Effect of THERMAL TREATMENT, MICROWAVE, and PULSED ELECTRIC FIELD processing on the antimicrobial potential of Açai (*Euterpe oleracea*), Stevia (*Stevia rebaudiana* Bertoni), and Ginseng (*Panax quinquefolius* L.) extracts

Maria Consuelo Pina-Pérez¹, Alejandro Rivas², Antonio Martínez² and Dolores Rodrigo^{2*}

¹Institute of Life Technologies, HES-SO Valais-Wallis, Route du Rawyl 47, CP 1950, Sion, Switzerland.

²Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Avda. Agustín Escardino, 7, 46980 Paterna (Valencia), SPAIN

*Corresponding author: lolesra@iata.csic.es

Phone: (+34) 96 390 00 22; Fax: (+34) 96 363 63 01

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Abstract

The antimicrobial potential of three functional ingredients, açai (*Euterpe oleracea*), stevia (*Stevia rebaudiana* Bertoni), and ginseng (*Panax quinquefolius* L.), was assessed against sporeforming/non-sporeforming foodborne pathogens before and after processing. *In vitro* inhibitory effect of tested functional ingredients was significantly affected by the extract concentration (1–10 % (w/v)) and the technology applied (microwave treatment (MW) (900 W-30 s), conventional thermal treatment (CT) (90 °C, 60 s) and non-thermal pulsed electric fields (PEF) (20 kV/cm, 270 µs)). Ginseng was the most stable ingredient with regard to CT, MW, and PEF application, maintaining or enhancing its antimicrobial capability after processing. The highest inhibition zone diameter of those obtained in the present study (15.11±0.11 mm) is reflecting the promising potential of MW treated ginseng extracts (10 % (w/v)) as antimicrobials against *E. coli* O157:H7. The PEF process significantly (p-value < 0.05) improved the antimicrobial bioactivity of stevia and açai against Gram (+) and Gram (–) bacteria even applied at the lowest concentration level (1 % (w/v)). New validation studies are required to assess the antimicrobial potential and the stability of other functional properties attributed to these ingredients to be integrated in novel formulated matrices processed by conventional and emerging food preservation technologies.

1. Introduction

Consumer demand for safe functional foods has increased in recent years. Among the most important groups of functional vegetable products with bioactive compounds that contribute to human health improvement, special interest is focused on three of them, (i) the so-called “superfruits” (Dastmalchi et al., 2012), mainly with effective anti-aging and antioxidant properties; (ii) medicinal plants (Lemus-Mondaca et al., 2012), mainly with anti-inflammatory, anticarcinogenic, and cardiovascular disease prevention properties, and (iii) recently known plants or extracts with cognitive improvement potential such as propolis, ginkgo biloba, lecithin, omega-3-fatty acids, isoflavones, cocoa, and ginseng (Field et al., 2011; Sokolov et al., 2013). These natural products and their bioactive compounds (including polyphenols, steviosides, ginsenosides, heterosides) are being used as ingredients in the formulation of innovative beverages, ready-to-eat foods, meals, and pharmaceutical supplements (Marete et al., 2011; Pina-Pérez et al., 2013; Tárrega et al., 2012).

Among “superfruits”, berries (blackberry, blueberry, açai), grapes, or pomegranate are attracting the interest of food industry due to their associated beneficial compounds, including vitamins, fatty acids, minerals, and mainly, antioxidants. *Euterpe oleraceae* Mart., known as açai, an Amazonian palm berry, has been become specifically interesting for the food scientists and food processors (Schauss, 2016; Seeram et al., 2008). This small black berry boasts high polyphenol content and an omega-6 to omega-9 ratio comparable to olive oil (Pacheco-Palencia et al., 2008). In addition, its anthocyanin content is up to 33 times higher than that of red wine. So, in this context, and due to the increasing market that is being opened around the açai anti-aging and beauty enhancing properties (Marcason, 2009), this berry is hailed as a superfood with potential applicability in human fed and pharmacological innovations.

