$) in $kJ \cdot mol^{-1}$ and the activation entropy variation ($\Delta S^{\ddagger}$), in $J \cdot mol^{-1}$. The Planck constant (h) equals to $6{,}626 \cdot 10^{-34}\ J \cdot K^{-1}$ and the Boltzmann constant (k_B) has a value of $1.381 \cdot 10^{-23}\ J \cdot K^{-1}$.

$$k = \frac{k_B T}{h} e^{-\left(\Delta G^{\ddagger}/RT\right)} \quad (4.1)$$

$$ln\left(\frac{k}{T}\right) = -\frac{\Delta H^{\ddagger}}{R} \cdot \frac{1}{T} + ln\left(\frac{k_B}{h}\right) + \frac{\Delta S^{\ddagger}}{R} \quad (4.2)$$

These formulas can be applied to deduce the effect of temperature on mushrooms in accelerated studies.

3.3. ACCELERATED SHELF-LIFE TESTING (ASLT)

Most kinetic studies can span a big period of time, which can be detrimental if the product has a short expiration date. A method known as Accelerated Shelf-Life Testing (ASLT) was developed to quickly determine the shelf-life of perishable products by subjecting them to somewhat extreme conditions of storage. It has been found that most processes that lead to the expiration of food are mainly driven by temperature[7] and, being relatively easy to control, it is widely used in these studies. By following how a measurable property reacts to said conditions, one can establish a linear dependency using the formulas from the kinetic theory to determine the time at which they expire[8]. Because of this, there is a parameter that links the different rate constants: the acceleration factor ($\alpha_{T2,T1}$). This factor allows for a quick conversion between predetermined rate constants at different temperatures. The acceleration factor is defined by (Eq. 5), where T_2 is higher than T_1 and k_T is the rate constant at that temperature.[6]

$$\alpha_{T2,T1} = \frac{k_{T2}}{k_{T1}} \quad (5)$$

This method has been utilized previously for different kinds of edible products, a few examples being salads[9], oils and fatty acids[10] or cured meat[11]. Despite that, this method presents its downsides: for complex samples whose overall quality is defined by multiple

sensory or chemical properties, ASLT is insufficient and can lead to inconsistent results depending on the chosen variable[7].

3.4. MULTIVARIATE ACCELERATED SHELF-LIFE TESTING (MASLT) AND PRINCIPAL COMPONENT ANALYSIS (PCA)

As a result of ASLT's inconsistencies, a new approach has been proposed: the Multivariate Accelerated Shelf-Life Testing (MASLT), a combination of several variables within a model of Principal Component Analysis (PCA)[6].

PCA processes multivariate data, samples from which several parameters have been measured, and presents it in a new reduced dimensionality using what's called Principal Components (PC). These components act as new orthogonal axes where data is represented in an easier to digest fashion without losing the important information. Each PC explains a portion of the original data's variance, with the first representing the biggest. By use of specialized software, one may build a model with the required PCs that can explain a plethora of variables within 2 or 3 data axes (in the best of cases). The data is split into Scores (S matrix), which is where it is located within the component, and Loadings (L matrix), which is the importance of each variable exerts in each sample[12].

Something important to take into consideration with PCA is that not every variable could have a comparable degree of change. For instance, the extreme values in one of them could have a difference of 100 units, but another could have a difference of 1 unit. That means that the former would have more weight in the model and could obscure the relevant information of the latter. To remedy that, the data can be preprocessed in two ways without losing relevant information. When the magnitude of the scale is too high, which is when the values of one variable are too big compared to the other, the data can be mean centered. To mean center the data, the means of the values are subtracted to each individual sample, which means that the scale is shrunk to only the amount that varies. This leads to the next problem one could encounter: the amplitude of scale, which is when the standard deviation of every variable is too different between them. In this case one can opt for autoscaling. In order to autoscale data, the mean is subtracted to each sample, as explained earlier, but then is divided by the standard deviation of the variable to normalize it. There are other kinds of mathematical algorithms that can be used to preprocess data, like normalization of spectra, base-line corrections or derivatives, but due to their complexity they will not be discussed[12].

MASLT method assumes that the samples quality dwindles due to the degradation reactions, because initially they have the same composition, and all the other external factors are controlled. As such, the first components should have a clear dependency with time, signifying the progress of the degradation. When the time-driven component is not within the first few, it can be either of 2 situations: there was a problem with the storage conditions or some uncontrolled variable is exerting its influence and can yield some insightful information. It also assumed that PCA can accept non-linear processes in its scores, which can be later evaluated using the loadings of each component[6].

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4. OBJECTIVES

The main objective is to assess the efficiency of the ASLT and MASLT methods to build a realistic and accurate model with the help of chemometric tools to determine the shelf-life of *A. bisporus* based on the study of its weight loss, the IR spectrum and color changes.

Secondary objectives include but are not limited to:

- Optimization of experimental conditions for sampling and instrumental analysis based on previously used methodology to evaluate mushrooms' deterioration with time.
- Applying the required knowledge of data treatment in ASLT and MASLT modeling.
- Interpreting and extracting the most important information regarding the studied variables by means of PCA chemometric techniques.
- Performing a preliminary shelf-life estimation at different storage temperatures with ASLT and compare them with MASLT and information available on the literature.

5. EXPERIMENTAL SECTION

5.1. SAMPLE PREPARATION AND STORAGE

The *A. bisporus* were obtained from a local fruit shop from Mercabarna moments after harvest to guarantee their freshness as much as possible. There was some prior testing in order to optimize the measurements, but they will not be further discussed. For each set of samples, five mushrooms that visually did not present noticeable damage were picked, numbered with a marker from 1 to 5 and placed over a paper towel on a carboard plate. It is worth noting that after prior testing, it was deemed important to cover the plates with plastic foil. Afterwards each set of samples would be stored in the required temperature, which could be 5, 15 or 25 ºC.

The storage equipment consisted in a regular lab fridge, which could preserve the samples at steady 5 ± 0.5 ºC, a Panasonic Versatile Environmental Test Chamber MLR-35H-PE that maintained the temperature at 25 ± 0.5 ºC and a cooler sustained by ice that unfortunately could only keep a temperature of 15 ± 5ºC. The plastic film that covered the samples ensured that every set was under the same humidity conditions, as the 25ºC chamber had 60% or lower relative humidity.

