Highlights

- Natureseal ® prevented browning of fresh-cut potatoes during 9-day storage at 4 ºC.
- Green tea extract helped in maintaining membrane integrity of sliced potatoes.
- Both solutions increased the antioxidant activity of the samples.
- Ultrasound (35 or 130 kHz) did not enhance the effects solutions had per se.
- No impact in dry matter, sugar content or natural microbiota was observed.

Combination of sonication with anti-browning treatments as a strategy to increase the shelf-life of fresh-cut potato (cv. Monalisa)

Antibrowning treatments for fresh-cut potato

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Abstract

Two antioxidant solutions, a patented mixture based on vitamin C and other compounds (Natureseal ®, MV) and green tea extract (GT), were proposed to prevent browning in sliced potatoes. Combination with ultrasound (US) at two frequencies (35 and 130 kHz) was also evaluated, but no significant enhancement of the effects was detected with US. In MV-treated samples, respiration rate and dry matter (19.7 %) were higher than they were in the control (CK) (17.3 %) or GT-treated samples (16.8 %). Neither membrane integrity nor malondialdehyde content were significantly affected by the addition of MV or GT, but in MV-samples the antioxidant activity was increased 6-fold at the beginning of the storage (39.1 mmol ascorbic acid equivalents/kg), but decreased after 9 days. A yellowish coloration on the surface of GT samples was observed (*L*a*b** values being 68.3, -6.4, and 25.3, respectively), attributed to the coloration of the tea concentrate. In the conditions studied, GT was not able to delay browning in potato slices. Contrarily, MV solution preserved the original colour of the sliced potatoes (69.6, -6.7, 27.7 for *L*a*b*,* respectively) during the 9-day storage at 4 ºC.

Keywords:

Sliced potato, antioxidant, shelf-life, green tea, ultrasound

Novelty impact statement

Sulphites are used as an additive to maintain food colour, shelf-life and prevent the growth of fungi or bacteria. The study proves the efficacy of vitamin C to substitute sulphites in preservation and browning prevention of fresh-cut potatoes, preventing, in turn, the allergenic responses sulphites may cause to the consumers.

1. Introduction

Potatoes are the most important tuber crop in the world and they hold the third place in the rank of important human consumption crops (Torero, 2018). In addition to their low-fat content, potatoes also supply dietary fibres, carbohydrates and high-quality proteins and minerals (Seijo-Rodríguez, Escuredo, Rodríguez-Flores, & Seijo-Coello, 2018). As an outcome of consumers' lifestyle changes, and in response to their demand for fresh, healthy and easy-to-prepare vegetables, a wide assortment of minimally processed fruits and vegetables have been developed, including sliced potatoes (Ramos, Miller, Brandão, Teixeira, & Silva, 2013).

The different culinary methods that exist for potatoes make these sliced potatoes a good product to be sold as ready-to-cook, but they have two main drawbacks: processing operations - peeling, cutting - may add susceptibility to quality deterioration and shelf-life is averagely limited to 5-7 days (Ierna, Rizzarelli, Malvuccio, & Rapisarda, 2017). It is known that once cut, potatoes undergo colour changes, induced by the formation of intensely coloured products, as a result of enzymatic browning (Mareçek et al., 2013). For decades, sulphites have been added to prevent colour deterioration. However, this may lead to reactions in sensitive consumers, so certain products such as ascorbic acid have been incorporated and recently, other compounds have been commercially developed. One example is Natureseal®, based on organic acids, vitamins and minerals, which claims to maintain the product colour during storage. Green tea extract has also been suggested for this purpose; due to its antioxidant capacity it has been reported to inhibit polyphenol oxidase (PPO) activity, which is the enzyme behind the browning reactions (Nirmal & Benjakul, 2011; Soysal, 2009).

On the other hand, ultrasound technology has been explored as a potential technology to induce an enhancement in the penetration of the antioxidant solutions in the vegetable tissue, and hence, increase their effect (Nicolau-Lapeña et al., 2019b). This effect is attributed to the cavitation bubbles created by the sonic waves. Their asymmetric implosions generate microjets in the direction of the solid surface, which can affect mass transfer, enhancing the penetration on the fruit surface (Carcel et al., 2012).

The aim of this study was to investigate a method consisting of a combination of antioxidant solutions and US technology to reduce the mechanisms that shorten sliced potato shelf-life. The paper focuses on this patented mixture of vitamins and minerals (primarily based on vitamin C) and green tea extract, which were proposed as antioxidant solutions. The effect of antioxidant treatments, combined or not with ultrasonication, was evaluated by physicochemical, microbiological, enzymatic and nutritional parameters during cold storage.

2. Materials and methods

2.1. Materials

Potatoes (cv. Monalisa) were purchased from a local supermarket. Natureseal® (mixture of vitamins and minerals, based on vitamin C and sulphite free) was kindly provided by Eurotech (Barcelona, Spain) and green tea was acquired from a local provider.

Peptone, plate count agar (PCA), and dichloran rose bengale chloramphenicol agar (DRBC), were obtained from Biokar Diagnostics (Allonne, France).

Ascorbic, and gallic acids, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), polyvinyl pirrolidone (PVPP), cistein, pyrocatechol, and guayacol were acquired from Sigma-Aldrich (Steinheim, Germany). Sodium hypochlorite, peroxide hydrogen, methanol, sodium chloride, potassium chloride, ferric chloride hexahydrate and Folin Ciocalteau's reagent were procured from Panreac (Llinars del Vallès, Spain).

2.2. Methods

2.2.1. Experimental design

Potatoes were processed and sliced as described below, and two solutions were used in order to delay surface browning during storage and to increase shelf-life. Natureseal® 7.5 % (w:v) (MV) and green tea 5 % (w:v) (GT) were used as potential antioxidants and enzyme inhibitors (Supplementary material 1), according to previous studies (Bobo, 2014). Tap water was used as a control (CK). Ultrasounds (US) at two frequencies, 35 or 130 kHz, were applied with the intention of increasing the penetration of the two solutions into the potato slice. A non-sonicated trial was carried out in order to ascertain whether ultrasound had an enhancing influence on the anti-browning effect of each solution (NS, GT or CK). In total, 9 treatments were carried out: CK with or without 35 or 130 kHz US (CK-0, CK-35, CK-130), and the same for MV (MV-0, MV-35, MV-130) and GT (GT-0, GT-35, GT-130).

Once processed, trays containing the sliced potatoes were stored at 4 ºC. Sampling was done on days 0, 2, 4, 7, and 9 (D0, D2, D4, D7, D9). Some determinations were made in the fresh product, including respiration rate, color, total aerobic mesophylls (TAM), total aerobic psychrophiles (TAP), yeasts and molds (Y&M), dry matter and membrane integrity. Aliquots of samples for each treatment were frozen with liquid nitrogen, milled using a MINIMOKA GR-020 grinder (Taurus Group, Barcelona, Spain) and stored at -80 °C for further biochemical analysis, which included antioxidant activity, total phenolic compounds (TPC), malondialdehyde (MDA) content and free sugar content.

2.2.2. Preparation and characterisation of antioxidant solutions

MV solution was prepared to reach a final concentration of 7.5 % (w:v) diluting the commercial product in distilled water. GT solution was prepared from a green tea concentrate brewed in the ratio of 1:6 (GT: water; w:v), which was prepared with distilled water at 75 °C and sonicated for 10 min, 75 kHz, continuous, 20 % power, to enhance antioxidant extraction (Das & Eun, 2018). After filtration and centrifugation to eliminate suspended solids, the extract was diluted to a final concentration of 5 % as Bobo (2014) established as the optimal concentration

to inhibit potato PPO. The solution was kept at 4 ºC until assay. To characterise the potential efficacy of both solutions, the antioxidant activity was determined by DPPH· assay, explained in section 2.2.10 and the ability to inhibit PPO activity was determined with a microplate colorimetric method, as described in section 2.2.12.

2.2.3. Potato processing

Potatoes were washed with tap water at 4 ± 1 ºC, disinfected in a 200 mg/L chlorine solution (pH 6.5) for 2 min in a ratio of 1:3 potato: solution (w:v), and rinsed with tap water for 2 min in the same proportion. After peeling using potato abrasive peeler PI-20 (Sammic, Spain) for 1.5 min, potatoes were cut into 5 mm width slices with automatic slicer Robot-Coupe CL-50 Ultra (Bourgogne, France). Excess of water was removed for 40 s using a centrifuge Marrodan PR47248 (Navarra, Spain). Then, slices were immersed in glass pots containing the treatment solutions – tap water (CK), 7.5 % Natureseal ® (MV) and 5 % green tea (GT) – in a ratio of 1:2 potato: solution (w:v) and agitated for 2 min. When required, continuous mode, 100 % power, 35 or 130 kHz US was applied simultaneously to the immersion. After treatment, the slices were manually centrifuged to drain the excess water for 40 s, and approximately 100 ± 2 g of product drained were placed in 350-cm^3 polypropylene trays and sealed with polypropylene film with an O_2 permeability of 110 cm^3 /m^2 · day · atm at 23 °C (film PP with a line of holes of 100 μm each and 100 mm apart from each other). The packaged product was stored at in a refrigerated chamber at 4 ºC and 95 % relative humidity.

