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Fortification of coconut water with microencapsulated grape pomace extract towards a novel electrolyte beverage: Biological, sensorial and quality aspects

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ABSTRACT

A bioactive grape pomace extract (GPE) rich in polyphenols was encapsulated into alginate (GPE-Alg) or chitosan (GPE-CS) microparticles, later incorporated into coconut water. Biological and sensory properties were evaluated. Storage was performed at 4 °C followed by quality assessment.

Evaluation of coconut water after gastrointestinal digestion on the growth pathogens and probiotic bacteria showed that the fortification with GPE-Alg and GPE-CS decreased the growth rate of pathogens when compared to non-fortified water, while promoted the growth of different bifidobacteria and lactobacilli strains. Sensory analysis allowed to conclude that the incorporation of GPE-Alg and GPE-CS did not promote significant differences in most of evaluated attributes, including aroma and flavor. The storage at 4 °C allowed a reduced degradation rate of total phenolics and anthocyanins for GPE-Alg and GPE-CS fortified beverage, with the half-life time of phenolic acids higher for GPE-Alg beverage and the half-life time of anthocyanins higher for GPE-CS fortified water.

This study opens the opportunity in the application of food by-products in the development of novel efficient functional foods and beverages.

1. Introduction

In the last decade, the increasing consciousness that diet and health are closely related and nutrition can have benefits in the mitigation of some diseases, has led to an increased demand for new healthier foods and beverages (Corbo et al., 2014; Nazir et al., 2019). Although, there is no a sole consensual definition of functional food, it is accepted that they should have some properties, including the enhance of a biological property or help in the prevention of disease, which means that it should have benefits beyond the nutritional function, and finally should be in the form of a common food or beverage, meaning that it can be consumed as part of the daily diet and routine (Nazir et al., 2019). Beverages are the most common category of functional foods as they are convenient to consume within the modern busy lifestyle, highly stable at shelf or refrigerated storage, and the incorporation of the bioactive ingredient is usually easier (Corbo et al., 2014; Nazir et al., 2019).

Together with the functional foods trend, consumers are also demanding for more sustainable food products, as it is even more evident the negative impact of the food production systems, including the agriculture and food industries, in the climate changes, mainly caused by the accumulation of by-products (Asioli et al., 2017). Currently, by-products applications include their use as fertilizers, compost, fuel or animal feed, and in the worst-case scenario, as wastes are incinerated or landfilled, which constitute a high environmental impact, without generating value and thus, leading to high management costs (Martin et al., 2016). These agroindustrial by-products are also a considerable source of bioactive compounds, such as carotenoids, phenolic compounds, dietary fibers and proteins, which can be applied as ingredients in functional foods, increasing their nutritional value and providing beneficial properties to the consumer. The development of new ingredients from agro-food by-products allows us to simultaneously address two imperative issues in food processing, namely to increase the sustainability of processes and supply new raw materials of interest.

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Coconut water is largely consumed in tropical countries, appreciated for its sweetness and freshness, rich in sugars and minerals, and it is taken directly from the inner part of the fruit (Walter et al., 2014; Prades et al., 2012). The World Health Organization highly recommends this drink for rehydration in the cases of cholera and diarrhea due to its high content in potassium, which makes it also a natural isotonic drink for athletes (Walter et al., 2014). Coconut water is also a major by-product in coconut processing plants, such in the processing of ready-to-eat coconut, namely fresh minimally processed or desiccated coconut (Prades et al., 2012). Nevertheless, due to its low pH (5.8) and high water activity, in addition to high sugar content, this product allows a fast microbial growth, which requires an extra care when using this product (Prades et al., 2012; Walter et al., 2014).

Grape pomace is also another major industrial by-product that has been described as bioactives source, due to the presence of polyphenols, oligosaccharides, minerals that provide its aqueous extract with antioxidant, antimicrobial and prebiotic potentials (Costa et al., 2019a). GP as a source of phenols and fiber has been applied in the formulation of a wide variety of functional foods, including fermented milks, yogurt, ice-creams, salad dressing and cheeses (Dos Santos et al., 2017; Karnopp et al., 2017; Hwang et al., 2009; Lucera et al., 2018; Tseng and Zhao, 2013). Nonetheless, its application in the formulation of coconut non-fermented beverages has never been exploit. Considering the nutritional properties and convenience of coconut water, makes this an ideal carrier to incorporate functional ingredients such as a bioactive grape pomace extract to develop a new functional beverage, which is able to inhibit the pathogens responsible for diarrhea and modulate the intestinal microflora, at the same time it can restore the body hydration and electrolytes.

Thus, the main objective of this study was to develop a new healthy coconut beverage through the incorporation of a bioactive grape extract encapsulated into polymeric particles, alginate (GPE-Alg) or chitosan (GPE-CS) with proven antioxidant, antimicrobial and prebiotic activities. In addition, evaluation of their bioactive properties, product stability and consumer acceptance were also studied.

2. Material and methods

2.1. Raw materials

Water from mature coconuts was provided by Nuvi Fruits S. A. (Torres Vedras, Portugal) as byproduct, vacuum-filtered and pasteurized at 80 ˚C for 5 min. Coconut water was characterized for brix degree, pH, minerals, titratable acidity and sugars profile. Grape pomace was provided by Ouro Verde Winery (Bahia, Brazil), and was oven-dried at 45 ˚C for 24 h, milled and sieved in a Bonina 0.25 df depulper (Itametal, Bahia, Brazil).