5.2. EXPERIMENTAL DESIGN

In this experiment, six different sets of samples (30 mushrooms total) were prepared and stored as previously mentioned, two at 5 ºC, two at 15 ºC and two at 25 ºC. The weight and image of every sample marked from one of the sets were obtained every day possible. Afterwards, in the same day, the IR spectrum of every sample of the other set stored at the same temperature was obtained. The samples were separated in this fashion as the weight and imaging are non-destructive tests, whereas the IR is a destructive one, which could complicate the first two determinations. The measurements were carried out on day 0, 1, 4, 5, 6, 7, 8, 11, 12, 13 and 15. The samples stored at 15 ºC had its final day on day 8, and the ones stored at 25 ºC ended on day 6. A total of 115 different weights, spectra and images were recorded.

5.2.1. Weight loss rate

Each sample was weighted over a paper towel in a KERN 440-35N electronic balance with a maximum weight range of 400g and a readout of 0.01g. This operation was done quickly so as not to let the temperature change much. Afterwards, the percentage of lost weight was calculated by use of (Eq. 6), where w_0 is the initial weight and w_i is the weight at day i.

$$\% = \frac{(w_0 - w_i)}{w_0} \cdot 100 \qquad (6)$$

5.2.2. Imaging

The images of each sample were recorded over the same blue sheet each time with a ViewScan Objektscanner using the software Bandicam and stored in individual image files. As there was not any available dark chamber, it had to be done in the same place with the window blinds closed to minimize the variation of light intensity. Furthermore, so that the mushrooms could stand at the same height each time, a little stand was crafted using the remains of a disposable plastic cup. An image of the set-up can be found in Figure 9 in the Appendix.

5.2.3. ATR-FTIR spectroscopy

The spectra of each sample were recorded via a Nicolet Avatar 360 FT-IR system from 650 cm^{-1} to 4000 cm^{-1} (1868 points) a total of in absorbance mode using the EZ OMNIC software. Based on previous experimentation, the best option to measure the ATR-FTIR spectrum of this mushroom without much complication is to cut out a small sample from every mushroom. This was done by making 2 very close, approximately 1 mm apart, incisions until they reached the core and then the fragment was extracted using a pair of tweezers and measured as is. The images of the process can be found in Figure 13 in the Appendix. The measurements were carried out one at a time after collecting the background, as cutting the samples too early would quickly oxidize the extracted sheet and give inaccurate results. That means that as quickly as each set of samples was determined, it was returned to their respective temperature so as not to impact the results in following days.

6. DATA TREATMENT

Out of the three chosen variables, the rate of weight loss is the only one that does not require any prior treatment before using it in the ASLT method to get the kinetic parameters. On the other hand, images and spectra are very complex and hold quite a bit of different information. To try and compress the aforementioned information in an easier to understand format, the use of PCA is required. All data treatment was done using MATLAB R2022b and the PCA was carried out using Eigenvector Research Solo version 9.2.

6.1. ATR-FTIR SPECTROSCOPY

Before the spectra can even be input in a PCA model, first the data must be arranged in order and preprocessed. The normal procedure in these cases is to first normalize the spectra, which for the purpose of this work the chosen method was the built-in Multiplicative Signal Correction, or "MSC (Mean)" option in Solo. Further preprocessing may be needed depending on the characteristics of each set and should be selected carefully. One option is to use a base-line correction or apply any number of derivatives to filter out certain undesirable signals. As a last resort when the spectra are too noisy, one can opt for smoothing them, which is normally done with the built-in Savitzky-Golay Smoothing option in Solo (Smoothing SavGol). This can have dangerous consequences, as it may smooth over a signal that is of high significance to the study. Finally, when the desired preprocessing methods have been chosen, normally spectral data is mean centered to eliminate the first component, which will always be the mean spectrum of every sample and explain most of the variance. That means that when not mean centering, the other components, which explain how the spectrum changes, will globally explain a very small amount of variance.

In this experiment, the spectra were sorted based on the day (0 to 15), then in order of sample (1 to 5) and finally based on temperatures (5, 15 or 25). The result was a 117x1868 matrix for the whole duration of the experiment. However, there were two spectra that were visually clearly outliers, as they did not show any resemblance to the general outline of the other samples. For that reason, spectra 106 and 109 (T25 d5) were removed before beginning the next step (matrix is now 115x1868).

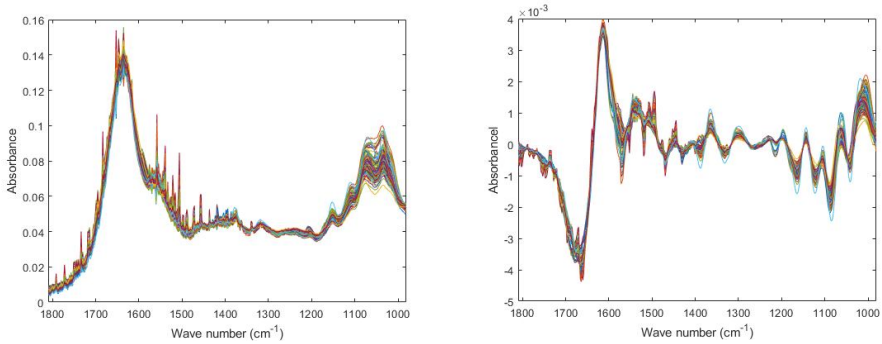

Figure 1. (Left) Normalized spectra for the mushrooms. (Right) Preprocessed spectra after calculating 1st derivative. Only shows the area between 981 cm-1 and 1810 cm-1.

Afterwards, the data was first normalized by using the "MSC (Mean)" option and was only preprocessed by using only a first derivative (Derivative SavGol: Filter width 15, polynomial order 2, derivative order 1). This method allowed the improved detection of important peaks, but also increased the unwanted scattering peaks, although fortunately it did not negatively affect the results. Following some trial and error and by checking with previous works [1,13], it was found that the best results only used the peaks relating to polysaccharides, proteins and water (due to it overlapping with the proteins signal). That meant that the matrix got reduced to 115x430, which contained the spectra from 981 cm^{-1} to 1810 cm^{-1}. The new normalized reduced spectra and their following derivatives are displayed on Figure 1.

Under these conditions, a model with 5 principal components was constructed, which had a cumulative variance of 99.88 %. The objective here is to find at least one component that has a clear dependency with time within each temperature. By analyzing the scores and loadings of each component, it was found that component 4 showed a clear dependency with time, making it the best one to represent the needed information. However, the 8th day for both T5 and T15, as well as the 4th day at T15 had a very erroneous placement, which may indicate some unidentified systematic error is present. For the purpose of this investigation, those samples were removed as well, leaving a 95x1868 matrix. The 4th component will be used later in ASLT and MASLT modeling to find the kinetic parameters. Its loading values indicate that the most relevant peaks for this components are those related to polysaccharides and proteins.

