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Enrichment and preservation of a vegetable smoothie with an antioxidant and antimicrobial extract obtained from beet by-products

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Abstract

This study aims to assess beet leaves extracts (BLE) potential for fruit and vegetable smoothie enrichment and/or preservation. BLE presented a total phenolic content (TPC: 132.43 ± 1.51 g kg^{-1} dry weight (DW) basis) and an antioxidant capacity (219.2 ± 23.4 mmol kg^{-1} DW for FRAP and 28.9 ± 0.5 mmol kg^{-1} DW for DPPH). An antimicrobial activity assay proved BLE effectiveness against *Listeria innocua*, *E. coli* and *Saccharomyces cerevisiae* on systems of pH 3.7 and 7, with initial counts of 4 or 7 log CFU/mL. Incorporation of the BLE into a fruit and vegetable (F&V) smoothie containing orange juice (59%), apples (15%), carrots (15%), BL (6%) and beet stems (5%), significantly increased (50%) TPC of the product as well as their antioxidant capacity. Additionally, extract-enriched smoothies achieved reductions between of 1-3 log cycles in smoothie´s native microflora allowing to obtain a one-week shelf-life extension, and presented greater nutritional retention during storage at 5 ºC. Therefore, BLE has great potential to be used for the enrichment and preservation of smoothies, bringing great advantages for food industry, consumers and producers.

Keywords: Natural antimicrobials, waste valorization, sustainable food production, value-adding, bioactives.

1. Introduction

In recent years, global efforts are being made to diminish food waste as a means of sustainable management, and to achieve an increase in profit for local and global economies. Research on waste valorization practices and recovery of agro-industrial by-products are of great importance to achieve this purposes (Kowalska, Czajkowska, Cichowska, & Lenart, 2017). The commonly used alternatives include its use as livestock feed or as an organic soil amendment or alternative growing substrate to peat (Llorach et al., 2003). More recently, the potential of these by-products as alternative sources of energy has been explored: biogas, bioethanol or biohydrogen (Domínguez-Bocanegra et al., 2015, Jaiganesh et al., 2014). Moreover, growing evidence that vegetable by-products are promising sources of high-value compounds such as fibers, antioxidants, essential fatty acids, antimicrobials, among others, led to the exploration of ways to extract and use them as healthy functional ingredients in food products (Amofa-diatuo, Anang, Barba, & Tiwari, 2017; Ba et al., 2016; Lai et al., 2017), with encouraging results. In this sense, the elaboration of foods with the addition of natural extracts containing phytochemicals of high biological value such as polyphenols and betalains, obtained from underutilized natural resources, is an excellent alternative to increase their nutritional quality (Galanakis, 2013) and add value to underutilized resources. Through this application, both the need for higher sustainability of the productive system (Veneziani et al., 2017; da Silva, Barreira & Oliveira, 2016) as well as growing consumer´s demand for healthier foods are meted.

In Argentina, beet leaves (BL) constitute a by-product that is not exploited and is discarded as waste (Fernandez, Agüero, & Jagus, 2017a). In recent studies, these authors have demonstrated that BL are a rich source of nutrients, phytochemicals and fiber presenting a high antioxidant capacity (Fernandez et al., 2017a). Furthermore, in previous studies, the extraction of bioactive compounds from beets by-products, including leaves and stalks, was optimized, obtaining extracts rich in phenolic

compounds and/or betalains (Bengardino, Fernandez, Jagus, & Agüero, 2017; Marán & Priya, 2016). However, the study of BLE application into food products is still vacant.

Nowadays, fruit and vegetable (F&V) beverages are considered one of the food industry sectors with the highest growth worldwide (Morales-de la Peña, Welti-chanes, & Martín-belloso, 2016). They are an excellent option to increase the intake of F&V nutrients and bioactive compounds as they are tasty, convenient and ready to drink, fulfilling all the current demands of consumers (Morales-de la Peña et al., 2016). However, untreated F&V beverages have a short shelf-life that generally can be attributed to microbial spoilage. Although they are usually highly acidic products (pH <4) and this condition inhibits the growth of most of the bacterial spores, some acid tolerant microorganisms could survive and grow (Bevilacqua et al., 2018). Traditional preservation processes often result in a loss of heat-labile nutrients such as certain vitamins or bioactive phytochemicals, among other compounds responsible for nutritional and organoleptic attributes (Bevilacqua et al., 2018), hence, modifying their natural nutritional and sensory characteristics. Thus, one of the main challenges of F&V beverages industry is to find an alternative to heat preservation method that allows to ensure product´s safety while maintaining their fresh characteristics. An alternative to responding to this need is the biopreservation, which seeks to ensure the safety of the food through the use of natural compounds with antimicrobial properties (Draughon, 2004). In this sense, extracts of several agro-industrial by-products have demonstrated good potential for microbial control (Kowalska et al. 2017; López-romero, Ayala-zavala, Adolfo, Peña-ramos, & González-ríos, 2018) and could be a good option for this type of products.