Stevia rebaudiana Bertonni and its steviosides has been used since ancient times for various medicinal purposes throughout the world (anti-hyperglycemic, antihypertensive, anti-inflammatory, anti-tumour, anti-diarrhoeal, diuretic, and immunomodulatory effects)

(Chatsudthipong & Muanprasat, 2009; Goyal et al., 2010). This branched bushy shrub of the Asteraceae family is the source of a number of sweet ent-kaurene diterpenoid glycosides (Prakash et al., 2008), responsible for its sweet taste, the main characteristic of Stevia leaves that is promoting is recently used e.g. in the formulation of a-caloric sweet beverages. Pól et al., 2007).

Nowadays special attention should be paid in the known as “new century diseases”, specifically in which are evolving brain and central nervous system disorders (depression, anxiety, hyperactivity, attention deficit disorders) that require innovative and non-aggressive solutions.

The consumption of supplements to improve neural and cognitive functions is reaching very high levels nowadays (Gomez-Pinilla and Gómez, 2012). Ginseng (*Panax quinquefolius* L.) is the product most frequently indicated by pharmacists for problems of “memory loss” and “absentmindedness” (Leung & Thuret, 2015). The major active constituents of the *Panax* genus are thought to be triterpenoid glycosides or saponins, also known as ginsenosides.

Taking into account the emergence of resistant bacterial strains in recent years, the search for alternative antimicrobial sources from natural origin is being boosted by the public health organizations and scientific researchers worldwide (WHO, 2017). To be at the forefront in the R&D of new healthier products, phytochemicals from natural extracts, and directly the integration of specific ingredients with proved bioactive properties are being tested regarding their antimicrobial capability as additional control strategies in food preservation, in order to develop safe and nutritionally valuable products (Gyawali and Ibrahim, 2014; Sanz-Puig et al., 2016; Pina-Pérez et al., 2017). In spite of this, to date, only a few research works have expanded the possibilities of using açai, ginseng, and stevia as food preservatives (Belda-Galbís et al., 2015; Lee et al., 2013).

To our knowledge, little information has been reported about the possible antimicrobial effects produced by the combination of any of these ingredients and other preservation technologies (Belda-Galbis, et al., 2016; Rivas et al., 2016). The application of novel non-thermal technologies is improving the nutritional and sensory profiles of final processed products compared to thermally processed commodities (Barba et al., 2017). Additionally, these technologies have demonstrated a great potential for increasing the bioactive compounds extracted from plant cells, consequently enhancing the functional potential of novel formulated matrices. However, the stability of these compounds with regard to maintaining their bioactivity before and after processing is of great concern to the scientific community and manufacturers (Barba et al., 2014; Carbonell-Capella et al., 2016; Criado et al., 2014; Jacobo-Velazquez et al., 2012; Oms-Oliu et al., 2012; Santhirasegaram et al., 2015). To scale up the introduction of these ingredients in complex food matrices important questions should be answered, including *“how could traditional and novel food processes affect the functional and technological properties of these ingredients?”*.

In this context the main objectives of the present study are (i) to determine the antimicrobial potential of açai, stevia, and ginseng, individually, at various concentrations, [1–10] % (w/v), against sporeforming/non-sporeforming foodborne pathogens; and (ii) to provide an overview regarding the extent to which thermal and non-thermal technologies could affect, and enhance or reduce, the antimicrobial effectiveness of these three functional ingredients.

2. Material and Methods

2.1. Non-sporeforming bacterial cultures

Pure cultures of *Listeria monocytogenes* (CECT 4032), *Salmonella* Typhimurium (CECT 443), *Escherichia coli* O157:H7 (CECT 5947), and *Cronobacter sakazakii* (CECT 858) were provided freeze-dried by the Spanish Type Culture Collection (CECT). Tryptic soy broth (TSB) (Scharlab

Chemie, Barcelona, Spain) was used for *E. coli* O157:H7, *S. Typhimurium*, *C. sakazakii*, and *L. monocytogenes* rehydration. After 20 minutes, the rehydrated culture was transferred to 500 mL of TSB, and incubated at 37 °C for the microorganisms studied, with continuous shaking at 200 rpm for 14 hours, to obtain cells in a stationary growth stage. Growth curves were obtained by both optical density and plate count. The cells were centrifuged twice at 4000 x g at 4 °C for 15 minutes and then resuspended in TSB. After the second centrifugation, the cells were resuspended in 20 mL of TSB, with 20 % glycerol, and then dispensed in 2 mL vials to a final concentration of 10¹¹ colony-forming units per milliliter (CFU/mL). The 2 mL samples were immediately frozen and stored at -80 °C until needed for the assays.