2.2.4. Respiration rate and analysis of internal gas composition

Respiration rate (RR) of potato slices was determined immediately after the processing. For this, 100 ± 2 g of sliced potatoes were put inside a hermetic plastic pot and stored at 4 ºC. After 4 and 24 h, O_2 and CO_2 concentrations were measured using headspace gas analyser CheckMate 3 (Dansensor, Spain). Respiration rate was calculated following Equation 1.

$$\textbf{RR (µmol / kg · s)} = \frac{[CO2]f - [CO2]i \cdot (Vt - V0) \cdot 0.01}{W \cdot (tf - t0)} \qquad \text{Eq. 1}$$

where $[CO_2]_f - [CO_2]_i$ is the change in concentration between measurements (moles of gas using the equivalence at standard conditions of 1 mole equals 22.4 L), V_t is the total volume of the container (600 mL), V_0 is the volume of the potatoes (mL), $t_f - t_i$ is the time difference between measurements (s), and W is the weight of the potatoes in the container (kg).

Evolution of internal gas composition in sample trays was followed by measuring O_2 and CO_2 concentration on each storage day (D).

2.2.5. Colour analysis

The surface colour of 4 slices per tray and per treatment was measured (n=12) on three random places of the surface of each slice by using a CR-200 Minolta Chroma Meter (Minolta, INC., Tokyo, Japan). Color was measured as CIE *L* a* b** coordinates, using a D65 illuminant and 10° observer angle. Chroma and Hue value were calculated by using Equation 3, and Equation 4, respectively (McGuire, 1992).

$$\textbf{Chroma} = (a^{*2}+b^{*2})^{1/2} \qquad \text{Eq. 3}$$

Hue angle = $\tan^{-1}$ (b*/a*) Eq. 4

2.2.6. Microbial quality

To study the evolution of alterative microbiota, 10 ± 1 g of sliced potatoes were placed in a sterile filter bag (80 mL BagPage®, Interscience BagSystem, Saint Nom, France) and diluted with buffered peptone water 1:10 (w:v). It was mashed in a paddle blender (MiniMix, Interscience, France) for 2 min at 9 strokes/s. Aliquots of the mixture were serially diluted on saline peptone and plated in duplicate on PCA for total aerobic mesophylls (TAM) and total aerobic psychrophiles (TAP) and on Dichloran-rose bengal chloramphenicol (DRBC) for yeasts and molds (Y&M). Plates were incubated at 30±1 ºC for 3 days for TAM, at 4±1 ºC for 10 days for TAP and at 25±1 ºC for 3 to 5 days for Y&M. Three repetitions were made for each treatment. Results were expressed as log CFU/g and the detection limit was 5 CFU/g.

2.2.7. Dry matter

Dry matter of samples was calculated using Equation 3 after drying dice of 5 mm^3 for 24 h at 105 ºC until a constant weight.

% dry matter = (dry weight / fresh weight) · 100 Eq. 3

2.2.8. Membrane permeability

Membrane permeability was expressed as electrical conductivity as previously reported by Liu et al. (2019) with some modifications. Briefly, a 12 mm diameter circle per slice was cut and washed with distilled water three times. Two slices per tray and in triplicate (n=6) were determined per each treatment. The circles were dried using filter paper and immersed in 30 mL of boiling distilled water for 15 min. After removing slices, the water was cooled down and its electrical conductivity was measured using a conductimeter Testo 240 (Tarragona, Spain), and the results expressed as mS / m.

2.2.9. Malondialdehyde (MDA) content

Determinations of MDA content in potato slices were carried out employing the supernatant obtained from a mixture of 1.0 ± 0.1 g with 8 mL 0.1 % TCA (w:v), followed by homogenisation for 10 min (Multivortex V-32, BioSan) and a centrifugation at 20,000 × *g* for 10 min at 20 ºC. For the reaction, 0.5 mL of the extracts were transferred to 1.5 mL of 20 % TCA (w:v) and to 0.5 % TBA (w:v) in 20 % TCA. Tubes were incubated for 30 min in a thermal plate at 90 ºC, and the reaction was stopped by immersing the tubes in ice for 5 min. Absorbance at 532 and 600 nm were read using GENESYS™ 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, MA, USA). Four replicates were determined for each treatment, only at D0 and D9, and MDA content, expressed as µmol/kg (in FW basis) was calculated following Equation 4.

MDA content (µmol / kg) = $(2 \cdot (m + V) \cdot (\Delta Abs_{TBA} - \Delta Abs_{TCA}) / (m \cdot b \cdot E)$ Eq. 4

where m is the mass of the sample (kg), V is the extract volume (mL), ΔAbs $_{TBA}$ is the difference between absorbance at 532 and 600 nm for samples that reacted with 0.5 % TBA in 20 % TCA. ΔAbs $_{TCA}$ is for samples mixed with 20 % TCA, b is the optical distance and E is the MDA extinction coefficient (15.5 M^{-1} m^{-1}).

2.2.10. Reducing sugar content

Total reducing sugars, includin, D-glucose and D-fructose, were determined on frozen slices (n=4). Reducing sugar content was determined spectrophotometrically with PowerWave HT (Biotek, Vermont, United States), following the instructions of the Kit 12819 from Biosystems (Barcelona, Spain) and expressed in g/kg FW.

2.2.11. Antioxidant activity (AC)

Antioxidant activity of the frozen samples was assessed using two methods: ferric reducing antioxidant power (FRAP) and DPPH· scavenging activity assays, as described previously in Nicolau-Lapeña et al. (2019a). Standard curves were prepared with ascorbic acid (AA) for both methods, and processed the same as with the samples. Results were expressed as AA equivalents (AAE) in mmol / kg (DW basis). Four repetitions were measured for each treatment (n=4).

2.2.12. Total phenolic content (TPC)

The TPC was determined by the Folin-Ciocalteau method, as described previously in Nicolau-Lapeña et al. (2019a). Standard curve with gallic acid (GA) was prepared, and results were expressed as GA equivalents (GAE) in mmol / kg (DW basis). Four repetitions were measured for each treatment (n=4).

2.2.13. Polyphenol oxidase (PPO) and peroxidase (POD) enzymatic activity

The enzymatic extraction was carried out by mixing 5.0 ± 0.5 g of the frozen product with 0.5 g PVPP and 10 mL 0.1 M phosphate buffer solution pH 6 (PBS) with 0.05 mM cysteine in an ultraturrax Ultra-turrax ® Tube drive P control (IKA, Staufen, Germany) for 1.5 min at 5,000 rpm. After filtration and centrifugation at 20,000 × *g* for 10 min at 4 ºC, supernatant was maintained in ice. The supernatant was the crude extract.

The capacity of MV and GT solutions to inhibit potato PPO activity (%) was determined *in vitro* by microplate assay. For this, 65µL of each antioxidant were poured into different wells, and 65 µL of potato crude extract were added to them. As a control (to calculate the 100 % enzymatic activity), 65 µL of distilled water were poured into different wells and mixed with 65 µL of potato crude extract. Then, 65 µL of 0.2 M pyrocathecol in PBS were added. After 10 min of incubation at 37 ºC, absorbance was read at 400 nm. Inhibition was expressed as a percentage of potato PPO inhibition (Bobo, 2014; Masuda et al., 2005).

To evaluate the effect of the treatments and US in potato PPO y POD activity *in vivo* (in food matrix), the following analyses were also done:

PPO activity determination was carried out by adding 20 µL of the sample to 300 µL of 0.2 M pyrocathecol in PBS. Absorbance at 400 nm was read every 9 s for 3 min using a microplate spectrophotometer.

POD activity determination was carried out by adding 20 µL of the sample to 200 µL of 10 mM guayacol solution in PBS and 100 µL of 10 mM H_2O_2 solution in PBS. Absorbance at 470 nm was read every 9 s for 10 min using the same spectrophotometer.

Their enzymatic activities (PPO and POD) were calculated based on the linear portion of the plotted curve. Four replicates were done, and results were expressed as the increment in optical density (ΔOD 10^6) / kg · s (in protein basis).

2.3.Statistical analysis

All data were checked for significant differences by applying analysis of variance test (ANOVA). The criterion for statistical significance was $p < 0.05$. When significant differences were observed, Tukey's Honest Significant Difference (HSD) of the means was applied. Correlations between paràmetres were calculated by linear regression R-square. Principal components analysis (PCA) was carried out to obtain correlations among all the parametres studied at D0 (immedately after the treatment) and D9 (after 9 days of storage) (Section 3.9 of the results). All statistical analyses were carried on using JMP 13 (SAS Institute Inc., Cary, USA).

3. Results and discussion

3.1. Antioxidant solution characterisation

Antioxidant activities of the proposed solutions MV and GT were determined by DPPH· inhibition assay, and MV solution antioxidant activity was found to be 3.8-fold higher than GT solution. Moreover, PPO inhibition was higher for MV solution than it was for GT solution, being 95.7 and 71.5 %, respectively. Despite the MV solution showing an inherent higher potential in *in vitro* studies, the two solutions were applied to the potato at the following concentrations: MV was prepared following the manufacturer's indications, and GT was prepared at 5 % according to Bobo (2014), as a higher concentration could have affected the sensorial properties of the samples negatively.

3.2. Respiration rate and O_2 and CO_2 concentrations during storage

Respiration rate (RR) of sliced potatoes averaged 1.8 ± 0.1, 2.8 ± 0.3, and 2.4 ± 0.1 μmol CO_2 / kg · s, for CK, MV, and GT treatments, respectively. These values are in agreement with the literature data for potatoes (Fennir et al., 2003).

