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Principal component and hierarchical cluster analysis to select hurdle technologies for minimal processed radishes

Rosario Goyeneche [a,b,*], Sara Roura [a,c], Karina Di Scala [a,c]

[a] Grupo de Investigación en Ingeniería en Alimentos, Facultad de Ingeniería, Universidad Nacional de Mar del Plata, Juan B. Justo 4302, 7600 Mar del Plata, Buenos Aires, Argentina
[b] Agencia Nacional de Promoción Científicas y Tecnológica (AGENCIA), Argentina
[c] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

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ABSTRACT

The application of principal component and hierarchical cluster analysis as a mathematical tool to select hurdle technologies (physical and chemical) to inhibit polyphenol oxidase (PPO) activity and color stability of minimally sliced radishes was presented. Results of PPO activity and color were reported initially (day zero) and after 4 d of refrigerated storage. Based on the mentioned tools and from the thirteen mono-hurdles technologies that were initially selected, eight hurdles were chosen as the best and combined in pairs. From those hurdles, the following double-hurdles were selected as those capable of retaining the product color: Ascorbic Acid (AA) + Acetic Acid (ACA-05), AA + NaCl-1, ACA-05+NaCl-1, AA + Heat Treatment (TT-501), ACA-05 + Ultrasound (US), Citric Acid (CA) + AA, CA + ACA-05, NaCl-1+US. When comparing the best mono-hurdles (AA, TT-501, NaCl-1) to the best double hurdles, the following hurdles: AA, TT-501, CA + ACA-05, NaCl-1+US, CA + AA and NaCl-1 were chosen as those capable of maintaining the typical color of radish slices with acceptable sensory parameters and enzyme inhibition after 4 d of storage.

1. Introduction

Diets rich in horticultural crops are associated with reduced risk of several diseases due to potent antioxidant properties of phytochemicals that decrease oxidative stress in consumers (Zhang et al., 2013).

Radish (*Raphanus sativus* L.) is a root crop pungent or sweet, rich in folic acid, Vitamin C and anthocyanins (Patil, Madhusudhan, Ravindra Babu, & Raghavarao, 2009). Although radishes are widely used in salad preparations, the rapid deterioration mainly due to slices browning decreases the marketability of these preparations (Andi et al., 2011; Gonzalez-Aguilar, Wang, & Buta, 2001). During processing of minimally-processed radishes operations like peeling, cutting, etc. induced the biosynthesis of enzymes associated with several biochemical reactions responsible for changes in color, aroma, texture and nutritional value (Albanese, Cinquanta, & Di Matteo, 2007; Saavedra del Aguila et al., 2008). Therefore, controlling the physiological response during preparation processes is the key factor to obtain a good quality minimally processed product (Saavedra del Aguila et al., 2008). Oxidative browning is usually caused by the enzyme polyphenol oxidase (PPO) which converts phenolic compounds of fruits and vegetables into dark colored pigments. Chemical methods to inhibit PPO activity consist of using different types of additives as reducing, acidulant, chelating, and complexing agents, or compounds that directly inhibit the enzyme (Amodio, Cabezas-Serrano, Peri, & Colelli, 2011). Ascorbic and citric acids have been shown to be effective anti-browning agents on different fresh-cut products (Gorny, Cifuentes, Hess-Pierce, & Kader, 2000; Lattanzio, Linsalata, Palmieri, & Van Sumere, 1989; Tortoe, Orchard, & Beezer, 2007). In addition, calcium treatments have been used to extend the shelf life of fruit and vegetables. Calcium is reported to maintain firmness by cross-linking with cell wall and middle lamella pectins forming calcium pectate (Grant, Morris, Rees, Smith, & Thom, 1973; Rico, Martín-Diana, Barat, & Barry-Ryan, 2007).

Regarding physical methods, ultrasound can have either destructive or constructive effects to cells depending on the sonication parameters employed. Microbial and enzyme inactivation (preservation), e.g. in fruit juices and sauces, are one application of ultrasound in the food processing (Rico et al., 2007). Thermal

* Corresponding author. Grupo de Investigación en Ingeniería en Alimentos. Facultad de Ingeniería, Universidad Nacional de Mar del Plata. Juan B. Justo 4302, 7600, Mar del Plata, Buenos Aires, Argentina. Tel.: +54 223 4816600; fax: +54 223 4810046.
E-mail address: rogoye@fi.mdp.edu.ar (R. Goyeneche).

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treatment is another useful physical method to improve post-harvest quality of several horticultural products, inhibiting enzymes activity as well as delaying phenolic compounds and tissue browning (Rico et al., 2007).

