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ORIGINAL ARTICLE

Chemometric studies of hops degradation at different storage forms using UV-Vis, NIRS and UPLC analyses

Estudos quimiométricos sobre a degradação de lúpulo em diferentes formas de estocagem usando UV-Vis, NIRS e análises por UPLC

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Abstract

Hops (*Humulus lupulus* L.) are one of the vital raw materials of brewing and their form of storage directly influences the final organoleptic sensations of beers. When hops are incorrectly stored, the degradation of important bitter compounds occurs fast. In the present work, it was used the ultraviolet/visible and near infrared regions, maximized by Ultra-Performance Liquid Chromatographic (UPLC) analyses, to identify the best way to store hops, by varying the *(i)* storage temperature, *(ii)* contact with atmospheric air, and *(iii)* storage time. For that, three different varieties of commercial hops were stored for six months (*Hersbrucker*, *Magnum* and *Zeus*). The chemometric results obtained with the Ultraviolet/ Visible (UV-Vis) and Near Infrared Spectroscopy (NIRS) data demonstrated the hop degradation kinetics under different storage conditions, while the chromatographic results provided the quantification of this degradation. Together, the results indicated that hops stored at low temperatures (≤ -10 °C) under a vacuum plastic bag presented the lower α - acids degradation rates over the months of the study.

Keywords: PCA; Variable selection; α - acids; β - acids; Humulones and Lupulones.

Resumo

O lúpulo é uma das matérias-primas vitais para a fabricação da cerveja e sua forma de armazenamento influencia diretamente nas sensações organolépticas finais das cervejas. Quando os lúpulos são armazenados incorretamente, a degradação de importantes compostos, responsáveis pelo amargor, ocorre de forma rápida. No presente trabalho, são apresentados estudos, nas regiões ultravioleta/visível e infravermelho próximo, maximizados por

Cromatográfica Líquida de Ultra Eficiência, para a identificação da melhor forma de armazenamento de lúpulos, através da variação da *(i)* temperatura de armazenamento, *(ii)* do contato com o ar atmosférico e *(iii)* do tempo de armazenamento. Para tanto, três variedades diferentes de lúpulos comerciais foram armazenadas por seis meses (*Hersbrucker, Magnum* e *Zeus*). Os resultados quimiométricos com os dados obtidos por UV-Vis e NIRS demonstraram claramente a cinética de degradação dos lúpulos em diferentes condições de armazenamento, enquanto os resultados cromatográficos promoveram a quantificação dessa degradação. Juntos, os resultados indicaram que os lúpulos estocados em sacos plásticos a vácuo em baixas temperaturas (≤ -10 °C) apresentaram a menor taxa de degradação dos α - ácidos durante os meses de estudo.

Palavras-chave: PCA; Seleção de variáveis; α - ácidos; β - ácidos; Humulonas e Lupulonas.

1 Introduction

Hop (*Humulus lupulus* L.), one of the vital components in brewing, is considered by many producers as the spice of this beverage. This plant is grown, almost exclusively, for beer production. Germany is the largest world producer, followed by the United States of America (USA), the Czech Republic, and China (BarthHaas, 2020). In Brazil, the hop was briefly introduced in the imperial era (1860-1870), and its culture was reborn a few years ago. Its short recent history has shown that hop culture is very promising due to some regions with proper climatic and geographical conditions (Simieli et al., 2021).

Brazil is the third largest beer producer in the world, and due to the impressive growth in the number of craft beer producers (MAPA, 2020, De-Souza et al., 2022), the demand for this raw material is increasingly high. Thus, the production of hops in the country has become an alternative strategy, in addition to the gains of the brewing industry, since it can use an input of greater freshness and stimulate the creation of a product with national identity (Freitas, 2021; Lopes et al., 2020).

In Brazil, research, in the last decade, was carried out on different varieties of hops with adapted and promising plantations. The state of Santa Catarina (SC) presented the first cultivators on the national scene (Silva, 1958) and is also the state with the highest percentage of producers, followed by Rio Grande do Sul (RS), São Paulo (SP), Paraná (PR), Minas Gerais (MG) and Rio de Janeiro (RJ). Other Brazilian regions, such as the Federal District and the mountains of Espírito Santo (ES), also show the beginning of small and medium-sized productions (Prefeitura Municipal de Viana, 2022; Silva, 2019).

Considering the lack of information regarding Brazilian cultivars (chemical, physiological and organoleptic characteristics), the development of research and the dissemination of their results were promoted (Almeida et al., 2021; Arruda et al., 2021). Due to this emerging demand, in November 2019 the 1st Brazilian Meeting of Researchers and Hop Producers was held in Botucatu City (SP) (Enbralúpulo, 2019). The objective of this meeting was to encourage national production to supply the domestic demand of the beer industry and the development of different biotechnological applications. In addition, in 2019, the Brazilian Congress of Beer Science and Technology (BRAU, 2019) took place, which aimed to address issues such as improving production processes and historical perspectives.