2.2. Production of coconut beverages with encapsulated grape pomace extract

2.2.1. Production of encapsulated GPE

GPE was obtained through enzymatic extraction of a GP flour, using an enzymatic cocktail with xylanase activity produced by *Aspergillus niger* 3T5B8, as previously described (Costa et al., 2019a). GPE was then encapsulated into alginate or chitosan capsules, as previously described (Costa et al., 2021). The sizes of alginate capsules were 523 nm and of chitosan capsules were 853 nm, safe and suitable for gastrointestinal delivery.

2.2.2. Production of functional coconut beverages

Functional coconut beverages were then produced through the incorporation of freeze-dried GPE-loaded capsules into the coconut water, at final concentration of 2% (w/v). The beverages were stored at 4 ˚C for stability studies.

2.3. Assessment of functional properties of the coconut beverage

2.3.1. In vitro simulation of gastrointestinal digestion

Simulation of the gastrointestinal digestion of the coconut beverages was performed following the method described by Madureira et al. (2011) with slight modifications.

Mouthdigestion: The pH value was adjusted to 6.9, using HCl 1 M. Artificial saliva was simulated by using α-amylase at 100 U/ mL and added at a rate of 0.6 mL/ min of digestion. Incubation was made during 2 min at 37 ˚C and 200 rpm.

Stomachdigestion: The pH value was adjusted to 2.0 using HCl 1 M. Gastric juice was simulated by dissolving pepsin 25 mg/ mL, and added at a ratio of 0.05 mL/ mL of sample. Incubation lasted 120 min (long digestion), at 37 ˚C and 130 rpm.

Gut digestion: Simulation of gut conditions was performed by initial adjustment of pH value to 6.0 using $NaHCO_3$ 1 M. The intestinal juice was simulated by dissolving 2 g/ L of pancreatin and 12 g/ L bile salts. This solution was then added at a concentration of 0.25 mL/ mL of sample. All samples were incubated during 1 h, at 37 ˚C and 45 rpm.

After gut digestion, samples were dialyzed using a 3kDa cut-off dialysis membrane and freeze-dried.

2.3.2. Antimicrobial activity

Antimicrobial activity of coconut beverages after digestion was determined upon *Staphylococcus aureus* (MSSA) ATCC 25923, *Listeria monocytogenes* (food isolate from ESB collection) and *Candida albicans*, as these microorganisms are representative of Gram-positive and Gram-negative pathogens, and yeasts.

For determination of growth inhibition curves, inocula were prepared by suspending each bacterial colony into Mueller-Hinton Broth (MHB), with a final concentration of ca. 10^8 CFU/ mL. Two microliters of each inoculum were transferred to a 96-well microplate, every well was filled (to final volume of 200 µL) with the coconut beverages dissolved in MHB, at final concentration of 2% (w/v). Microplate was incubated in a microplate reader (Multiskan GO, Thermo Scientific) at 37˚C for 24 h, with absorbance measurements at 620 nm registered every hour. Three controls were also performed: the first one containing inoculum and MHB (positive control), the second one containing the coconut beverages (negative control) and the third one containing only MHB.

2.3.3. Impact on probiotic bacteria

Impact on probiotic bacteria of coconut beverages after digestion was determined for *Bifidobacterium animalis* Bo (CSK, Ede, Netherlands), *Bifidobacterium longum* BG3 (Cell Biotech, Hellerup, Denmark), *Bifidobacterium animalis* spp. *lactis* Bb12, *Lactobacillus casei 01* (Chr. Hansen, Hørsholm, Denmark), *Lactobacills rhamnosus R11* (Lallemand, Montreal, Canada), and *Lactobacillus plantarum* 299v (Probi AB, Lund, Sweden). Strains were stored at −80˚C in de Man–Rogosa–Sharpe (MRS) broth (Biokar Diagnostics, Beauvais, France) with 30% (v/v) glycerol.

L. casei, L. rhamnosus, and L. plantarum inocula were prepared by suspending each bacterial colony into MRS broth, achieving a concentration of 10^7 CFU/ mL. The microplate wells were filled with 245 µL syringe-filtered (0.22 µm) coconut beverages diluted in basal MRS broth without glucose, at concentration of 2% (w/v), and 5 µL of the inoculum. Microplate was incubated (Multiskan GO, Thermo Scientific) at 37˚C for 24 h with agitation, and cellular growth was monitored by measuring the Optical Density (OD) at 620 nm.

B. animalis Bo and *B. lactis* BB12 inocula were prepared under anaerobic atmosphere, by suspending each bacterial colony into MRS broth supplemented with 0.05% (v/v) L-cysteine-HCl, achieving a concentration of 10^7 CFU/ mL). The microplate wells were fulfilled with 245 µL syringe-filtered (0.22 µm) coconut beverages diluted in basal MRS broth without glucose, at concentration of 2% (w/v), 5 µL of the inoculum, and 50 µL of paraffin. Microplate was incubated (Multiskan GO,

Thermo Scientific) at 37°C for 48 h with agitation and cellular growth was monitored by measuring the OD at 620 nm.

For the evaluation of the prebiotic effect of the coconut beverages, growth rates of the bacteria tested were calculated in order to compare with the growth obtained with MRS basal media. This calculation was made by determination of the slope of the trend line of the OD620 over log phase of the growth curves (Sousa et al., 2015).

2.4. Sensory evaluation

Sensory evaluation was performed only at day zero, by a group of nine semi-trained panelists. Participants were informed about the general aim of the work and test procedure.