Hurdle technologies provide a framework for combining a number of milder preservation techniques to achieve an enhanced level of product safety and stability (Gupta et al., 2012). In addition, the application of chemometric tools for characterization and quality control of food products has recently become a very active research area. Multivariate mathematical approaches are powerful tools which often permit a relatively simple representation of similarities between samples on the basis of more-or-less complex analytical data (Patras et al., 2011). Principal Component Analysis (PCA) is one of the most popular multivariate techniques because it reduces the dimensionality, compresses the noise and correlates measurements in a simple informational sub-space of the data set (Brereton, 2009, chap. 1).

The present study aimed to analyze and compare the effects of different hurdle technologies (physical and chemical) on the color and the inhibition of PPO of minimally processed sliced radishes during refrigerated storage based on PCA and hierarchical cluster analysis (HCA).

2. Theoretical considerations

2.1. Principal component analysis

PCA is the most preferred multivariate technique due to the reduction in the original dataset dimensionality by explaining the correlation amongst a large number of variables in terms of a smaller number of underlying factors without losing much information (Sarbu et al., 2012). PCA transforms the original measured variables into new uncorrelated variables called Principal Components (PC). The first component describes most of the variation in the data. The second principal component is orthogonal and covers much of the remaining variation and so on (Keenan, Valverde, Gormley, Butler, & Brunton, 2012). The PCs are useful tools for examining the relationships between objects, looking for groups and trends, sorting out outliers.

2.2. Cluster analysis

Cluster analysis is a well-known and widely used unsupervised clustering procedure with its hierarchical and non-hierarchical approaches. Cluster analysis is a multivariate analysis technique used to sort samples (in our case treatments applied) into groups. The Ward's method as the amalgamation rule and the squared Euclidean distance as metric were used to establish clusters (Sarbu et al., 2012).

Hierarchical clustering (HCA) uses a defined metric to form clusters sequentially; grouping the most similar objects first and these initial groups are merged due to their similarities. As the similarity decreases, all groups are fused together into a single cluster (Keenan et al., 2012).

3. Materials and methods

3.1. Plant material and sample preparation

Radishes were purchased from a local market in Mar del Plata, Argentina. They were kept at 5 ± 1 °C in darkness prior to processing. Radish roots were separated from leaves. The roots were washed in tap water to eliminate any surface contamination and cut with a vegetable cutter (HLC-300, Dynam-h, SYSTEL S.A., Argentine) into slices of 4 mm. Then, they were washed again in tap water using a sliced radish to water ratio of 1:10. The slices were dried by a manual centrifuge, and then the hurdles were applied.

3.2. Treatments

Two experiments were consecutively conducted. First, the application of physical and chemical treatments as mono-hurdles was carried out. Anti-browning agents were chosen among the most effective in preventing browning of several fresh-cut products, as reported in the literature. Regarding physical hurdles, ultrasound (180W, TestLab) and heat treatment (Lauda-GMBH & CO. KG) were applied. Samples called "CO" were those without treatments. Table 1 shows the treatments, the concentration or condition and the codes of the thirteen selected mono-hurdles. Results of these treatments were presented in the section called "Experiment 1". In order to test the combination effects of the most effective compounds, a second set of experiments were conducted. After mono-hurdles application and PCA and HCA analysis was conducted, some hurdles were selected as feasible, and therefore they were applied combined in pairs. The results of this second analysis were reported in the section called "Experiment 2".

In all hurdles where immersion was involved, the time of immersion was 5 min with periodical stirring. The ratio between the solids (sliced radish) and the soaking solutions was 1:10. After each dipping, the slices were dried by a manual centrifuge and samples of 50 g were packaged in polyethylene bags (25 cm × 30 cm) of 25.4 μm thickness (with an O_2 transmission rate of 600 $cm^3\ m^{-2}\ d^{-1}$, CO_2 transmission rate of 4000 $cm^3\ m^{-2}\ d^{-1}$, and water vapor transmission rate of 4 $cm^3\ m^{-2}\ d^{-1}$; $P = 101{,}325$ Pa, $T = 25$ °C) using a manual impulse sealer (HL, FS-300, Argentina). Two bags for each treatment were analyzed at 0 and 4 d of storage at 9 ± 1 °C.

3.3. Measurement of the enzyme activity

PPO activity was measured by the colorimetric method according to previous work of Goyeneche, Di Scala, and Roura (2013). 10 g of radishes were homogenized with a commercial mixer at 1:2 ratio with 0.5 mol L^{-1} phosphate buffer (pH = 7.0) in the presence of 50 g L^{-1} polyvinylpyrrolidone (ICN Biomedicals, Inc. OH) and centrifuged at 12.700 × *g* for 30 min. The supernatant, which contained PPO activity, was used as the experiment enzyme source (PPO crude vegetable extract). Crude extract was maintained at 0 °C until use. Gallic acid (4 $mmolL^{-1}$) on phosphate buffer (pH = 7) was used as the substrate solution. The reaction cuvette contained 2.9 mL of substrate solution and 0.1 mL of PPO crude vegetable extract, and the reference cuvette contained substrate solution.