Considering the above mentioned, the objective of this study was to assess the antioxidant and antimicrobial potential of BLE to be used as a preservative and/or for the enrichment of a fruit and vegetable smoothie.

2. Materials and methods

2.1. Plant material and extract preparation

Beet plants (*Beta vulgaris* L. var. *Conditiva*) were harvested in Escobar, Argentina, and immediately transported on refrigerated containers to the laboratory. Once there, leaves were washed with run tapper water, drained and stored at 5 ± 1 ºC until further processing.

The extract was prepared according to previously optimized conditions (Bengardino et al., 2017). Briefly, fresh leaves were manually cut into pieces of roughly 1 mm^2. Then, a mixture 1:50 of processed BL and solvent (ethanol solution at 80%) were prepared in an Erlenmeyer and adjusted to pH 6. Flasks were sealed with a film to avoid solvent evaporation. Extractions were carried out in an agitated thermostatic bath (Vicking mod. Dubnoff, Germany) during 40 min at 80 ºC and an agitation speed of 60 min^{-1}. After the extraction process, samples were centrifuged at 5 ºC (15,557 rcf) for 15 min (Eppendorf Centrifuge 5804R, Germany) and the supernatant was used as the source of bioactives (ethanolic extract). Then, 350 mL of ethanolic extract was evaporated under vacuum, using a rotavapor (R-124, Büchi, Switzerland) at 40 ºC until dryness, and then resuspended in 40 mL of sterile distilled water. The aqueous extract obtained is the hereafter called “BLE”.

2.2. Total phenolic compounds and antioxidant activity determinations

The **total phenolic content** was determined using the Folin–Ciocalteu method expressed as milligrams of Gallic acid equivalent per liter of BLE (mg L^{-1}) or gram of Gallic acid equivalent per kilogram of extract in dry weight (DW) basis (g kg^{-1}). The **antioxidant capacity** was evaluated through the DPPH and FRAP assays, as described by Fernandez et al. (2017a) and expressed as micromole equivalent of Trolox per liter of BLE (µmol L^{-1}) or as milimole equivalent of Trolox per kilogram of extract in DW basis (mmol kg^{-1}).

2.3. *In vitro* antimicrobial activity

In vitro antimicrobial activity was assessed according to the methodology proposed by Fernandez, Jagus, & Agüero (2018a). The assessed microorganisms

were *Listeria innocua* (strain ATCC33090), representing Gram-positive bacteria; *Escherichia coli* (strain ATCC8739) representing Gram-negative bacteria and *Saccharomyces cerevisiae* (strain CBC1171) as representative of yeast group. Additionally, the first two are surrogates of *Listeria monocytogenes* and *Escherichia coli* O157: H7, respectively, two pathogens of great interest in fruit and vegetable products, while *Saccharomyces* is a recognized spoilage and human illness associated microorganism (Alighourchi, Barzegar, Sahari, & Abbasi, 2014).

In order to assess the antimicrobial potential of the extract, covering a large amount of food products, two different pH matrices (3.7 and 7) were used in this study. Moreover, since it is well known that the level of contamination of the product has a main role on the efficacy of the treatment, a high and a medium inoculum level were tested.

2.3.1. Culture preparation

A mixed culture of *L. innocua*, *E. coli* and *S. cerevisiae* was used. Each of the microorganisms was cultivated separately in its corresponding broth. For both bacteria, trypticase soy broth enriched with 0.6% yeast extract (TSBYE, Biokar Diagnostics, France) was used according to the procedure detailed by Fernández et al. (2018a). While *S. cerevisiae* was grown in 150 mL of Sabouraud broth at 28 °C in a continuously agitated temperature-controlled shaker until early stationary phase was achieved. In order to obtain the mixed culture, an aliquot of each individual broth was mixed together to obtain a broth containing approximately 10^8 CFU/mL of each microorganism.

