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ORIGINAL RESEARCH

Ultrasound and Its Combination with Natural Antimicrobials: Effects on Shelf Life and Quality Stability of a Fruit and Vegetable Smoothie

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Abstract

Fruit and vegetable natural beverage market is growing around the world, but their intrinsic characteristics lead to a high microbial and enzymatic activity, showing a short shelf life when not treated. The present work aims to evaluate the effect of ultrasound (US) treatments on the main spoilage factors and some quality indicators of a mixed fruit and vegetable smoothie (F&VS). Likewise, the advantages of combining US with green tea extract (GTE), nisin (Ni), or natamycin (Na) on the shelf life and stability of the F&VS were assessed. Temperature control was needed to avoid high temperatures during treatment and consequent nutrients and organoleptic degradation. Length of treatment lower than 15 min was required to avoid polyphenoloxidase activation. Ultrasound-selected treatment (UST) was of 70% of amplitude during 4 min, since presented the greatest reduction of peroxidase activity (71.6%) and well performance on microbial control. Both UST and UST + GTE treatments extended F&VS microbial shelf life by 1 week, while UST + Na and UST + Ni by at least 2 weeks. UST greatly increased betalain extractability (55–80%). Its combination with GTE significantly increased the TPC and the antioxidant capacity of the product, as well as enhanced betalain stability during storage. Finally, remarkable results on *Listeria* initial reductions were obtained with UST + Ni (> 4 log) and on *Escherichia coli* with UST + GTE (1.7 log) when contamination was simulated. Indeed, the potential of US and their combination with natural antimicrobials on quality preservation as well as on shelf life extension and safety assurance of the F&VS was probed with very promising results.

Keywords Non-thermal technology · Nisin · Green tea · Natamycin · *Listeria* · *Escherichia coli*

Introduction

Today's consumers are increasingly demanding more natural, nutritious, and healthier foods (Research and Markets, 2019). Accordingly, they are prioritizing those plant-based and avoiding ultra-processed foods containing added sugars, sodium, trans-fats, and/or synthetic additives due to the growing evidence about their adverse health outcomes (Baker et al., 2020; Hosni et al., 2017; Research and Markets, 2019). Nonetheless, the current busy lifestyle has led to an increase in the demand for foods that are easy to prepare or ready for consumption, stimulating the expansion of the minimally processed fruit and vegetable (F&V) market (Huang et al., 2017), including products as natural F&V beverages (Fernandez et al., 2018). Among these products, mixed F&V smoothies combine the sensory and nutritional properties of two or more F&V, creating novel flavors and taste while are an excellent way to intake the nutrients and bioactive compounds responsible for health effects, including their natural fibers (Fernandez et al., 2018; Jolayemi & Adeyeye, 2018).

The intrinsic characteristics of F&V beverages are optimal for enzymatic and microbial activity, especially for species resistant to acidity, which leads to a very short shelf life when not properly treated (Fernandez et al., 2018). Thermal pasteurization is not compatible with consumers' requirements seeking products with nutritional content and

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sensory characteristics similar to their natural fresh equivalents, since it generates nutritional losses and significant organoleptic alterations (Perera & Alzahrani, 2021). Non-thermal technologies, such as high hydrostatic pressures, gamma irradiation, and ultrasound (US), manage to inactivate spoilage-causing factors and preserve, even improve, nutritional and sensory characteristics (Bevilacqua et al., 2018). Among these technologies, US is gaining popularity due to its low energy consumption, accessibility, and feasibility for being included in production lines (Fu et al., 2020a; Perera & Alzahrani, 2021). When high-power ultrasound propagates, cavitation bubbles appear due to pressure variation. The collapse of these bubbles generates heat and many extreme conditions in the microenvironment: thermal effect, shock waves, shear forces, free radical production, and mechanical stress causing microbial destruction and enzyme inactivation (Cao et al., 2018; Nadeem et al., 2018; Nicolau-Lapeña et al., 2019). Moreover, US improves the quality of processed foods and results in characteristics (color, consistency, flavor, and nutrients) similar to the fresh product (Gallo et al., 2018), thus representing a highly promising technology. Additionally, by the hurdle technology concept (Khan et al., 2017), the use of natural antimicrobials, in replacement of traditional synthetics, could complement or enhance the effects of non-thermal technology while keeping the product's natural characteristics. Particularly, nisin, a bacteriocin produced by *Lactococcus lactis*, is a generally recognized as safe (GRAS) compound (FDA, 1988), highly effective in the inactivation of a wide range of Gram-positive bacteria and spores resistant to high temperatures. Natamycin (pyramycin) is an antifungal produced by *Streptomyces natalensis* (Delves-Broughton & Weber, 2011) designed as a natural preservative by the European Union (EEC N° 235), and green tea extracts (GTE) have demonstrated antibacterial, antiviral, antifungal, and antioxidant activity and promote numerous health benefits, particularly the prevention of various types of cancer and cardiovascular diseases (Perumalla & Hettiarachchy, 2011). The effectiveness of these treatments has been proven in several fruit juices (Bevilacqua et al., 2012; Bi et al., 2020; Cassani et al., 2017; Lafarga et al., 2019; Noguera et al., 2021; Park et al., 2021). However, their effect on matrices like mixed F&V smoothies, more complex than juice matrices, remains largely unexplored.

In this context, the present work aimed to evaluate the effect of ultrasound treatments on the main spoilage-causing factors and some quality indicators of a mixed fruit and vegetable smoothie. Likewise, the advantages of combining this technology with natural antimicrobials on the shelf life and stability of the smoothies were assessed.

Materials and Methods

Smoothie Preparation

The raw material was purchased in a local market in Buenos Aires, Argentina. Once in the laboratory, the selected raw material was washed and disinfected by immersion in chlorinated water (200 mg kg^{-1}) for 5 min and dried. The composition (by weight) of the ingredients was: orange juice 59%, apples 15%, carrots 15%, beet greens 6%, and beet stems 5%. Orange juice was extracted using a home squeezer (Oster, USA), and carrots and apples were peeled and chopped into small pieces. Then, all ingredients were mixed in a homogenizer (JTC OmniBlend, Guangdong, China) for 60 s.

Exploration of Amplitude and Temperature Effects

In a first assay, ultrasound treatments were applied to explore the effects of amplitude and temperature of treatment on the main spoilage-causing factors and some quality indicators of a mixed fruit and vegetable smoothie.

Smoothie was prepared according to 2.1 and portions of this untreated smoothie were reserved as controls (C). For ultrasonic treatment, an ultrasonic processor (model VCX 750, Vibra-Cell Sonics, Newtown, Connecticut, USA), equipped with a 13 mm (1/2 inch) high-grade titanium alloy probe threaded to a 3 mm tapered microtip, was employed. The frequency of the ultrasound processor is 20 kHz and their highest power is 750 W. The sonication probe was immersed in a beaker containing 0.2 L of smoothie, with or without the use of jacketed glassware with an ice bath (IB) to control the temperature. Three fixed amplitudes (100, 50, and 30%) were evaluated, giving place to six treatments: A100, A50, A30, A100 + IB, A50 + IB, and A30 + IB. For thermal profile, the temperature was registered at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 7, 9, 10, 12, and 15 min after sonication started. Immediately after treatment, the treated samples were transferred to a 0.033-L polyethylene terephthalate (PET) flask, closed with rubber caps, cooled to 5 ± 1 °C, and subsequently stored under refrigeration until further analysis. On the same day, triplicates of each treatment were taken, and the following quality indicators were determined:

Native microflora counts: Mesophilic aerobic bacteria (MAB), enterobacteriaceae (EB), and mold and yeast (M&Y) counts were determined according to Fernandez et al. (2018a, b). The detection limit (DL) of the method was 2.7 log CFU mL^{-1}, and the end of microbiological shelf life was settled when 6.0 log CFU mL^{-1} of MAB or M&Y was achieved (Fernandez et al., 2019b).

Enzymatic activity: The enzyme activity of polyphenoloxidase (PPO) and peroxidase (POD) was determined as described by Fernandez et al. (2018a, b). Residual activity was calculated as the specific activity of the enzyme on the treated sample per their specific activity on the control sample and expressed as a percentage.

Total soluble solids (TSS) and pH: The TSS were determined with a Milwaukee MA871 Refractometer (Milwaukee Instrument, Rocky Mount, USA) and the results were expressed as the percentage of soluble solids on the solution (%); the pH was measured with a digital pH meter (Hanna, HI99163, Romania, with FC232D electrode, Italy).