Coconut beverages were evaluated using Attribute difference-from-control tests (Meilgaard et al., 2007). Each panelist received a portion of plain coconut water labeled as "control sample" and three coded samples: a second portion of plain coconut water (blind control sample) plus the GPE-CS and GPE-Alg test samples. The blind control and test samples were coded with three-digit random numbers and were presented to panelists in a balanced order. Panelist were asked to compare and rate the samples for general appearance, color, aroma, flavor and texture using a continuous line scale (0 = similar to the control sample, 10=very different from the control sample), apparent viscosity and coconut odor intensity using a continuous bipolar line scale (-5 = much weaker than to the control sample, 0 = similar to the control sample, 5=much stronger than the control sample).

2.5. Assessment of coconut beverages stability

Stability of coconut water functionalized with GPE-Alg and GPE-CS was evaluated through microbiological control and stability of phenolic compound. Measurements were performed in triplicate at 0, 2, 7, 14, 30 and 60 days. Plain coconut water was used as control.

2.5.1. Microbiological control

Microbiological stability was assessed through direct plating into Plate Count Agar (PCA) for quantification of total viable cells, Potato Dextrose Agar (PDA) for quantification of molds and yeasts, De Man, Rogosa and Sharpe Agar (MRS) for quantification of lactic acid bacteria (LAB), MacConkey Agar for quantification Gram-negative enteric bacteria, and Mannitol Salt Agar (MSA) for quantification of *Staphylococcus aureus*. Microorganisms enumeration was performed by decimal dilutions in 0.1% (w/v) peptone water, and plated 100 μL in the different media through spread plate technique. PCA, MacConkey Agar and MSA plates were incubated for 24 h at 37°C, MRS plates were incubated for 48 h at 37°C and PDA plates were incubated for 5 days at 30°C.

2.5.2. Total phenolic compounds

Total phenolic compounds in coconut beverages was determined by Folin–Ciocalteu method (Singleton and Rossi, 1965). Quantification was done at 750 nm (UV mini 1240, Shimadzu, Tokyo, Japan) with gallic acid as standard in the range of 0.015–1.00 mg/ mL.

The degradation of coconut beverages was monitored by phenolic and anthocyanins degradation kinetics using first-order models, as described by Chung et al. (2016). The first order reaction was expressed by Eq. (2), where C_t is the phenolics content at t days of storage, C_0 is the initial phenolic content, k is the reaction rate constant, and t is the days of storage.

$$\ln\left(\frac{C_t}{C_0}\right) = -kt \tag{2}$$

The half-life time ($t_{\frac{1}{2}}$) for a first order reaction can be calculated using Eq. (3).

$$t_{1/2} = \frac{\ln(2)}{k} \tag{3}$$

2.6. Statistical analysis

Statistical analysis was performed with IBM SPSS Statistic Program v 23.0 (Illinois, USA), using analysis of variance (ANOVA) with Tukey post-hoc test, and Wilcoxon test for non-parametric data. All assays were performed in triplicate. Differences were considered significant at a level of $p < 0.05$.

3. Results

Coconut water obtained from mature coconuts from dried fruits industry presented 9.0 ± 0.1 °Bx of total soluble solids, pH value of 5.79 ± 0.13 and titratable acidity of 0.14 ± 0.01 g/ 100 mL, expressed as citric acid. These values are slightly higher than the values described by Nambiar and co-workers, which were 6.5 °Bx, pH 4.5 and titratable acidity of 0.09 g citric acid/ 100 mL, although these values are regarding tender coconuts and the increase of soluble solids, pH and acidity increases with aging (Nambiar et al., 2017). Regarding the pH value, it is in accordance with Prades and co-workers, who studied the pH of different coconut waters from mature coconuts and determined pH values ranged between 5.1 and 6.1, and with the results obtained by Halim and colleagues, who determined pH values in the range of 5.3 to 6.3 for mature coconuts (Prades et al., 2012; Halim et al., 2018). The ratio between total soluble solids and acidity is 64.3 is in accordance with other values reported for coconut water (between 60 and 70), indicating the beverage quality as low pH values and high sugar concentration promote the development of yeasts (Nambiar et al., 2017; Costa et al., 2015).

Coconut water presented high concentration of minerals, 4.78 ± 0.04 g/ 100 mL, and sugars: 1.26 ± 0.07 g/ 100 mL of glucose, 1.20 ± 0.05 g/ 100 mL of fructose, 0.96 ± 0.11 g/ 100 mL of sucrose, 0.15 ± 0.01 g/ 100 mL of xylose and 1.08 ± 0.01 mg/ mL of cellobiose, which contribute to the high sweetness of the coconut water. The concentration of total sugars, 4.65 g/ 100 mL, is slightly higher than the average of total sugars present in the different coconut varieties analyzed by Prades and co-workers, which ranged from 1.9 to 4.4 g/ 100 mL (Prades et al., 2012). Nevertheless, the concentrations of glucose and fructose are in accordance with the values described for these sugars, which ranges 1.5 to 1.7 g/ 100 mL for glucose and around 1.4 g/ 100 mL for fructose (Prades et al., 2012; Vigliar et al., 2006).

Raw coconut water presented some microbial contaminations, in the range of 10^2 cfu/ mL, and specifically, 10^1 cfu/ mL for Gram-negative bacilli (MacConkey Agar medium) and 10^2 cfu/ mL for yeasts and molds (PDA). The European Regulation on microbiological quality in foods and drinks (WHO, 2016) demands that the presence of thermotolerant coliforms, determined through MacConkey medium, can not be present, thus the coconut water was pasteurized before further use. After pasteurization, no viable cells were found in the different media.