US application involves cell wall disruption and it may imply certain stress. However, no significant differences were observed in RR between sliced potatoes regardless the sonication conditions. Regarding changes in the internal atmosphere (Data not shown), potato slices treated with MV showed significant changes in O_2 (12.7 ± 1.2, 6.0 ± 0.7, 2.1 ± 1.3, and 1.0 ± 0.9 % at D2, D4, D7, and D9, respectively) and CO_2 (5.7 ± 0.4, 12.2 ± 0.6, 17.1 ± 0.6, and 18.2 ± 1.0 % at D2, D4, D7, and D9, respectively) concentrations, respectively. Despite the low concentrations of O_2 present inside the package, the holes performed in the film prevented anaerobic conditions. Moreover, absence of fermentation odours was checked when packages were opened. Contrarily, in GT and CK samples O_2 and CO_2 exchange was slower. In CK samples, the internal O_2 (14.3 ± 0.4, 11.1 ± 0.5, 10.2 ± 0., and 9.7 ± 0.7 % at D2, D4, D7, and D9, respectively) and CO_2 (3.6 ± 0.2, 6.8 ± 0.3, 8.6 ± 0.2, and 10.1 ± 0.5 % at D2, D4, D7, and D9, respectively) in CK samples changed gradually and achieved balance at D9. Regarding GT potatoes, composition balance in trays was reached between D4 and D7, and composition in O_2 (13.6 ± 0.3, 10.8 ± 0.3, 6.8 ± 1.5, 6.9 ± 1.0 %, at D2, D4, D7, and D9, respectively) and CO_2 (3.9 ± 0.2, 7.1 ± 0.2, 9.8 ± 0.8, and 10.8 ± 0.7 %, at D2, D4, D7, and D9, respectively) reached a balance between D4 and D7. The stability values reached in gas composition inside the trays with CK and GT treated potatoes were close to the recommendations given by Farber et al. (2003), who stablished that percentages of 1-3 % O_2 and 6-9 % CO_2 were optimal for potato storage. This internal gas combination is reported to be the most suitable for products like tubercles, in order to maintain quality and delay the processes that typically degrade the product, such as colour alterations, changes in texture or microbial growth. Petri et al. (2008) reported that immersion in ascorbic acid solutions (0.5 %) decreased the RR of fresh-cut potato by scavenging oxygen, which affected the enzymes of the oxidative phosphorylation pathway. The differences between samples form GT or CK treatments and MV behaviour could be explained in part by the different pH values of the solutions and their effect on the potato metabolism. It is suggested that ascorbic acid content in MV and the pH of the solution (3.2) could be related to the higher RR of these samples. The stress posed by immersion of MV samples in such acidic solution could have quickened their metabolic and respiration processes (Tudela, Espín, & Gil, 2002).

3.3. Microbial quality evolution

The evaluation of epiphytic microbiota in potato slices revealed that populations of Y&M were below the detection limit for all the antioxidants and US frequencies used, both immediately after the treatments and during storage. Populations of TAM and TAP in the sliced product before the treatments were 2.2 ± 0.1 and 1.8 ± 0.3 log CFU / g, respectively. After the treatments (Figure 1), populations were maintained in CK samples, whereas MV treatment showed a slight sanitizing effect. Conversely, after GT application, populations increased in the same numbers for both TAM and TAP. For all the treatments and days, a strong correlation was observed (0.7293 – 0.8879 R^2) between TAM and TAP counts – except for MV-35 and MV-130 treatments. A bacteriostatic effect of MV was patent mostly against TAP, maintaining such populations below 1.84 ± 0.6 and 2.4 ± 0.6 log CFU / g for MV-35 and MV-130, respectively. A possible explanation of this effect involves the low pH of the MV solution (2.6 ± 0.2) and the subsequent acidification of the surface of sliced potatoes. At such pH conditions, that are lower than the growth range of most TAM (between 4.2–7.5), most microorganisms common in food products are not able to grow (Ray, 2004). Few reports have been found regarding antimicrobial activity of MV, as it is described as an antioxidant product and it is therefore used for this purpose. *Salmonella enterica* was not found on fresh-cut cantaloupes washed with hot water (76 ºC) for 3 min with MV 8.5 % (w/v) during the 21 days of study (Alicea, Annous, Mendez, Burke, & Orellana, 2018). In CK-0, a growth was observed during storage, and counts at D9 were 4.5 ± 0.5 log CFU / g. No significant differences were observed when comparing US application, either at 35 or at 130 kHz. A similar growth trend was detected in potato slices treated with GT, achieving values averaging 4.0 ± 0.5 log CFU / g for both TAM and TAP. The antimicrobial characteristics of GT extract in food have been previously described (Perumalla and Hettiarachchy, 2011). However, in this study and at concentrations used, GT did not exert a significant effect against epiphytic microbiota.

3.4. Dry matter and reducing sugar content

Dry matter and reducing sugar content are important parameters of potato quality, as they are used as reference values for further processing, especially frying (Wayumba et al., 2019). Figure 2 presents the results obtained for these two parameters at D0 and D9. Sugar content was only evaluated at the beginning and the end of the storage, and dry matter values did not present practical differences between samples or days during storage. At the end of the storage, dry matter was maintained at 17.3 ± 1.1, 19.7 ± 1.2, and 16.8 ± 1.6 % for each treatment (CK, MV and GT, respectively), and differences between sonication conditions were not significant. Dry matter was slightly higher in samples in which MV was applied, possibly because of the higher concentration in soluble solids of the MV solution. Reducing sugar content did not significantly differ between treatments, but it was affected by storage time. While reducing sugars at D0 were under 8.2 ± 0.5 g / kg (except for MV 130 in which values of 9.3 ± 0.1 g / kg were determined), at D9 there was an increase ranging from 1.2- to 1.8-fold of their initial sugar content, which reached values between 10.6 ± 0.4 to 12.1 ± 0.3 g / kg. Storage at temperatures lower than 4 ºC or higher than 8 ºC is related to sugar accumulation or sweetening for sprout, respectively (Medeiros Vinci et al., 2012).

3.5. Malondialdehyde (MDA) content and membrane integrity

Slicing operations may affect membrane integrity of cell tissue, and subsequent oxidative damages may occur. MDA is a product of lipid peroxidation in plant membranes and indicates

the deterioration of the metabolism of plant cells (Møller, Jensen, & Hansson, 2007). For this, it is a good indicator of the degree of oxidative stress of the plant cell (Hodges et al., 2004), and electrical conductivity is usually correlated with the integrity of the cell membrane (Jiankang et al., 2007). In the present study, initial values of MDA content ranged from 14.7 ± 1.2 to 21.7 ± 0.6 µmol/kg (Figure 3). No pattern was observed involving antioxidant solutions and US frequencies that can explain variations in initial content. After 9-day storage, MV treated potatoes showed a higher increase in MDA values, when compared to CK and GT. Contrary to what Liu et al. (2019) reported, there was a sharp rise in the conductivity of CK treated potatoes, even though conductivity immediately after the processing was lower in these samples (Figure 3). The treatments MV-35, and GT-35 helped in maintaining membrane conductivity below 100 mS/m), which can be related with a better maintenance of membrane integrity when using this frequency (35 kHz) in combination of both antioxidants.

3.6. Antioxidant capacity (AC) and total phenolic content (TPC)

AC was calculated by the methods of DPPH· and FRAP. As a strong correlation has been found between the two variables (R^2 = 0.9622), only DPPH· results are shown in Figure 4, which also presents TPC results. Initial AC and TPC values of CK potatoes averaged 5.5 ± 1.0 and 1.7 ± 0.1 mmol / kg, respectively. These values differ to what was observed by Serpen and Gökmen (2009) and Albishi et al. (2013). Variations were attributed to differences in potato cultivars, the maturity of the tubers or previous storage conditions (Seijo-Rodríguez et al., 2018). Statistical differences were found between MV treated potatoes and the other treatments (initial AC and TPC contents in such samples was significantly higher), but regarding US conditions, no differences were observed. Initial AC and TPC values in MV samples averaged 39.1 ± 1.1 and 3.8 ± 0.4 mmol/kg, respectively. GT treated potatoes did not reach such levels of AC, the values (averaging at the beginning of the storage 6.6 ± 0.8 mmol/kg) being 6 times lower than they were for MV. Differences in TPC figures were mainly attributed to an overestimation of TPC in MV samples rather than biological variances. In fact, MV is primarily composed of organic acids, including ascorbic acid, which is a reducing compound (non-phenolic antioxidant) that also reduces the Folin Ciocalteau reagent to form a blue colouring alkaline pH (Lester et al., 2012). A decrease in TPC values for MV treatments was observed for all US treatments, which could also be attributed to the reduction of ascorbic acid present in MV to dehydroascorbic acid during time, reducing, in turn, the interferences caused in Folin-Ciocalteau method. For CK and GT samples, AC and TPC values were well maintained during storage.