Table 1
Physical and chemical mono-hurdles technologies applied to sliced radishes.

Hurdle	Concentration or condition	Code
Citric acid	0.6% w/w	CA
Ascorbic acid	1% w/w	AA
Lactic acid	1% w/w	LA
Acetic acid	1% w/w	ACA-1
Acetic acid	0.5% w/w	ACA-05
Chitosan on acetic acid	5 g/L	QUI-ACA
Chitosan on lactic acid	5 g/L	QUI-LA
Sodium chloride	5% w/w	NaCl-5
Sodium chloride	1% w/w	NaCl-1
Calcium chloride	2% w/w	$CaCl_2$
Ultrasound	5 min	US
Heat treatment	50 °C, 1 min	TT-501
Heat treatment	60 °C, 1 min	TT-601

The enzyme activity was defined as a 0.001 change in absorbance at 350 nm between 0 and 60 s under the assay conditions, according to previous experiments (Goyeneche et al., 2013). Each solution was tested in triplicate.

3.4. Color measurements

The color development was measured on the sliced surfaces with a colorimeter (Lovibond, RT Series, England). The colorimeter had been standardized against a white tile ($L^* = 97.63$, $a^* = 0.3133$, $b^* = 0.3192$). The measurements were made in triplicate over each surface sample at 0 and 4 d of storage at 9 ± 1 °C. Color was recorded using a CIE – L^* a^* b^* uniform color space (Lab), where L^* indicates lightness (whiteness or brightness/darkness), a^* indicates chromaticity on a green (−) to red (+) axis, and b^* chromaticity on a blue (−) to yellow (+) axis (CIE 1978). Numerical values L^*, a^*, b^* were converted into total color difference (ΔE) (Eq. (1)), according to:

$$\Delta E = \sqrt{(a^* - a_o)^2 + (b^* - b_o)^2 + (L^* - L_o)^2} \quad (1)$$

Total color difference (ΔE) is generally used to inform the difference between two colors as indicated by the following scale: Trace level difference $\Delta E^* = 0$–0.5, Slight difference $\Delta E^* = 0.5$–1.5, Noticeable difference $\Delta E^* = 1.5$–3.0, Appreciable difference $\Delta E^* = 3.0$–6.0, Large difference $\Delta E^* = 6.0$–12.0, Very obvious difference $\Delta E^* > 12.0$ (Chen & Mujumdar, 2008 , pp. 26–29).

3.5. Sensory evaluation

Sensory acceptability of radish slices was assessed in a taste panel suite by a trained sensory panel (5 members) using a descriptive test, as described by Alegria et al. (2009) with some modifications. Color (two scales, brown scale to observe browning -ColorM- and violet scale to observe pigment diffusion –ColorV-) as well as texture was subjectively evaluated on three samples per treatment and evaluation date. The analysis was performed on day zero and on day four. For each index (color and texture) the scale used rated from 0 (best quality) to 5 (worst) and scores above 3 indicated the rejection of the product.

3.6. Statistical analysis

Pattern recognition methods such PCA and HCA were applied to the data using PC-ORD software (McCune & Mefford, 2011). Experiments were performed in triplicate. Values are expressed as means ± standard deviations.

4. Results and discussion

4.1. Experiment 1

Fig. 1A and B shows a representation of different hurdle technologies for zero time and four days of storage based on principal components as well as the hierarchical cluster analysis of sliced radish data quality. The covariance matrix is used to measure how much the dimensions vary from the mean with respect to each other.

Initially (at day zero), axis 1 (PC1) representing the color changes, and explains 83% of the total variance in the data set and axis 2 (PC2) expresses texture changes and explains 10%. From these results, the variation of the natural color of radish slices after the application of physical and chemical mono hurdles have more impact on sensory acceptance respect to texture changes. Furthermore, the inhibition of the PPO enzyme activity has no significant impact on the acceptance or rejection of the hurdle.

After 4 d of storage, Axis 1 (PC1) explains 91% of the total variance, which correspond for color changes and Axis 2 (PC2) only explains 8% of the total variance (corresponding with textural changes).