Hops used in beer production can be divided, basically, into two groups as follows: *(i)* those of bitterness; and *(ii)* the aromatic ones (Homini Lúpulo, 2018). The main species of aromatic hops are Cascade, Citra, Fuggle, Saaz, Golding, etc. Among the hops of bitterness, are Hallertauer Magnum, Zeus, Chinook, Admiral, etc. In addition, hops can help in the color of the drink (complexing with proteins), promote foam stability, and finally assist in supporting the sensory characteristics of the final product (Hieronymus, 2012; Boulton, 2013). This diversity, concerning the characteristics of hops, and their different chemical compositions is due to several factors, such as species, varieties, the form of cultivation, climate, soil composition, altitude, photoperiod, agricultural management, etc (Almaguer et al., 2014).

The chemical composition of hop flowers is complex, they can be grouped according to their secondary metabolites as their total resins (15 - 30% (w/w)), polyphenols (4% (w/w)), essential oils (0.5 - 3% (w/w)), proteins (15% (w/w)), among others. Inside the total resins, 10 to 25% (w/w) are the soft resins composed by α-acids (5 – 13% (w/w) and β-acids (3 – 8% (w/w) (Almaguer et al., 2014; Durello et al., 2019).

The α-acids, composed of five humulone isomers (humulone, cohumulone, ad-humulone and analogues), in general, are responsible for the compounds that generate the bitterness of beers (Durello et al., 2019), much appreciated in some styles, such as variations of Indian Pale Ale (IPA). The bitterness intensity depends on the hop mass added, level of α-acids (cohumulone % (w/w)) and how long hops are boiling (Malowicki & Shellhammer, 2005).

The β-acids, on the other hand, composed of five lupulone isomers (lupulone, colupulone, ad-lupulone, and analogs) present a relatively lower value in beer production, because they have low solubility in aqueous systems. However, they have compounds with important biological activities (Karabín et al., 2016) as antimicrobiological agents (on gram-positive bacteria), essential for the death of microorganisms present during storage (Schönberger & Kostelecky, 2011). In addition, these β-acids are sensitive to oxidation, becoming great oxidizing agents in beer (Karabín et al., 2016; HrnčIč et al., 2019).

Recently, a study regarding Brazilian hops has reported the presence of bioactive compounds (phenolic compounds such as isoquercitrin and quercetin) and promising antioxidant activities from ethanolic extracts (Almeida et al., 2019). Another study from the same research group presented chemical composition and antioxidant activity from a cascade variety of essential oil (Almeida et al., 2021).

As it is a flower, the how the hops are stored directly affects their quality, since their degradation or oxidation occurs quickly, and the previously desired sensory characteristics become undesirable. This degradation is one of the main problems faced by beer producers. To understand it, some studies have been carried out over the years (Danenhower & Baker, 2008; ASBC, 2010; Taniguchi et al., 2013; Canbas et al., 2001; Srečec et al., 2009; Carpenter, 2014).

Considering that the vast majority of hops used in Brazil are imported (pellets under vacuum or nitrogen atmosphere) and that Brazil, in recent years, has started to produce its hops, hop producers and craft brewers must learn how to store this raw material in the most correct and possible conditions.

Thus, this work aimed to evaluate the degradation of hops under different storage conditions using spectrophotometry in the UV-Vis region, with Near Infrared Spectroscopy (NIRS) data, Ultra-Performance Liquid Chromatography (UPLC) analysis, and chemometric tools.

2 Materials and methods

2.1 Reagents

Sodium hydroxide (≥ 99% of purity), was supplied by Sigma-Aldrich (Munich, Germany), while Methanol was acquired from JT Baker (Phillipsburg, USA). International Calibration Extract 2 (ICE-3) was obtained by American Society of Brewing Chemists (ASBC) (St. Paul, USA).

2.2 Hop samples

Three hop samples from BarthHaas® (Nuremberg, Germany), in pellet under nitrogen atmosphere, were acquired at Brew Shops in Vitória City (ES). The varieties were selected due to the α-acids (cohumulone) contents found on their labels and newest batches. Thus, the hops chosen for the study were: *(i) Hersbrucker*, with an α-acid content of 2.5% (w/w); *(ii) Magnum* with 11% (w/w) of α-acid content; and *(iii) Zeus*, with an α-acid content of 16% (w/w).

2.3 Storage conditions

The three hop samples studied were divided in four different conditions, absence of light: *(i)* At room temperature (27 °C), closed in polyethylene plastic bag (a); *(ii)* at room temperature (27 °C), closed in polyethylene vacuum plastic bag (b); *(iii)* at low temperature (≤ -10 °C), closed in polyethylene plastic bag (c); and *(iv)* at low temperature (≤ -10 °C), closed in polyethylene vacuum plastic bag (d). All samples were stored for six months and studied every two months *(0 - initial, 1 – two months later, 2 – four months later and 3 – six months later)*.