6.2. IMAGING

A picture contains a plethora of information, but not all of it is useful, which complicates its analysis. Every pixel of photo contains a value from 0 to 255 depending on what the detector perceives as red (700 nm), green (546.1 nm) and blue (435,8 nm), that's the RGB space. An image will contain a matrix of r pixels high, c pixels long and 3 parameters wide (RGB values). By using Calvini et al.[14]'s Colourgram_GUI, these matrices can be decomposed on one-dimensional parameters, called colorgrams, that can be further investigated by use of PCA. In essence, colorgrams are histograms containing the frequency of each value, which makes the changes easier to understand. The different colorgrams this tool can output are: absolute red, blue and green, lightness (L = addition of the RGB values), relative red, blue and green (R, G or B divided by L), and hue, saturation and intensity (by transforming from the RGB space to the HSI space).

The obtained images were quite large, 2592x1944 pixels that contained not only the mushroom but also the whole blue paper used as background. Every single photo was reduced to a 550x550 area centered on the mushroom to aid with the colorgrams calculations. First, following the instructions by Cavini et al.[14] the images were loaded as is to find the best value to erase the background through the use of the image reconstruction function. The colorgram for relative blue showed two distinct peaks, the lower one would belong to the normal "blue" contribution from the mushrooms (around 0.33), while the higher one would belong to the signal from the background (around 0.4). That meant that, when generating the colorgrams, instead of choosing "normal", "keep only" relative blue below 0,34 was selected. This meant that the program would only consider the pixels with less than 34 % of blue, in other words, the pixels relating to the mushrooms.

With one problem solved, the next one was to find a way to normalize the images, because, as stated before, the light conditions were not the same every day. The different techniques were: normalized by dividing each pixel's values by the pixel's lightness value, grayscale transformation and adjusted by transforming the data to the HSI space, equalizing the Intensity values and then transforming back to the RGB space. The first showed no clear dependency with time upon performing PCA, likely due to the normalization smoothing over the changes. The second one did not work, as the program cannot process greyscale pictures, although this

set would see some use later. Finally, the last set did show some dependency with time in the green component (Figure 1) as the red and blue signal variations were minimized.

Upon performing PCA, while mean centering the signal, the first component (28.25 % explained variance) showed the time-correlated change. This component will later be used in the ASLT and MASLT models.

On the other hand, the greyscale images were used to determine the dark to white pixels ratio. When transformed to greyscale and after removing the background, the intensity values from 0 to 255 can be interpreted as dark when their values are below 110 or as white when their values are above 220. The grey pixels in between are considered to be mostly affected by the changes in light conditions between data collections. With this into account, a ratio between dark and white pixels was found using a MATLAB script that counts how many pixels in the picture are above or below the aforementioned thresholds. This value increases with time due to the darkening of the mushroom and thus is useful for ASLT and MASLT modeling.

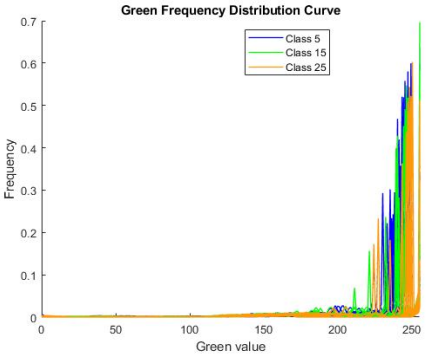

Figure 2. Colorgram for the green contribution of the images. Note that as temperature increases, the distribution has a tendency for the higher values.

6.3. ASLT MODELING

To predict the shelf-life of mushrooms using ASLT modeling, the variables must fit into one of the kinetic equations (Eq 2.1-3). The rate of lost weight, the fourth component of the IR spectra, the first component of the green contribution and the dark to white pixel ratio are fitted into order 0 equations so that the kinetic parameters can be obtained. The 0^{th} order kinetics was used as it gives the best fit for each of the variables and temperatures. The shelf life can be

obtained by isolating the time variable and deciding on some acceptable cut-off criteria. The results will be discussed later.

6.4. MASLT MODELING

In MASLT, the data must follow a certain placement inside a matrix. The required steps to construct a valid MASLT model are very nicely described by Pedro and Ferreira[6]:

1. All data is collected and placed in a $n \times k$ matrix, which will be called X_i, where n is the number of samples for one temperature i and k is the number of different variables.
2. The different X_i matrices are stacked on top of each other, with the lowest temperature being at the top, and the highest, at the bottom.
3. If required, the data can be preprocessed accordingly either by mean centering or autoscaling.
4. The PCA is performed to obtain the Scores (S) and Loadings (L) matrixes. The PCs with bigger explained variance are evaluated.
5. The main X matrix is split into the different X_i matrices for their posterior analysis.
6. The Score values are ploted against time to identify which components are time dependent.
7. For each time-related component, the desired kinetic parameters are calculated using the scores as the measured property.
8. The parameters chosen as cut-off criteria are placed in a row vector in the same order as in the MASLT model and preprocessed in the same way.
9. This preprocessed vector is multiplied by the Loadings column vector to yield the score at which these criteria are met.
10. The score value is substituted in the MASLT linear regressions to find the shelf life at every temperature.

In this work, the matrix is built using the mean values of every variable for each day in the same order as in Figure 3. When there is no available data, the gap is filled with “NaN” so that the model does not take it into account. The resulting matrix has 23 rows (11 for 5ºC, 7 for 10ºC and 5 for 25ºC) and 4 columns (%weight loss rate, ATR-FTIR spectra PC4, Green colorgram PC1 and dark to white pixel ratio).

As explained in the introduction, the cut-off criteria have been selected as the mean values for each of the four variables when the mushrooms had a noticeable loss of organoleptic properties, which is at day 11 for the 5 ºC set. To get the autoscaled values, the raw data was input into (Eq. 7) where P_a is the autoscaled value of the variable k, P is the raw value, P_k is the mean of variable k and s_k is the standard deviation for variable k. The raw and autoscaled values that serve as the cut-off criteria are shown in Table 1.

$$P_{a_k} = \frac{P_k - \overline{P_k}}{s_k} \quad (7)$$

	% weight loss	Score ATR-FTIR PC4	Green PC1	D/W pixel ratio
Raw (x)	0.2088	$9.44 \cdot 10^{-4}$	0.0118	0.0011
Autoscaled (x_a)	0.3465	0.8069	0.1256	-0.5990

Table 1. Raw and autoscaled cut-off criteria.

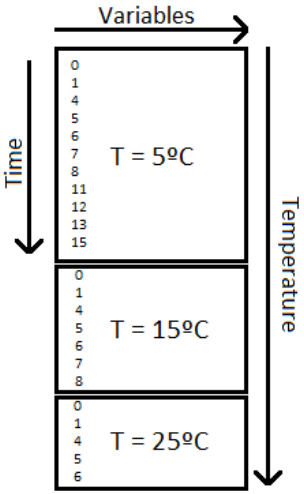

Figure 3.Schematic of the placement of data inside the MASLT matrix. Based on the works of Pedro and Ferreira[6] and Li et al[7].