Immediately after hurdle applications, through PCA and HCA analysis, the samples are divided into five groups (Fig. 1A): Group 1 – represented for the mono-hurdle NaCl-5, located at one end of the axis 2, with excessive texture softening (texture value higher than 4) and PPO inhibition of 20%; Group 2 – represented for the mono-hurdles ACA-1 and LA, with ΔE values higher than 10, caused by diffusion of the skin pigment (ColorV higher than 3) and PPO inhibition of 30–40%; Group 3- represented for the mono-hurdles QUI-ACA and TT-601, with similar behavior than samples of Group2, but less strong; Group 4 – represented for the mono-hurdles TT-501, QUI-LA, US, NaCl-1, and AA, with a general global acceptance, all sensorial parameters below 3, ΔE values lower than 3 and PPO inhibition between 10 and 40%; and finally, Group 5 – conformed by the mono-hurdles CO, $CaCl_2$, CA, ACA-05 whose samples not significantly differ to control, i.e. treatments not affected the samples.

After a storage period of four days, PCA and HCA analysis allows to divide the hurdles into five groups (Fig. 1B). Group 1 – located at one extreme, the hurdles NaCl-5, ACA-1 and QUI-ACA, with very high ΔE (values up to 25) and with excessive texture softening (texture value higher than 4). All these hurdles are discarded for combined application. The two central groups (near the axis 2) are represented for treatments with ΔE values higher than 15, caused by different factors; while Group 2 – QUI-LA, CA, US, LA present high value of sensorial parameter ColorM (higher than 3), with presence of browning on the slices, texture softening (texture value over 3) and no inhibition of PPO; the other group (Group 3), represented for ACA-05 and TT-601, show pigment diffusion toward the white part of the slices (ColorV > 4) but texture retention (texture value below 3) and 30% of PPO inhibition. In the left of the graph are two groups: one conformed for CO and $CaCl_2$ (Group 4), and the other composed AA, TT-501 and NaCl-1 (Group 5). This last group show percentages of PPO inhibition between 10 and 40%, and ΔE values lower than 6, with all sensorial parameters below 3. Consequently, the mono-hurdle $CaCl_2$ does not improve (or worse) the behavior respect control sample, so it is selected as a hurdle feasible to be implemented.

Based on the premise that the hurdles must cause minimal effect on color and texture as well as to minimize PPO activity, the following hurdles are discarded: ACA-1, QUI-ACA, QUI-LA, NaCl-5 and TT-601. ACA-1 due the unacceptable color and texture changes observed at day four. QUI-ACA and QUI-LA are discarded because addition of chitosan does not improve the effect caused by the acids applied alone (ACA and LA). NaCl-5 was eliminated due to the unacceptable texture observed at day four (slices were very soft); however at lower concentration (1%) the hurdle performed better. TT-601 is discarded because the thermal treatment applied caused an immediately significant discoloration of the slices. Moreover, after 4 d, the slices are dyed pink and they exuded liquid inside the bags.

Finally, from the eight hurdles that are selected as suitable to be applied as a double hurdles is possible to select those having better retention of radish sliced natural color: AA, TT-501 and NaCl-1. Similar results were obtained by Perez-Gago, Serra, and Del Rio (2006) and Son, Moon, and Lee (2001) when applying ascorbic acid on fresh-cut apples. However, other authors did not report satisfactorily results for shredded radish (Saavedra del Águila et al., 2008). Inhibition of enzymatic browning was not observed on sunflower with NaCl applications (De Leonardis, Lustrato, Macciola, & Ranalli, 2010). Finally, Roura, Moreira, Ponce, and Del Valle (2003) found that 50 °C/1 min could be useful for short time preservation of minimally processed lettuce.