2.3.2. Treatment scheme and sample preparation

Different systems were prepared on sterile falcon tubes containing in all cases 50% v/v of TSBYE double concentrated. Systems with normal pH (7) and at reduced pH (3.7) were evaluated. To obtain samples of pH 3.7, a corresponding aliquot of HCl 0.5N were added. For treated samples (E) BLE, prepared as detailed on section 2.1, were added (25% v/v) on falcon system. For both control (C) and treated samples the

remaining volume was completed with sterile water. Falcon tubes capacity was selected in order to avoid the presence of a headspace on the system. Finally, all the systems (E7, C7, E3,7, and C3,7) were inoculated with an aliquot of the mixed culture. As previously mentioned, a high (7 log CFU/mL) and a medium (4 log CFU/mL) inoculum level were tested.

2.3.3. Sampling and storage

Samples were stored during 144 h at 15 ± 1 °C, temperature suggested for an accelerated test (Fernandez, Agüero, & Jagus, 2017b). Periodically, at 0, 6, 24, 48, 96, 144 h three samples of each system were taken for analysis.

2.4. Fruit and vegetable smoothie enrichment and preservation

2.4.1. Smoothie´s elaboration and sample preparation

For this study a smoothie containing orange juice (59%), apples (15%), carrots (15%), BL (6%) and beet stems (5%) was used. The smoothie was prepared according to the procedure described by Fernandez, Denoya, Agüero, Jagus, & Vaudagna (2018b). Briefly, orange (cv. Salustiana, Argentina) juice was extracted using a home squeezer (Oster, USA). Apples (cv. Granny Smith, Argentina), carrots (cv. Flakee, Argentina) and beets (cv. Detroyt, Argentina) were obtained from a local retailer. Before processing, all fruits and vegetables were washed and disinfected by dipping in 200 ppm chlorinated water for 5 min and dried. The carrots and apples were then manually peeled and chopped into small pieces. Finally, all the ingredients were blended in a homogenizer (JTC OmniBlend, Guangdong, China) for 60 s. The smoothie was packed into polyethylene terephthalate (PET) tubes of 33 mL of capacity. On treated samples, BLE, prepared as detailed on section 2.1, were added on smoothies (30%v/v), while on control samples, the same volume was replaced with sterile distilled water. The added volumes of smoothie, BLE and/or sterile distilled water in the tubes were determined in order to avoid the presence of headspace in the

system, thus reducing detrimental action associated with the diffusion of oxygen into the samples.

2.4.2. Sampling and storage

All the samples were stored for 21 d at 5 ± 1 °C. Periodically, at day 0, 7, 14 and 21, three samples were taken from each treatment for analysis.

2.4.3. Quality parameters determination

2.4.3.1. Microbiological counts

Mesophilic aerobic bacteria (MAB), Enterobacteriaceae (EB) and **molds and yeasts (M&Y)** counts were determined according to the described by Fernandez et al. (2018b). The detection limit of the methods was 1.00 log CFU/g.

2.4.3.2. pH and total soluble solids (TSS)

They were determined at 20 ± 1 °C. For pH determination, a digital pH-meter (Hanna, HI99163, Rumania) with an amplified pH electrode with a built-in temperature sensor (FC232D, Italy) was used. For TSS determination, a Milwaukee MA871 Refractometer (Milwaukee Instrument, Rocky Mount, USA) was used, and informed as percentage of soluble solids on the solution (%).

2.4.3.3. Betaxanthins and betacyanins

Betaxanthins (Bx) and betacyanins (Bc) determination were carried out according to the method described by Fernandez et al. (2018b). The results were expressed as milligrams of Bx or Bc per liter of fresh smoothie.

2.4.3.4. Total phenolic content and antioxidant capacity

The extraction and determination of total phenolic compounds by Folin-Ciocalteau methodology, and of antioxidant capacity by FRAP and DPPH assays, were carried out according to Fernandez et al. (2018b).

2.5. Statistical analysis

Results were expressed as the mean of all the repetitions together with the standard deviation. The statistical analysis was performed with Origin® 8 software

(OriginLab®, USA). The data were subjected to an analysis of variance (ANOVA) using as sources of variation: TIME, TREATMENT and interaction TIME-TREATMENT. Differences were determined using the Tukey comparison test. A 95% confidence level was used to determine significant differences.