Total phenolic content (TPC) and antioxidant capacity: The extraction and determination of total phenolic compounds by Folin-Ciocalteu methodology, and of antioxidant capacity by FRAP and DPPH assays, were carried out according to Fernandez et al. (2018a, b). TPC results were expressed on milligrams of gallic acid equivalents per kilogram of smoothie (mg GAE kg^{-1}), and FRAP and DPPH results were expressed on micrograms of trolox equivalent antioxidant capacity per kilogram of smoothie (μTEAC kg^{-1}).

Betalain content: Betaxanthin and betacyanin determination was carried out according to the method described by Fernandez et al. (2018a, b). The results were expressed as milligrams of Bx or Bc per liter of fresh smoothie (mg L^{-1}).

Selection of Ultrasound Process Parameters

In a second assay, the effects of ultrasound process parameters, amplitude, and time of treatment, on the main spoilage-causing factors (enzymatic and microbial activity), were assessed to select the most promising combination of factors.

Smoothie was prepared according to the "Smoothie Preparation" section and portions of this untreated smoothie were reserved as controls (C). Samples of 0.2 L of smoothie were treated with the ultrasound probe considering a factorial design with two levels of amplitude (30% and 70%) and three levels of time (2, 4, and 8 min) using the ice bath in all cases to avoid thermal effect involvement, keeping temperature during treatment below 30 °C in all cases. Thus, the resulting treatments were as follows: 30%-2 min, 30%-4 min, and 30%-8 min and 70%-2 min, 70%-4 min, and 70%-8 min. Immediately after the treatment, the samples were transferred to a 0.033-L PET flask, closed with rubber caps, cooled to 5 ± 1 °C, and subsequently stored under refrigeration until analysis. On the same day, triplicates of each treatment were taken and native microflora counts, enzymatic activity, pH, and TSS were determined according to those described in the "Exploration of Amplitude and Temperature Effects" section.

Ultrasound Treatment Combined with Natural Antimicrobials

In a third assay, the ultrasound treatment with the previously selected parameters was combined with natural antimicrobials and the effect on the quality stability and shelf life of the smoothie was evaluated.

Moreover, one of the main challenges of F&V beverage industry is to find alternatives to heat preservation method that allows not only to maintain their fresh characteristics and extend their shelf life but also to ensure product's safety. Previous studies show that the survival of *Escherichia coli* and *Listeria*, the main contaminants in this type of food product, can decrease gradually when they have high acidity (Fernandez et al., 2019a). Therefore, when contamination occurs during the process, the effective reduction of the initial load is decisive to guarantee the safety of the product. Based on this, the initial effectiveness of treatments against contamination with *E. coli* and *Listeria* was evaluated.

Effect on Shelf Life and Quality Stability of the Smoothie

Smoothie was prepared according to the "Smoothie Preparation" section and portions of this untreated smoothie were reserved as controls (C). Ultrasound treatment was conducted according to the "Exploration of Amplitude and Temperature Effects" section, with the conditions selected in the previous studies: 70%-4 min with an ice bath. After treatment, some samples (UST) were reserved for further evaluation, while others were added with natural antimicrobials: nisin (UST + Ni), natamycin (UST + Na), and green tea extract (UST + GTE) at doses selected according to previous study results (Nieva et al., 2022) and bibliographical references. Concentrated antimicrobial solutions were prepared immediately before use and dosed according to the desired concentration in the product: nisin (Ni; Nisin®, DSM) at 6.25 mg kg^{-1}, natamycin (Na; Delvocid® Salt, DSM) at 100 mg kg^{-1}, and green tea extract (GTE; Sunphenon 90LB, TAIYO®, Japan) at 0.2%. The samples were stored at 5 ± 1 °C and periodically (0, 7, 14, 21, 28 days) triplicates of each treatment were taken and native microflora counts, TPC and antioxidant capacity, betalain content, pH, and TSS were determined according to those described in the "Exploration of Amplitude and Temperature Effects" section.

Initial Effect Against *Listeria innocua* and *Escherichia coli*

Samples prepared as described in the "Smoothie Preparation" section were inoculated with a mixed culture of *Listeria innocua* (CIP 80.11 and ATTC 33,090) and *Escherichia coli* (ATCC 3526 and ATCC 8739), prepared as described by Fernandez et al. (2019a), to achieve an initial bacterial count

of around 10^6 CFU mL^{-1}, simulating contamination during the process. The selected strains are used commonly as *Listeria monocytogenes* and *E. coli* 0157:H7 surrogates, respectively, since they have shown similar behavior and resistance (Evrendilek et al., 1999; Omac et al., 2015). Samples were inoculated before treatment with US and natural antimicrobials, described in the "Ultrasound Treatment Combined with Natural Antimicrobials" section. *Listeria* spp. and *E. coli* counts were determined according to the one described by Fernandez et al. (2019a). Results were expressed as log CFU mL^{-1} and DL was 2.00 log CFU mL^{-1}.

Statistical Analysis

Results were analyzed using the Origin®8 statistical software (OriginLab®, USA). The statistical analysis was performed using the analysis of variance (ANOVA) and significant differences were determined using the Tukey test ($p < 0.05$).

Results and Discussion

Exploration of Amplitude and Temperature Effects

Although ultrasound is considered a non-thermal technology, it is well known that intense and/or long treatments can generate an increase in the macro-temperature of the system (Bevilacqua et al., 2018). Indeed, thermal profiles obtained during the treatment of smoothies under different conditions (Fig. 1) showed that systems without ice bath presented linear temperature increases. While the initial temperature of the smoothie was 17.3 ± 1 °C, mean rates were 7.8, 3.5, and 1.2 °C/min for A100, A50, and A30 treatments, respectively, causing a rise in system temperature during treatment. In all cases, the 30 °C was surpassed at 1.5, 3, and 15 min, respectively. Above this temperature, a thermal effect must be considered, since it is well known that the deactivation of enzymes and microorganisms, as well as destruction of thermolabile compounds, can occur (Khandpur & Gogate, 2015). Moreover, in the case of treatment A100, the process was interrupted at minute 9 since boiling signs were observed. This increase in temperature of the medium caused by ultrasound is attributed to the cavitation phenomenon, resulting from the formation, growth, and collapse of gas and vapor microbubbles with the consequent release of thermal energy in the form of heat that is absorbed by the medium, increasing its temperature (Bevilacqua et al., 2018; Nutter et al., 2020). In regards to samples with an ice bath, with A50 and A30 treatments, it was possible to maintain temperatures below 30 °C throughout treatment, ensuring that only cavitational effects play a role on the different quality indicators under evaluation (Khandpur & Gogate, 2015). For these samples, thermal profiles also showed a linear behavior with slopes of 0.5 and −0.2 °C/min for A50 + IB and A30 + IB, respectively. The exception was the treatment with the greatest amplitude applied (A100 + IB), in which temperature was maintained during the first 4 min, then increasing rapidly reaching 52 °C at the end of treatment. Hence, if treatment at maximum amplitude is needed, for keeping temperatures below 30 °C, it would be necessary to optimize the temperature control system.

Physicochemical indicators were not significantly affected by treatment, showing in all cases a pH of 3.92 ± 0.08 and TSS of 11.24 ± 0.32%. According to Nadeem et al. (2018), F&V beverage processing should not affect properties like pH and TSS, as they are important parameters for quality and shelf stability that affect the taste and other organoleptic properties of the product. In this sense, many authors also showed that US is an adequate technology to preserve these attributes (Bhat & Sharma, 2016; Lafarga et al., 2019; Nadeem et al., 2018; Nicolau-Lapeña et al., 2019).