2.2. Sporeforming bacterial cultures

Freeze-dried *Bacillus circulans* (CECT 10T) and *Bacillus cereus* (CECT 131) cultures were provided by the Spanish Type Culture Collection and were activated overnight in nutrient broth (NB) (Scharlab Chemie, Barcelona, Spain) at 37 °C. After that, the strains were streaked on nutrient agar (NA) (Scharlab Chemie, Barcelona, Spain), followed by incubation at 37 °C for 12 h. Strains were activated overnight aerobically in Brain Heart Infusion Broth (BHIB) (Scharlab Chemie, Barcelona, Spain) at 30 °C. Spore suspensions of the strains were prepared by plating 0.1 mL from fresh overnight cultures on BHI agar (BHIA) (Scharlab, S.A., Barcelona, Spain) additionally supplemented with 10 mg of MnSO₄·H₂O unless otherwise stated. The agar plates were incubated until 90 to 99 % of the cells contained phase-bright spores, as determined by phase-contrast microscopy. Spores were collected from the plates by flooding the surface of the plate with 10 mL of cold sterile distilled water. After harvesting, the spore suspensions were washed four times by centrifugation at 3000 x g for 15 min at 5 °C, resuspended in sterile distilled water (10¹⁰ CFU/mL), and stored at 5 °C until used.

2.3. Preparation of aqueous plant suspensions

Stevia rebaudiana Bertoni dried leaves and commercial powders of açai and ginseng were used in the present study. Stevia dried leaves were provided by ANAGALIDE, S.A., (Huesca, Spain) and were macerated separately with 100 mL of sterile distilled water at boiling temperature for 30 min (Criado et al. 2014). The macerate was first filtered through muslin cloth at room temperature. Stevia infusion was obtained from the supernatant filtered through Whatman No. 1 filter paper. Suspensions of 1, 5, and 10 % (v/v) were prepared from the infusion obtained and used as potential antimicrobial substrates.

Açai powder (Nature's Way Products Inc., Green Bay, USA), standardized with a 10 % polyphenols content, and Ginseng powder (Natur Lider Import Export, S.L., Ciudad Real, Spain), standardized at 10 % of ginsenosides, were used in the present study. Açai and ginseng were added at concentrations of 1, 5, and 10 % (w/v) to sterile distilled water to obtain matrix substrates. All matrices were prepared just before their use in the *in vitro* antimicrobial assays.

2.4. Thermal and non-thermal technologies applied

Thermal technologies, conventional (Conventional Thermal treatment, CT) and microwave (MW), and a non-thermal technology, pulsed electric fields technology (PEF), were applied at fixed conditions (see Table 1) to prepared vegetable suspensions. Treatment conditions considered for each of the technologies applied correspond to slightly intensive pasteurization treatments (Benlloch-Tinoco et al., 2014, 2015; Mohideen, 2011; Pina-Pérez et al., 2013).

A household microwave oven (3038GC, Norm, China) was used to treat the substrates studied. For each treatment, a 200 mL sample was tempered to an initial temperature of 25 °C and then heated in the microwave oven in a standard-size glass beaker (BKL3-1K0-006O, Labbox, Spain). Treated samples were immediately cooled in ice-water to 25 °C.

Conventional thermal treatment (CT) was applied in a thermostatic water bath (Precistern, Selecta, Spain). Each natural suspension was placed in TDT stainless steel tubes (13 mm inner diameter and 15 cm length) and closed with a screw stopper. A thermocouple that was connected to a data logger was inserted through the sealed screw top in order to record the time-temperature history of the sample during treatment. Three replicates were carried out to define the average temperature profile of the process. Previously, the samples were preheated to 25 °C to shorten and standardize the come-up time.