3. Results and discussion

3.1. *In vitro* antioxidant potential and total phenolic compounds content of BLE

BLE presented a total phenolic compounds (TPC) content of 206.6 ± 2.4 mg L^{-1} (132.43 ± 1.51 g kg^{-1}), while their antioxidant capacity was of 438.42 ± 46.74 µmol L^{-1} (219.2 ± 23.4 mmol kg^{-1}) for ferric reducing capacity and of 45.62 ± 1.15 µmol L^{-1} (28.9 ± 0.5 mmol kg^{-1}) for antiradical scavenging capacity. These values are in the order, or in some cases even higher, of those informed by other authors for different by-products extracts that were used successfully as a functional ingredient in food products. For example, Amofa-diatuo et al. (2017) reported a TPC content of 105 mg L^{-1} in a cauliflower by-product extract, Ndayishimiye, Jum, & Soo (2018) obtained 43.64 g kg^{-1} in a citrus peel extract, Tyug, Prasad, & Ismail (2010) informed 6.27 g kg^{-1} in a soy husk powder extract and Babbar, Oberoi, Uppal, & Patil (2011) found values of 24.6 y 37.4 g kg^{-1} in litchi pericarp and grape seed extracts. In general, all these authors observed that these extracts exhibited high antioxidant potential. Even though the direct comparison of the antioxidant capacity of the different extracts is difficult, given the existence of different methods of determination and the use of different standards and units to express the results, worth mentioning the presence of values of 60 mmol kg^{-1} for DPPH and 200 mmol kg^{-1} for FRAP in citrus peel extract (De Moraes Barros, Aparecida Pinto de Castro Ferreira, & Genovese, 2012), a Trolox equivalent antioxidant capacity (TEAC) of 63.50 mmol kg^{-1} in soy husk powder extract (Tyug et al., 2010), and values of 146 y 169 mmol kg^{-1} TEAC in litchi pericarp and grape seed extracts (Babbar et al., 2011).

It is well known that F&V phenolic compounds exhibit a wide range of physiological properties like cardioprotective, anti-cancer, and neuroprotective activities; as well as they are effective natural food preservatives against oxidative deterioration and microbial contamination (Lai et al., 2017). According to this analysis, BLE has good potential as a bioactive food ingredient.

3.2. *In vitro* antimicrobial activity of BLE

Changes in microbial counts during the storage of different treatments are presented in **Figure 1** for a 7 log and a 4 log CFU/mL initial inoculum of the mixed culture. In all cases, an interaction between TIME and TREAT was significant ($p<0.05$), indicating that changes registered along storage were different depending on the treatment applied.

In regards to ***Listeria innocua*** counts their already known sensitivity to low pH matrices (Buchrieser et al., 2003) was also manifested in our results. Moreover, this effect was highly notable in systems with medium inoculum level since values under detection limit were achieved during storage, even in control samples. In relation to treatment effect, 1-3 log reductions were observed in samples treated with BLE respect control, been these differences greater during the first hours of storage. Hence, the addition of BLE to a matrix of low pH showed the most promising results on *Listeria* control, especially in case of lower contamination.

Regarding ***E. coli*** counts, even though some strains of this microorganism have shown to be acid-resistant, surviving for long periods in acidic foods (Noma, Tomita, Shimoda & Hayakawa, 2004), in this study, a bacteriostatic behavior was observed on samples of low pH for both tested inoculum levels. Samples treated with BLE presented significant reductions regarding control, been this differences more remarkable in samples with lower contamination. These are outstanding results since it is very difficult to find effective antimicrobials against Gram-negative bacteria, due to the characteristic protective outer membrane of lipopolysaccharides of these microorganisms (Fernandez, et al., 2018a).

In ***Saccharomyces cerevisiae*** case, changes in counts were similar in both matrices (at different pH). It is well known that M&Y are resistant to low pH (Arroyo-López, Orlic, Querol, & Barrio, 2009), which is probably why the growth was independent of the pH of the matrix, showing both controls (C7 and C3.7) similar behavior. For both tested inoculum levels, significant reductions were showed in samples treated with BLE, especially during the first hours of storage, been these reductions higher on samples with lower contamination.

According to Kowalska et al. (2017) several fruit and vegetables by-product extracts exhibited antibacterial activity against various Gram-positive and Gram-negative bacteria as well as antifungal activity. This activities have been mainly associated to the antioxidants compounds, such as phenolic compounds, terpenes and saponins which alter the membrane properties of Gram-negative and Gram-positive bacteria, affecting changes in hydrophobicity, surface charge and membrane integrity, followed by leakage of intracellular constituents and subsequent cellular death (López-romero et al., 2018).