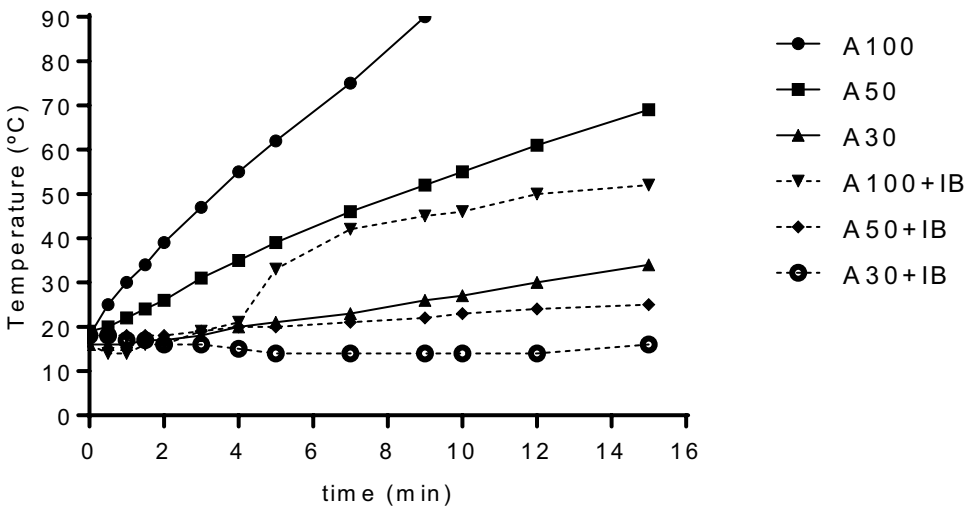

Fig. 1 Thermal profile obtained during ultrasound treatment of smoothies under different conditions

On the other hand, treatments had an important impact on the main F&V beverage deterioration factors, such as enzymatic activity and microbial counts, as shown in Fig. 2. Treatments presented a decisive impact on residual enzymatic activity. In this regard, the greatest POD reduction (99.5%) was obtained with the highest amplitude and without ice bath, A100, followed by A50, A100 + IB, and A30. Conversely, treatments with temperature control and lower amplitudes did not achieve significant POD reductions. It is well known that during US treatment, enzyme inactivation is mainly attributed to physical (cavitation, mechanical effects, micro-mechanical shocks) and/or chemical (formation of free radicals due to sonochemical reaction) principles (Mawson et al., 2011), and can be increased by combination with factors like pressure, temperature, and pH (Mawson et al., 2011; Perera & Alzahrani, 2021). However, in the present study, temperature effect was determinant for POD inactivation since for treatments with the same amplitude applied with or without ice bath, the results were significantly different, with the last ones showing very limited effects. Similar results were observed by Cao et al. (2018), working with a bayberry juice treated with US, with or without temperature control, finding that temperature increase was the major factor affecting enzyme denaturation. In the case of PPO, treatment A100 also presented the greatest reduction, followed by A50 and

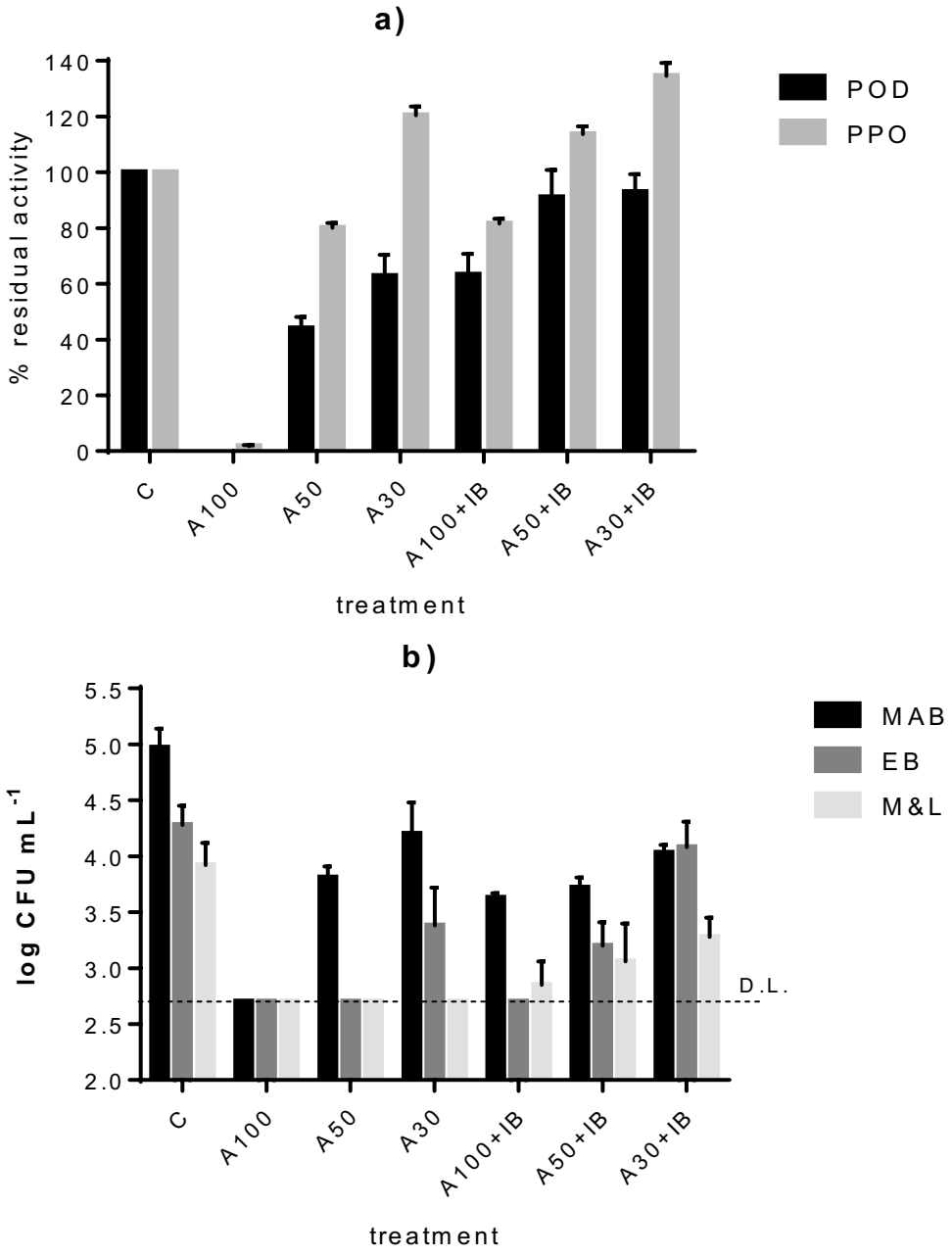

Fig. 2 Effect of ultrasound treatment on smoothies' detrimental factors. **a** Enzymatic activity. **b** Native microbial counts

A100 + IB. Again, by comparison of treatments A100 vs. A100 + IB and A50 vs. A50 + IB, the preponderant effect of temperature is demonstrated since greater reductions were observed in the treatments without ice bath. Interestingly, significant activations in the A30, A30 + IB, and A50 + IB samples were observed. According to Mawson et al. (2011), US treatments at certain frequencies and intensity levels can lead to an increase of enzyme activity due to different effects: physical effects such as an enhancement of mass transfer due to micro-mixing, resulting in an increase of substrate availability and enzyme release due to cell break-up; and biochemical effects such as stimulation of reactions within cell tissues to enhance the production of specific enzymes. Moreover, Engmann et al. (2015) working with mulberry juice observed that PPO activity increased with extension for all frequency levels and suggested that this phenomenon may be associated with activation of latent PPO isoenzymes during treatment. Indeed, according to Plazzotta and Manzocco (2018), cell wounding could promote the release of proteases, responsible for the activation of latent PPO which, differently from the free soluble one, is bound to the cellular membrane. In the present study, the treatments that showed reductions were those in which the sonication was carried out at temperatures near or above the enzyme denaturation temperature. Undoubtedly, again, the temperature was a decisive factor. Nonetheless, considering Engmann et al.'s (2015) observations, if treatment at a controlled temperature is carried out, shorter treatment times may improve PPO activity results.