For non-thermal treatment, an OSU-4D bench-scale continuous PEF system, designed at Ohio State University (Columbus, OH 43210-1007, USA), was used to treat the samples. Eight co-field treatment chambers with a diameter of 0.23 cm and gap distance of 0.293 cm were connected in series. The pre- and post-treatment temperatures at the inlet and outlet of each pair of treatment chambers were monitored by type T thermocouples located at the entrance and exit chambers. All treatment chambers were kept at 20 °C for all treatment intensities, with thermo-regulation via a heat exchanger, which could be regulated to the desired working temperature. The pulse width was fixed at 2.5 µs. Pulse wave form, voltage, and current in the treatment chambers were recorded with a digital oscilloscope (Tektronix TDS 210, Tektronix Inc., OR, USA). The PEF treatments remained below a temperature of 27±2 °C in all cases.

2.5. Determination of antimicrobial activity

The antimicrobial assay of the three natural products against foodborne pathogens was carried out by means of the agar diffusion method on Müller Hinton Agar (MHA) (Scharlab, S.A., Barcelona, Spain). Bacterial 100 µL inoculums of 10⁵ and 10⁷ CFU/mL were spread on the MHA plates with a sterile swab. The agar plates were allowed to dry for 15 min before use in the assay. Sterile filter paper disks (7 mm in diameter) were arranged in the plates (4 disks per plate) and then impregnated with 50 µL of the treated/untreated vegetable extracts, at various

concentrations, [1–10] % (w/v). The extracts were substituted by buffered peptone water (Scharlab, S.A., Barcelona, Spain) as negative controls. The plates were labeled and incubated overnight at 37 °C. Finally the microbial growth was determined by measuring the diameter of the inhibition zone including the filter disks. The inhibition zone was measured in (mm) with a slide gauge and the results were recorded (± 0.01). Studies were carried out in triplicate and the average and standard deviation of the three values were calculated. An ANOVA analysis was carried out using STATGRAPHICS Centurion XV (version 15.1.03; STATGRAPHICS, Warrenton, VA) to test significant differences in the antimicrobial potential of the extracts studied depending on the conditions studied.

3. Results and Discussion

3.1. *In vitro* antimicrobial effectiveness of AÇAÍ, STEVIA, and GINSENG natural extracts against sporeforming/non-sporeforming bacteria

The effectiveness of natural açai, stevia, and ginseng extracts against selected sporeforming and non-sporeforming bacteria was tested *in vitro* at different concentrations in the range [1–10] % (w/v) (see Table 2). Açai added at concentration levels in the range [5–10] % (w/v) was effective in inhibiting the growth of Gram (+) and Gram (–) 10^7 CFU/mL bacterial populations, showing a maximum inhibition zone of 11.94 ± 0.26 mm against *B. circulans* (10^5 CFU/mL). Açai showed the most widely inhibitory potential, being effective against all the bacteria studied with the exception of *E. coli* O157:H7.

To our knowledge, no previous research work has been conducted regarding the antimicrobial effect of açai against a wide variety of vegetative and sporeforming food-related microorganisms. The effectiveness of açai against Gram (+) bacteria, with a minimum inhibitory concentration close to 10 mg/mL, is the most reported antimicrobial property of this “superfruit”, with the research works of Belda-Galbis et al. (2015) and Espitia et al. (2014)

about the effectiveness against *Listeria innocua* and *Listeria monocytogenes*, respectively, being among the most relevant ones. The antimicrobial effect of açai has been attributed to its high content of polyphenols (Biswas et al., 2012; Ellong et al., 2015; Kang et al., 2011), including anthocyanins. The fact that Gram-positive bacteria are more sensitive than Gram-negative bacteria to the effect of plant polyphenols could be explained by the differences in cell wall structure in the two groups (Cetin-Karaca, 2011). The mechanism of polyphenol antimicrobial capacity is mediated by cellular enzyme inactivation and membrane permeability changes that can hardly affect Gram-positive bacterial structures (Lambert, 2002). In spite of this, few data on the chemical composition of açai are available at present, and more research studies are required linking bioactive compounds present in this fruit and the effective antimicrobial potential that it exerts.