Effect of BLE was similar for both tested pH. Effectiveness over a wide pH range is an important characteristic for antimicrobials, which are generally more effective at lower pH (Davidson, Cekmer, Monu, & Techathuvanan, 2015). Moreover, although when working with lower initial counts, a better performance of BLE was observed, as usual when working with natural antimicrobials and different inoculum levels (Siroli et al., 2015), significant reductions were obtained in all cases. These results indicate that BLE not only has great potential for their use as antimicrobial against a wide group of microorganism, but also in a wide group of food products.

3.3. Fruit and vegetable smoothie enrichment and preservation

The smoothie formulated in our work is rich in bioactive and antioxidants compounds as it was demonstrated in a previous work. Also, the main cause of deterioration of this smoothie is the microbial activity (Fernandez, Denoya, Jagus,

Vaudagna, & Agüero, 2019), thus the stability of smoothies enriched with BLE was evaluated through microbiological and physicochemical indices.

3.3.1. Microbiological counts

Changes in mesophilic aerobic bacteria (MAB), enterobacteria and molds and yeast (M&Y) in control and treated smoothies are presented in **Table 1**. It is noteworthy that treated samples presented significant lower counts in all the microbial groups tested, showing, in general, differences between 1 and 3 log cycles along storage. This is consistent with the observations during *in vitro* test regarding the antimicrobial potential of BLE. Moreover, if the usually accepted microbial limit of 6.0 log CFU/mL for MAB and M&Y is considered (Varela-Santos et al., 2012), the treated smoothie has a microbial shelf-life of at least 14 days while the control samples of 7 days.

Similar results were observed with the application of other by-products extracts on different food products. In this sense, Ba et al. (2016) applied a shitake mushrooms by-product extract on fermented sausages and observed that extract-treated samples had MAB values below the maximum limits for meat products, while the control was in the limits. Indeed, by-products extracts have been frequently applied in the preservation of meat products, presenting interesting results for MAB, acid lactic bacteria, M&Y, coliforms, and some pathogenic bacteria control as detailed by Nikmaram et al. (2018). Application of by-products extracts in F&V products have been very limited, on this line Son, Kang, & Song (2017) studied the antimicrobial activity of a safflower seed meal extract against MAB and *Listeria monocytogenes* on lettuce leaves, observing a microbial reduction of 1.55 and 1.58 log CFU/g regarding control, respectively.

3.3.2. pH and total soluble solids (TSS)

No significant differences on pH (4.09 ± 0.03) and TSS (6.96 ± 0.70 %) were observed with treatment nor with the time of storage. Hence no differences were

introduced by the use of BLE, one of the desired attributes of a good antimicrobial agent (Davidson et al., 2015).

3.3.3. Betacyanins (Bc) and betaxanthins (Bx)

Changes in Bc and Bx during the storage of different treatments are presented in **Table 2.** BLE contains Bc and Bx (Bengardino et al., 2017), nonetheless, their content is very low, so it was not expected a relevant increase on smoothie´s content. Major changes over time were neither expected since it is well known that betalains are stable at refrigeration temperatures (Azeredo, 2009). Indeed, in general, no significant differences were observed with treatment nor with storage time, except in the case of Bc that presented a significantly lower value in control samples at day 21, showing significant differences with treated samples. These observations could indicate a better preservation of the pigment with treatment. Betalain stability has been reported to be improved by antioxidants (Azeredo, 2009), explaining the higher value of Bc content observed on treated samples towards the end of storage. Indeed, according to López-romero et al. (2018) by-products extracts could be used to inhibit food oxidative process and inhibit the activity of oxidative enzymes, preserving the quality and maintaining the nutritional value of food products.