3.1. Bioactive properties

3.1.1. Antimicrobial activity

Growth inhibition curves of GPE-Alg and GPE-CS coconut beverages were performed for MSSA, *Listeria monocytogenes*, and *Candida albicans*. Growth inhibition curves for selected microorganisms, in the presence of the digested coconut water control and coconut beverages with GPE-Alg or GPE-CS, at concentration of 2.5% (w/v), as measured by turbidity at 630 nm, are presented in Fig. 1.

As previously described, the GPE has inhibitory effect upon different microorganisms, due to the presence of different polyphenols, including anthocyanins, minerals and organic acids (Costa et al., 2019b). Antimicrobial properties of chitosan are also well described and the encapsulation within this polymer may enhance its antimicrobial activity (Zheng and Zhu, 2003; Qin et al., 2006; Du et al., 2009). As observed in Fig. 1, digested coconut beverages with GPE-Alg and GPE-CS

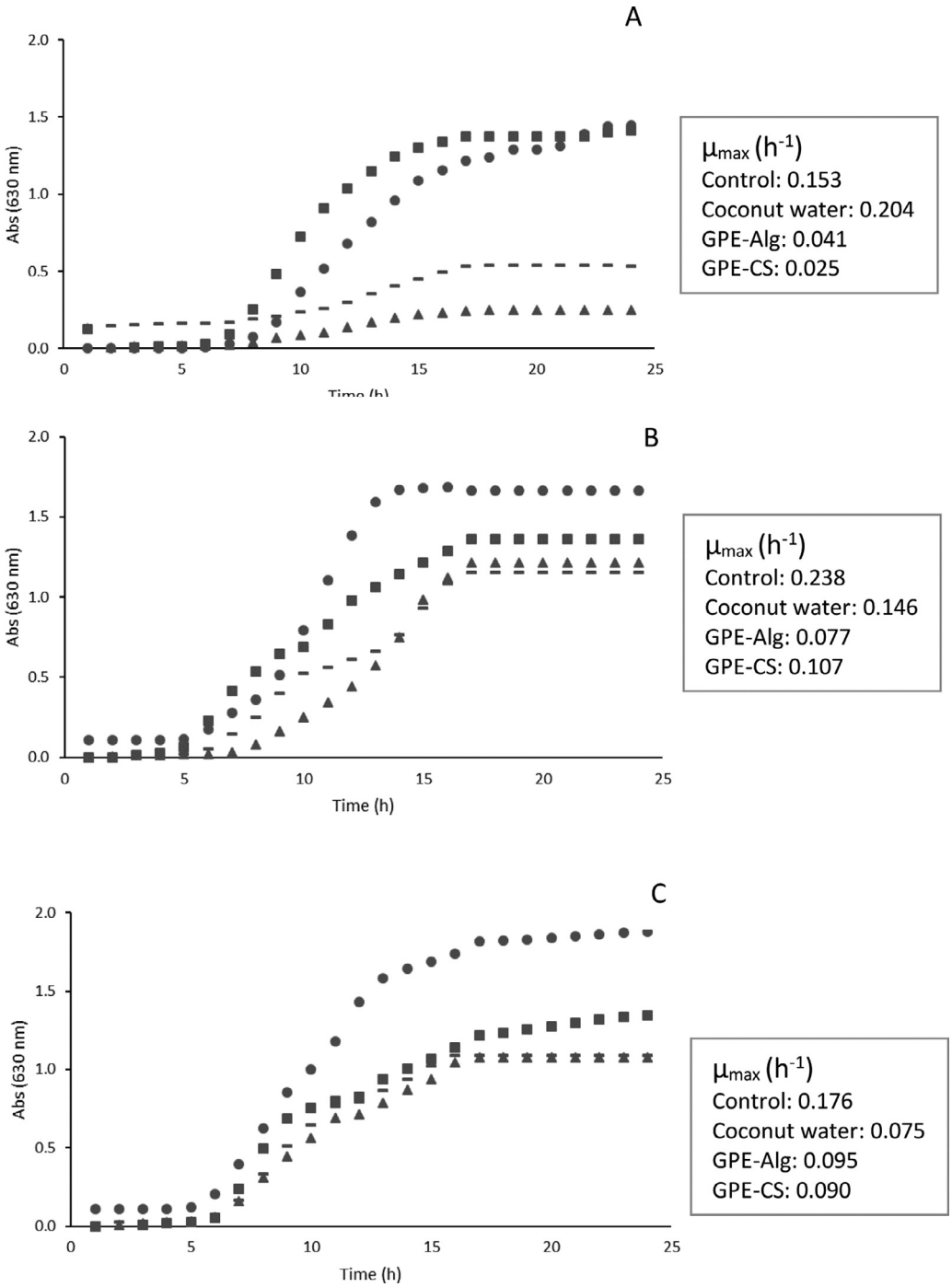

Fig. 1. Growth inhibition curves of coconut water (□), GPE-Alg coconut beverage (-) and GPE-CS coconut beverage (△) against *Staphylococcus aureus (A), Listeria monocytogenes (B)* and *Candida albicans (C)* growth curves.

had similar antimicrobial behavior upon *C. albicans* and *L. monocytogenes*, but the activity of digested beverage with GPE-CS upon *S. aureus* was higher than GPE-Alg beverage. Using a concentration of only 2.5% (w/v), both functional coconut beverages decreased the growth of *S. aureus* after 24 h of incubation and the growth of *C. albicans* and *L. monocytogenes* after 12 h of incubation. Although GPE-Alg and GPE-CS beverages could not inhibit the growth of *L. monocytogenes* and *C. albicans*, they were able to retard the microbial growth. For L. *monocytogenes*, the growth rate decreased from 0.238 h^{-1} to 0.077 h^{-1} and 0.107 h^{-1} in the presence of GPE-Alg and GPE-CS, respectively. Regarding *C. albicans*, the growth rate decreased from 0.176 h^{-1} to 0.095 and 0.090 with the incorporation of GPE-Alg h^{-1} and GPE-CS h^{-1}, respectively. Nevertheless, it is possible to observe that the control coconut water also had effect on the growth of *C. albicans*, even without the incorporation of GPE. This inhibition could be explained by the high diversity and concentration of flavonoids present in coconut, including epicatechin, epigallocatechin, catechin, apigenin, rutin, kaempferol, quercetin and myricetin, which specifically showed antifungal activity against *C. albicans* (Shahzad et al., 2014; Arivalagan et al., 2018).