Previous research studies (Ferrer et al., 2009) based on the antimicrobial potential of olive powder, a natural ingredient rich in polyphenols, support the results obtained regarding the capability of açai to inhibit growth of sporeforming bacteria. The specific effect of olive powder in reducing *Bacillus cereus* spore growth was observed by Ferrer et al. (2009). The results revealed that the bacteriostatic effect observed depended on the olive powder concentration ([0–5] % (w/v)) and the incubation temperature applied [7–30] °C. In the present study, the observed antimicrobial effect of açai powder against *Bacillus* spp. spores was also dependent on the concentration. Additionally, several reports have also indicated the high effectiveness of polyphenols and oleuropein from olives in inhibiting the germination of *Bacillus cereus* T spores (Sakanaka et al., 2000; Tassou et al., 1991). According to the studies of Sakanaka et al. (2000), *B. stearothermophilus* growth and survival capability were also restricted by exposure to tea polyphenols.

Ginseng extract showed growth inhibitory potential at all of the concentration levels studied, [1–10] % (w/v), but was only effective against vegetative Gram (–) bacteria. Ginseng extract at 10 % (w/v) was able to inhibit *E. coli* O157:H7 growth (10^5 – 10^7 CFU/mL), providing the largest

inhibition halo of those obtained in the present study (14.11 ± 0.12 mm) in unprocessed functional substrates.

The lowest antimicrobial potential can be attributed to stevia, with a visible inhibition area close to 9 mm when it was applied at 10 % (w/v) against *C. sakazakii*, *S. Typhimurium*, and *L. monocytogenes* inoculated at a level of 10^5 CFU/mL. Furthermore, stevia did not exert any inhibitory capability against 10^7 CFU/mL inoculums of the food-related bacteria studied.

An ANOVA analysis was carried out to determine the extent to which (significant or not) the bacterial inhibition response depended on (i) the plant extract, (ii) the concentration at which it was applied, (iii) the bacterial strain considered, and (iv) the bacterial inoculum. The ANOVA results revealed that the bacterial inhibitory potential of açai and ginseng was significantly (p -value ≤ 0.05) dependent on the concentration level at which the extract was applied, between 1 % (w/v) and [5–10] % (w/v), and was not influenced by the initial inoculum value (10^5 CFU/mL or 10^7 CFU/mL). Similarly, the effectiveness of the plant ingredients in inhibiting bacterial growth was significantly dependent ($p \leq 0.05$) on the bacterial strain considered, with Gram (–) bacteria being generally more sensitive to ginseng than Gram (+), whereas the opposite was true with exposure to açai.

Although many studies have demonstrated the effect that conventional and novel technologies have on quality and nutritional factors inherent in various functional ingredients (Albarici & Pessoa, 2012; Ellong et al., 2015; Patras et al., 2010), little information is available regarding the impact that thermal and non-thermal processes have on the functionality exerted by stevia, açai, and ginseng ingredients incorporated in innovative formulated products. The impact of processing methods on the antimicrobial potential of these natural plant extracts is described in the following section.

3.2. *In vitro* antimicrobial effectiveness of AÇAÍ, STEVIA, and GINSENG processed by thermal and non-thermal technologies against sporeforming/non-sporeforming bacteria

Table 3 shows the antimicrobial results obtained for thermally (heat and MW) and non-thermally (PEF) processed plant ingredients. Açaí antimicrobial capability was specifically affected by thermal treatment at 90 °C, 60 s. Under these treatment conditions, only the 10 % (w/v) açaí concentration was effective in inhibiting bacterial growth of *L. monocytogenes*, *C. sakazakii*, and *S. Typhimurium*, significantly reducing the inhibition zone diameter against *L. monocytogenes* (8.5 ± 0.15 mm) in relation to the diameter obtained with the unprocessed açaí suspension. This lost antimicrobial capability is probably associated with degradation of the phytochemicals contained in the raw plant material, such as anthocyanin, ascorbic acid, and carotenoids (Chen et al., 2013; Esteve & Frigola, 2008). According to the studies of Patras et al. (2010), thermal treatment at 70 °C, 2 min, significantly affected loss of blackberry and strawberry anthocyanins, at rates close to 30–80 %, and heat-labile factors such as endogenous enzymes also accelerate anthocyanin degradation during heat processing (Albarici & Pessoa, 2012).