2.4 Sample preparation for ultraviolet and visible spectrophotometric analysis

The preparation of the samples for the Ultraviolet/Visible (UV-Vis) spectrophotometric analysis was followed according to Egts et al. (2012) with modifications. Thus, solutions of hop samples were prepared in 125 mL capped Erlenmeyers containing 1.25 g of crushed hops and 25 mL of methanol and kept under stirring, using a magnetic stir bar, for 30 min at room temperature (27 °C). After extraction, the solutions were left resting for 10 minutes followed by gravity filtration to remove solid particles. Then, 20 µL aliquots of the filtrates were placed in 10 mL of volumetric flasks, with their volumes adjusted with alkalized methanol (0.5 mL of 6 M NaOH in 250 mL of methanol). To prepare the blank, 20 µL of methanol in 10 mL of alkaline methanol solution were measured. All extractions were performed in triplicate. The three samples were analyzed every two months, according to the four conditions.

2.5 Ultraviolet and visible spectrophotometric (UV-Vis) analysis

The UV-Vis scanning analyses of the samples prepared in the previous section were performed on Agillent Cary 60 (UV-Vis Routine Spectrophotometer, Santa Clara, USA) between 200 and 400 nm, with resolution of 2 nm. Three aliquots of each replicate were analyzed in quartz capped cuvettes. The spectrophotometric data was acquired on Cary WinUV software.

2.6 Near infrared spectroscopy (NIRS) analysis

Diffuse reflectance spectra were obtained by using near-infrared on Qinterline model DairyQuant FT-NIR (Stengårdsvej, Denmark). The samples' spectrum of crushed pellets was profiled with 32 scans in a range of 1100 to 2500 nm and resolution of 4 nm. Six spectra were recorded for each sample. The spectroscopic data was acquired on InfraQuant software.

NIRS analysis was performed only at the beginning (*0 – initial*) and at the end of the storage time (*3 – six months later*).

2.7 Chemometric treatments

Two spectroscopic original data profiles were arranged into matrices format $\mathbf{X}_{\text{uv-vis}}$ (IxJ), $\mathbf{X}_{\text{nirs}}$ (NxM), each replicate was considered as one sample. Data analyses were carried out using Matlab v.2017 software (The MathWorks, Co., Natick, MA, USA) with the PLS_Toolbox computational package (Eigenvector Research, Inc. – PLS_Toolbox version 8.61.) (Wise et al., 2004).

Three pre-treatments were applied to both original data matrices ($\mathbf{X}_{\text{uv-vis}}$, $\mathbf{X}_{\text{nirs}}$) to minimize the effect of the analyses on different days, signal-to-noise ratio and baseline problems: *(i)* normalization by unit area; *(ii)* Savitzky-Golay smoothing (Savitzky & Golay, 1964) with a window size of 7 and 11 points, respectively, with first derivative; and *(iii)* mean centered pre-treatment. Principal Component Analysis (PCA) was used as the multivariate exploratory method (Ferreira, 2015). Variables were selected according to the Ordered Predictors Selection (OPS) method (Roque et al., 2019) followed by visual inspection. Other statistical

analyses (linear fit, means, standard deviations, etc) were performed in Origin Pro 2018 software (64 bit) (Northamton, USA).

2.8 Ultra-Performance Liquid Chromatography (UPLC)

2.8.1 Sample preparation

Hop samples from final storage time (*3 – six months later*) and storage conditions (a and d) were crushed with a mortar and pestle. Then, 0.25 g of each sample was accurately weighed and added to a 150 mL capped Erlenmeyer. First, the compounds were extracted with 20 mL of the extraction solution (85% Methanol/15% Water, acidified with 0.025% (v/v) formic solution), stirred for one hour using a magnetic stir bar and filtered through medium porosity filter paper. Then, they were washed by 10 mL of extraction solution, diluted to a 50 mL volumetric flask with extraction solution, filtered again using a 0.45 µm nylon syringe filter and quantified according to Danenhower & Baker (2008) with modifications. The volume of 50 µL of the final filtered solutions was injected into the UPLC system. The analyses were made in triplicate.

2.8.2 UPLC system

The quantification of cohumulone, ad-humulone + humulone, colupulone, ad-lupulone + lupulone contents % (w/w) was performed by an UPLC Acquicty UPLC® (Milford, USA) using a C-18 reverse phase column (Acquity UPLC® BEH C-18 1.7 µm, 2.1 x 50 mm) with an UV-Vis spectrophotometric detector at 326 nm. The analytical conditions used were: *(i)* flow of 0.8 mL min^{-1}; *(ii)* mobile phase (filtered and degassed): 85% of methanol, 15% of ultrapure water acidified with 0.025% formic acid; *(iii)* ambient temperature (27 °C); *(iv)* wavelength of 326 nm; and *(v)* injection of 50 µL (Danenhower & Baker, 2008 with modifications). The chromatographic data was acquired on Waters Empower® 3 software.