7. RESULTS AND DISCUSSION

7.1. GENERAL OVERVIEW

Overall, there was a clear worsening of qualities over time, which was sharper the higher the storage temperature was. For instance, at 25 ºC the weight was lost quicker, or the mushroom darkened at a faster pace than at 5 ºC. This is in line with the bibliography[7], which stablishes that at high temperatures, the darkening, putrid smell and corrupted physiology appear sooner. More on this in the respective section below.

Although the experiment did not stop exactly upon arrival of these phenomena, the day at which they were observed was noted. At day 11 for 5 ºC, 7 for 15 ºC and 5 for 25 ºC the mushroom started to emit a lightly pungent smell, uncharacteristic of fresh mushrooms, the pileus was dried and seemed more fragile, and the membrane that covered the gills started opening. In order to get some points past their shelf-life, the measurement was stopped when the putrefaction smell had worsened significantly, which translated in an end point of 15 days for 5 ºC, 8 days for 15ºC and 6 days for 25 ºC.

7.2. ASLT MODEL RESULTS

7.2.1. Weight loss rate

Figure 4 shows that the rate at which the mushroom's weight sinks fits very nicely into the 0^{th} order reaction equation, yielding an R^2 of 0.9992, 0.9959 and 0.977 for 5, 15 and 25 ºC respectively. A rather curious occurrence is that the slope for 15 ºC is slightly sharper than that at 25 ºC, which is not the expected behavior. It is possible that some uncontrolled storage condition has affected the way weight is lost, although other variables do not seem to be affected. On the other hand, the weight loss did not fit too well into the Arrhenius and Eyring models, which yielded R^2 of 0.69 and 0.66 respectively, which could be a consequence of the strange slope behavior. The energy of activation is in line with what is expected in food kinetics, which usually spans a range from 30 to 120 kJ/mol[15]. The calculated kinetic parameters are shown in Table 2. As for the shelf-life, after isolating the time variable from the linear regression equation using 20.88 % weight loss as the reference value, the obtained univariate shelf-life times for weight loss are 10.88 days for 5 ºC, 4.13 days for 15 ºC and 4.52 days for 25 ºC. As mentioned earlier, it does not make sense for the 25 ºC to have a lower shelf-life estimation, so

it's possible there is some kind of mistake on the 15 ºC value, based on other parameters' result.

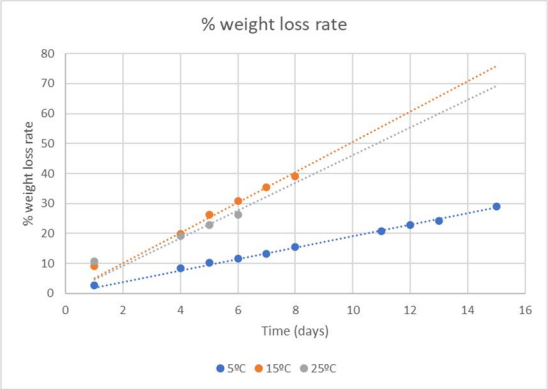

Figure 4. 0th order kinetics model in percentage of lost weight.

%weight loss				
Temperature	k	a	R^2	$\alpha_{T2,5ºC}$
5	0.0192	0	0.9992	1.00
15	0.0505	0	0.9959	2.63
25	0.0461	0	0.977	2.40
Arrhenius	E_a [kJ/mol]	k_{ref}	R^2	
	30.6	0.0359	0.6914	
Eyring	ΔH [kJ/mol]	ΔS [J/K·mol]	R^2	
	28.2	-174.4	0.6550	

Table 2. Univariate kinetic data for the percentage of lost weight.

7.2.2. ATR-FTIR Spectroscopy

Regarding the IR spectra of the mushrooms, while it is true that only an interval of wave numbers have been used for the calculations, the spectra as a whole can give an idea of the composition of the mushrooms. The mean spectrum of all samples and the corresponding assignations can be found on Figure 5.a.

The spectra present several peaks closely related to the composition of the *A. bisporus.* First of all, the first few peaks at lower wave numbers: 1033.71, 1074.21, 1108.92 and 1151.35

cm^{-1} all relate to polysaccharides[13], the most abundant of which in this species is mannitol[16]. Following them, there is a sharp peak at 1635.42 cm^{-1} and a very subtle and overlapped peak at 1556.35 cm^{-1}, which originate due to the proteins[17]. It is important to note that the peak at 1635.42 cm^{-1} is mostly overlapped by the bending vibration from water[1], in the same way the alkyl (2931.41 cm^{-1})[17] and hydroxyl group (3200+ cm^{-1}) are overlapped by the main stretching vibration from water[1]. This may be due to the high moisture content of these type of mushroom, which can represent up to 95% of its weight[1], as their peaks overlap other important peaks. As for the unidentified peaks around 2300 cm^{-1}, their slow appearance could signify it is some degradation product. Unfortunately, it could not be assigned as there was not easily accessible information on it, although that wave number is usually CO_2. On the matter of C=O, the characteristic band for lipids[18,19] could not be found in this spectrum, as it falls near 1745 cm^{-1}, a region too overlapped by the water bending movement, which combined with its very low concentration compared to other nutrients[16], it means it is unlikely to be seen.

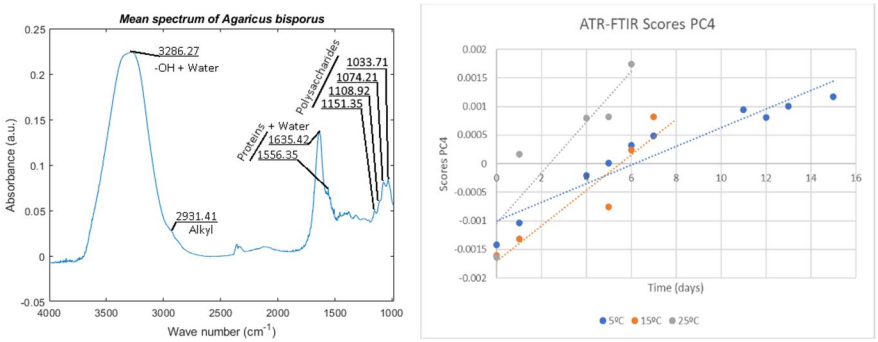

Figure 5. (Left) Mean spectrum of A. bisporus with assignations based on bibliography. (Right) 0th order kinetics model with the scores for PC4 from the ATR-FTIR spectra.