Unlike the potential described for other coconut components, such as oil, control coconut water did not have antimicrobial potential, mostly due to the high content in water, it is severely prone to contaminations (Oliveira et al., 2018). Nevertheless, these results are somehow in accordance with the antimicrobial properties of a fermented coconut

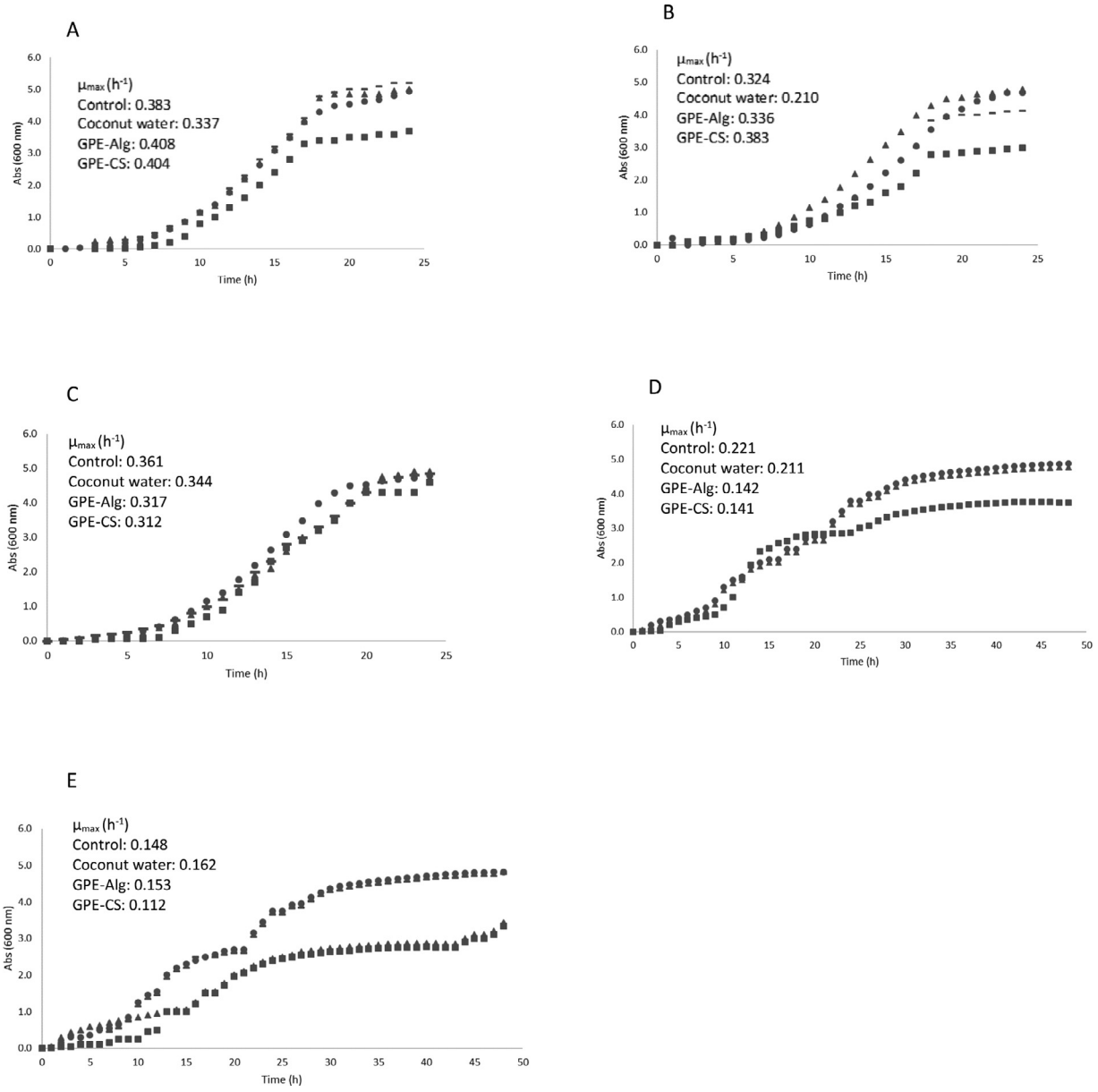

Fig. 2. Growth curves of *Lactobacillus plantarum* (A), *Lactobacillus rhamnosus* R11 (B), *Lactobacillus casei* 01 (C), *Bifidobacterium animalis* subsp. *lactis* BB-12 (D) and *Bifidobacterium animalis* Bo (E) with FOS (●), coconut water (■), GPE-Alg coconut beverage (-) and GPE-CS coconut beverage (▲)

beverage developed by Kantachote et al. (2017) that successfully inhibited *Staphylococcus aureus, Bacillus cereus and Listeria monocytogenes*, although the characteristic low pH of fermented beverages could additionally increase this effect. Furthermore, Prado and co-workers isolated lactic acid bacteria from coconut water and tested for antimicrobial capacity against different intestinal pathogens, observing high inhibitory capacity against *Staphylococcus aureus* but no significant effect against *Listeria monocytogenes* (Prado et al., 2015).