No significant reduction in stevia inhibitory potential (p -value > 0.05) was observed after the thermal process (CT 90 °C, 60 s) applied. Ginseng showed the highest antimicrobial potential among the thermally processed ingredients, maintaining its inhibiting capability against Gram (–) bacteria, *C. sakazakii*, *E. coli* O157:H7, and *S. Typhimurium* in the concentration range [1–10] % (w/v). No significant differences in the diameter of the inhibition zones were observed between the application of thermally processed ginseng and unprocessed extract. A recent study has confirmed the antimicrobial potential of heat treated (130°C for 3 h) ginseng extracts (HTG) (natural ginsenosides converted to low-polarity ginsenosides by deglycosylation at high temperature) against *Fusobacterium nucleatum*, *Clostridium perfringens*, and *Porphyromonas*

gingivalis bacteria indicating that for these ginsenoside-enriched fractions a dose – dependent antimicrobial potential was exerted (HTG MIC = 1.25 µg/mL)

Under MW processing, açai lost its antimicrobial capability against *S. Typhimurium*. On the other hand, its inhibition capability against *C. sakazakii* was enhanced at a concentration level of 10 % (w/v). No differences were observed in the antimicrobial potential of MW-processed/-unprocessed açai against *L. monocytogenes* in the [5–10] % (w/v) concentration range. MW processing of stevia significantly enhanced the antimicrobial potential of this extract, which was effective against Gram (–) bacteria at all of the concentrations studied, [1–10] % (w/v). It is noteworthy that, under the conditions studied, MW-processed stevia exerted antimicrobial properties against *E. coli* O157:H7, whereas the unprocessed extract lacked this property. The MW-processed ginseng was the most effective ingredient against *E. coli* O157:H7, with the largest inhibition zone (15.11±0.11 mm), at 10 %, (w/v), of those obtained in the present study.

After the application of PEF technology (20 kV/cm, 270 µs), the inhibition capability of açai, ginseng, and stevia increased against all the bacteria studied. PEF-treated extract of açai was effective in inhibiting *L. monocytogenes* and *C. sakazakii* in the range 5–10 % (w/v). *E. coli* O157:H7 and *S. Typhimurium* were inhibited by PEF-treated açai extracts, even at a 1 % (w/v) concentration. Sporeforming bacteria were sensitive to PEF-treated açai extract at all the concentrations studied, and the higher the PEF-treated açai concentration, the higher the inhibition zone observed.

PEF-processed stevia was the extract most affected by non-thermal processing, and the antimicrobial potential of this plant ingredient was significantly enhanced at all the concentrations applied, [1–10] % (w/v). The processed stevia was slightly effective against all the bacterial strains, even at the lowest concentration studied. No significant differences were

observed between the antimicrobial potential of raw ginseng and the PEF-processed ginseng extracts in the range [1–10] %.

Several studies have suggested that PEF technology has a great capability for extracting bioactive compounds with high efficiency rates (Barba et al., 2015; Carbonell-Capella et al., 2017a; Kannan, 2011; Kadam et al., 2013). Ginsenosides and polyphenols (Buniowska et al., 2016) have been successfully extracted by means of PEF technology. Taking into account that both polyphenols in açai (Gonçalves et al., 2010) and ginsenosides in ginseng (Ludwiczuk et al., 2006) have been reported to be the major antimicrobial compounds, it seems reasonable to consider that the high levels of these compounds extracted by PEF (Vallverdú-Queralt et al., 2012) enrich the processed matrices.