3.7. Colour

Colour of potatoes was expressed by *Chroma and Hue angle* (Table 1). MV treated potatoes were clearer and brighter to eyesight (Supplementary material 2). Luminosity (*L*)*, which is not represented in Chroma and Hue angle calculations, is also presented (Table 1). In general, *L** values, did not show statistical differences between MV and CK samples, which averaged 69.5 ± 0.5 (Table 1) indicating that no significant differences throughout storage were observed in luminosity of the samples. Chroma of MV (28.1 – 28.9) was slightly higher than it was in CK and GT samples (26.1-28.1). The Hue angle indicates the simple colour in a 360 ° space (McGuire, 1992). Initial Hue angle of sliced potatoes ranged from 165.7 to 166.4, and no remarkable differences were observed between treatments. During storage, in the case of potatoes treated with GT, Hue angle increased at day 2 to 171.8 to 174.4, and these values were maintained higher than those presented by MV and CK. When Hue and Chroma varied, this

was mainly attributed to variations in a* values (Table 1), whereas b* values were maintained (26.1 ± 1.1). At the beginning, *a** values ranged from -6.3 ± 0.2 to -6.9 ± 0.1, there was homogeneity between all the treatments and no differences in *a** values were found between US conditions. These values increased during storage, separating in two marked groups at D4. On one hand, CK and MV, in which the *a** change was slight, averaged -4.5 ± 0.4. The Hue angle in this samples was relatively well maintained until D7, in which it ranged from 166.7 to 167.1). On the other, GT, in which a significant increase in *a** occurred, reaching -3.2 ± 0.4, turned into a more brownish colour, rather than yellow-green. As can be seen in the photos (Supplementary material 2) and also reflected in the lower luminosity and higher *a** values of the samples, GT slices acquired a brownish colour. That was attributed to the natural pigmentation of the green tea solution that imbibed on the potato slice surface and remained slightly visible on it. As aforementioned, the composition of the patented antioxidant is based mainly on ascorbic acid, which has already been used successfully in potato slices to prevent browning by Abubakr (2016). Ierna et al. (2017) also applied a mix of ascorbic acid and citric acid on fresh-cut potatoes, preventing the browning until 9-day of storage. The proposed mechanism involves the acids, that could have reverted the oxidized quinones and their derivatives into phenolic substances, preventing their subsequent polymerization to form brownish melanoid pigments (Li et al., 2017). Regarding green tea, it is rich in polyphenols (including catechins, teaflavins and tearubigines), substances that can possess strong antioxidant properties (Amarowicz & Shahidi, 2003) and have been reported to inhibit PPO activity, and further browning in fruits (e.g. in apple and apple juice up to 96% when using 3 g/L)(Klimczak and Gliszczyńska-Świgło, 2017; Soysal, 2009). Even so, in the present study, GT solution was not able to control browning in potato slices in the conditions established.

3.8. Polyphenol oxidase (PPO) and peroxidase (POD) activity

Initial values of PPO activity ranged between 461.9 ± 32.5 and 855.5 ± 56.4 $\Delta OD\ 10^6$ / kg · s (Figure 5). The higher values presented in PPO activity of GT samples were attributed to the increased content of phenolic compounds after the addition of GT, which act as a substrate for PPO (Tsouvaltzis and Brecht, 2017). MV treated samples showed the lowest PPO activity, which started ranging between 461.9 ± 32.5 and 763.5 ± 45.5 $\Delta OD\ 10^6$ kg · s at D0, and ended ranging from 236.2 ± 10.7 to 312.1 ± 29.4 at D9. This could be explained by the bonding of the organic acids in MV with the phenolic compounds or with PPO, thus creating inactive complexes. The acids that are present in MV could also contribute to a pH decrease, making PPO less effective in its suboptimal pH (Tsouvaltzis and Brecht, 2017).

POD activity at D0 ranged between 5,696.1 ± 323.6 and 8,443.0 ± 821.6 $\Delta OD\ 10^6$ / kg · s. A clear effect of antioxidants or US conditions could not be identified. Even though initial activities were significantly different between the samples, a general decrease of such values was observed during storage, making POD activities homogeneous in all samples at the end of the 9-day storage. The initial higher values of POD and PPO activity in GT-130, and also in PPO in MV-130, could be attributed to the sonication and the subsequent liberation of the enzymes (O'Donnell, Tiwari, Bourke, & Cullen, 2010). The posterior decrease could be explained by the effect of MV – by means of pH decrease - and GT – for its antioxidant compounds – as explained before.

3.9.Overall view

A Principal Component Analysis (PCA) was performed in order to provide an overall view of the effects that the different treatments, CK, MV, and GT, and the different US conditions applied, 0, 35, or 130 kHz, relating all the studied variables. In both PCAs, D0 and D9, scores were distributed similarly. There was one separated group, formed by MV samples, and another two groups that overlap, CK and GT.

At D0 (Supplementary material, 3A), the sum of variability explained by principal component 1 (PC1) and principal component 2 (PC2) was 73.4 %. MV samples were located in one side of the PC1, and CK and GT samples were located in the other side of the PC1, in two groups that had an intersection, meaning that a similar trend was found in those samples. MV samples were characterized by a high dry matter content and high antioxidant activity expressed as DPPH· inhibition and FRAP. A direct connection between membrane conductivity and MDA content in PC1 was also observable, as the more stressed and disrupted the potato tissue was, the higher those values were. An indirect relationship was found between these parameters and TAP and TAM counts, which were higher for GT and CK samples. Variances found between MV and GT could be attributed to the differences of both antioxidants. MV solution is mainly composed of ascorbic acid, whose antimicrobial effects relay on the free radicals formation during autoxidation of the acid (Tajkarimi and Ibrahim, 2011), and on the low pH, that makes it difficult for bacteria to survive as it alters some metabolic cycles (Angós, Vírseda, & Fernández, 2008). GT extract consists mainly of phenolic compounds, which basically interact with membrane-dependent processes (cell signaling cycle, arachidonic acid metabolism, cell proliferation, apoptosis and mitochondrial functionality) (Taylor, Hamilton-Miller, & Stapleton, 2005).

At D9 (Supplementary material, 3B), the sum of variability explained by PC1 and PC2 was 74.1 %. Groups were distributed similarly to D0. Gas concentration appeared to be a significant variable, and related with TAM and TAP counts. Indeed, a reduced O_2 pressure in the package acts as a reducer of metabolism rate of microorganisms (Zahra et al., 2016). As happened in D0, GT was positively related to a higher a* value, indicating a more brownish colour, given by the solution. Also, MV was characterized by high antioxidant values expressed by DPPH· inhibition, and higher content in dry matter, which was attributed to the organic acids present in the solution. High L* values also correlated with MV samples, which verified that MV could maintain potato slices brighter after the 9-day storage. As a general rule, the more O_2 concentration in contact with the surface of potatoes, the more polyphenols will be oxidized by PPO and converted into brownish molecules that will change the colour of the samples (Deng, Yang, Capanoglu, Cao, & Xiao, 2018). However, some authors discussed the direct implication of the main components that are usually related to browning: PPO, POD, hydrogen peroxide, ascorbic acid content, and initial phenolics content as well as total and individual phenolics accumulation. According to Cantos et al. (2002), none of these parameters per separate was able to fully explain the browning behavior of fresh-cut potatoes. Moreover, they suggested that to completely understand the possible limiting factors in the development of browning in fresh-cut potatoes, more studies involving other important aspects (lipid composition, calcium content, protease activity, agronomic practices, etc.) are required.

4. Conclusions

In this study, two anti-browning solutions, were evaluated to prevent browning and prolong the shelf-life of packaged fresh-cut potatoes stored at 4 ºC. The effect of ultrasound in increasing the penetration of the antioxidant in the potato tissue could not be elucidated.

Despite the *in vitro* efficacy of green tea (GT) in decreasing polyphenol oxidation activity, its performance *in* vivo was affected by the surface darkening of potato caused by the coloration of the antioxidant solution *per se*. Moreover, no significant improvement in microbial counts, antioxidant activity, or reducing sugars, was observed when compared to the control.

Conversely, the visual quality of potatoes immersed in a commercial mix of vitamins and minerals (MV) was acceptable after 9 days of storage, for no browning had developed. Antioxidant capacity and total phenolic content values were higher in MV samples than in the control samples. Furthermore, this treatment was able to exert a higher control on the psychrophilic microorganisms, which otherwise, could grow at 4 ºC. Overall, NatureSeal® proved to be a good product to prevent sliced potato deterioration.

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Conflict of interests

The authors declare no conflict of interests.

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Table 1. Time evolution of L* and a* values, and Hue angle and Chroma values of potatoes processed with antioxidants combined with US, expressed as the mean of 10 replicates ± standard deviation. Different lowercase letters mean significant differences (p-value < 0.05) between treatments in the same D, and different capital letters mean significant differences (p-value < 0.05) between days within the same treatment.