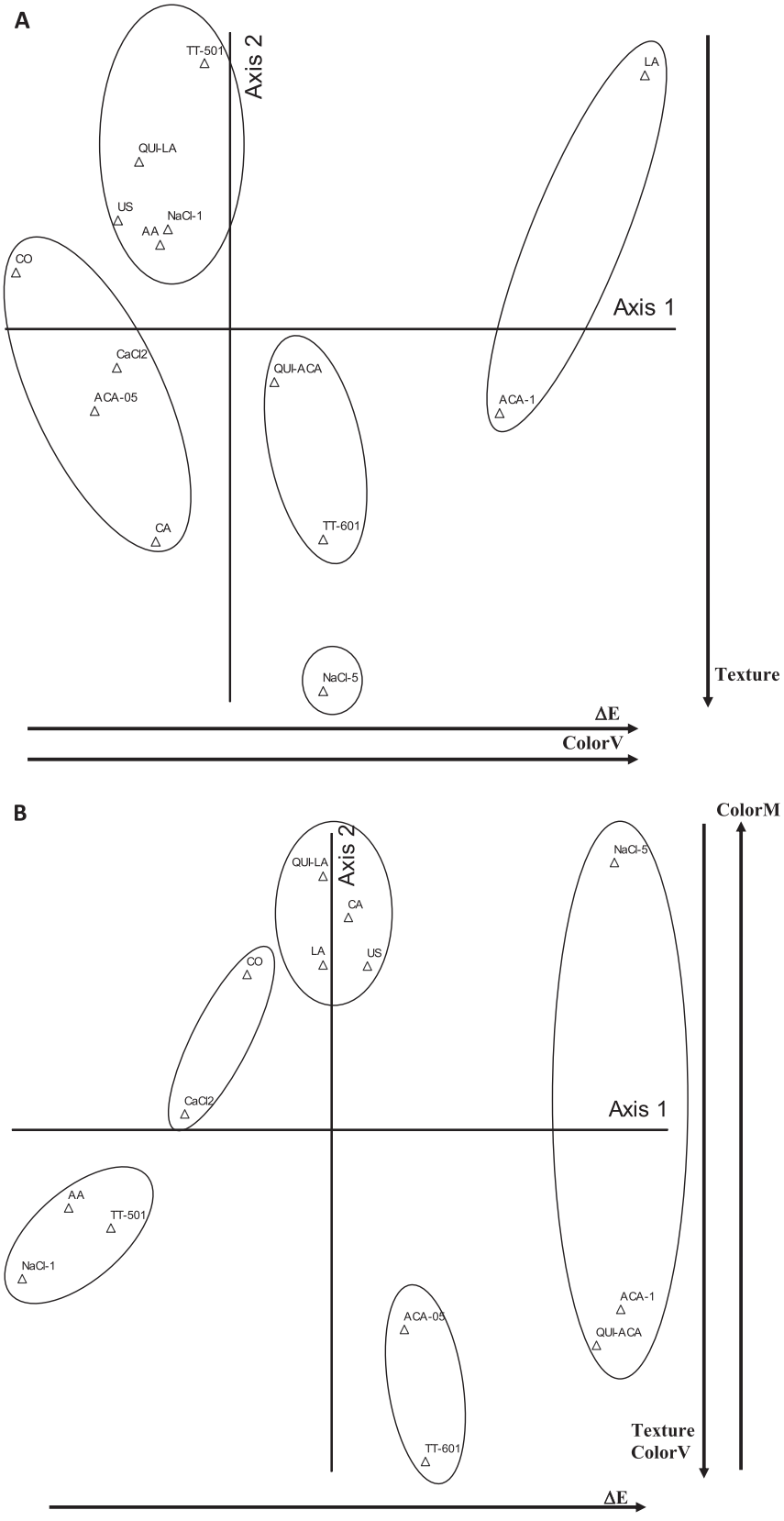

Fig. 1. PCA and HCA for mono-hurdles applied to radish slices at zero time (A) and after 4 d of storage (B).

4.2. Experiment 2

In a second stage, the eight selected hurdles were combined in pairs, resulting in 28 possible treatments. Fig. 2 shows a representation of the data with two principal components. Data for zero time and four days of storage are presented in Fig. 2A and B, respectively.

Immediately after the application of the combined hurdles, axis 1 (PC1) which corresponds to ΔE variation, explains 92% of the total variance in the data set and axis 2 (PC2), which corresponds to changes in texture and violet pigment diffusion, explains only 4%. At the end of storage, axis 1 (PC1, corresponding to ΔE variation) remains the most significant and explains 85% of the total variance in the data set and axis 2 (PC2), changes in texture and violet pigment diffusion, explain 9%.

At day zero, immediately after treatment application, the samples are divided into six groups (Fig. 2A): Group 1 – represented for samples that did not differ from the control, with all sensorial parameters below 3 and ΔE values lower than 3 (CO, AA + TT-501, CA + AA, AA + $CaCl_2$, CA + $CaCl_2$, AA + US, CA + US, ACA-05 + $CaCl_2$); Group 2 – represented for samples with ΔE values around 5 and 30% of PPO inhibition (CA + ACA-05, AA + NaCl-1, LA + ACA-05, LA + NaCl-1, ACA-05 + NaCl-1, NaCl-1+$CaCl_2$, ACA-05 + TT-501, $CaCl_2$ + TT-501); Group 3 – represented for AA + ACA-05, CA + NaCl-1, CA + TT-501, NaCl-1 + TT-501, ACA-05 + US, NaCl-1 + US, $CaCl_2$-US, US + TT-501; which presents ΔE values around 10, sensorial acceptance (all sensorial indices values below 3) and PPO inhibition between 0 and 40%; Group 4 – represented for samples with 30–40% of PPO inhibition, but ΔE values upper 10, corresponding with pigment diffusion and violet dye of slices (ColorV values above 3) (AA + LA, LA + $CaCl_2$, LA + TT-501). The last two groups are composed by one element each, Group 5 – represented for LA + US, with ΔE values around 18, pigment diffusion (ColorV values above 3) and no PPO inhibition, and finally Group 6 – represented for samples with 30% PPO inhibition, but ΔE value higher than 25 and sensorial rejection because they show violet dye (CA + LA).