Results on microbial counts (Fig. 2b) showed that the A100 treatment presented the greatest reduction, followed by A50, with significant reductions of >2.3 (values under D.L.) and 1.2 log in MAB, respectively, and reaching values below D.L. in EB and M&L for both treatments. These results were expected, considering the high temperatures developed during treatments. Samples of A100 + IB and A50 + IB treatments also showed significant reductions of 1.3 and 1.1 log on MAB counts compared to control, and non-significant differences with A100 and A50 result, respectively, on EB and M&Y reductions, which means a significant effect of US beyond the thermal effect. Indeed, a trend towards greater reductions when using higher potencies (or % amplitude) was observed. Moreover, while A30 only achieved a significant reduction on M&Y (values under D.L.), treatment A30 + IB did not present significant reductions in any of the evaluated microbial groups. It is well known that the effect of US depends on the type of microorganism, the amplitude of ultrasonic waves, the exposure times and treatment temperatures, and the volume of food being processed as well as food composition (Chen, 2017). Although US is a technology recognized for its effectiveness for microorganism inactivation, many authors reported reductions between 1 and 2 log, or less, when applying this technology individually (Aadil et al., 2015; Alighourchi et al., 2014; Bevilacqua et al., 2014; Tomadoni et al., 2016; Zafra-rojas et al., 2013). Moreover, it is accepted that it is necessary to combine it with other factors such as heat, low pH, antimicrobials, or other technologies (high pressures, UV radiation, pulsed electric fields, among others) to achieve reductions equivalent to those reached with traditional heat treatments (Brilhante de São José et al., 2014; Chen, 2017; Nicolau-Lapeña et al., 2019; Perera & Alzahrani, 2021).

Regarding the effect of the treatments on the nutritional indicators (Table 1), the total phenolic content (TPC) of control samples was 589 ± 2 mg GAE kg^{-1} and all the treated samples showed higher values, increasing with the amplitude applied, but only 100% and 100% + IB were significantly different from control, with increases of 19 and 12%, respectively. Phenolic compounds are bound to cellulose, hemicellulose, and pectin or are present in soluble form in the vacuole (Li et al., 2019). According to Nadeem et al. (2018), it is possible that ultrasound enhances the release of these compounds from the cell wall, through the collapse via cavitation, into the surrounding medium. While some authors observed better results on TPC of samples when treatments with higher temperatures were applied due to better diffusivity of the extracted molecules

Table 1 Effect of ultrasound treatment on smoothies' nutritional indicators

Treatment	DPPH (μTEAC kg^{-1})	FRAP (μTEAC kg^{-1})	TPC (mg GAE kg^{-1})	Bc (mg L^{-1})	Bx (mg L^{-1})
C	2147 ± 36^{a}	2654 ± 81^{a}	589 ± 2^{a}	10.1 ± 0.1^{a}	6.9 ± 1.3^{a}
A100	2450 ± 36^{b}	3282 ± 23^{b}	699 ± 12^{b}	$15.6 \pm 0.3^{b,d}$	14.7 ± 0.3^{b}
A50	$2216 \pm 58^{a,c}$	2666 ± 75^{a}	$629 \pm 5^{a,c}$	12.8 ± 0.3^{c}	$9.5 \pm 0.2^{c,d}$
A30	2136 ± 13^{a}	2584 ± 63^{a}	$617 \pm 18^{a,c}$	$13.9 \pm 1.3^{b,c}$	11.2 ± 1.0^{c}
A100 + IB	$2322 \pm 23^{b,c}$	3078 ± 58^{b}	$657 \pm 12^{b,c}$	17.6 ± 0.3^{d}	17.5 ± 0.5^{b}
A50 + IB	$2246 \pm 58^{a,c}$	2702 ± 138^{a}	$638 \pm 21^{a,c}$	$13.9 \pm 0.4^{b,c}$	11.2 ± 0.3^{c}
A30 + IB	2159 ± 13^{a}	2536 ± 51^{a}	613 ± 3^{a}	$12.1 \pm 0.9^{a,c}$	$8.4 \pm 0.6^{a,d}$

TPC total phenolic content, *DPPH* antiradical antioxidant activity, *FRAP* ferric-reducing antioxidant activity, *Bc* betacyanin content, *Bx* betaxanthin content

into the medium, and increased polyphenol solubility (Nutter et al., 2020), others observed that cooling during ultrasound treatment was beneficial to retain phenolic compounds. (Cao et al., 2018). In this study, non-significant differences were observed on TPC among samples treated at the same amplitude with or without temperature control.

Results on the content of betalains (Table 1) showed that these compounds were significantly affected by treatments, all of them presenting significantly higher values than control, except for the mildest treatment with an ice bath (30% + IB) in which the differences were non-significant. The highest values were observed for the 100% + IB treatment, followed by 100%, 50% + IB, and 50%, which indicates that greater extraction is achieved at higher potencies and the convenience of using temperature control. Betalains are hydrosoluble vacuolar pigments, and their enhanced extractability during US treatments was observed by many authors (Maran & Priya, 2016; Nutter et al., 2020) associating this observation with cellular disruption caused by cavitation (Maran & Priya, 2016; Nutter et al., 2020). Moreover, betalains are thermolabile molecules and, while increases in treatment temperature between the range of 25 and 50 °C can favor their extractability, by increasing the permeability of the cell membranes, improving the solubility of the pigment and increasing the diffusion coefficient (Prakash Maran et al., 2013); their decomposition rate also increases with temperature (>50 °C), causing hydrolysis of aldimine bound (Herbach et al., 2006). Moreover, according to Maran and Priya (2016), the heating effect and exposure of ultrasound treatment for long times can cause the structural destruction and decomposition of pigments. Hence, neither high temperature nor long treatments are recommended if their preservation is wanted.

Concerning antioxidant capacity, control samples presented mean values of 2147 ± 36 μTEAC kg^{-1} for DPPH and 2654 ± 81 μTEAC kg^{-1} for FRAP, all the treated samples showed higher values than control, increasing with the amplitude applied. Only treatments 100% and 100% + IB were significantly different from control (but not between them), with increases of 24 and 16% for FRAP and 14 and 8% for DPPH, respectively. Indeed, behavior was similar to the one observed for TPC, which could mean a major participation of these compounds in the total antioxidant capacity of the product, at least compared with Bc and Bx. Indeed, the relation between TPC and antioxidant capacity was demonstrated in many products (Fernandez et al., 2019a; Plazzotta & Manzocco, 2018; Yikmiş, 2019; Luo et al., 2015). Moreover, by the bioactive compound increase achieved with US treatment, increased antioxidant activity is usually observed (Bhat & Sharma, 2016; Keenan et al., 2012; Nadeem et al., 2018; Nicolau-Lapeña et al., 2019).

Taking into account the thermal effects observed in the preliminary evaluation, the temperature control with an ice bath was adopted for the following tests. Moreover, shorter treatments were explored since they could benefit PPO inactivation and/or achieve better bioactive retention.

Selection of Ultrasound Process Parameters: Amplitude and Time of Treatment

Non-significant changes were observed on physicochemical indicators of smoothies with different treatments, showing in all cases a pH of 4.09 ± 0.07 and TSS of 11.66 ± 0.42%.

The effect of the treatments on the POD activity (Fig. 3a) shows significant differences with control in all cases. When a 30% of amplitude was applied, the reductions increased as the treatments were longer. When a 70% of amplitude was applied, a higher level of inactivation was achieved than at 30%, but unlike that amplitude, a maximum level of inactivation was found at intermediate times. This could be related to the previously mentioned effect: Long treatment time can favor the activation of latent enzymes or give place to more active structures (Engmann et al., 2015; Mawson et al., 2011). In the case of PPO activity, while the mildest treatments (30%-2 min and 30%-4 min) presented the greatest reductions ($p < 0.05$), the strongest treatments (long time, high amplitude) presented very low reductions, without differences with the control. Again, this could be due to the structural changes of some enzymes towards more active structures when more intense US treatments are applied. Rojas et al. (2016) suggested that a general conclusion about the effect of treatment on enzymatic activity cannot be specified, since properties of both product (pH, activity of water/vapor pressure, ionic strength, composition) and process (kind of equipment, volumetric power, frequency, intensity, amplitude, reactor geometry, and wave distribution) influence the enzyme activity. Remarkably, with the conditions used in this trial, activation was not observed as in the previous trial, probably due to the limitation of the treatment extension.

Results on the native microflora (Fig. 3b) showed a clear trend of reduction with greater intensity of the treatments. However, the reductions obtained in all cases were less than 1 log cycle, following the one observed in the previous assay. Significant differences with the control were only obtained in samples corresponding to the 70%-8 min treatment, with reductions of around 0.7 log for MAB, EB, and M&L counts. Moreover, no significant differences were observed between treatment 70%-8 min and 70%-4 min, even when the latter only achieved significant differences from control in M&Y counts.