Following this analysis of the spectra, the results from the ASLT method still need to be discussed. As seen by Figure 5.b and Table 3, the scores from the 4th component of the ATR-FTIR spectra fit best into a 0th order kinetic reaction, with R^2 of 0.9073, 0.8785 and 0.8295. Unlike the weight loss, the rate constants increase with temperature, as what would be expected. Regarding the activation energy, enthalpy and entropy, those parameters are rather similar to the ones calculated by the weight loss and are within expected values. The difference here is that the rate constants fit better into Arrhenius' and Eyring's models, with R^2 of 0.9793 and 0.9759 respectively. The shelf-life for the IR spectra has been calculated by isolating the

time variable from the linear regression formula using $9 \cdot 10^{-4}$ as the reference value for the score, which yields 12,09 days for 5 ºC, 8.53 days for 15 ºC and 4.51 days for 25 ºC. These values are slightly above the ones calculated from the weight loss, which further exemplifies the need for the multivariate design.

ATR-FTIR Scores PC4				
Temperature	k	a	R^2	$\alpha_{T2,5ºC}$
5	0.00016	-0.00099	0.9073	1.00
15	0.00031	-0.00170	0.8785	1.90
25	0.00044	-0.00104	0.8295	2.71
Arrhenius	E_a [kJ/mol]	k_{ref} [$\cdot 10^{-4}$]	R^2	
	34.6	2.85	0.9793	
Eyring	ΔH [kJ/mol]	ΔS [J/K·mol]	R^2	
	32.2	-200.9	0.9759	

Table 3. Univariate kinetic data for the ATR-FTIR spectra.

7.2.3. Green colorgram

Due to the normalization process, the only color that showed a clear contribution to change was the color green. Based on experimentation, *A. bisporus* develop some brown spots (combination of all three colors) and also turn yellower over time (combination of green and blue), so it makes it the most useful channel of the RGB space to study the change in coloration. As seen in Table 4 and Figure 6, the change in color varies vastly with temperatures, being sharper as it rises. The physical meaning of this increase is, according to the loadings, that with past of time there are more and more pixels with a high value for green, which translates in the darkening of the sample. Despite the seemingly negative slope for 5 ºC, the reality is that color changes so little that all variations registered by the scanner are most likely the result of noise. It is also somewhat concerning that the y intercept for 25 ºC is incredibly low compared to the other temperatures. To further complicate matters, this variable does not fit into any of the kinetic models, the highest R^2 were obtained at 0^{th} order with 0.1498, 0.2621 and 0.6877 for each temperature. The activation energy of 179.5 kJ/mol is well over the expected

value in food kinetics, even though the R^2 for the Arrhenius' and Eyring's models are over 0.9, which means that the main problem lies within the rate constants.

All in all, these results indicate that this parameter is not fit for ASLT modeling, so the calculated parameters are not to be trusted. Besides that, the univariate shelf-life could not be obtained based in the poor model.

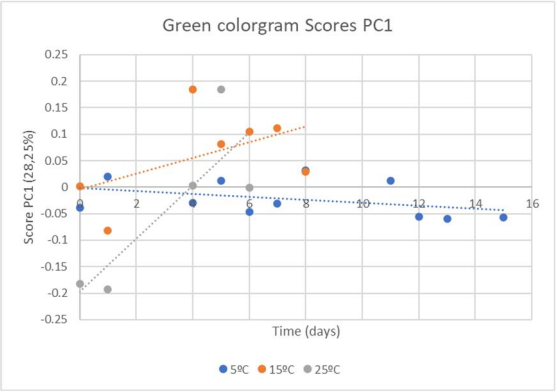

Figure 6. 0th order kinetics model with the scores for PC1 from the green colorgram.

Green colorgrams Score PC1				
Temperature	k	a	R^2	$\alpha_{T2,5ºC}$
5	-0.0027	-0.0018	0.1498	1.00
15	0.0239	-0.0249	0.5106	-8.85
25	0.0713	-0.2248	0.9012	-26.41
Arrhenius	E_a [kJ/mol]	k_{ref}	R^2	
	191.1	0.0090	0.8953	
Eyring	ΔH [kJ/mol]	ΔS [J/K·mol]	R^2	
	188.9	371.9	0.8928	

Table 4.Univariate kinetic data for the green colorgram.

7.2.4. Dark to white pixel ratio

The final parameter to be discussed in this ASLT section is the dark to white pixel ratio. As this value increases, there will be more dark pixels in the image, which translates to the darkening effect they suffer from. Table 5 and Figure 7 demonstrate that the darkening speed increases faster with temperature and fit best into 0th order equations with questionable R^2: 0.0636, 0.6647 and 0.7981. Like the previous parameter, at 5 ºC the slope is very close to 0, and its slight negative deviation is most likely noise, meaning it barely darkens at this temperature. This time the starting position, or y intercept, of all 3 storage conditions are relatively close, and the change in the slope between temperature seem reasonable. In regards to the activation energy, just as with the green colorgram, it is too high for regular food kinetics, although this time the R^2 are slightly worse, near 0,86. Most likely this stems from wrong rate constant values, meaning this variable is also unfit for univariate shelf-life determination.

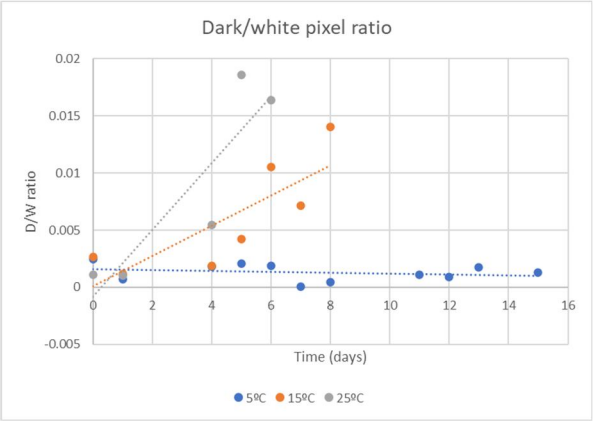

Figure 7. 0th order kinetics model with the scores for PC1 from the dark to white pixel ratio.

Dark to white pixel ratio				
Temperature	k	a	R^2	$\alpha_{T2,5ºC}$
5	-0.000038	0.0016	0.0636	1.00
15	0.00132	0.00011	0.6647	-34.74
25	0.00291	-0.00079	0.7981	-76.58
Arrhenius	E_a [kJ/mol]	k_{ref}	R^2	
	197.6	0.00036	0.8649	
Eyring	ΔH [kJ/mol]	ΔS [J/K·mol]	R^2	
	195.2	367.1	0.861922	

Table 5. Univariate kinetic data for the dark to white pixel ratio.