2.8.3 Preparation of the standards and analytical curves

A 0.15 g sample of ICE-3 (International Calibration Extract 3) was weighed accurately and diluted to a 25 mL volumetric flask with extraction solution. The standard (ICE-3) had a mixture of 13.88% cohumulone, 30.76% ad-humulone + humulone, 13.44% colupulone and 10.84% ad-lupulone + lupulone, totaling 44.64% of α-acids and 24.28% of β-acids (ASBC – American Society of Brewing Chemists).

Using the composition information provided by the ICE-3 standard label, the concentration of cohumulone and of all the other acids (ad-humulone + humulone, colupulone, and ad-lupulone + lupulone), in standards solution, could be determined in units of mg mL^{-1}.

A portion of the standard solution was filtered using a 0.45 µm nylon syringe filter and a set of 5 calibration standard with concentrations of cohumulone between 0.08 and 0.250 mg mL^{-1} was prepared.

3 Results and discussion

3.1 Chemometric study from UV-Vis data

The original data set (Figure 1A), obtained with the spectrophotometric measurements (200 and 400 nm), after being pre-treated (Figure 1B) was submitted to a PCA, aiming to observe the distribution of the samples in multivariate dimension. The normalization of the data was necessary due to different months of analysis, while the first derivative was applied to minimize the baseline problems of the original data. However, with the derivative, data became noisier so a smoothing by moving average with a window size of seven was used. Finally, the data was mean centered to translate the axis system along the mean vector, to the center of the data set.

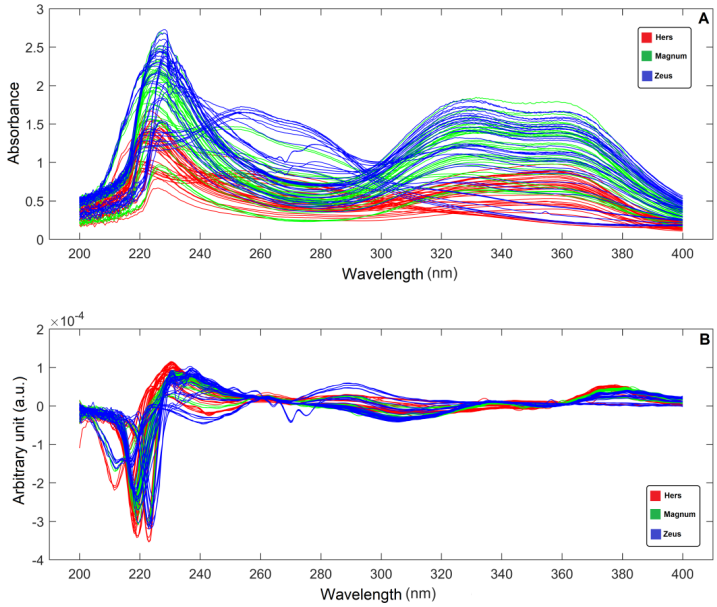

Figure 1. Original (A) and pre-treated (B) data of the three types of hops studied, in all storage conditions (**a**, **b**, **c** and **d**) and storage times (*0*, *1*, *2* and *3*), by UV-Vis spectrophotometry between 200 and 400 nm. Samples of *Hersbrucker* (—), *Magnum* (—) and *Zeus* (—) hops.

A PCA was calculated using variables between 230 and 340 nm, selected as the most important variables by OPS algorithm (Roque et al., 2019), and the accumulated variance of the first two principal components (PC1 and PC2) presented 94.05% of the original data information. The scores plot of PC1 *versus* PC2 (Figure 2) demonstrated the distribution of the three varieties of hop samples Hersbrucker (H), Magnum (M) and Zeus (Z), in addition to all the storage forms (a, b, c and d) and storage times (0, 1, 2 and 3).

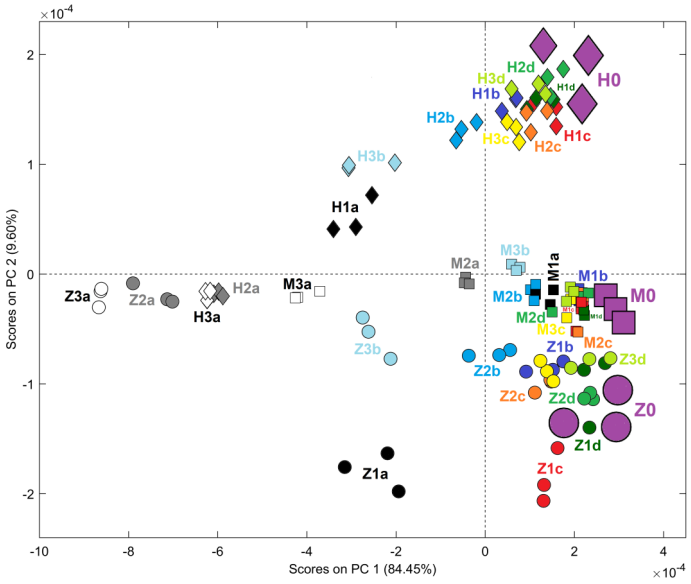

Figure 2. Score plot PC1 *vs*. PC2 showing the hops samples in all studied storage conditions (**a**, **b**, **c** and **d)** and storage times (*0, 1, 2* and *3)*. **H** indicates *Hersbrucker*, **M** *Magnum* and **Z** *Zeus*.