Results of this section showed that none of the evaluated treatments affected physicochemical indicators, and the effect on PPO was very limited, but without activations observed. The highest reduction of POD (71.6%) was obtained with 70%-4 min treatment, a very promising

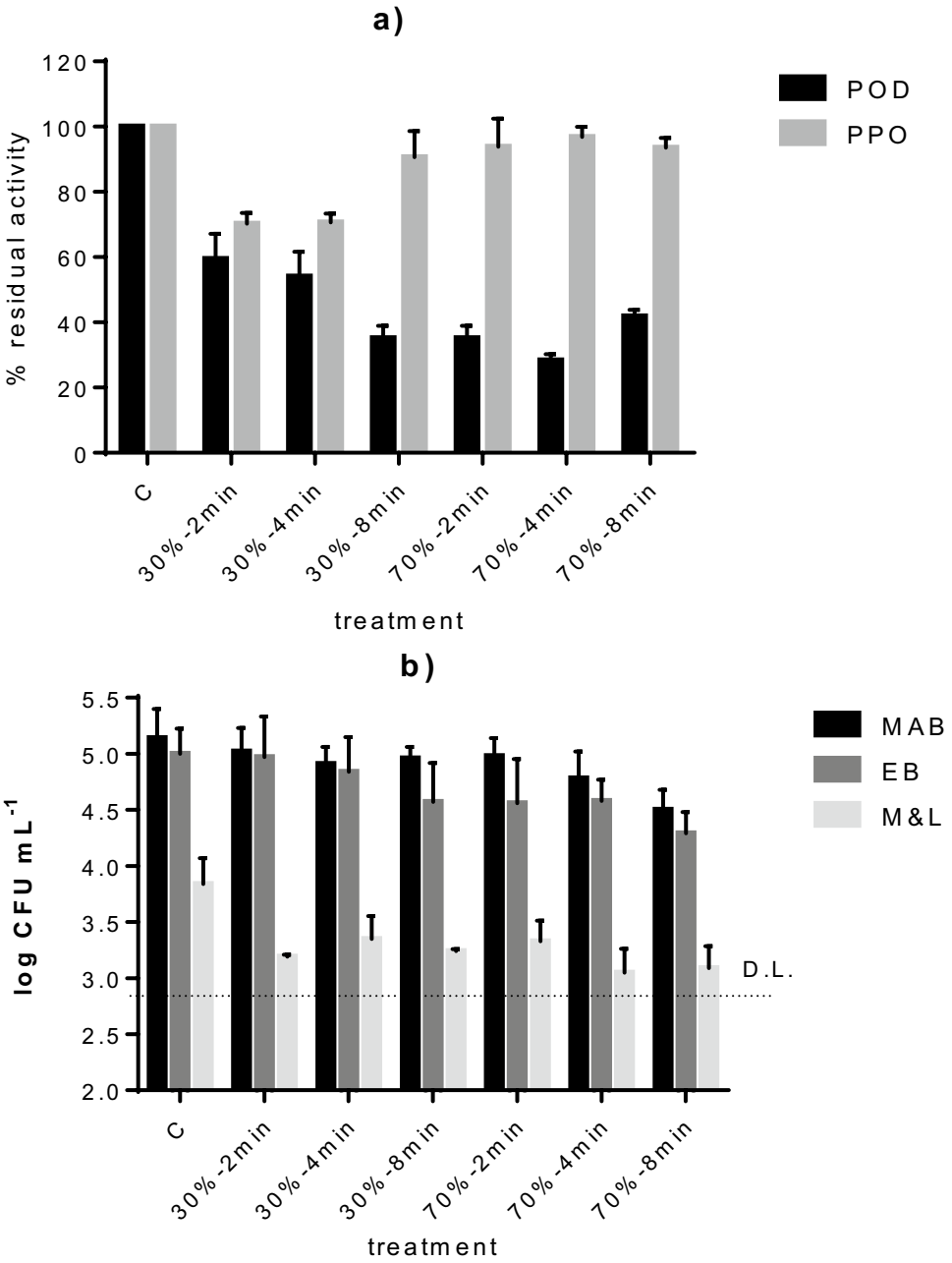

Fig. 3 Effect of time of treatment and amplitude on peroxidase (POD) and poliphenoloxidase (PPO) enzymatic activity (**a**) and native microflora counts (**b**)

result considering that this is a crucial enzyme involved in quality deterioration during the processing and storage of foods (Lee et al., 2021). For microbial control, better results were obtained for the more intense treatment, but no significant differences were observed between 70%-8 min and 70%-4 min. Based on these results and for energy consumption saving, the 70%-4 min treatment was selected for the next assays in which quality stability and shelf life of the smoothie were addressed. Nonetheless, as microbial reductions obtained were very limited, lower than 1 log, the combination of the ultrasound-selected treatment (UST) with natural antimicrobials was proposed to, by hurdle concept application, enhance treatment effect on microbial reduction.

Effect of Ultrasound Treatment Combined with Natural Antimicrobials on Smoothie Shelf Life and Quality Stability

The physicochemical indicators were not significantly affected neither with treatment nor with storage time, showing in all cases a pH of 4.04 ± 0.11 and TSS of $10.79 \pm 0.13\%$.

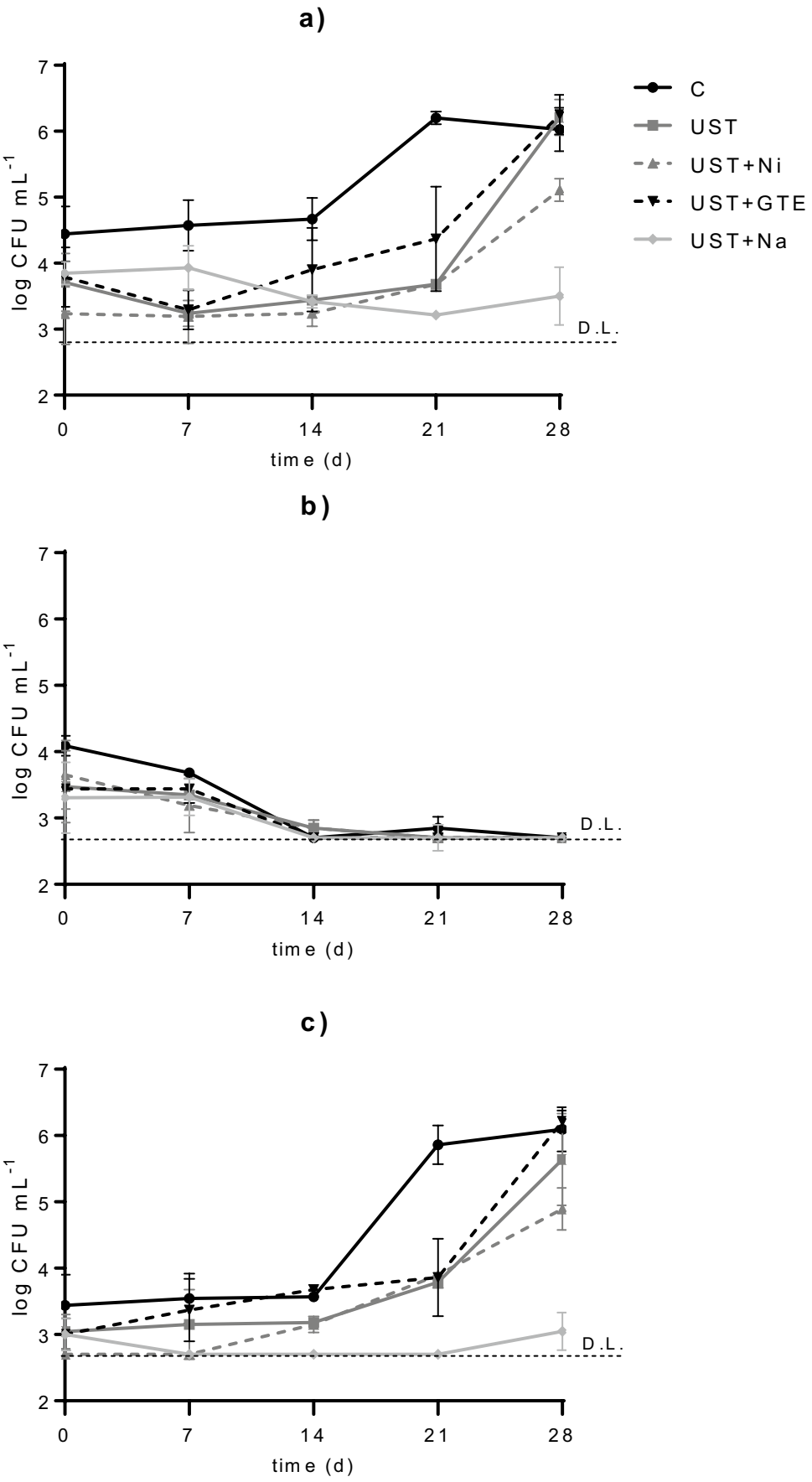

Fig. 4 Effect of US treatment and its combination with natural antimicrobials on mesophilic aerobic bacteria (**a**), enterobacteriaceae (**b**), and molds and yeasts (**c**)