	L*				
Treatment	**D0**	**D2**	**D4**	**D7**	**D9**
CK-0	69.8 ± 1.3 ^{aA}	70.1 ± 0.6 ^{aA}	71.4 ± 0.6 ^{aA}	69.1 ± 0.8 ^{aA}	69.7 ± 2.1 ^{aA}
CK-35	70.4 ± 1.8 ^{aA}	71.2 ± 0.9 ^{aA}	71.1 ± 0.7 ^{aA}	70.0 ± 1.3 ^{aA}	69.4 ± 0.7 ^{aA}
CK-130	69.3 ± 0.2 ^{aA}	69.3 ± 0.5 ^{aA}	70.5 ± 0.9 ^{aA}	69.8 ± 1.2 ^{aA}	69.9 ±1.9 ^{aA}
NS-0	69.6 ± 0.6 ^{aA}	67.8 ± 1.1 ^{bA}	70.8 ± 1.2 ^{aA}	68.4 ± 2.4 ^{aA}	70.9 ± 0.9 ^{aA}
NS-35	69.0 ± 0.3 ^{aA}	66.8 ± 0.8 ^{bB}	69.1 ± 0.9 ^{aA}	68.8 ± 1.2 ^{aAB}	70.2 ± 0.4 ^{aA}
NS-130	69.6 ± 0.7 ^{aA}	66.8 ± 1.2 ^{bB}	70.4 ± 1.2 ^{aA}	70.2 ± 0.8 ^{aA}	70.1 ± 0.5 ^{aA}
GT-0	68.3 ± 1.5 ^{bA}	67.8 ± 1.0 ^{bA}	68.5 ± 0.5 ^{bA}	67.5 ± 1.2 ^{aA}	67.6 ± 1.4 ^{bA}
GT-35	67.6 ± 1.3 ^{bA}	68.2 ± 1.2 ^{bA}	69.6 ± 0.4 ^{bA}	68.6 ± 1.1 ^{aA}	66.6 ± 2.4 ^{bA}
GT-130	68.2 ±0.8 ^{bA}	68.0 ± 1.5 ^{bA}	67.6 ± 1.7 ^{bA}	67.6 ± 2.3 ^{aA}	68.2 ± 0.9 ^{bA}
	a*				
Treatment	**D0**	**D2**	**D4**	**D7**	**D9**
CK-0	-6.5 ± 0.2 ^{abcA}	-5.9 ± 0.1 ^{cdAB}	-5.4 ± 0.4 ^{bABC}	-5.1 ± 0.2 ^{bBC}	-4.5 ± 0.9 ^{bC}
CK-35	-6.9 ± 0.1 ^{bA}	-6.3 ± 0.3 ^{cdeB}	-5.2 ± 0.2 ^{bC}	-5.6 ± 0.2 ^{bC}	-4.2 ± 0.2 ^{bD}
CK-130	-6.5 ± 0.1 ^{abcA}	-5.7 ± 0.1 ^{cAB}	-5.1 ± 0.2 ^{bBC}	-5.3 ± 0.4 ^{bBC}	-4.7 ± 0.7 ^{bC}
NS-0	-6.9 ± 0.1 ^{bA}	-6.9 ± 0.1 ^{eA}	-6.8 ± 0.3 ^{cA}	-5.9 ± 0.6 ^{bB}	-4.0 ± 0.1 ^{bC}
NS-35	-6.8 ± 0.2 ^{bcA}	-6.5 ± 0.1 ^{eAB}	-6.4 ± 0.1 ^{cAB}	-5.9 ± 0.1 ^{bAB}	-4.9 ± 0.6 ^{bC}
NS-130	-6.7 ± 0.1 ^{abcA}	-6.6 ± 0.1 ^{deA}	-6.7 ± 0.3 ^{cA}	-5.9 ± 0.6 ^{bAB}	-5.0 ± 0.4 ^{bB}
GT-0	-6.4 ± 0.2 ^{abA}	-2.5 ± 0.4 ^{abC}	-3.4 ± 0.4 ^{aB}	-3.7 ± 0.4 ^{aB}	-3.7 ± 0.1 ^{aD}
GT-35	-6.3 ± 0.2 ^{aA}	-3.7 ± 0.3 ^{bBC}	-3.8 ± 0.3 ^{aB}	-3.6 ± 0.1 ^{aBC}	-2.9 ± 0.4 ^{aC}
GT-130	-6.5 ± 0.3 ^{abcA}	-3.9 ± 0.5 ^{bB}	-3.5 ± 0.4 ^{aB}	-3.6 ± 0.6 ^{aB}	-2.9 ± 0.4 ^{aB}

Hue Treatment	D0	D2	D4	D7	D9
CK-0	165.9 ± 0.1 ^{abA}	166.8 ± 0.4 ^{cA}	168.2 ± 0.4 ^{aA}	168.1 ± 0.4 ^{aA}	170.8 ± 1.9 ^{aB}
CK-35	165.7 ± 0.1 ^{bA}	166.6 ± 0.8 ^{cdAB}	168.1 ± 0.6 ^{aC}	167.4 ± 0.2 ^{aBC}	170.6 ± 0.4 ^{aD}
CK-130	165.7 ± 0.2 ^{bA}	166.8 ± 0.1 ^{cAB}	168.3 ± 0.3 ^{aBC}	167.7 ± 0.3 ^{aABC}	169.6 ± 1.7 ^{aC}
MV-0	166.2 ± 0.3 ^{abA}	165.2 ± 0.3 ^{deA}	166.5 ± 0.1 ^{bA}	166.7 ± 0.5 ^{aA}	173.8 ± 0.4 ^{aA}
MV-35	166.0 ± 0.1 ^{abA}	164.7 ± 0.2 ^{dE}	166.2 ± 0.2 ^{bA}	166.7 ± 0.7 ^{aA}	169.3 ± 1.6 ^{aB}
MV-130	166.4 ± 0.2 ^{aB}	164.8 ± 0.3 ^{dE}	166.1 ± 0.2 ^{bAB}	167.1 ± 0.5 ^{aB}	168.7 ± 1.0 ^{aC}
GT-0	165.8 ± 0.2 ^{bA}	174.4 ± 0.7 ^{aD}	172.9 ± 0.5 ^{cC}	171.9 ± 0.7 ^{bC}	170.0 ± 0.2 ^{aB}
GT-35	166.1 ± 0.2 ^{abA}	172.3 ± 0.4 ^{bB}	172.4 ± 0.6 ^{cB}	172.2 ± 0.5 ^{bB}	173.8 ± 0.6 ^{aC}
GT-130	165.8 ± 0.2 ^{bA}	171.8 ± 0.7 ^{bB}	172.6 ± 0.6 ^{cBC}	172.2 ± 0.9 ^{bB}	174.1 ± 0.6 ^{aC}
Chroma **Treatment**	**D0**	**D2**	**D4**	**D7**	**D9**
CK-0	26.9 ± 0.9 ^{abA}	25.7 ± 1.1 ^{cA}	26.5 ± 0.7 ^{aA}	24.6 ± 1.1 ^{aA}	28.0 ± 0.5 ^{aB}
CK-35	28.1 ± 0.7 ^{bA}	27.0 ± 0.4 ^{cdAB}	26.0 ± 0.3 ^{aC}	25.7 ± 1.2 ^{aBC}	25.6 ± 0.7 ^{aD}
CK-130	26.5 ±0.8 ^{bA}	25.0 ± 0.3 ^{cAB}	25.3 ± 0.8 ^{aBC}	24.8 ± 1.6 ^{aABC}	26.1 ± 0.8 ^{aC}
MV-0	28.9 ±1.1 ^{abA}	27.0 ± 1.0 ^{deA}	29.0 ± 1.0 ^{bA}	25.6 ± 1.7 ^{aA}	25.2 ± 1.1 ^{aA}
MV-35	28.1 ±0.3 ^{abA}	24.9 ± 0.2 ^{eA}	27.0 ± 0.7 ^{bA}	25.7 ± 1.7 ^{aA}	26.4 ± 1.2 ^{aB}
MV-130	28.5 ±0.8 ^{aB}	25.3 ± 0.9 ^{eA}	28.1 ± 1.6 ^{bAB}	26.8 ± 1.8 ^{aB}	25.6 ± 0.4 ^{aC}
GT-0	26.1 ±0.8 ^{bA}	24.8 ± 1.2 ^{aD}	27.3 ± 1.5 ^{cC}	26.0 ± 0.6 ^{bC}	27.5 ± 0.4 ^{aB}
GT-35	26.2 ±1.3 ^{abA}	27.1 ± 0.6 ^{bB}	28.2 ± 0.2 ^{cB}	26.6 ± 1.3 ^{bB}	27.0 ± 1.4 ^{aC}
GT-130	26.4 ± 0.3 ^{bA}	26.5 ± 1.5 ^{bB}	27.2 ± 1.3 ^{cBC}	26.2 ± 1.8 ^{bB}	27.8 ± 1.3 ^{aC}

Figure 1. Microbial counts (log CFU / g) of total aerobic mesophylls (TAM, ■), total aerobic psychrophiles (TAP,■), of control (CK, **A**), mix of vitamins (MV, **B**), and green tea (GT, **C**) without ultrasound 0 kHz (**1**) or with ultrasound at 35 kHz (**2**) or 130 kHz (**3**). Values are the mean ± standard deviation (n=3). Different lowercase letters mean significant differences between treatments on the same day ($p < 0.05$). Different capital letters mean significant differences between days within the same treatment ($p < 0.5$).

Figure 2. Reducing sugars (g / kg, in FW basis) (**A**) and dry matter (%) (**B**) at day 0 (■) and day 9 (■). Values are the mean ± standard deviation (n=3). Different lowercase letters mean significant differences between treatments on the same day ($p < 0.05$). Different capital letters mean significant differences between days within the same treatment ($p < 0.5$)

Figure 3. Malonildihaldeyide content (MDA, µmol / kg) (**bars**) and conductivity (mS / m) (**lines**) of control (CK, **A**), mix of vitamins (MV, **B**), and green tea (GT, **C**) without ultrasound 0 kHz (**1**) or with ultrasound at 35 kHz (**2**) or 130 kHz (**3**). Values are the mean ± standard deviation (n=4). Different lowercase letters mean significant differences between treatments on the same day ($p < 0.05$). Different capital letters mean significant differences between days within the same treatment ($p < 0.5$).

Figure 4. Antioxidant capacity (ascorbic acid equivalents, mmol / kg, in DW basis) (**bars**) and total phenolic content (gallic acid equivalents, mmol kg^{-1}, in DW basis) (**lines**) of control (CK, **A**), mix of vitamins (MV, **B**), and green tea (GT, **C**) without ultrasound 0 kHz (**1**) or with ultrasound at 35 kHz (**2**) or 130 kHz (**3**). Values are the mean ± standard deviation (n=4). Different lowercase letters mean significant differences between treatments on the same day ($p < 0.05$). Different capital letters mean significant differences between days within the same treatment ($p < 0.5$).