At day four, seven groups are selected (Fig. 2B): Group 1 – located at the lower right corner, represented for samples with ΔE value above 20, corresponding with pigment diffusion and violet dye of slices (ColorV values above 4) and texture softening (LA + TT-501); Group 2 – represented for samples with PPO concentration double to fresh control samples, with ΔE values around 20, and ColorM values higher than 3, showing browning of the slices (CA + US, LA + $CaCl_2$, LA + US, $CaCl_2$+US, US + TT-501); Group 3 – (CA + LA, LA + ACA-05, LA + NaCl-1) and Group 4 – (CA + TT-501, ACA-05 + TT-501, NaCl-1 + TT-501, $CaCl_2$ + TT-501) represented for samples with ΔE values higher than 15 but while Group 3 is sensory rejected by browning (ColorM higher than 4) and 10% of PPO inhibition is observed, Group 4 have no PPO inhibition; Group 5 – represented for samples that not differed from control stored samples (CO, AA + $CaCl_2$, CA + $CaCl_2$, ACA-05 + $CaCl_2$, CA + NaCl-1, AA + LA, NaCl-1 + $CaCl_2$), that means that the application of these hurdle combinations do not provide any improvements over the control sample; Group 6 – represented for samples that despite having ΔE values higher to 10, they are acceptable from the sensorial point of view (all sensory parameters below 3) and has no inhibition of PPO at zero time (AA + ACA-05, AA + NaCl-1, ACA-05 + NaCl-1, AA + TT-501, ACA-05 + US). Finally, Group 7 is represented for samples with ΔE values around 5, sensorial acceptable scores and 15% of PPO inhibition (CA + AA, CA + ACA-05, NaCl-1 + US).

From the results obtained after double hurdles application at 0 and 4 d, the following hurdles were selected for better retention of radish natural color: AA + ACA-05, AA + NaCl-1, ACA-05 + NaCl-1, AA + TT-501, ACA-05 + US, CA + AA, CA + ACA-05, NaCl-1 + US. Roura et al (2003) found beneficial effects on lettuce with ascorbic acid + citric acid.

4.3. Best hurdles selection

In order to analyze the efficiency of all the applied treatments, the selected best hurdles (eight double and three simple hurdles) were analyzed together (PCA and HCA analysis), to determine the best treatments. For both zero and four days samples are located along axis 1, which explained at zero time 95% of the variation, while at four time explains 83%. These axes are strongly affected by changes in ΔE values.

At day zero, five groups are conformed (Fig. 3A). Group 1 is control sample (CO); that sample was taken as reference. The remaining groups mainly differed on ΔE value, although from a sensory point of view were all acceptable. The changes observed in ΔE are mainly due to the decrease of L^* parameter, meaning a darkening of radish slices. However, these objective color changes were not perceived by the sensory panel. Group 2 is composed by ACA-05 + US, AA + ACA-05, with ΔE values near 9. Group 3 is represented by NaCl-1 + US hurdle, which have ΔE value around 7 and without inactivating the PPO enzyme. The Group 4 formed by TT-501, ACA-05 + NaCl-1, CA + ACA-05, AA + NaCl-1 hurdles, presenting ΔE values between 4 and 5, and showing a PPO inhibition percentage between 10 and 30%. Finally, Group 5 is composed by AA + TT-501, NaCl-1, AA, CA + AA hurdles, which correspond to the best set of hurdles at zero time, with ΔE values about 3, and average inhibition of PPO of 50%.

At the end of storage (4 d), four groups are conformed (Fig. 3B). Group 1, represented by the control sample (CO), is separated from the rest due to PPO activity was twice the fresh sample and also because is sensory rejected by browning (ColorM greater than 3). Group 2, formed by ACA-05 + NaCl-1, AA + TT-501, AA + ACA-05, ACA-05 + US, AA + NaCl-1 hurdles, is located to the right side of the axis 2 and presented ΔE values greater than 10, and is therefore discarded, despite being suitable from a sensory point of view. The others two groups are located on the left side of the axis 2. Group 3 is formed by AA, TT-501, CA + ACA-05, NaCl-1 + US, present ΔE values less than 6, are acceptable from a sensory point of view and presented PPO activity similar to fresh control or even lower. Group 4, conformed for CA + AA and NaCl-1, present ΔE values less than 2, are acceptable from a sensory point of view and presented PPO activity similar to fresh control.