According to Figure 2, PC1 demonstrated the hops degradation trend in the different storage forms (a, b, c and d), moving the samples from the positive part of PC1 to the negative part of this component (degraded samples).

Under acutely evaluation, it could be noticed that the hops stored in plastic bag at room temperature (27 °C) (a), in the end of the 6 months of analysis, were arranged in more negative scores of PC1, the same was observed for those stored at room temperature (27 °C) and closed in vacuum (b) appearing in the central part of PC1 and finally, the samples stored at low temperature, regardless of the presence of oxygen or not (c and d) were arranged in positive PC1 scores.

The points represented by samples Z3a, H3a and M3a were the most degraded, demonstrating that all the samples stored in this condition did not keep their properties (a). In contrast, the points represented by Z3d, H3d and M3d were associated with the samples that kept their original characteristics better kept/maintained their characteristics over time (d), representing that they suffered less during storage. With minor differences, samples from condition 3c (Z3c, H3c and M3c) could appear near the 3d samples, a little less shifted to the left from PC1. This information indicates that temperature is more significant for degradation than the presence of atmospheric air in the packaging.

In PC1, it was also possible to notice that the rate of degradation of α-acids in the three hop samples was similar in the first months of the study (right part of PC1). However, the samples with higher levels of α-acids (*Zeus* and *Magnum*) continued degradation for a longer period (left part of PC1), while the degradation of α-acids from *Hersbrucker* sample had already occurred completely (central part).

The separation in PC2 occurred due to the original content of α-acids in the samples. In this component, on the positive side, there are the samples of *Hersbrucker* hops (initial content of α-acids of 2.5% (w/w)), negative/central part (*Magnum*, 11% (w/w)) while on the negative part of PC1 are *Zeus* samples (16% (w/w)).

Analyzing the loadings in PC1 (Figure 3A) and the original data (Figure 1A), it was possible to notice that the region around 275 nm started to show higher absorbance for the degraded compounds, while regions between 325 and 355 presented higher absorbance for α- and β-acids that have not yet undergone oxidation. A simpler visualization of the behavior of the loadings in PC1 can be made from the *Zeus* hops data in Figure 1A, because when the degradation of α - and β-acids occurs, the absorbance near 325 and 355 nm decreases, while the absorbance near 275 nm increases (Egts et al., 2012).

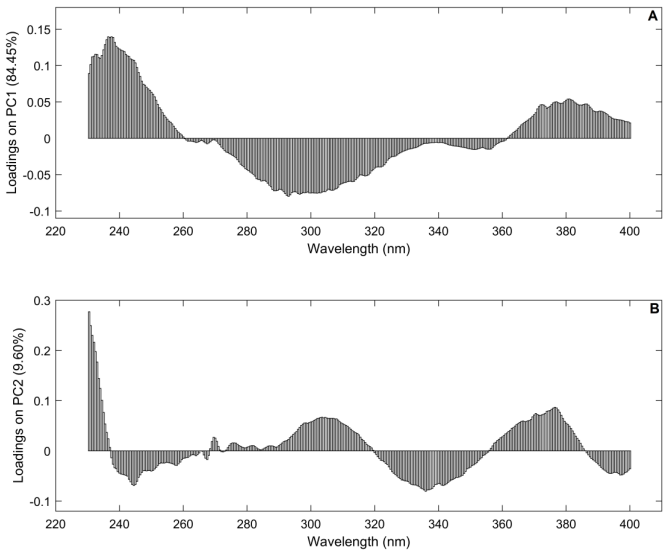

Figure 3. Loadings from PC1 and PC2 of the variables used to calculate the PCA.

On the other hand, it was possible to notice in loadings of PC2 that the region close to 325 nm demonstrated the discrimination according to the content of α-acids since the samples with higher content of these compounds (*Zeus*) could be found in the negative part of this component, *i.e.*, going to the positive part, where the samples with lower levels were found (*Hersbrucker*).

The aforementioned observation as well as the UPLC results corroborated with the information described by Canbas et al., (2001), in which when the hops are stored at low temperatures, they still undergo degradation, but it slows down the oxidation rate.