The changes of the native microflora counts on the mixed fruit and vegetable smoothie with different treatments are presented in Fig. 4. The initial counts in sample C were 4.4 ± 0.4, 4.1 ± 0.2, and 3.4 ± 0.4 log CFU mL^{-1} for MAB, EB, and M&Y, respectively. Treatment UST showed non-significant initial reductions of 0.7, 0.6, and 0.4 log on MAB, EB, and M&Y, respectively. Similar results were observed for treatments UST + GTE and UST + Na. Conversely, while UST + Ni treatment showed a similar initial EB reduction than the other treatments, it achieved a 1.2 log reduction of MAB and a 0.75 log reduction of M&Y, differing statistically from the control in both cases.

During storage, MAB counts of control samples remained around 4.5 log CFU mL^{-1} until day 14, then showing considerable growth, surpassing the generally accepted limit for this type of product (6 log CFU mL^{-1}; Varela-Santos et al., 2012) at day 21. UST showed better performance than control, with counts between 1 and 2 log below C until day 21 of storage, then showing a rapid growth exceeding the limit at day 28 of storage. Treatment UST + GTE presented a behavior similar to UST, probably due to the low concentration (0.2%) of GTE used in this treatment. In previous studies (Nieva et al., 2022), concentrations of 1% of GTE were needed to achieve significant reductions of around 1 log in native microflora of this smoothie, but at 0.2% which is the maximum tolerated dose, above which negative impacts on sensory attributes appear (Nieva et al., 2022), no antimicrobial effect was observed. Meanwhile, treatment UST + Ni presented counts around 3.5 log CFU mL^{-1} until day 21, then showing a growth until 5.1 ± 0.2 log CFU mL^{-1} on day 28, while samples treated with UST + Na showed values around 3.5 log CFU mL^{-1} throughout storage. Hence, neither treatment UST + Ni nor UST + Na exceeded the MAB microbial limit during the entire storage. In previous studies in which this smoothie was treated with individual treatment of nisin 12.5 mg kg^{-1} or natamycin 200 mg kg^{-1} (in both cases double concentration than used in the present work), no significant reductions for MAB were observed (Nieva et al., 2022). Considering this precedent and the results of the present study when only UST was applied, an enhanced effect of the US treatment by its combination with Ni or Na is evidenced. This effect could be attributed to an increased permeability of the cells by ultrasonic waves that favors the lethality of antimicrobial compounds increasing the rate of reaction between the antimicrobial and cell components (Ross et al., 2003). In fact, many authors studying the combination of US with antimicrobial compounds, such as essential oils (Cassani et al., 2017; Guo et al., 2020; He et al., 2021), carbon dots (Fan et al., 2020), citrus extract and sodium benzoate (Bevilacqua et al., 2012), ascorbic acid (Park et al., 2021), organic acids and citral (Zhang et al., 2020), vanillin (Cassani et al., 2017; Gastélum et al., 2012), nisin (Bi et al., 2020), and nisin and oregano (Takundwa et al., 2021), showed promising results. In regards to the remarkable effect of natamycin on MAB obtained in this study, similar results were observed by Yikmiş (2019) when studying the effect of a US treatment with natamycin and their combination on MAB and M&Y of pomegranate juice. Both, US and US + natamycin treatments, were highly effective, reducing the microbial count to values under D.L., maintaining these values until day 30 of storage at 4 °C. In the study conducted by Yikmiş (2019), natamycin treatment showed reductions of around 1.5 and 5 log on MAB and M&Y, respectively, with no regrowth observed at day 30 of storage. Natamycin is well known to be effective against a wide range of yeasts and molds, since acts binding specifically to ergosterol, an essential component of membranes of yeasts and molds, blocking fungal growth (te Welscher et al., 2010). However, their activity on bacteria was probed in some studies. Mohamed et al. (2005) observed a small but significant effect on soil bacteria and Kallinteri et al. (2013) in Galotyri cheese lactobacilli and lactococci population. Although none of these authors hypothesized about the mechanism of action, Kallinteri et al. (2013) associated this effect with an unknown factor (a metabolite produced by the bacteria or yeasts) showing a synergistic effect with natamycin, presumably causing cell injury and making cells natamycin-sensitive. Evidently, in the present work, combination of natamycin with US resulted in an enhanced effect in MAB control.

Regarding changes in EB counts during storage, all treatments, including the control, showed reductions with time, reaching values below D.L. from day 14 onwards. These results are consistent with previous observations when working with this smoothie (Fernandez et al., 2020, 2019a) and were related to a sensitivity of the native EB to the low pH of the smoothie. Hence, this microbial group would not be determinant of the microbial quality of this smoothie.

Finally, M&Y count changes during storage showed similar tendencies to MAB. Control remained on values around 3.5 log CFU mL^{-1} for M&Y until day 14, then showing considerable growth, with counts around 6 log CFU mL^{-1} (accepted limit for M&Y; Varela-Santos et al., 2012) from day 21 onwards. UST and UST + GTE presented similar behavior between them, remaining at values of M&Y around 3.5 log CFU mL^{-1} during an additional week compared to C, but exceeding the limit of 6 log CFU mL^{-1} at day 28 of storage. Treatment UST + Ni presented counts below 3.5 log until day 21, then reaching 4.9 ± 0.3 log CFU mL^{-1} on day 28, while UST + Na showed the best performance with values under D.L. until day 21 and counts of 3.1 ± 0.2 log CFU mL^{-1} on day 28. As previously mentioned, an improved effect on M&Y reductions was expected by combining US with natamycin. On the other hand, nisin is active against Gram-positive bacteria but has little or no effect against Gram-negative bacteria, yeasts, and

molds (Delves-Broughton & Weber, 2011). Nonetheless, in combination with other factors, nisin can exhibit a wider spectrum of action. Any treatment that can disrupt the cell wall of yeast facilitates the access of nisin to the membrane and could lead to cell rupture (Dielbandhoesing et al., 1998) which can explain the results obtained in this work.

In short, and considering the limits established for MAB and M&Y, the UST and UST + GTE treatments were able to extend the microbiological shelf life of the smoothie by 1 week, while the UST + Ni and UST + Na did so in at least 2 weeks, compared to control.

Changes in nutritional indicators of smoothies with different treatments are presented in Table 2. In general, all the samples treated with UST presented non-significant increases of around 10% of the TPC, DPPH, and FRAP immediately after the treatment. The exception was observed in the sample containing GTE that presented values 11, 14, and 12 times higher than the control for TPC, DPPH, and FRAP, respectively. This was associated with the high content of polyphenols of green tea and its recognized antioxidant activity (Ananingsih et al., 2013; Perumalla & Hettiarachchy, 2011). Reductions of 30, 67, and 50% of TPC, DPPH, and FRAP at day 28 were observed in control samples due to deterioration during storage. On the same day, samples UST, UST + Ni, and UST + Na showed reductions of around 38, 76, and 57%, respectively, clearly higher but not significantly different from control. Again, the sample containing GTE presented different behavior with reductions of 50, 40, and 30% at day 28, yet with values significantly higher above all the other samples. The higher reductions during storage observed on samples treated with UST can be due to the oxygen reactive species generated during sonication which promote the degradation of polyphenols by triggering radical chain reactions (Carrera et al., 2012; Nutter et al., 2020). Moreover, cellular disruption caused by cavitation and homogenization of the smoothie would place oxygen and intercellular oxidation enzymes in direct contact with antioxidant compounds, leading to a most rapid auto-oxidation of these bioactives (Keenan et al., 2012). The different behavior of these indices during storage (between samples with or without GTE) could

Table 2 Changes on nutritional indicators on samples with different treatments during storage at 5 ± 1 °C