The potential of functional GPE-Alg and GPE-CS coconut beverages to inhibit the growth of S. aureus and hold back the growth of other microorganisms responsible for the proliferation of intestinal infections, possibly reducing its severe symptoms such as diarrhea and consequently, dehydration.

3.1.2. Impact on probiotic bacteria

The impact of the coconut beverages on probiotic bacteria was studied using five strains in basal MRS medium without glucose, at concentrations of 2% (w/v) of each pre-digested coconut beverage. Fig. 2 presents the growth of evaluated bifidobacteria and lactobacilli strains, as measured by turbidity at 660 nm. Frutooligosaccharides (FOS) at the same concentration were also used as positive control at 2% (w/v). All the probiotic microorganisms grew in the presence of both coconut beverages, increasing their growth (OD at 660 nm) along the fermentation period. FOS proved to be the most efficient prebiotic component, except for *Lactobacillus plantarum*, in which digested beverage with GPE-Alg and GPE-CS promoted a higher OD. In the case of *L. rhamnosus* and *L. casei* growth, coconut water control and incorporating GPE-Alg and GPE-CS differences between themselves neither from FOS. In the case of BB-12, both functional beverages presented a prebiotic potential similar to FOS, while the growth in presence of coconut water control was lower. At last, for *B. animalis* Bo, it was verified a decrease of growth after 20 h, in the presence of FOS, which was not observe in the presence of functional beverages incorporating GPE-Alg and GPE-CS. Although coconut water is rich in sugars that are able to stimulate the growth of probiotic microorganisms, the incorporation of the GPE-Alg and GPE-CS promoted an enhanced prebiotic potential effect, due to the presence of

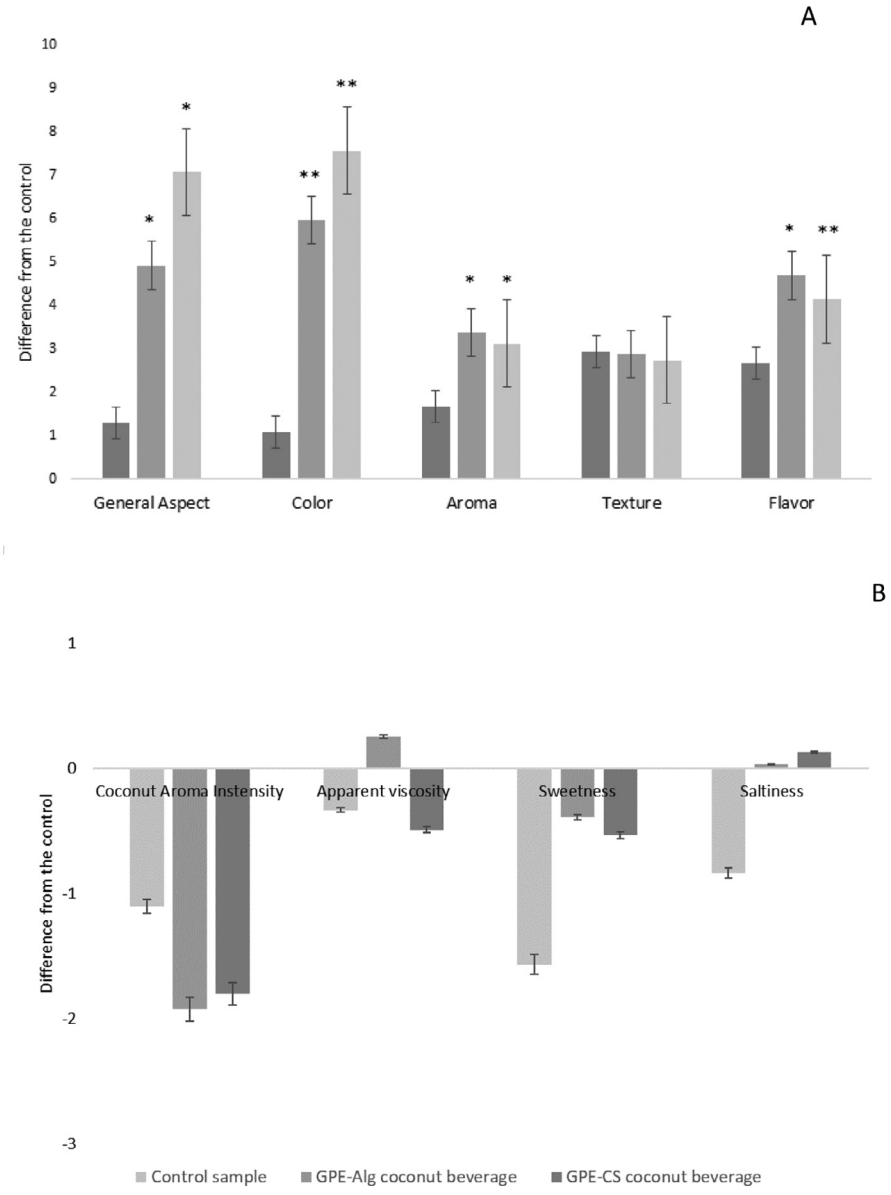

Fig. 3. Comparison of sensory attributes between control beverage and the blind control, GPE-Alg and GPE-CS coconut beverages evaluated using a (A) continuous line scale from 0 to 10 or (B) continuous bipolar line scale. Differences considered significantly at $p < 0.05$.* means differences at $p < 0.05$ and ** $p < 0.01$ attribute differences from the control evaluated.

xylooligosaccharides that are able to change the composition of short chain fatty acids, increased faecal weight and mineral absorption, and decreased colonic pH values (Costa et al., 2019b).