However, as discussed by Carpenter (2014), in comparison to those results, the oxygen (responsible for hops degradation, causing losses not only on their bitterness properties but also on their aroma) is not the main harmful substance for hops when samples are stored at low temperature (≤ -10 °C). In this case, the degradation rate of the compounds decreases significantly when stored at low temperatures (≤ -10 °C) even in the presence of oxygen.

In another work, the authors also reported that the lowest loss of α-acids occurred at low temperatures (4 to 7 °C), but the bags had to be well-closed with an inert gas (N_2) (Srečec et al., 2009).

3.2 Chemometric results of NIRS data

Analysis by NIRS had been carried out only at storage conditions (a - d) in the last stage of analysis (*3*). The original data presented in Figure 4A was pre-treated as indicated in section *2.8. Chemometric treatments*. After choosing the most adequate variables according to both visual inspection and OPS algorithm criteria of selection (Roque et al., 2019), the PCA was created with 15 spectral regions indicated in Figure 4B and described in Table 1.

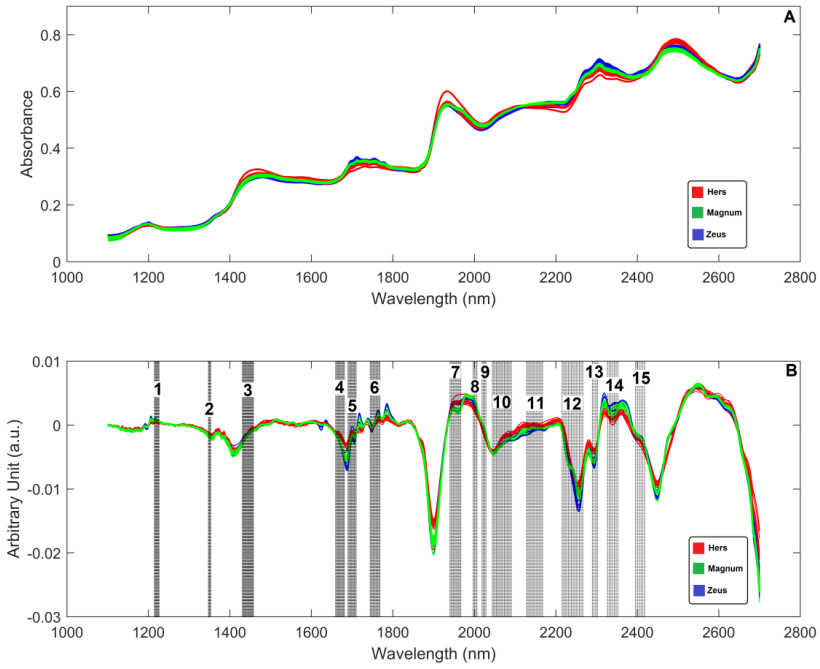

Figure 4. Original (A) and pre-treated (B) diffuse reflectance spectra of hops stored at different conditions (**a-d**) at final storage time (*3*). Samples of *Hersbrucker* (—), *Magnum* (—) and *Zeus* (—) hops. The vertical lines are the selected variables used to calculate de PCA. Figure constructed with the data from all conditions and at the initial and final storage time.

Table 1. Regions selected by OPS for the PCA construction.

Regions selected	General region ranges (nm)	Vibrational modes*	Charts
1[a,b]	1215 - 1226	2nd overtone of C–H	CH and CH_2
2[a,b]	1347 - 1353	2nd overtone of C–H	-
3[b]	1431 - 1458	2nd overtone of O–H C-H, N–H	ArOH, CH, CH_2, H_2O, ROH, $CONH_2$
4[a]	1660 - 1681	1st overtone of C–H	CH_3
5[a,b]	1690 - 1710	1st overtone of C–H	CH, CH_2, CH_3
6[a,b]	1745 - 1768	1st overtone of C–H	CH, CH_2, SH_3
7[b]	1941 - 1967	1st overtone of C–H	$CONH_2$, RCO_2R'
8[b]	1997 - 2007	1st overtone of C=O and O–H combination bands	-
9[b]	2019 - 2029	1st overtone of C=O and O–H combination bands	-
10[a]	2045 - 2091	1st overtone of C=O and O–H combination bands	$CONH_2$(H)
11[a]	2128 - 2168	N–H and O–H combination bands	ROH, $CONH_2$(R), RNH_2
12[a]	2216 - 2266	N–H and O–H combination bands	RNH_2 CC CHO
13[a,b]	2290 - 2302	C–H + C–H combination bands	H_2O, CH_2, CH_3
14[a,b]	2327 - 2353	C–H + C–H combination bands	CH, CH_2, CH_3
15[a,b]	2396 - 2418	C–H + C–H combination bands	CH, CH_2, CH_3

**Vibrational modes extracted from* Ribeiro et al., (2021); [a]. α- acids absorption; [b]. β-acids absorption (Halsey, 1987).