7.3. MASLT MODEL RESULTS

The mean values for each parameter (weight loss rate, ATR-FTIR spectra, green colorgram and dark to white pixel ratio) and for each temperature (5, 15 and 25 ºC) is shown in Table 7. PCA is performed in this table's data, resulting in a two component model that explains 83.07 % of data variance with a RMSECV value of 1.24. The first component (Figure 8.a) follows a clear growing trend that fits into 0^{th} order kinetic equations with good R^2: 0.9445, 0.9628 and 0.9693 for 5, 15 and 25 ºC respectively.

The kinetic parameters shown in Table 6 indicate that it is a superior method to predict the shelf life of *A. bisporus*, with ever increasing rate constants and an energy of activation of 59,1 kJ/mol, it follows the expected behavior and falls within the range of food kinetics. Furthermore, this energy is quite similar to the one obtained in another MASLT analysis, albeit with *Lentinula erodes*, a different unrelated type of mushroom[7].The shelf life was calculated as stated in the data treatment section, which yielded a score value of 0.3755, marked in Figure 8.a as a green straight line. When substituting this value in each of the linear regression equations, the obtained time is the shelf life: 12,6 days at 5 ºC, 4.1 days at 15 ºC and 3.4 days at 25 ºC. At first glance it is somewhat similar to what the univariate analysis indicated, but in this case it is clear that the higher the temperature, the shorter the shelf-life is (which the rate of weight loss did not follow) and this model could also take into account both variables that could not be used in the univariate determination. Unfortunately, there is no comparable information on the shelf life results, as each research proposes its own cut-off criteria.

The biplot of scores and loadings of the model (Figure 8.b) is interesting to understand the weight each parameter has in the degradation reactions. All 5º C samples are gathered forming a line perpendicular to the green colorgram PC1, which means they do not have significant change in color. However, they are also somewhat parelel to weight loss and the IR spectra, which means they are mostly affected by them. On 15 and 25ºC however, one can see that the line they form is more tilted towards the change in color, meaning that the higher the temperature, the more important is this phenomenon. Lastly, all 4 variables seem to have almost equal importance for the first component, with only the green colorgram being a bit behind.

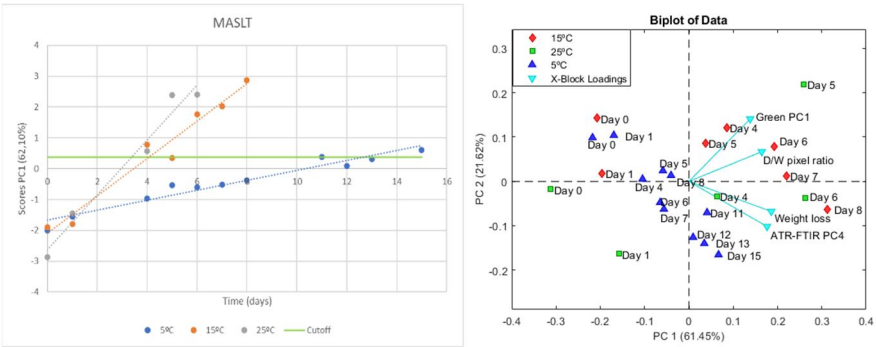

Figure 8. (Left) 0th order kinetic using the scores of the MASLT method. (Right) Biplot showing the scores and loadings of the MASLT method of PC1 against PC2.

Multivariate Accelerated Shelf-Life Test				
Temperature	k	a	R^2	$\alpha_{T2,5ºC}$
5	0.1607	-1.67	0.9445	1
15	0.6074	-2.11	0.9628	3.78
25	0.8829	-2.64	0.9693	5.49
Arrhenius	E_a [kJ/mol]	k_{ref}	R^2	
	59.1	0.4506	0.9164	
Eyring	ΔH [kJ/mol]	ΔS [J/K·mol]	R^2	
	56.7	-54.5	0.909723	

Table 6. 0th order kinetics for the multivariate analysis.

Temp.	Day	% weight loss	ATR-FTIR Score PC4 [$\cdot 10^{-3}$]	G Score PC1 [$\cdot 10^{-2}$]	D/W pixel ratio [$\cdot 10^{-3}$]
5 ºC	0	0	-1.42	-3.83	2.45
	1	2.8	-1.03	2.00	0.71
	4	8.5	-0.20	-2.98	1.79
	5	10.4	0.01	1.25	2.10
	6	11.6	0.32	-4.65	1.88
	7	13.32	0.49	-3.13	0.07
	8	15.6	-0.03 (NaN)	3.16	0.42
	11	20.9	0.94	1.18	1.07
	12	22.8	0.81	-5.59	0.93
	13	24.3	1.00	-6.01	1.73
	15	29.0	1.17	-5.73	1.31
15 ºC	0	0	-1.60	0.15	2.66
	1	9.2	-1.32	-8.16	1.18
	4	19.8	0.23 (NaN)	18.39	1.88
	5	26.2	-0.76	8.18	4.25
	6	30.7	0.24	10.43	10.55
	7	35.4	0.82	11.13	7.18
	8	39.2	1.56 (NaN)	2.90 (NaN)	14.03
25 ºC	0	0	-1.63	-18.18	1.10
	1	10.6	0.17	-19.30	1.02
	4	19.2	0.80	0.35	5.46
	5	14.9	0.82	18.49	18.60
	6	26.3	1.74	-0.05 (NaN)	16.38

Table 7. Mean values of every variable for each day and storage temperature. Note that except the percentage, the other variables are dimensionless. Numbers marked with "NaN" were outliers and were not taken into account when making the MASLT model.

8. CONCLUSIONS

Overall, the Accelerated Shelf-Life Testings (ASLT) and its multivariate (MASLT) version proved to be extremely helpful tools in the determination of the shelf life of *Agaricus bisporus* mushrooms using the rate of weight loss, the changes in the ATR-FTIR spectra and the changes in color. Both methodologies show that when temperature increases, the mushrooms deteriorate at a faster rate. The univariate approach was too complex to accurately describe and estimate the shelf-life of these mushrooms, giving relevant results depending on the variable, either weight loss rate or the IR spectra (10.88-12.09 days at 5 ºC, 4.13-8.53 days at 15 ºC and 4.51-4.52 days at 25 ºC). However, ASLT was unable to yield meaningful results when using image data. On the other hand, the multivariate analysis (MASLT) using all four variables, based on PCA, was more useful to calculate the shelf-life (12.6 days at 5 ºC, 4.1 days at 15 ºC and 3.4 days at 25 ºC), since it followed a marked kinetic growth that fits well into the kinetic model.