3.3.4. Total phenolic content (TPC) and antioxidant capacity (FRAP and DPPH)

Changes in TPC, FRAP, and DPPH during the storage of different treatments are presented in **Table 2.** Without doubt, the enrichment achieved with BLE was denoted on the increased TPC and FRAP of the treated smoothie, since significant differences were observed regarding control samples. Moreover, an initial increase of 50% in both TPC and FRAP values was shown, while no significant initial differences were observed on DPPH values. In fact, it was not surprising to find differences between the values of FRAP and DPPH since, although both are methods used for the determination of antioxidant capacity, they target to compounds with different

mechanisms of action (Huang, Ou & Prior, 2005). Specifically, DPPH method measures radical scavenging activity while FRAP measures ferric reducing antioxidant power. Considering that the extract is compose by several bioactive compounds which exert their activity by different mechanisms (de Kok, van Breda, & Manson, 2008), it is not strange to find different values and behavior on the different indices. In this case, the initial increase on phenolic compounds could explain the results observed on ferric reducing activity, while radical scavenging activity is probably mainly given by others bioactives, such as ascorbic acid, flavonoids or even betalains (Azeredo, 2009; Lai et al., 2017). Whereas for control samples TPC values were maintained during storage, for treated samples a slight but significant decrease was observed, presenting nonetheless at day 21 a value 20% higher than controls. FRAP values in control samples presented a significant decrease with time retaining only 47% of their initial value. Same behavior with time was observed for treated samples, although a 63% retention was observed in this case. DPPH values also decreased with storage time, with retentions at day 21 of 64 and 74% for control and treated samples, respectively.

Phenolic compounds and antioxidants, in general, are considered good additives to increase the nutritional value of food products (Kowalska et al., 2017). Certainly, many authors have explored this field of application. In this sense, Llorach, Tomás-barberán, & Ferreres (2005) found that cauliflower by-products extract (34 g kg^{-1} of polyphenols) added in chicken soup presented an increase of 3.5 times in antioxidant activity compared to control soup. Bobinaité et al. (2016) studied the addition of a raspberry marc extract (2%) on two mixed fruit purees, resulting in a 2-3 fold increase in total phenolic content and a radical scavenging increase from 3.5 to 13.5 mmol kg^{-1} and from 6.1 to 15.9 µm mmol kg^{-1}, respectively. Mildner-szkudlarz, Zawirska-wojtasiak, Szwengiel, & Pacynsky (2011) applied a grape by-product extract (58.95 g kg^{-1}) in a sourdough mixed rye bread, finding that this addition (4%) greatly enhanced phenolic content (2 fold increase) and antioxidant properties (6 and 2 fold increase on FRAP and DPPH, respectively) of the product. Moreover, many authors have

389 evaluated the application of by-products extracts on meats products in order to inhibit
390 lipid and protein oxidation (Echegaray et al., 2018) and to prevent the oxidation of
391 different types of oils (Anal, Jaisanti, & Noomhorm, 2012) with really encouraging
392 results.

393 Taking into account these results, undoubtedly the treatment with BLE was
394 successful in achieving an antioxidant enrichment of the product. Moreover, it is
395 important to note that by using a more concentrated extract, even better results may be
396 achieved, since these are usually dependent on extract bioactive´s concentration as
397 was demonstrated by several authors (Amofa-diatuo et al., 2017; Bobinaité et al., 2016;
398 Llorach et al., 2005).

399 Finally, it is interesting to mention that no differences between treated and
400 control samples were noted on smoothie´s color and flavor. Nonetheless, future studies
401 could be directed to deepen aspects such as color and sensory analysis, in order to
402 better characterize BLE's effect.

403

404 **Conclusions**

405 Beet leaves extract presented a total phenolic content and an antioxidant
406 capacity in the order, or even higher, of those informed for other by-products extracts
407 applied as a functional ingredient in several food products.

408 Results of *in vitro* antimicrobial activity test showed that beet leaves extract
409 could be effective for a wide group of foods, types and levels of contamination.
410 Moreover, incorporation of the beet leaves extract into a fruit and vegetable smoothie
411 significantly enriched (50%) phenolic content of the product with the concomitant
412 increase in their antioxidant capacity. Additionally, extract-treated smoothies presented
413 greater nutritional retention during storage and a one-week extension in their microbial
414 shelf-life.

415 Therefore, BLE has great potential to be used as a bioactive food ingredient,
416 acting as a value-adding by-product, bringing great advantages for consumer's health

as well as profits for the producers, besides representing a step forward towards a sustainable food chain from an environmental and economic point of view.