With regard to stevia, little information has been published that can explain how steviosides are extracted or affected by MW and PEF technologies (Buniowska et al., 2016; Carbonell-Capella et al., 2017b; Rivas et al., 2016). According to the studies of Buniowska et al. (2016), the application of PEF caused a significant increase in the extraction of all steviol glycosides (rebaudioside A, stevioside, and rebaudiosides F and C). According to the studies of Carbonell-Capella et al. (2017b) PEF applied at 20kV/cm – 10 µs treatment conditions close to used ones in the present study was the most efficient technology in the the extraction of stevioside (44.260.1 mg/g) and rebaudioside A (22.460.3 mg/g) from stevia leaves compared to MW or ultrasounds treatments. Additionally, and according to the studies of Rivas et al. (2016), a synergistic effect was observed against *L. monocytogenes* by means of the combined effect of stevia and PEF in a beverage mixture of oat milk and fruit juices. In the present study, PEF processing of stevia enhanced the antimicrobial potential of this ingredient at 10 % (w/v) against *L. monocytogenes* by means of a 70 % increase in the inhibition zone in comparison with the inhibition obtained with unprocessed stevia.

4. Conclusions

This study reveals the antimicrobial potential of functional ingredients to be added in new formulated products processed by CT, MW and PEF technologies. Although the results included in the present study point out the sensitiveness of bioactive açai potential after CT (90 °C, 60 s), PEF and MW technologies are enhancing the antimicrobial capability of studied ingredients against both vegetative and sporulated microorganisms. Meanwhile ginseng antimicrobial potential remained stable after PEF and CT treatments, MW processing of ginseng extracts promisingly improved its inhibitory growth result against *E. coli* O157:H7, achieving the maximum inhibitory halo among the obtained ones in the present study. After MW processing, the antimicrobial capability of all the plant extracts studied increased showing all of them inhibitory potential even at 1 % (w/v) concentration level. Among the processes studied, under PEF treatment açai and stevia exerted the most effective antimicrobial capability, even against sporeforming microorganisms. New lines of research should be opened regarding validation of the antimicrobial potential of these ingredients (processed/not processed by conventional and novel technologies) when integrated in real food matrices.

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Conflict of Interest

No conflict of interest declared.

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Table 1. Thermal and non-thermal treatments applied to Açaí (*Euterpe oleracea* M.), Stevia (*Stevia rebaudiana* Bertoni), and Ginseng (*Panax quinquefolius* L.) suspensions in sterile distilled water at concentrations in the range [1–10] % (w/v).

	Thermal technologies		Non-thermal technologies
	CT	MW	PEFs
Treatment conditions	90 °C – 60 s	900 W – 30 s	20 kV/cm – 270 µs

CT: conventional thermal treatment; MW: microwave treatment; PEFs: Pulsed electric fields.

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662 **Table 2.** *In vitro* antimicrobial effectiveness of açai, stevia, and ginseng extracts at concentration levels of [1–10] % (w/v) against non-sporeforming and
663 sporeforming bacteria. Inoculum levels studied: 10⁷ CFU/mL and 10⁵ CFU/mL.

Bacterial Inoculum				10 ⁵ CFU/mL					
Açaí				Stevia			Ginseng		
Concentration %	1	5	10	1	5	10	1	5	10
(w/v)									
Non-sporeforming									
<i>L. monocytogenes</i>	n.d.	9.22±0.55	10.92±0.15	n.d.	n.d.	7.84±0.52	n.d.	n.d.	n.d.
<i>C. sakazakii</i>	n.d.	n.d	11.26±0.15	n.d.	n.d.	8.11±0.23	9.51±0.52	9.01±0.81	11.64±0.12
<i>E. coli</i> O157:H7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	9.83±0.95	12.46±0.41	14.11±0.12
<i>S. Typhimurium</i>	n.d.	n.d.	8.46±0.05	n.d.	n.d.	7.74±0.21	8.65±0.23	8.88±0.21	9.67±0.36
Sporeforming									
<i>B. cereus</i>	n.d.	9.08±0.07	10.41±0.37	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<i>B. circulans</i>	n.d.	10.35±0.07	11.94±0.26	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Bacterial Inoculum				10 ⁷ CFU/mL					
Açaí				Stevia			Ginseng		
Concentration %	1	5	10	1	5	10	1	5	10
(w/v)									
Non-sporeforming									
<i>L. monocytogenes</i>	n.d.	8.12±0.25	9.25±0.13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<i>C. sakazakii</i>	n.d.	n.d.	9.59±0.11	n.d.	n.d.	n.d.	9.23±0.31	9.40±0.24	11.21±0.05

<i>E. coli</i> O157:H7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	9.60±0.34	11.36±0.11	13.81±0.22
<i>S. Typhimurium</i>	n.d.	n.d.	8.12±0.10	n.d.	n.d.	n.d.	8.15±0.09	8.32±0.27	9.18±0.11
<i>Sporeforming</i>									
<i>B. cereus</i>	n.d.	8.27±0.10	9.12±0.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<i>B. circulans</i>	n.d.	9.15±0.11	10.33±0.22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

664 n.d.: no inhibition detected.