Because all the selected hurdles at this point of analysis have acceptable sensory parameters and enzyme inhibition, a selection criteria based on those hurdles with ΔE values less than 6 (noticeable or appreciable difference, according to Chen & Mujumdar, 2008, pp. 26–29) is proposed and those hurdles would be the best. Therefore, treatments corresponding to Groups 3 and 4 (AA, TT-501, CA + ACA-05, NaCl-1 + US, CA + AA and NaCl-1) are chosen as those with the best color characteristics (like typical radish slices).

5. Conclusions

Application of principal component and hierarchical cluster analysis to select hurdle technologies (physical and chemical) for inhibition of PPO activity and color stability of sliced radishes are reported in this investigation. From the results, after double hurdles application at 0 and 4 d, the following hurdles are selected for better retention of radish natural color: AA + ACA-05, AA + NaCl-1, ACA-05 + NaCl-1, AA + TT-501, ACA-05 + US, CA + AA, CA + ACA-05, NaCl-1 + US. However, at the end of the storage (day four) the hurdles: AA, TT-501, CA + ACA-05, NaCl-1 + US, CA + AA and NaCl-1 are chosen as those capable of maintaining the characteristic fresh-like color of radish slices with acceptable sensory parameters and enzyme inhibition.

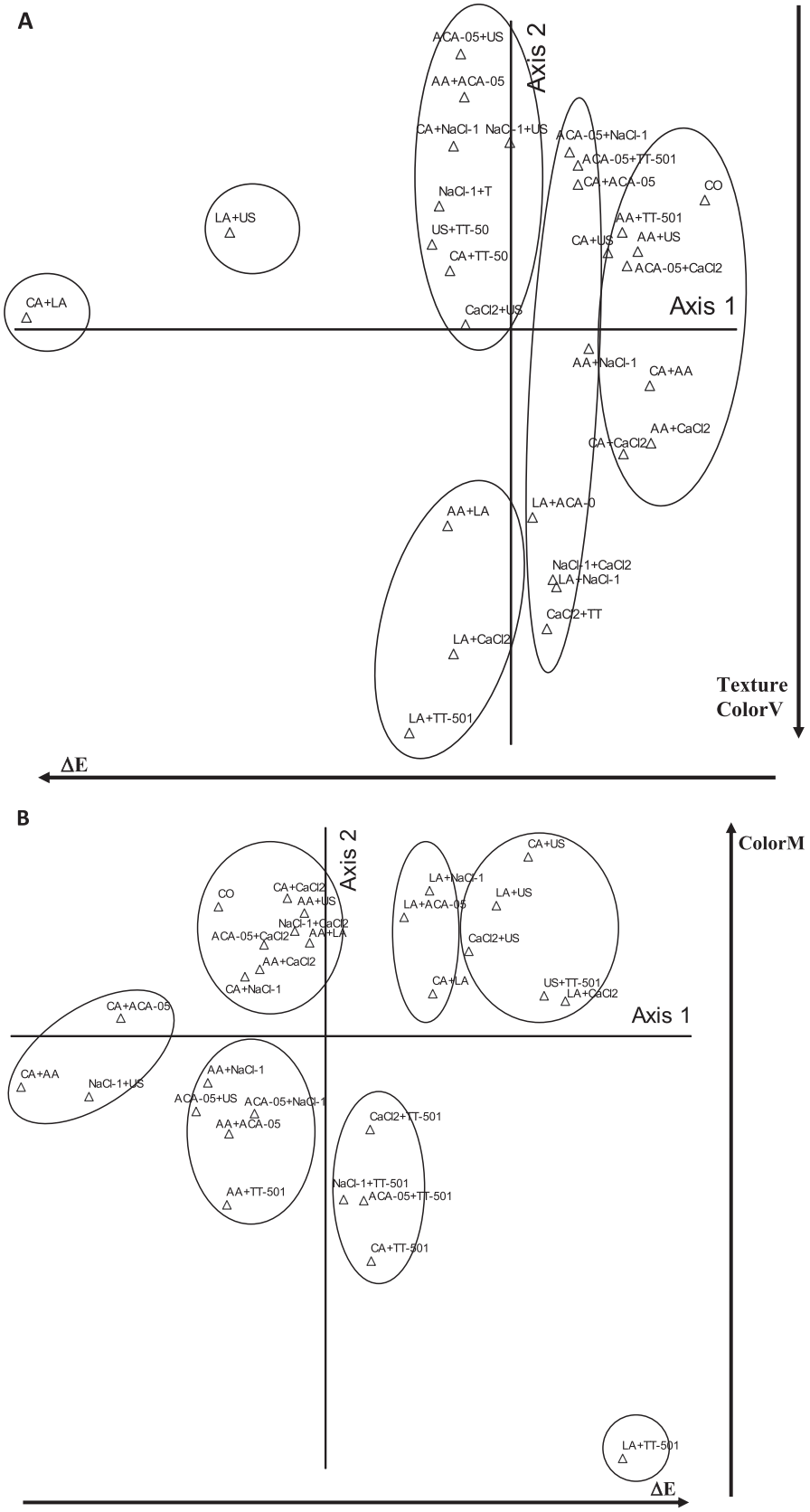

Fig. 2. PCA and HCA for double-hurdles applied to radish slices at zero time (A) and after 4 d of storage (B).