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Declarations of interest: none

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Figures captions:

Figure 1- Changes during storage at 15 ºC in *Listeria innocua* (a), *Escherichia coli* (b), and *Saccharomyces cereviseae* (c) counts when a high mixed culture inoculum is used and in *Listeria innocua* (d), *Escherichia coli* (e), and *Saccharomyces cereviseae* (f) counts when a medium mixed culture inoculum is used. Dotted lines: pH 3.7, full lines: pH 7. Black: control, grey: BLE

Table 1- Changes in microbial counts (log UFC/mL) in control and treated smoothies during storage at 5 ± 1ºC

		Control	Treated
Mesofilic aerobic bacteria	0	3.71 ± 0.23 ^{a,A}	2.37 ± 0.04 ^{b,A}
	7	5.44 ± 0.06 ^{a,B}	4.03 ± 0.09 ^{a,B,C}
	14	7.10 ± 0.06 ^{a,C}	5.31 ± 0.66 ^{b,B}
	21	6.32 ± 0.05 ^{a,B,C}	3.78 ± 0.12 ^{b,C}
Enterobacteria	0	3.39 ± 0.30 ^{a,A}	2.24 ± 0.34 ^{b,A}
	7	4.95 ± 0.20 ^{a,B}	4.03 ± 0.10 ^{b,B}
	14	3.15 ± 0.21 ^{a,A}	1.70 ± 0.10 ^{b,A}
	21	3.04 ± 0.06 ^{a,A}	2.30 ± 0.14 ^{a,A}
Molds and yeast	0	2.17 ± 0.12 ^{a,A}	< 1.00 ^{b,A}
	7	4.26 ± 0.12 ^{a,B}	< 1.00 ^{b,A}
	14	6.49 ± 0.11 ^{a,C}	5.74 ± 0.37 ^{a,B}
	21	7.40 ± 0.36 ^{a,D}	6.13 ± 0.10 ^{b,B}

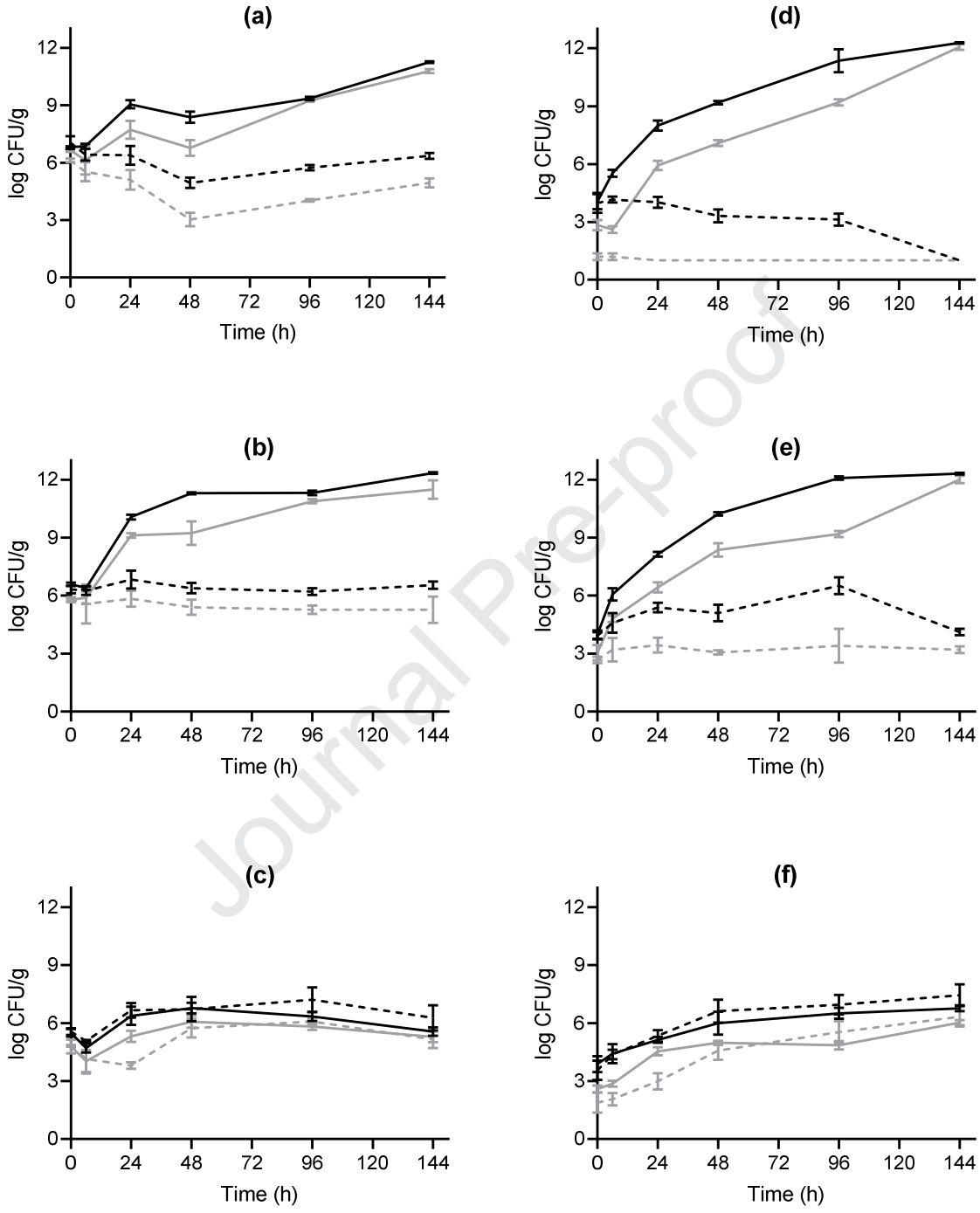
Different lowercase letters indicate differences between treatments and different capitals indicate differences over time. Data expressed as means ± standard deviation (n=3).