Figure 5. Polyphenol oxidase (PPO, **A**) and peroxidase (POD, **B**) activities in potato ($\Delta OD\ 10^3$ / kg · s, in protein basis) of (CK, 1), mix of vitamins (MV, 2), and green tea (GT, 3) combined with US at 0 kHz (■), 35 kHz (■), 130 kHz (■).Values are the mean ± standard deviation (n=4). Different lowercase letters mean significant differences between treatments on the same day ($p < 0.05$). Different capital letters mean significant differences between days within the same treatment ($p < 0.5$).

Supplementary material 1. Experimental design

Supplementary material 2. Images of the fresh-cut potatoes in control (CK), with mix of vitamins (MV) or green tea (GT) with or without ultrasound at 35 or 130 kHz, during storage at 4 ºC for 9 days (D0, D2, D4, D7, D9).

Supplementary material 3. Principal component analysis of the relationship between variables studied, at day 0 (**A**) and day 9 (**B**).

Fig. 1

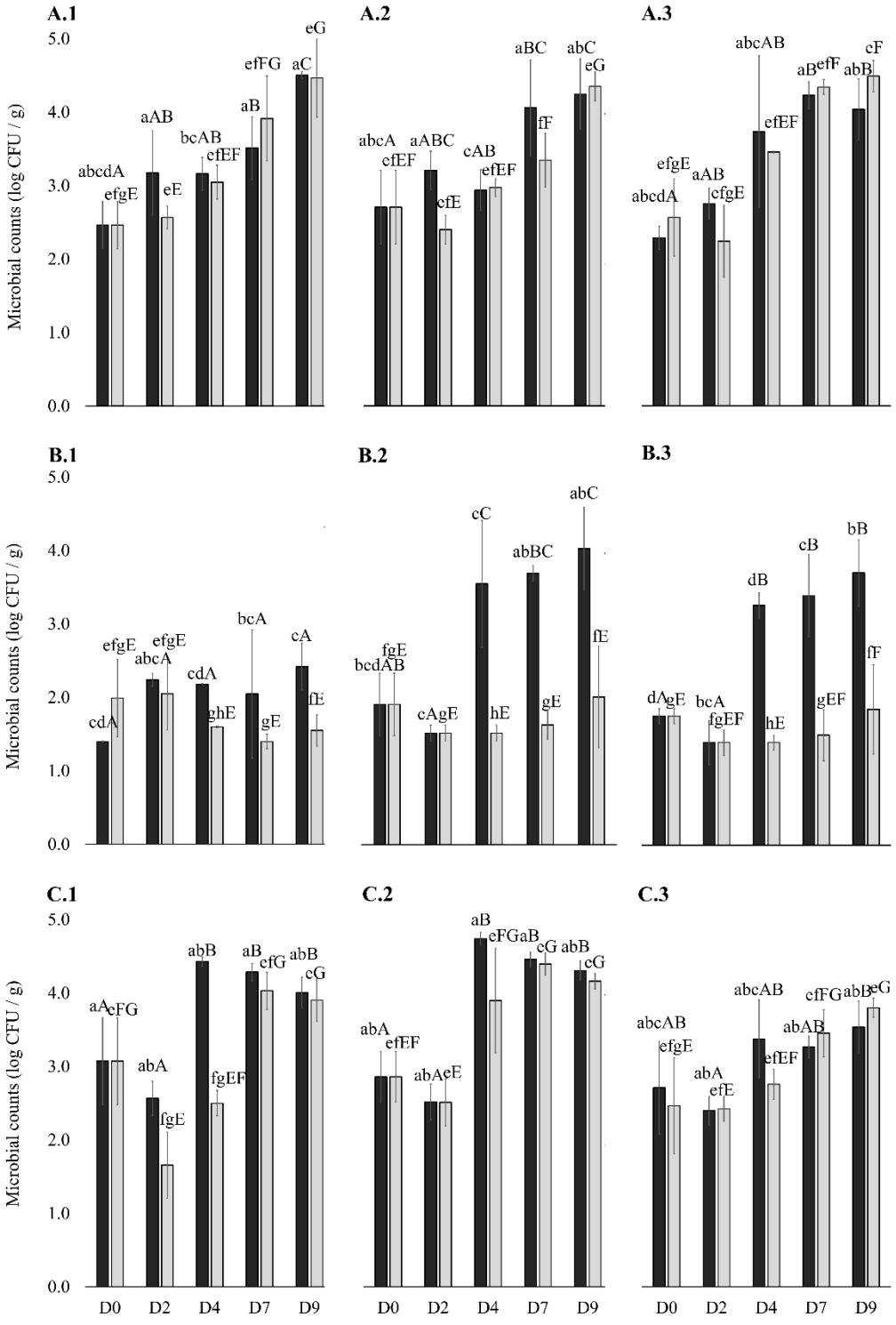

A.1
A.2
A.3
B.1
B.2
B.3
C.1
C.2
C.3
Microbial counts (log CFU / g)
5.0
4.0
3.0
2.0
1.0
0.0
D0
D2
D4
D7
D9

Fig 2

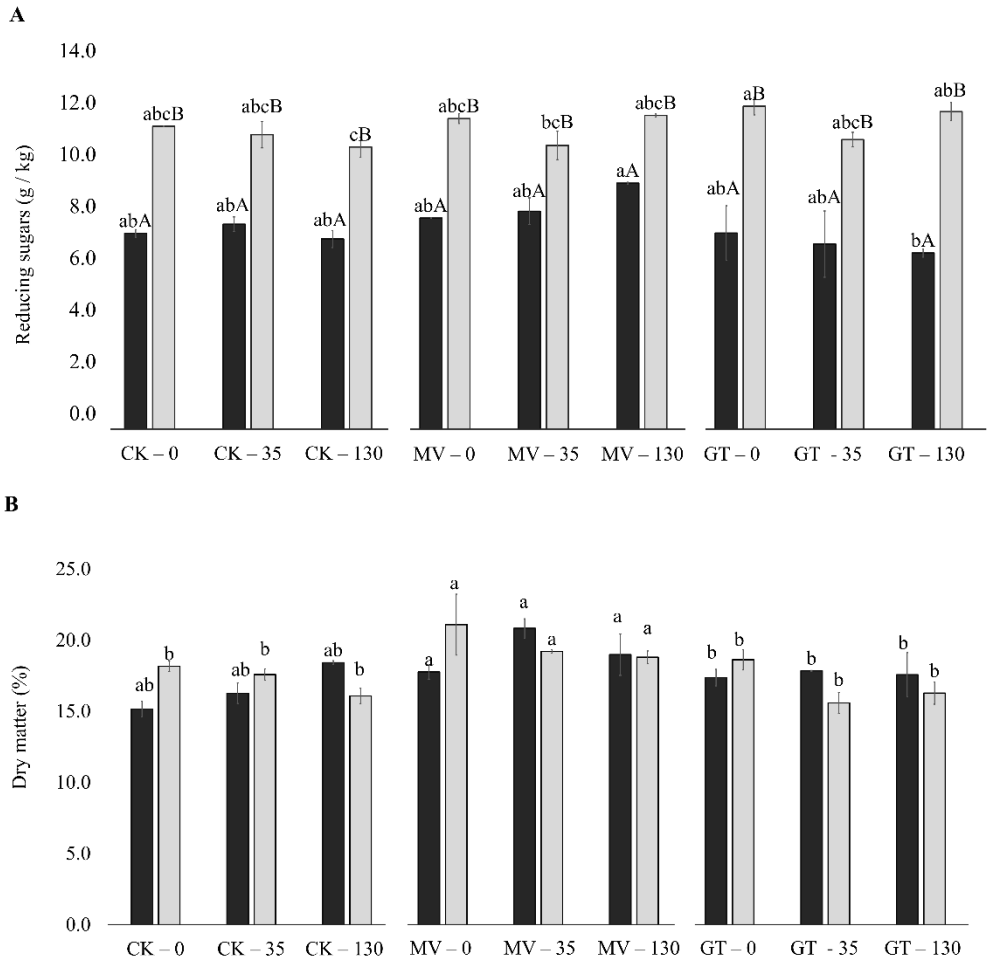

A
Reducing sugars (g / kg)
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
abA
abcB
abA
abcB
abA
cB
abA
abcB
abA
bcB
aA
abcB
abA
aB
abA
abcB
bA
abB
CK – 0
CK – 35
CK – 130
MV – 0
MV – 35
MV – 130
GT – 0
GT - 35
GT – 130
B
Dry matter (%)
25.0
20.0
15.0
10.0
5.0
0.0
ab
b
ab
b
ab
b
a
a
a
a
a
a
b
b
b
b
b
b
CK – 0
CK – 35
CK – 130
MV – 0
MV – 35
MV – 130
GT – 0
GT - 35
GT – 130