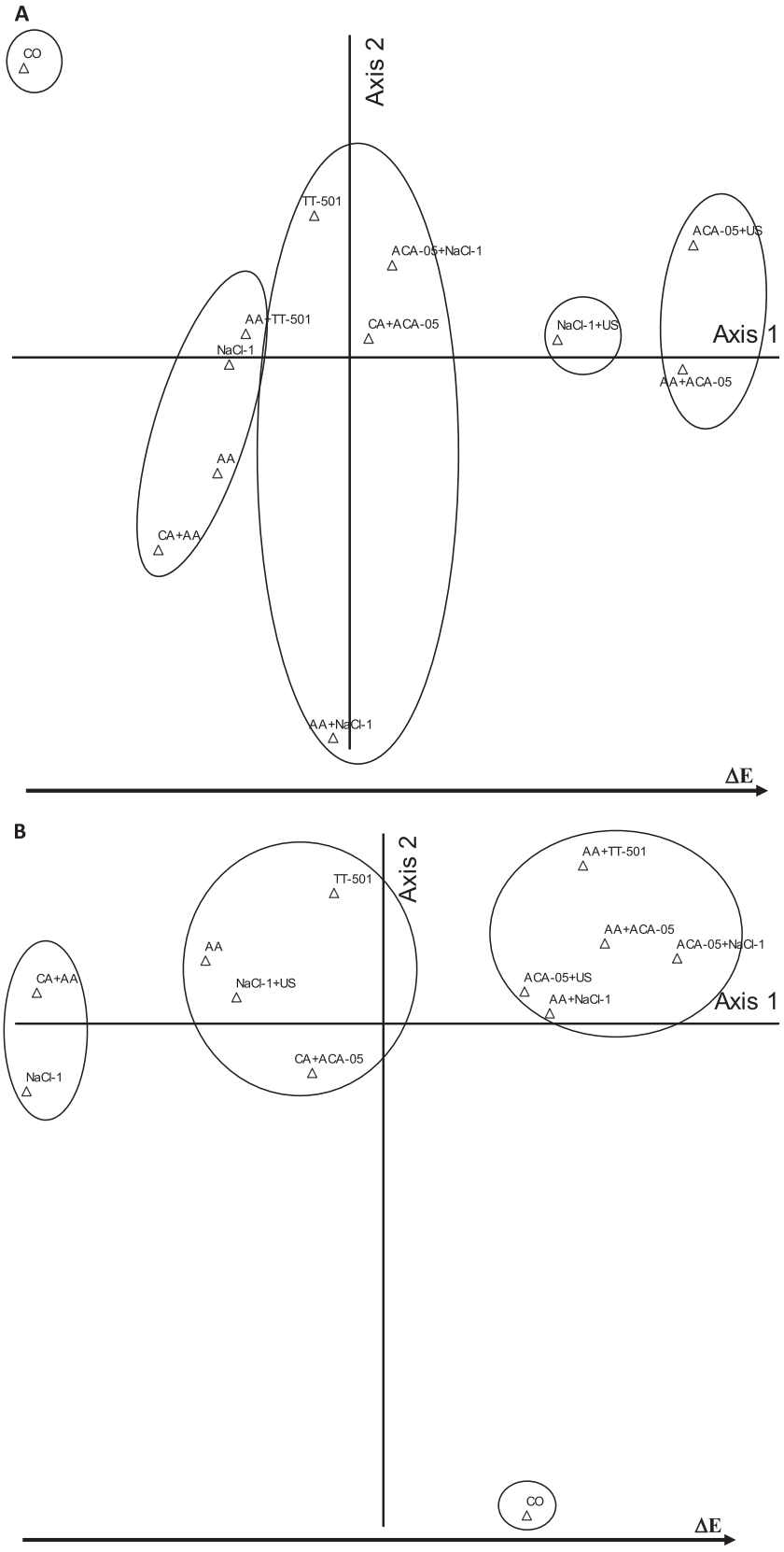

Fig. 3. PCA and HCA for best simple and double-hurdles applied to radish slices at zero time (A) and after 4 d of storage (B).

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