The calculated PCA presented an accumulated variance of 83.18% considering the first two principal components. Figure 5 shows the score plot between them (PC1 *vs*. PC2) and the distribution of the samples into the multidimensional space.

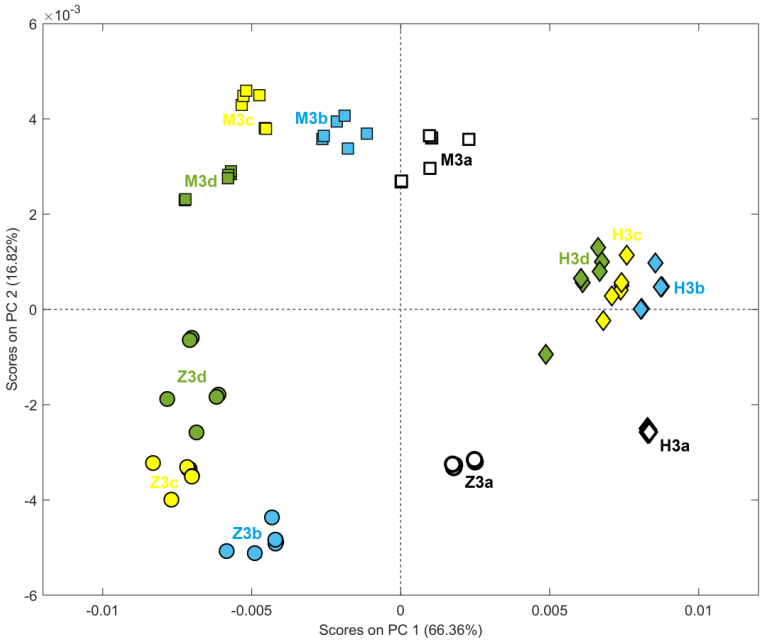

Figure 5. Score plot PC1 *versus* PC2 showing the hops samples in all studied conditions **(a, b, c** and **d)** and the final storage time (*3*). **H** indicates *Hersbrucker*, **M** *Magnum* and **Z** *Zeus* Hops.

At Figure 5, PC1 separated hops due to their α-acid contents. Presenting *H* samples more displaced to the right of PC1 and samples responsible for hops *M* and *Z* distributed over the central and left parts of this component. It is also possible to observe the separation by storage conditions (a-d), always following the trend of displacement from right to left of PC1.

The loadings on PC1 (Figure 6A) analyzed together with the original data (Figure 4A) indicated that spectral regions 4, 5, 12 and 13 (Table 1) were the main regions responsible for the separation of *H* from *M* and *Z* samples. On the other hand, spectral regions 6, 8, 9, 14 and 15 were important for *M* and *Z*, comparing to *H*. According to Halsey (1987) regions 4 and 12 could appear as specific bands for α-acids and regions 8 and 9 for β-acids.

On PC2 (Figure 6B), the main spectral regions with opposite high loading values were 12 and 7 (specific for α-acids and β-acids, respectively) (Halsey, 1987) separating samples of *M* from *Z*, passing throw *H*.