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676 **Table 3.** *In vitro* assessment of the antimicrobial potential of açai, stevia, and ginseng extracts, [1–10] % (w/v), processed by thermal (conventional and MW)
677 and non-thermal technologies, against 10⁵ CFU/mL inoculums of Gram (+) and Gram (–) bacteria.

Processed substrates	Açaí			Stevia			Ginseng		
Concentration %(w/v)	1	5	10	1	5	10	1	5	10
<i>CT 90 °C-60 s</i>									
<i>Non-sporeforming</i>									
<i>L. monocytogenes</i>	n.d.	n.d.	8.51±0.15	n.d.	n.d.	7.53±0.08	n.d.	n.d.	n.d.
<i>C. sakazakii</i>	n.d.	n.d.	10.78±0.10	n.d.	n.d.	8.24±0.13	9.11±0.12	9.23±0.10	11.45±0.12
<i>E. coli</i> O157:H7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	9.45±0.22	11.36±0.12	13.22±0.14
<i>S. Typhimurium</i>	n.d.	n.d.	8.12±0.09	n.d.	n.d.	7.66±0.10	8.24±0.22	8.67±0.45	9.34±0.24
<i>Sporeforming</i>									
<i>B. cereus</i>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<i>B. circulans</i>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<i>MW 900 W-30 s</i>									
<i>Non-sporeforming</i>									
<i>L. monocytogenes</i>	n.d.	9.11±0.12	10.45±0.13	n.d.	n.d.	7.89±0.22	n.d.	n.d.	n.d.
<i>C. sakazakii</i>	n.d.	n.d.	12.36±0.12	7.34±0.33	8.56±0.12	9.12±0.21	9.76±0.15	9.88±0.11	12.56±0.34

<i>E. coli</i> O157:H7	n.d.	n.d.	n.d.	7.13±0.11	7.56±0.23	8.45±0.23	10.34±0.12	13.45±0.23	15.11±0.11
<i>S. Typhimurium</i>	n.d.	n.d.	n.d.	7.35±0.12	7.45±0.21	8.67±0.15	8.45±0.12	9.16±0.11	10.78±0.23
<i>Sporeforming</i>									
<i>B. cereus</i>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<i>B. circulans</i>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<hr/> <i>PEF 20 kV/cm-270 µs</i>									
<i>Non-sporeforming</i>									
<i>L. monocytogenes</i>	n.d.	9.78±0.11	12.89±0.22	8.25±0.33	9.15±0.21	12.11±0.13	n.d.	n.d.	n.d.
<i>C. sakazakii</i>	n.d.	9.45±0.09	12.33±0.07	8.11±0.23	8.78±0.23	9.45±0.06	9.89±0.14	10.45±0.12	12.33±0.09
<i>E. coli</i> O157:H7	8.15±0.23	8.78±0.08	11.56±0.08	8.09±0.11	8.67±0.08	10.15±0.22	9.75±0.23	9.88±0.11	12.38±0.05
<i>S. Typhimurium</i>	8.23±0.15	8.65±0.09	10.56±0.07	7.71±0.14	7.91±0.06	8.39±0.11	9.12±0.09	9.76±0.07	11.34±0.05
<i>Sporeforming</i>									
<i>B. cereus</i>	8.56±0.22	9.36±0.08	10.12±0.05	7.23±0.06	7.75±0.11	7.95±0.04	n.d.	n.d.	n.d.
<i>B. circulans</i>	9.10±0.23	9.56±0.05	10.45±0.07	8.25±0.09	9.76±0.15	10.34±0.06	n.d.	n.d.	n.d.
<hr/> n.d.: no inhibition detected.									