Table 2- Changes in betacyanins (Bc), betaxanthins (Bx), total phenolic content (TPC), antiradical scavenging capacity (DPPH) and ferric reducing antioxidant potencial (FRAP) during storage at 5± 1ºC on control and treated samples

	Days	Control	Treated
Bc (mg L^{-1})	0	6.94 ± 0.51 ^{a,A}	6.47 ± 0.10 ^{a,A}
	7	6.37 ± 0.25 ^{a,A}	7.51 ± 0.62 ^{a,A}
	14	6.97 ± 0.14 ^{a,A}	6.95 ± 0.58 ^{a,A}
	21	5.34 ± 0.15 ^{a,B}	7.57 ± 0.13 ^{b,A}
Bx (mg L^{-1})	0	5.10 ± 0.29 ^{a,A}	5.99 ± 0.37 ^{a,A}
	7	6.06 ± 0.05 ^{a,A}	6.18 ± 0.76 ^{a,A}
	14	6.00 ± 0.14 ^{a,A}	6.28 ± 0.47 ^{a,A}
	21	5.36 ± 0.65 ^{a,A}	6.95 ± 0.22 ^{a,A}
TPC (mg kg^{-1})	0	334.8 ± 31.0 ^{a,A}	496.3 ± 8.3 ^{b,A}
	7	326.0 ± 13.1 ^{a,A}	421.3 ± 5.7 ^{b,B}
	14	377.7 ± 3.9 ^{a,A}	443.1 ± 32.2 ^{a,A,B}
	21	341.6 ± 3.2 ^{a,A}	426.2 ± 1.5 ^{b,B}
DPPH (µmol kg^{-1})	0	1551.1 ± 86.9 ^{a,A}	1473.6 ± 5.8 ^{a,A}
	7	1473.6 ± 43.2 ^{a,A}	1454.7 ± 35.8 ^{a,A}
	14	1142.8 ± 13.2 ^{a,B}	1100.9 ± 11.6 ^{a,B}
	21	995.9 ± 6.0 ^{a,B}	1087.9 ± 5.4 ^{a,B}
FRAP (µmol kg^{-1})	0	4212.3 ± 302. ^{a,A}	6210.5 ± 245.8 ^{b,A}
	7	3526.4 ± 146.4 ^{a,A,B}	5361.3 ± 223.3 ^{b,A,B}
	14	2683.9 ± 91.9 ^{a,B,C}	4741.7 ± 307.7 ^{b,B,C}
	21	2000.6 ± 334.0 ^{a,C}	3928.4 ± 343.4 ^{b,C}

Different lowercase letters indicate differences between treatments and different capitals indicate differences over time. Data expressed as means ± standard deviation (n=3).

Figure 1



Highlights

- Beet leaves extract (BLE) presented good phenolic content and antioxidant capacity
- BLE presented significant antibacterial and antifungal *in vitro* activity
- Beet leaves extract significantly enriched (50%) phenolic content of a vegetable smoothie
- Extract-treated smoothies showed greater nutritional retention after 21 d at 5 °C
- Extract-treated smoothies presented a 1 week extension on their microbial shelf-life

Conflict of Interest and Authorship Conformation Form

Please check the following as appropriate:

- o All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.
- o This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.
- o The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript
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