Based on maximum specific growth rates (μ_{max}) achieved, lactobacilli exhibited higher capacity to growth and specifically, *Lactobacillus plantarum* exhibited the best capacity to growth, achieving the fastest growth in the presence of coconut water supplemented with GPE-Alg (0.408 h^{-1}). GPE-CS presented a slightly lower μ_{max} value of 0.404 h^{-1}, but without statistical differences ($p > 0.05$), and both functional beverages presented significant higher ($p < 0.05$) μ_{max} than FOS. *L. rhamosus* was grew faster in the presence of GPE-CS (μ_{max} 0.383) than with GPE-Alg (μ_{max} 0.336), but both presented better results than FOS, which μ_{max} was 0.324. La01 and Bb12 grew faster with FOS than with the functional beverages. Finally, for *Bifidobacterium* B0 the growth rates in the presence of GPE-Alg (0.153 h^{-1}) and FOS (0.148 h^{-1}) were similar and significantly higher than the μ_{max} with GPE-CS (0.112 h^{-1}). Coconut fermented beverages are a novel alternative to fermented dairy beverages, coconut functional beverages incorporating GPE-Alg and GPE-CS can be a potential alternative to these fermented drinks, as they can also play a relevant role in the modulation of intestinal microflora (promoting gut healthy bacteria and inhibiting gut pathogens), avoiding the inherent difficulties associated to the stabilization of probiotic strains on food matrices.

3.2. Sensory evaluation

In order to evaluate the sensorial differences in coconut water beverage triggered by the introduction of GPE-Alg or GPE-CS, a sensory evaluation test was performed by nine semi-trained panelists. Fig. 3 displays the averages of differences at day 0 between evaluation of coconut water control and coconut water beverage with GPE-Alg or GPE-CS.

No significant differences ($p > 0.05$) in texture, coconut aroma intensity, sweetness and saltiness, were observed between the (blind) the coconut water control and the coconut water beverage with GPE-Alg or GPE-CS. Significant differences ($p < 0.05$) were found for general appearance and aroma for both the coconut water beverage with GPE-Alg and GPE-CS and for flavor of beverage with GPE-Alg. Differences between the flavor of beverage with GPE-CS and the control were also considered significant ($p < 0.01$), which could be related with the slight bitter flavor of chitosan and with its inability to mask the bitterness of GPE provided by some phytochemicals. The major difference ($p < 0.01$) was regarding the color of the beverage, which was somehow expected