Treatment	Day	TPC* (mg GAE kg^{-1})	DPPH* (μTEAC kg^{-1})	FRAP* (μTEAC kg^{-1})	Bc (mg L^{-1})	Bx (mg L^{-1})
Control	0	$597 \pm 6^{a,A}$	$2483 \pm 48^{a,A}$	$3194 \pm 26^{a,A}$	$5.4 \pm 0.4^{a,A}$	$6.2 \pm 0.7^{a,A}$
	7	$541 \pm 39^{a,A,B}$	$1323 \pm 147^{a,B}$	$2807 \pm 597^{a,A,B}$	$5.3 \pm 0.6^{a,A}$	$5.4 \pm 0.5^{a,A}$
	14	$471 \pm 10^{a,B,C}$	$1212 \pm 161^{a,B,C}$	$1756 \pm 115^{a,B,C}$	$4.4 \pm 0.9^{a,A,B}$	$5.2 \pm 0.5^{a,A}$
	21	$437 \pm 24^{a,B,C}$	$817 \pm 45^{a,C}$	$1708 \pm 156^{a,B,C}$	$4.3 \pm 0.1^{a,B}$	$6.0 \pm 0.3^{a,A}$
	28	$419 \pm 42^{a,C}$	$829 \pm 49^{a,B,C}$	$1577 \pm 23^{a,C}$	$4.5 \pm 0.3^{a,B}$	$6.0 \pm 0.5^{a,A}$
UST	0	$665 \pm 31^{a,A}$	$2653 \pm 24^{a,A}$	$3405 \pm 78^{a,A}$	$8.3 \pm 0.2^{b,A}$	$8.0 \pm 0.2^{b,A}$
	7	$519 \pm 34^{a,A,B}$	$1255 \pm 19^{a,B}$	$2256 \pm 176^{a,B}$	$8.2 \pm 0.4^{a,b,A}$	$8.9 \pm 0.4^{b,A}$
	14	$513 \pm 44^{a,A,B}$	$1199 \pm 30^{a,B}$	$1701 \pm 61^{a,C}$	$6.9 \pm 0.1^{b,A,B}$	$9.0 \pm 0.2^{b,A}$
	21	$469 \pm 75^{a,B}$	$668 \pm 31^{a,C}$	$2034 \pm 64^{a,B,C}$	$6.3 \pm 0.1^{b,B,C}$	$8.1 \pm 0.1^{b,A}$
	28	$408 \pm 3^{a,B}$	$653 \pm 59^{a,C}$	$1408 \pm 26^{a,D}$	$6.3 \pm 1.0^{b,C}$	$7.9 \pm 0.7^{b,B}$
UST + Ni	0	$645 \pm 13^{a,A}$	$2675 \pm 35^{a,A}$	$3415 \pm 122^{a,A}$	$8.7 \pm 0.2^{b,A}$	$8.2 \pm 0.3^{b,A}$
	7	$536 \pm 1^{a,B}$	$1384 \pm 92^{a,B}$	$2184 \pm 162^{a,A,B}$	$8.6 \pm 1.1^{b,A}$	$9.0 \pm 1.0^{b,A}$
	14	$479 \pm 14^{a,B,C}$	$1423 \pm 57^{a,B}$	$1817 \pm 139^{a,B}$	$7.1 \pm 0.4^{b,A}$	$9.2 \pm 0.4^{b,A}$
	21	$499 \pm 1^{a,B}$	$669 \pm 40^{a,C}$	$1728 \pm 52^{a,B}$	$6.3 \pm 0.1^{b,A}$	$8.2 \pm 0.1^{b,A}$
	28	$405 \pm 9^{a,C}$	$656 \pm 58^{a,C}$	$1463 \pm 40^{a,B}$	$7.4 \pm 0.6^{b,A}$	$9.3 \pm 0.8^{b,A}$
UST + GTE	0	$6505 \pm 107^{b,A}$	$34,149 \pm 56^{b,A}$	$38,713 \pm 721^{b,A}$	$8.1 \pm 0.3^{b,A}$	$8.0 \pm 0.5^{b,A}$
	7	$5756 \pm 39^{b,A}$	$20,210 \pm 2266^{b,B}$	$32,696 \pm 404^{b,B}$	$8.5 \pm 0.6^{a,b,A}$	$8.3 \pm 1.1^{a,b,A}$
	14	$3077 \pm 159^{b,B}$	$32,518 \pm 459^{b,A}$	$27,414 \pm 1068^{b,C}$	$8.9 \pm 0.3^{b,A}$	$8.6 \pm 0.3^{b,A}$
	21	$4812 \pm 743^{b,A,C}$	$19,475 \pm 662^{b,B}$	$25,027 \pm 115^{b,C}$	$8.6 \pm 0.5^{c,A}$	$8.4 \pm 0.5^{b,A}$
	28	$3060 \pm 568^{b,C}$	$20,563 \pm 32^{b,B}$	$27,822 \pm 1817^{b,C}$	$7.5 \pm 0.1^{b,B}$	$8.0 \pm 0.6^{b,A}$
UST + Na	0	$692 \pm 38^{a,A}$	$2745 \pm 43^{a,A}$	$3458 \pm 59^{a,A}$	$8.1 \pm 0.3^{b,A}$	$7.9 \pm 0.5^{b,A}$
	7	$523 \pm 45^{a,B}$	$1287 \pm 58^{a,B}$	$2258 \pm 75^{a,B}$	$8.0 \pm 0.2^{b,A}$	$8.6 \pm 0.2^{b,A,B}$
	14	$478 \pm 14^{a,B}$	$1452 \pm 97^{a,B}$	$1554 \pm 09^{a,C}$	$7.1 \pm 0.3^{b,A,B}$	$9.2 \pm 0.2^{b,B}$
	21	$534 \pm 20^{a,B}$	$632 \pm 15^{a,C}$	$1609 \pm 188^{a,C}$	$6.4 \pm 0.6^{b,B}$	$8.2 \pm 0.6^{b,A,B}$
	28	$426 \pm 14^{a,B}$	$610 \pm 29^{a,C}$	$1554 \pm 16^{a,B,C}$	$6.5 \pm 0.4^{b,B}$	$8.2 \pm 0.6^{b,A,B}$

TPC total phenolic content, *DPPH* antiradical antioxidant activity, *FRAP* ferric-reducing antioxidant activity, *Bc* betacyanin content, *Bx* Betaxanthin content

*Different lowercase letters indicate differences between treatments, and different capital letters indicate differences between day 0, 7, 14, 21, and 28. Data expressed as means ± standard deviation

be attributed to the different composition of polyphenols in these samples being epigallocatechin gallate highly predominant in samples containing GTE, while in the other samples (without GTE), the pools of polyphenols and antioxidants are the natives from F&V of the smoothie. The stabilities of the different phenolic compounds and other antioxidants with parameters such as temperature, pH, light, and dissolved oxygen content depend on their chemical structure and will be different for each compound (Ananingsih et al., 2013; Friedman & Jürgens, 2017).

In concern to the effect of treatments on betalain content, in general, all samples treated with UST showed increases of around 55% in the Bc content and 80% in the Bx content, a phenomenon already observed in the exploration study presented previously (the "Exploration of Amplitude and Temperature Effects" section). On day 28, losses of around 20% in Bc were noted on control samples due to deterioration with time, similarly to previously observed when this smoothie was stored at 5 °C (Fernandez et al., 2019a). Similar losses over time were observed for UST, UST + Ni, and UST + Na treatments, while the sample containing tea (UST + GTE) presented greater stability over time with losses of only 8% at day 28. This result can be associated with the high antioxidant power of the tea polyphenols, which may have exerted a protective effect avoiding oxidative processes that lead to the deterioration of these compounds (Fu et al., 2020b; Herbach et al., 2006). They are known to have inhibitory effects on the main betalain deterioration enzymes polyphenoloxidase, peroxidase, and glucosidases (Herbach et al., 2006; Klimczak & Gliszczynska-Swigło, 2017). Nonetheless, considering the effects of UST on POD, greater stability of Bc was expected in samples with this treatment compared to control, which was not observed in this study. A possible explanation may be related to the reactive oxygen species that are generated during US treatment, like H_2O_2, whose participation as a catalyst in the degradation processes of betanin has been demonstrated (Herbach et al., 2006). Therefore, the beneficial and detrimental effects that are triggered because of treatment with US may, in this case, have been compensated, not observing significant differences in the stability of the pigment in comparison with the control samples. On the other hand, the Bx contents were more stable than Bc during storage, even with small increases being observed in some cases. Control samples showed a reduction of 3% on day 28 compared to the value obtained at day 0, while the other treatments showed less than 1% or no reductions. The deterioration of these compounds during storage depends on many factors, such as pH. In the case of this smoothie, whose value is 4.2, and according to what some authors report, betaxanthins should present very good stability, while betacyanins present greater stability at higher pHs (5–5.8) (Fu et al., 2020b; Herbach et al., 2006). Likewise, some studies suggest that Bc would be more susceptible to the action of PODs than Bx (Herbach et al., 2006). On the other hand, because of the degradation of betacyanins by hydrolysis of the aldimine bond, bethalamic acid may become available and, in the presence of amino compounds in the matrix, lead to the formation of betaxanthins (Herbach et al., 2006), which could explain the small increases observed in some cases.