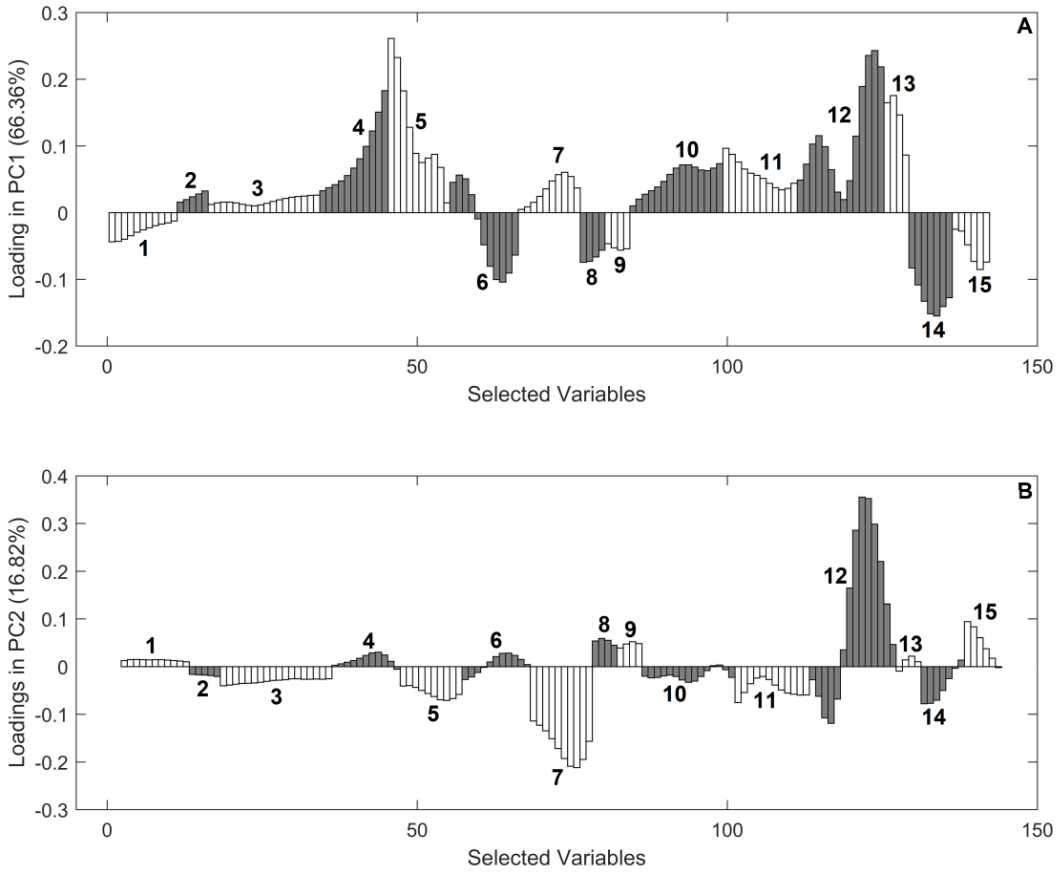

Figure 6. Loadings from PC1 and PC2 of the variables selected to calculate the PCA from NIRS data.

3.3 UPLC results

Through the five standard solution concentration values (mg mL^{-1}) and the means of the corresponding peak areas (Arbitrary Unit – a.u.) of each compound at the chromatograms, it was possible to calculate linear regressions using the least squares method (Table 2). Table 2 also contains the values of the intercept, slope, and correlation coefficient (r^2) calculated from the regressions.

Table 2. Integrated areas for each of the studied compounds and numerical values obtained from the linear regression by least squares.

	Peak Area (a.u.)			
Solutions	**Cohumulone**	**ad + humulone**	**Colupulone**	**ad + lupulone**
P1	102880	11644513	4716395	3369960
P2	2412916	9802961	3972689	2838808
P3	3198338	7650438	3111270	2220743
P4	4089386	5770281	2357392	1680893
P5	4861365	4083973	1725069	1310139
Intercept	*59938*	*128929*	137383	173086
Slope	$1.91x10^7$	$2.08x10^7$	$1.88 x10^7$	$1.62 x 10^7$
r^2	*0.998*	*0.998*	0.997	0.994

After performing the calibration steps and obtaining the necessary information from the peak areas of the six compounds of interest, it was possible to calculate their concentrations in mg mL^{-1} and convert the values to % (w/w) (Table 3).

Table 3. Concentration of the six main compounds studied in the three hops samples. Storage conditions (**a**, **b**, **c** and **d**) and storage time (*0*, *1*, *2*, and *3*).

	Samples	**Cohumulone % (w/w)***	**ad + humulone % (w/w)**	**Colupulone % (w/w)**	**ad + lupuline % (w/w)**
Initial condition (*0*)	*Hersbrucker*	2.5	-	-	-
	Zeus	16	-	-	-
	Magnum	11	-	-	-
Final (*3*) from **d** condition	*Hersbrucker*	0.95	1.11	6.00	13.90
	Zeus	14.15	16.08	9.20	8.04
	Magnum	7.76	25.79	9.37	15.94
Final (*3*) from **a** condition	*Hersbrucker*	*nd***	*nd*	*nd*	*nd*
	Zeus	*nd*	*nd*	*nd*	*nd*
	Magnum	0.38	*nd*	0.36	0.28

* Information extract from packing label. ***nd* means not detected.

The UPLC results presented in Table 3 demonstrated the same tendency of PC2 loadings (Figure 6B) where ad-lupulone + lupulone were higher for *M*, intermediate for *H* and lower for *Z*.

The degradation of hops revealed a decrease in α-acid (humulone, cohumulone and ad-humulone) and β-acid peaks (lupulone, colupulone and ad-lupulone), and the appearance of larger quantities of many oxidized compounds as humulinones and hulupones (cis and trans-humulinic acids, tricyclodehydroisohumulone, Ashurst's compound, tricyclic β-acid among others) (Taniguchi et al., 2013).

4 Conclusion

The chemometric results obtained from the two spectroscopic techniques (UV-Vis and NIRS) demonstrated that it is possible to use them to follow the degradation of hop samples, and the quantitative analyses of UPLC, indicated that the best method of hops storage occurs at low temperatures (≤ -10 °C) keeping the samples in vacuum plastic bags (**d** storage condition). It is important to point out that the presence of oxygen at low temperatures (≤ -10 °C) did not demonstrate a significant factor for the degradation of the samples (**c** storage condition).

After six months of storage (*3*) the three hop samples (*Hersbrucker, Magnum and Zeus*) in this condition (d), presented absolute degradation of the cohumulone (α-acid for bitterness) contents of 1.85%, 3.24% and 1.55% (w/w).

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