Fig. 3

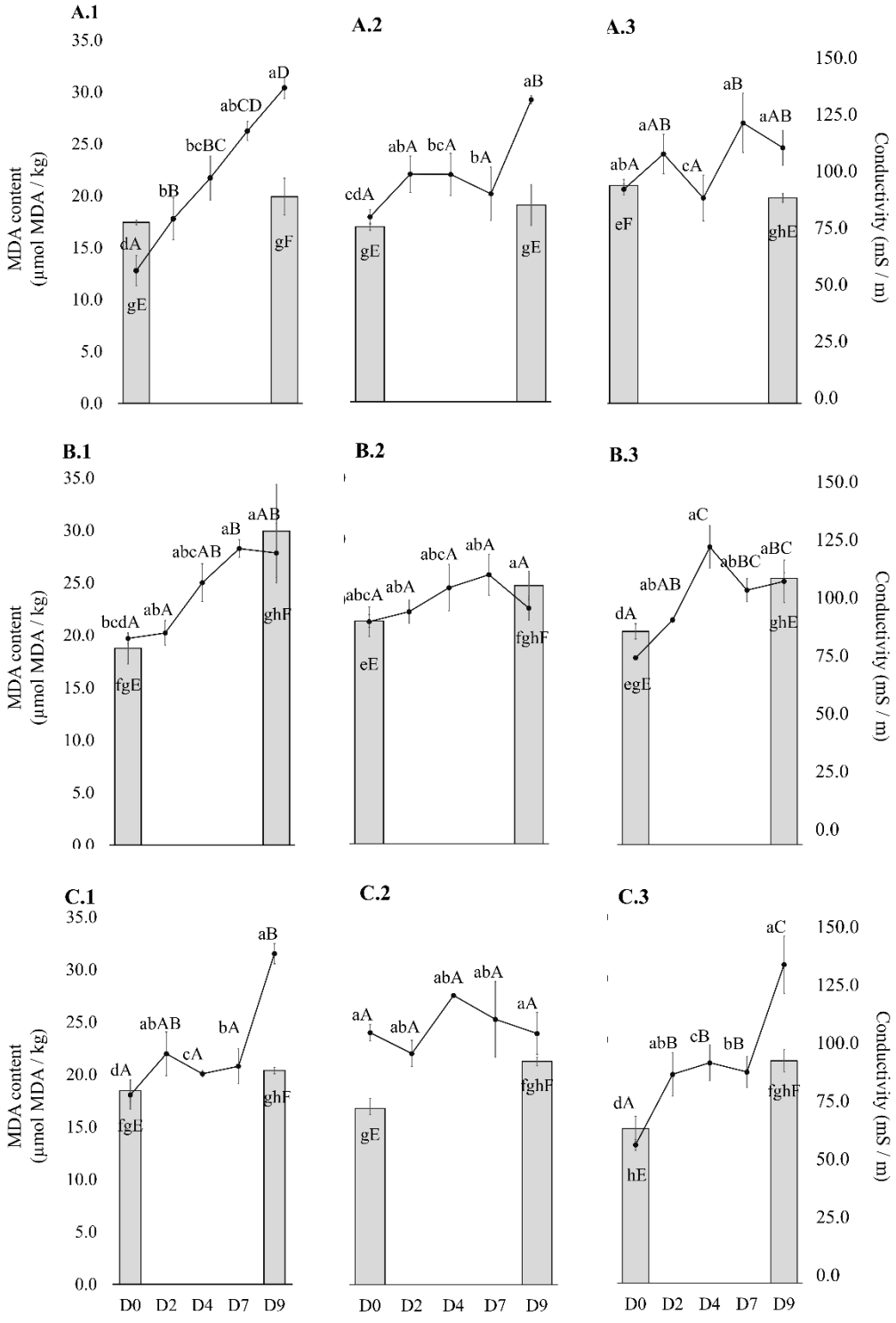

A.1
A.2
A.3
B.1
B.2
B.3
C.1
C.2
C.3
MDA content (µmol MDA / kg)
Conductivity (mS / m)
D0 D2 D4 D7 D9

Fig 4

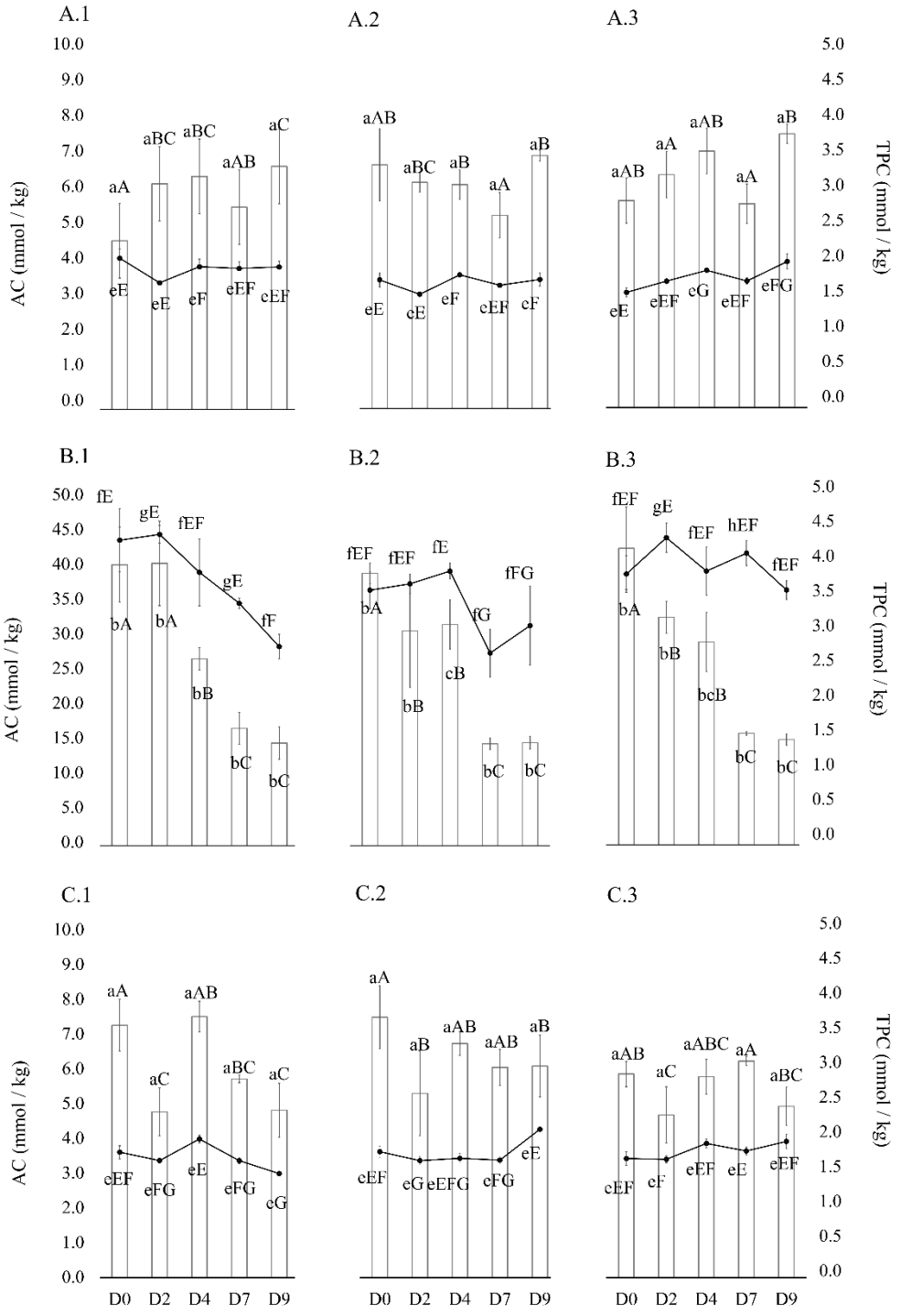
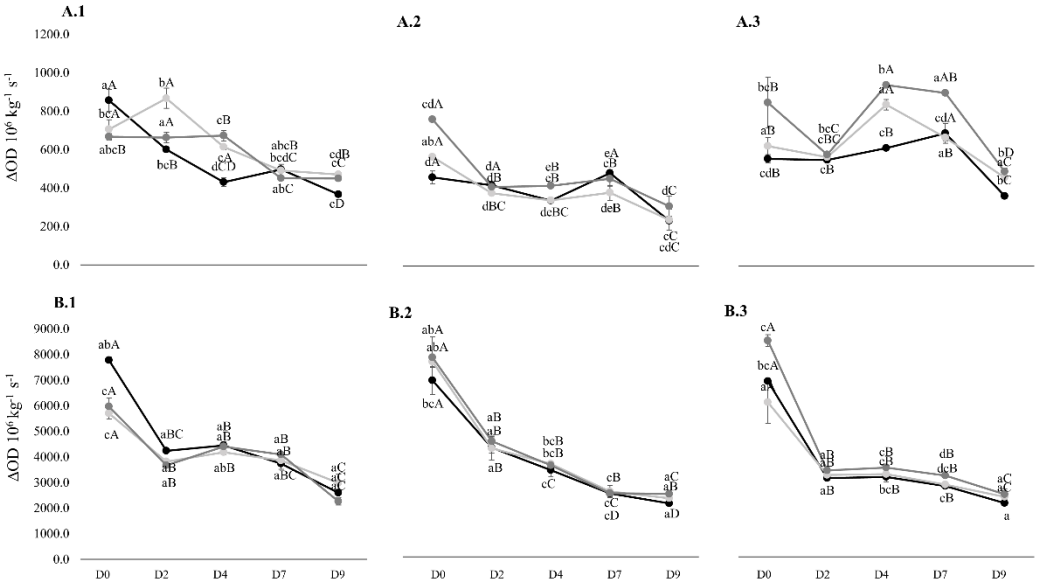
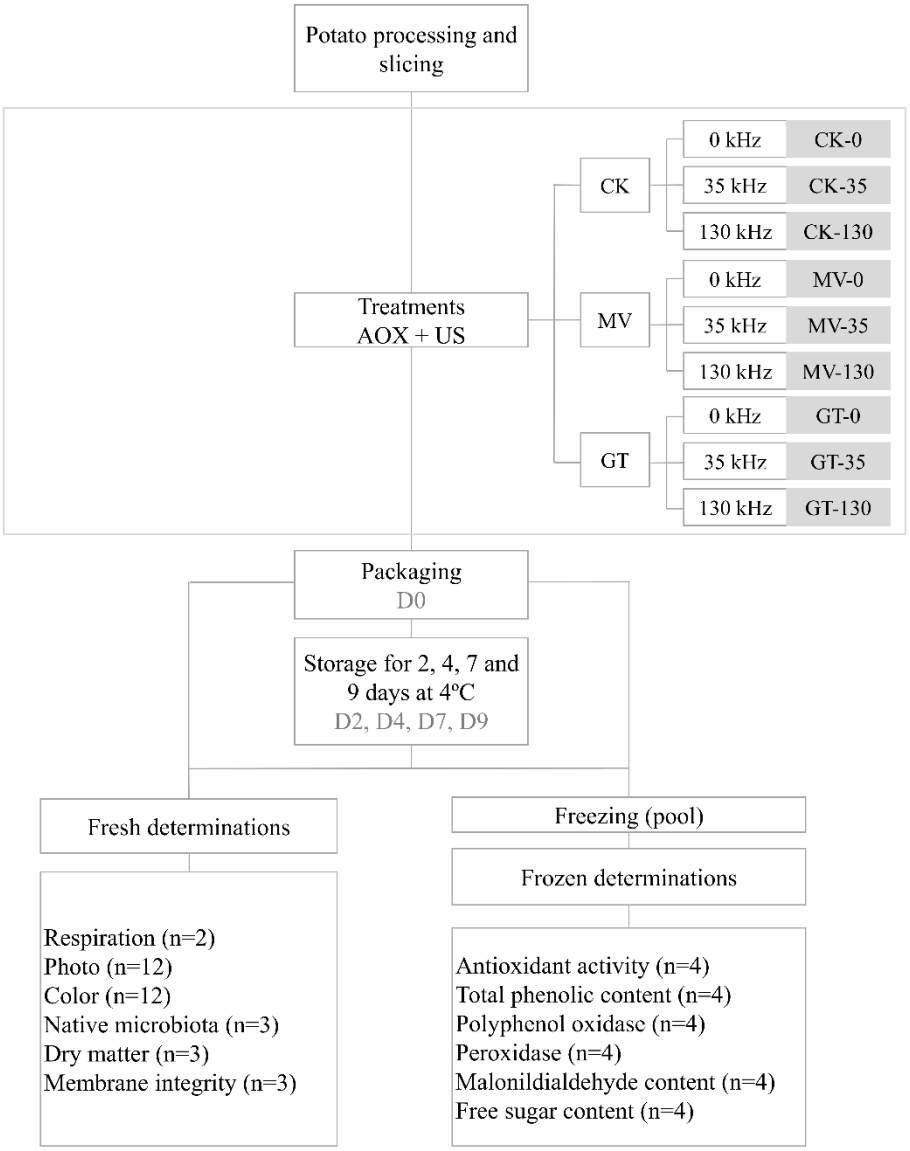