Initial Effect of Treatments Against *Listeria innocua* and *Escherichia coli*

The initial counts of *L. innocua* and *E. coli* on the mixed fruit and vegetable smoothie with different treatments

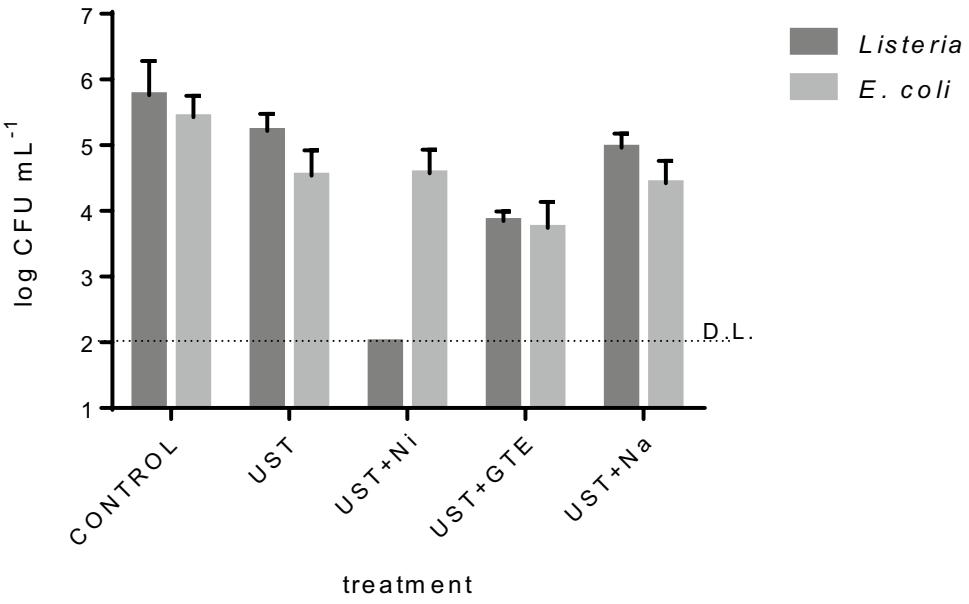

Fig. 5 Initial effect of US treatment and its combination with natural antimicrobials on *Listeria innocua* and *Escherichia coli* counts

are presented in Fig. 5. In the control samples, the initial *Listeria* count was 5.8 ± 0.5 log CFU mL^{-1}. Treatment with UST achieved a non-significant reduction of 0.5 log, while all the combined treatments were statistically different from the control with reductions of >4, 2, and 0.8 log for UST + Ni, UST + GTE, and UST + Na, respectively. It is noteworthy that the treatment with Ni obtained counts below the D.L. The initial count of *E. coli* in control samples was 5.4 ± 0.3 log CFU mL^{-1}. Treatment UST showed a significant reduction of 1 log, while UST + Ni and UST + Na presented similar results than UST. Hence, nisin and natamycin did not achieve an additional effect on *E. coli*, which was expected since, as previously mentioned, Gram-negative bacteria are not the main target of these antimicrobials. On the other hand, the combination of UST with GTE showed a 1.7 log reduction differing statistically from the other treatments. The effect of GTE on *E. coli* reduction was observed by other authors, using considerably higher concentrations than the one applied in the present work (Fernandez et al., 2017; Perumalla & Hettiarachchy, 2011). The proposed mechanism of action of GTE on Gram-negative bacteria involves damage to the cell wall, such as pore-like lesions, and further membrane degradation (Perumalla & Hettiarachchy, 2011), processes that can be accelerated or enhanced by US action.

To the best of our knowledge, there is no information on the effect of US treatment in combination with antimicrobials for *Listeria* or *E. coli* control on F&V mixed smoothies. Nonetheless, in the last years, interesting in vitro studies and some studies on vegetable products were presented and enhanced effects of the combination of these preservation methods were informed. In this sense, Dolan et al. (2018) determined in vitro efficacy of an ultrasound treatment (20 kHz, 43–45 W, 8 min) combined with zinc oxide 40 mM on reducing a 6 log CFU mL^{-1} *Listeria innocua* load. They observed that, while individual treatments caused < 1 log CFU mL^{-1} reduction, the combined treatment achieved > 5 log CFU mL^{-1} reduction. Wu and Narsimhan (2017) studying the in vitro deactivation of *L. monocytogenes* using 0.78 g L^{-1} of melittin (a naturally occurring antimicrobial peptide) or ultrasound (20 kHz, 40 W, 30 min), alone or in combination, observed < 1 log reduction for US alone, <2 log reduction for melittin alone, and a >4 log reduction when combined. Takundwa et al. (2021) found that a combination of 19.3 mg kg^{-1} nisin, 0.185% v/v oregano, and ultrasound (50 kHz, 600 W, 14.65 min) was the most effective treatment for *E. coli* and *L. monocytogenes* control on lettuce, showing reductions of 3.43 and 9.20 log CFU mL^{-1}, respectively, while the lowest and middle concentrations of the tested factors could not significantly reduce the microbial counts. Similarly, He et al. (2021) found synergistic effects when combining ultrasound (20 kHz, 167 W/L, 9 min) with thyme essential oil nanoemulsion (0.0625 g L^{-1}) which reduced the *E. coli* populations by 5.43 log CFU mL^{-1} on the surface of cherry tomatoes, while individual treatments achieved 1 log and 3 log reductions, respectively.

Although the reductions obtained in this work were not as high as the those obtained in several of the aforementioned studies, we consider that there is great potential in the combination of ultrasound with all nisin, natamycin, and GTE, so it would be interesting in future studies to test other concentrations and combinations to improve their effectiveness.

Conclusions

In the present work, the effects of ultrasound treatment on the main spoilage-causing factors and some quality indicators of a mixed fruit and vegetable smoothie were evaluated. It was found that temperature control is needed, to avoid high temperatures during treatment, in which nutrients and organoleptic degradation can occur. Moreover, time of treatment lower than 15 min was required to avoid PPO activation. Treatment with 70% of amplitude during 4 min presented a great reduction of POD (71.6%) and showed well performance on microbial control, compared with the other evaluated treatments. Then, evaluation of shelf life and stability of the smoothies when treated with ultrasound-selected treatment (UST: 70% + 4 min) alone or combined with the natural antimicrobials green tea extract (GTE), nisin (Ni), or natamycin (Na) was evaluated. The UST + GTE treatment extended the shelf life by 1 week (as well as UST treatment), increased the extractability and stability of Bc and Bx, and greatly increased the TPC and the antioxidant capacity of the product. Additionally, this treatment achieved 0.8 log and 1.7 log reductions in *Listeria* and *E. coli* counts when a 6 log CFU mL^{-1} contamination was simulated. The treatment UST + Na and UST + Ni extended the shelf life at least 2 weeks, while UST + Na achieved reductions between 0.8 and 1 log of the contaminant microorganisms, and UST + Ni achieved reductions > 4 log (to values below the D.L.) for *Listeria* and of 1 log for *E. coli*. Nutritionally, the effect of UST + Na and UST + Ni was similar to those obtained with UST, observing an increase in the extractability of Bc and Bx, while the rest of the evaluated indicators were not affected. Indeed, the potential of combining ultrasound technology with antimicrobials for the preservation of the smoothie was probed, and future studies combining other intensities or antimicrobial doses can be promisingly projected.

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Data Availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Competing Interests The authors declare no competing interests.

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