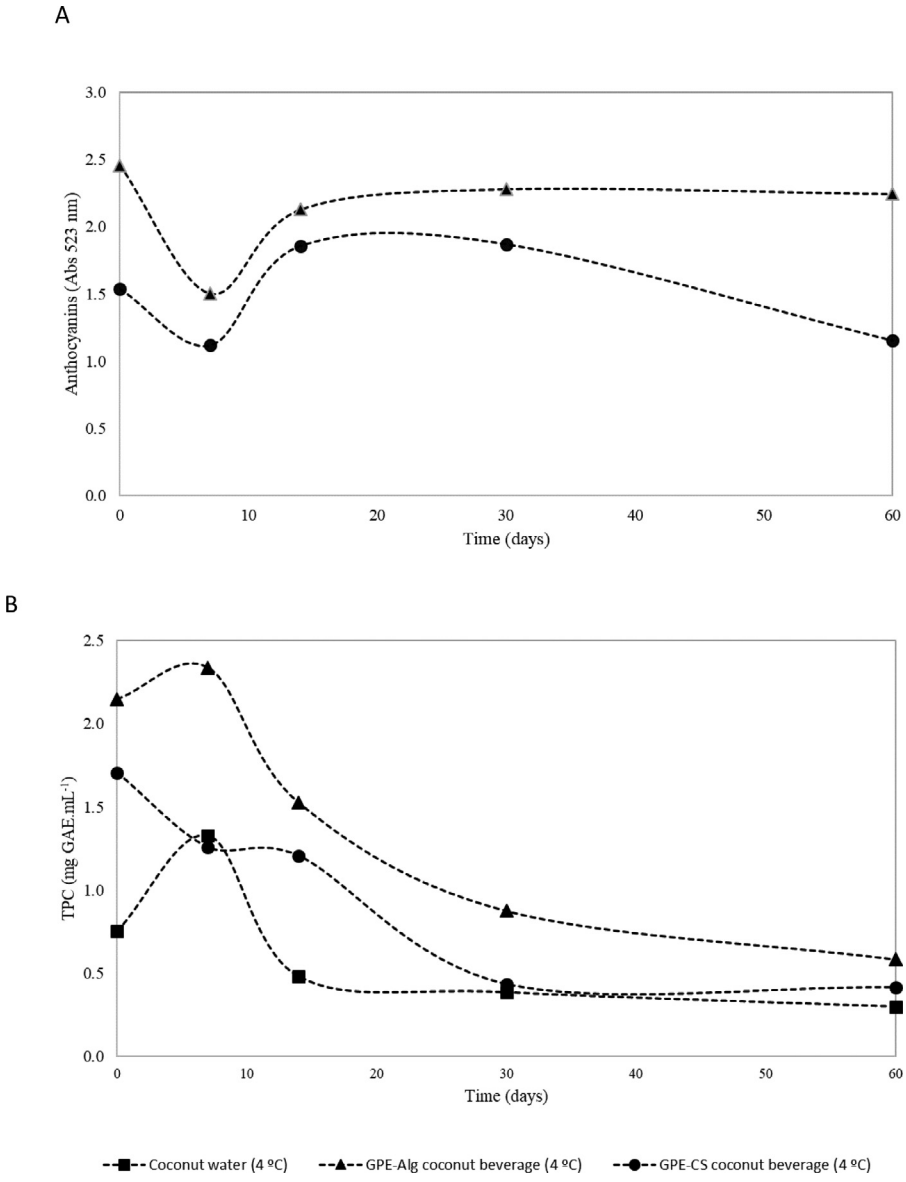

Fig. 4. Stability of (A) anthocyanins as absorbance at 523 nm and (B) total phenolic compounds present in coconut water control, and GPE-Alg and GPE-CS coconut beverages along 60 days of storage.

due to the GPE content in anthocyanins, which are able to provide a pinkish color and corroborates the results for color evaluation.

These results allow to conclude that the incorporation GPE-Alg or GPE-CS into coconut water did not affect most of the coconut water sensory attributes, although some optimization process could be performed to decrease the differences in flavor. A consumer study should also be performed in future to analyze the acceptance of this type of functional beverages.

Table 1
Kinetic reaction parameters for phenolic compounds stability in all coconut beverages.

Formulation	Rate constant k	R^2	t $\frac{1}{2}$ (days)
Coconut Water	0.0272	0.72	25.5
GPE-Alg coconut beverage	0.0272	0.99	25.5
GPE-CS coconut beverage	0.0379	0.60	20.3

3.3. Stability studies

Microbiological control performed during the storage showed that the beverages were stable along the 60 days of storage as no viable cells were detected in all culture media used.

The degradation profile of phenolic compounds is presented in Fig. 4a and the parameters of the kinetic reaction are provided in Table 1. Coconut water and coconut water beverage with GPE-Alg followed a similar profile of TPC degradation, with a slight increase of TPC after 7 days of storage, under both conditions, followed by an accentuate degradation until 14 days and a slight decrease until the end of the storage period. Coconut water beverage with GPE-CS presented a continuous degradation of TPC, presenting the main reduction between the 0 and 7 days of storage. Coconut water beverage with GPE-Alg presented the higher concentration of TPC during the first seven days of storing, but after 14 days the beverages stored at 4 °C presented the highest concentration of those compounds. After the 60 days of storage, all the systems presented similar concentrations.

The kinetic reaction order of TPC degradation was calculated using a first-order model ($0.46 < R^2 < 0.99$) for all the formulations, as described by Chung et al. (2016). The degradation profile of anthocyanins is presented in Fig. 4b and the parameters of the kinetic reaction are provided in Table 2. The kinetic reaction order of anthocyanins degradation was calculated using a first-order model ($R^2 = 0.99$) for all the

Table 2
Kinetic reaction for anthocyanins stability in coconut beverages containing GPE-Alg or GPE-CS

Formulation	Rate constant k	R^2	t $\frac{1}{2}$ (days)
GPE-Alg coconut beverage (4°C)	0.0286	0.99	24.3
GPE-CS coconut beverage (4°C)	0.0078	0.99	89.3

formulations, as described by Chung et al. (2016). The coconut water beverage with GPE-Alg presented higher half-life time, 25 days, than the coconut water beverage with GPE-CS that presented a half-life of 20 days. Regarding anthocyanins, coconut water beverage with GPE-CS presented higher half-life time of anthocyanins than coconut water beverage with GPE-Alg. When in solution media, like liquid beverages, anthocyanins may undergo different degradative reactions due to the high water activity, gradually reducing with other phenolic compounds into polymeric pigments (Monteiro et al., 2017). Furthermore, the origin and type of anthocyanin as well as the matrix where they are included can influence the stability of the anthocyanin, although there are scarce studies on the stability of polyphenols in fruit-derived beverages neither the potential of their encapsulation. Nonetheless, the degradation of anthocyanins occurred at a similar rate than the other polyphenols, when encapsulated into alginate particles, and at a slower rate when encapsulated into chitosan, confirming that, under the optimal storage conditions, the anthocyanin content in fruits and fruit-extracts remains unchanged or eventually increase during the storage (Beer et al., 2012).

4. Conclusions

Coconut water as a relevant by-product from ready to eat or processed fruit industry, may act as a sustainable beverage matrix for the incorporation of encapsulated bioactive grape pomace extract to produce a functional coconut beverage. The incorporation of GPE encapsulated into alginate or chitosan microparticles to produce for the first time functional coconut beverages, allowing the bioactive molecules to reach the intestine with minimal losses during the gastrointestinal digestion without affecting most sensory attributes of coconut water. The potential antimicrobial activity of digested functional coconut water beverages against intestinal pathogens was validated and related to the high level of total phenolics and total anthocyanins.

This work allows to demonstrate the potential of a coconut-based functional beverage to modulate the intestinal microflora, which in addition to the hydrating capacity and high mineral content of the coconut water, can be used as a co-adjuvant in the treatment of severe diarrheas, protecting the intestine and restoring hydration. As non-dairy functional beverages, coconut beverages incorporating grape pomace extract encapsulated into alginate or chitosan nanoparticles can also be consumed by vegetarians and lactose-intolerant consumers.

Declaration of Conflict Interest

We confirm that this work is original and has not been published elsewhere nor is it currently under consideration for publication elsewhere. The authors declare that there is no conflict of interest.

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