The results show that there are still possible improvements regarding storage and data analysis. The abnormal kinetic slope behavior in the univariate tests showed that there was some uncontrolled systematic error that should be corrected. The chosen techniques proved to be really useful in the determination of the shelf-life, as they are very easy to carry out and contribute meaningful information to the multivariate model.

When it comes to each individual technique, there is room for improvement as well. The unexpected slope behavior in weight loss rate could be due to the plate not being properly sealed by the plastic film, or maybe due an unsteady relative humidity. The ATR-FTIR spectra has proven that only using the range for the polysaccharides and proteins was a good decision and properly explained the variance. In the future a methodology to remove the high humidity could prove to be key into improving the signal, as the high moisture content masks the relevant peaks. Color change was also a good idea, but it came with its share of problems. For once, preprocessing the images is quite complex, as one has to decide how to normalize it. This would most likely not be required if the sampling was done in a dark chamber with reference colors. Such chamber would remove the light variations that were encountered in this experiment and made it more difficult to analyze. On the other hand, Colourgrams_GUI really eased the work required to process the information from the images, and should totally be used

for image analysis going forward. Finally, the dark to white pixel ratio, despite being calculated from the same images as the change in color, proved to contribute sensitively different information, as evidenced by the loadings of the MASLT method. This parameter is easier to use than the change in color and it should be studied whether it can be used in place of the green colorgram.

Ultimately, MASLT is quite the useful method and could potentially save a lot of time and resources when applied to other kinds of samples like other types of food or pharmaceutical compounds. This line of research should be pursued by future experiments, while also introducing other changes, like using MCR-ALS instead of PCA or choosing other meaningful parameters.

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10. ACRONYMS

- ASLT: Accelerated Shelf-Life Testing
- HSI: Hue, Saturation, Intensity color space
- MASLT: Multivariate Accelerated Shelf-Life Testing
- MCR-ALS: Multivariate Curve Resolution – Alternating Least Squares
- MSC: Multiplicative Signal Correction
- PCA: Principal Component Analysis
- RGB: Red, Blue, Green color space
- SavGol: Savitzky-Golay

APPENDICES

APPENDIX 1: ADDITIONAL IMAGES

Figure 9. Set-up used to take the mushroom's images.

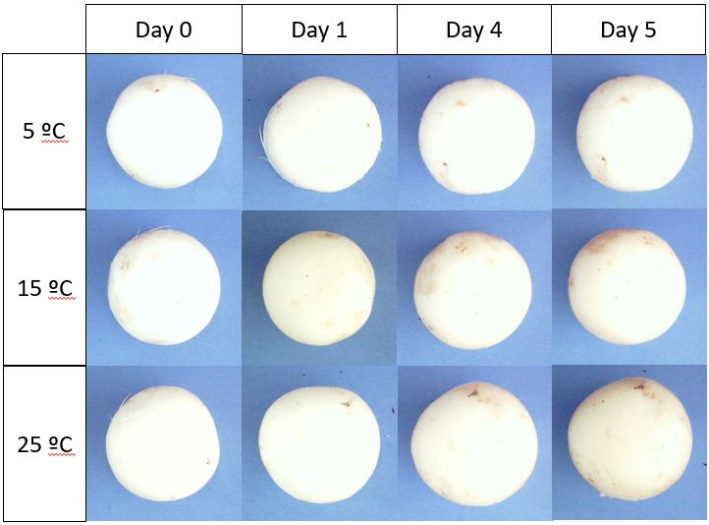

Figure 10. Comparison of the original mushroom images for the first 5 days.

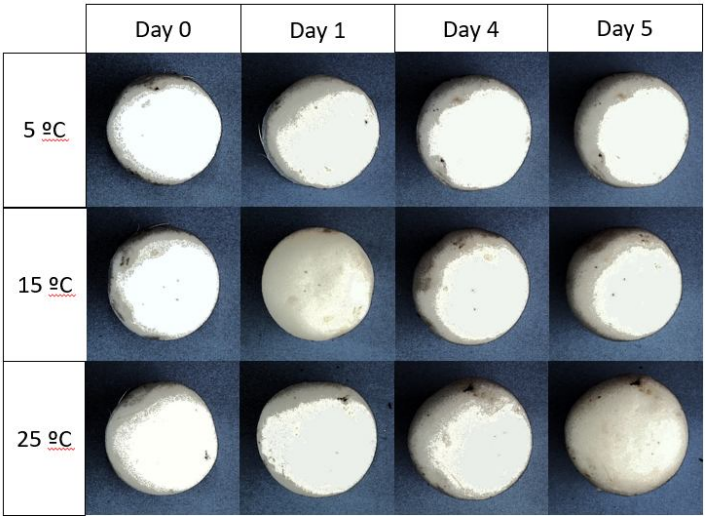

Figure 11. Comparison of the colod adjusted mushroom images for the first 5 days.

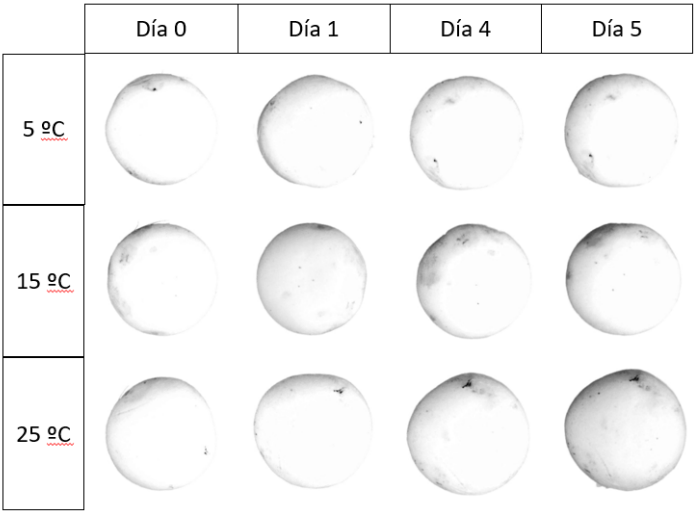

Figure 12. Comparison of the greyscale mushroom images without ackground for the first 5 days.