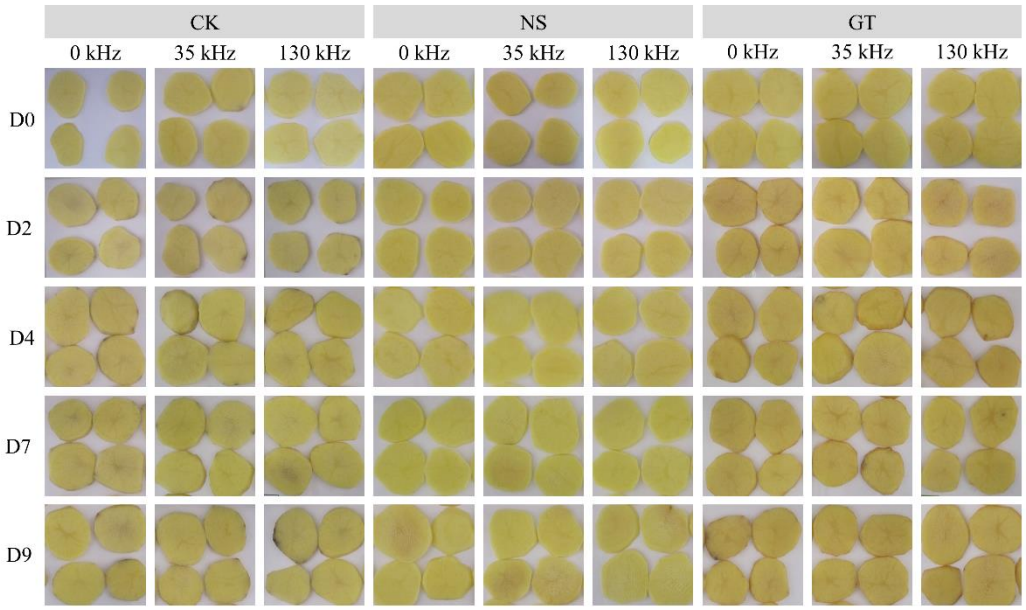
Fig 5



Supl. 1

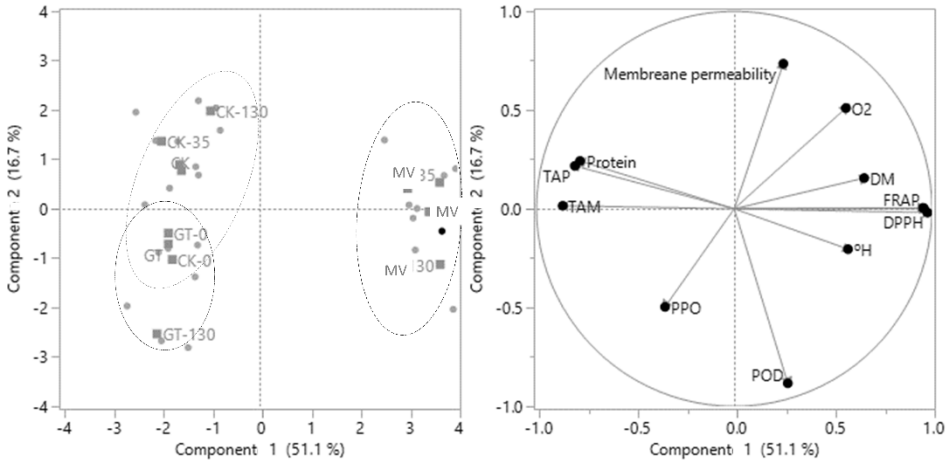

Potato processing and slicing
Treatments AOX + US
CK
MV
GT
0 kHz
35 kHz
130 kHz
CK-0
CK-35
CK-130
MV-0
MV-35
MV-130
GT-0
GT-35
GT-130
Packaging D0
Storage for 2, 4, 7 and 9 days at 4ºC D2, D4, D7, D9
Fresh determinations
Freezing (pool)
Frozen determinations
Respiration (n=2)
Photo (n=12)
Color (n=12)
Native microbiota (n=3)
Dry matter (n=3)
Membrane integrity (n=3)
Antioxidant activity (n=4)
Total phenolic content (n=4)
Polyphenol oxidase (n=4)
Peroxidase (n=4)
Malonildialdehyde content (n=4)
Free sugar content (n=4)

Supl 2

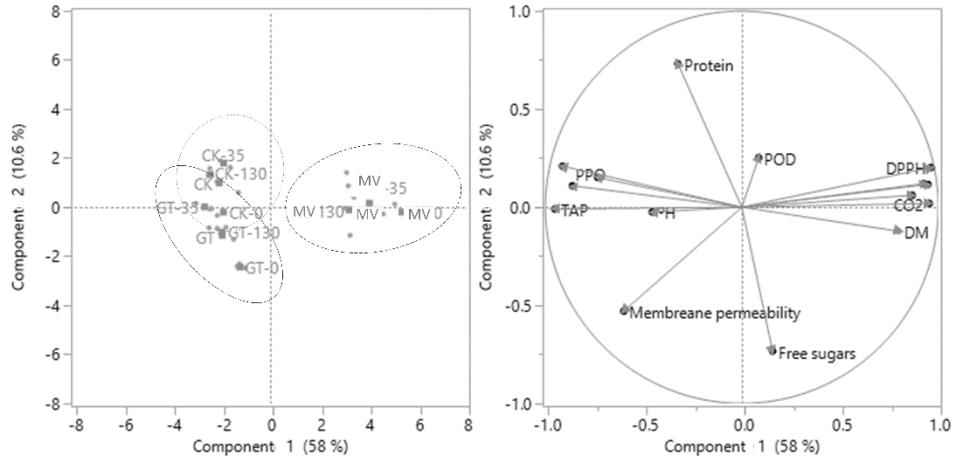

CK
NS
GT
0 kHz
35 kHz
130 kHz
0 kHz
35 kHz
130 kHz
0 kHz
35 kHz
130 kHz
D0
D2
D4
D7
D9

Supl 3

A


CK-130
CK-35
CK
GT-0
GT
CK-0
GT-130
MV 35
MV
MV 130
Component 2 (16.7 %)
Component 1 (51.1 %)
Membreane permeability
O2
Protein
TAP
DM
FRAP
TAM
DPPH
°H
PPO
POD

B


CK-35
CK-130
CK
GT-35
CK-0
GT
GT-130
GT-0
MV 35
MV 130
MV
MV 0
Component 2 (10.6 %)
Component 1 (58 %)
Protein
POD
PPO
DPPH
TAP
°H
CO2
DM
Membreane permeability
Free sugars