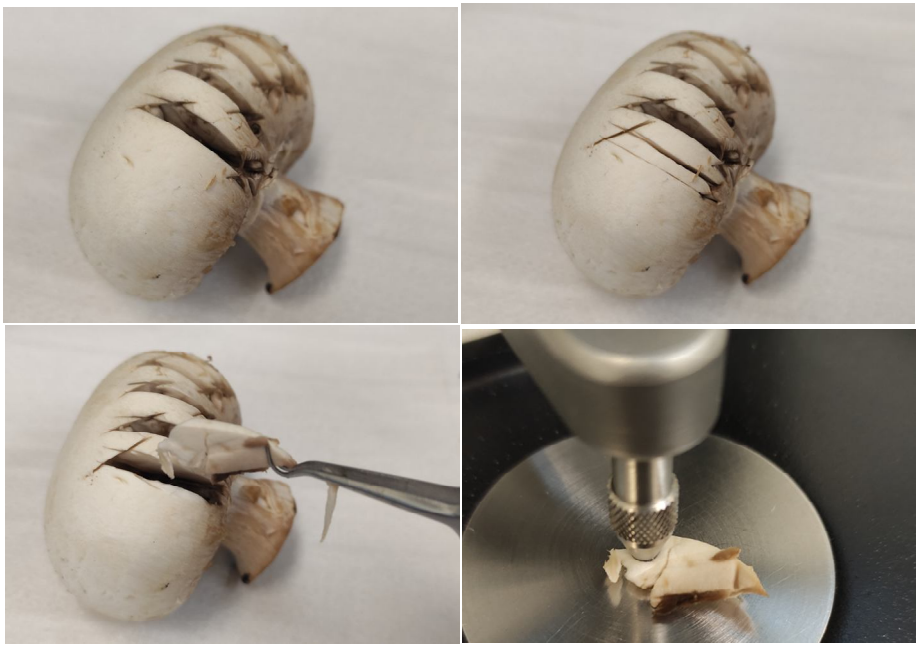

Figure 13. Process of sampling and measurment of ATR-FTIR spectrum.

APPENDIX 2: PCA ADDITIONAL DATA

1.ATR-FTIR spectra PCA model data:

Included: [1-95] [1-430]
Included (in axis units): [n/a] [981.638-1808.99]
Preprocessing: MSC (Mean), 1st Derivative (order: 2, window: 15 pt, tails: polyinterp)
Num. PCs: 5
Algorithm: SVD
Cross validation: venetian blinds w/ 10 splits and blind thickness = 1
RMSEC: 3.52366e-05
RMSECV: 4.57402e-05

Percent Variance Captured by PCA Model

Principal Component Number	Eigenvalue of Cov(X)	% Variance Captured This PC	% Variance Captured Total
1	6.13e-04	98.73	98.73
2	3.80e-06	0.61	99.34
3	2.23e-06	0.36	99.70
4	1.06e-06	0.17	99.87
5	2.48e-07	0.04	99.91

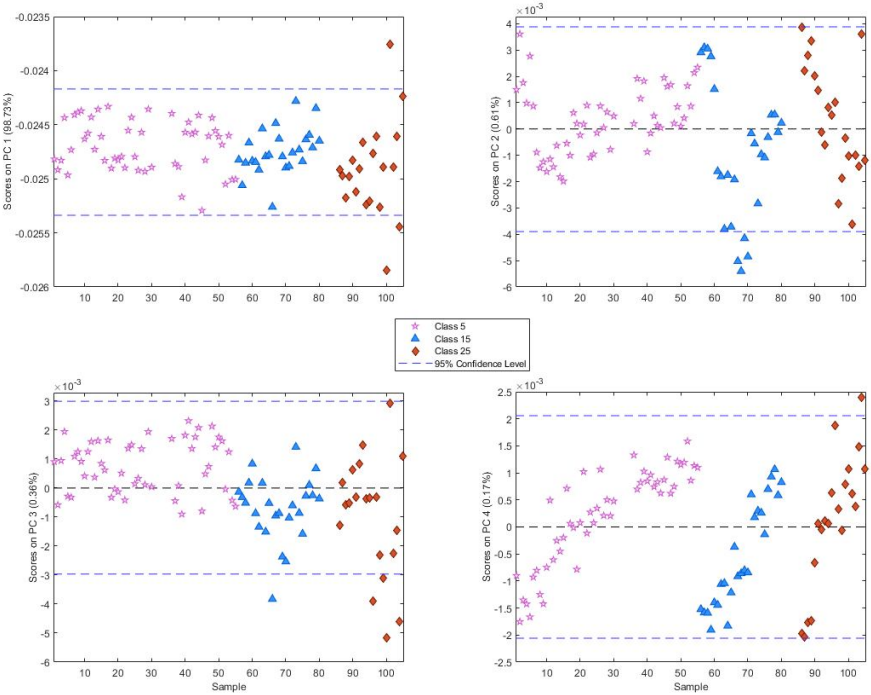

Figure 14. Score values for the first 4 components of the ATR-FTIR spectra.

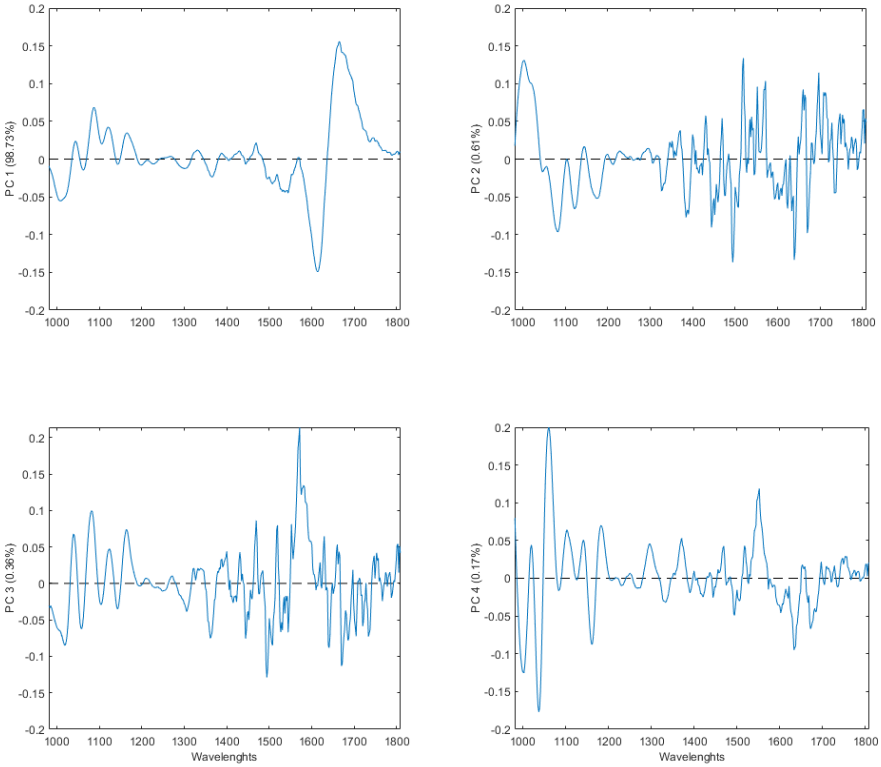

Figure 15. Loadings for the first 4 components of the ATR-FTIR spectra