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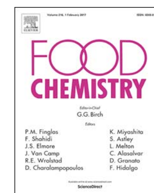

Effect of nanoencapsulation using PLGA on antioxidant and antimicrobial activities of guabiroba fruit phenolic extract

Marina C. Pereira[a], Daniela A. Oliveira[b], Laura E. Hill[b], Rui Carlos Zambiazi[a], Caroline D. Borges[a], Marcia Vizzotto[c], Susanne Mertens-Talcott[d], Stephen Talcott[d], Carmen L. Gomes[b,*]

[a] Department of Food Science and Technology, Federal University of Pelotas, Faculdade de Agronomia Eliseu Maciel, Campus Capão do Leão, Pelotas, RS 96010-900, Brazil

[b] Department of Biological & Agricultural Engineering, Texas A & M University, College Station, TX 77843-2117, United States

[c] Embrapa Temperate Agriculture, Pelotas, RS 96010-971, Brazil

[d] Department of Nutrition and Food Science, Texas A & M University, College Station, TX 77843-2253, United States

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ABSTRACT

Guabiroba fruit has been highlighted for its high phytochemical content, particularly of phenolic compounds. The stability, bioavailability, and bioactivity of these compounds can be enhanced by nanoencapsulation, to improve functionality. Poly(D,L-lactic-co-glycolic) acid (PLGA) nanoparticles containing phenolic extract of guabiroba (GPE) were synthesized by an adapted emulsion-evaporation method and their physico-chemical and functional properties were studied at two lactic to glycolic acid ratios (50:50 and 65:35). Higher ($P < 0.05$) or equivalent antioxidant capacity compared to free GPE were observed for GPE-loaded nanoparticles. Free extract and PLGA nanoparticles were effective inhibitors of *Listeria innocua*, with lower ($P < 0.05$) GPE concentrations required for inhibition when nanoencapsulated. Also, reduction of ROS generation in non-cancer cells was achieved with lower GPE concentrations ($P < 0.05$) after encapsulation. These results suggest that PLGA nanoparticles can be used as a delivery system for phenolic compounds at lower levels than originally required for enhanced functional properties.

1. Introduction

The Southern regions of Brazil show great richness in wild fruits, among which the botanical family of Myrtaceae stands out for presenting the greatest number of species with food and pharmaceutical potential still underexplored. In particular, guabiroba fruit (*Campomanesia xanthocarpa* O. Berg.), showed the highest antioxidant activity among three Brazilian native fruits from the Myrtaceae family in a recent study, mainly due to its high content of phenolic compounds, vitamin C and carotenoids (Pereira, Steffens, Jablonski, Hertz, & Rios, 2012). The major phenolic compounds in the guabiroba fruit are epicatechin, gallic acid, ellagic acid, ferulic acid and p-coumaric acid (Hass, 2011). However, little information is available on the potential application of extracts of these fruits for use as natural value-added preservatives and antioxidants by the food and pharmaceutical industry. More recently, another study by our research group reported that PLGA nanoparticles containing the hydrophobic extract of guabiroba with carotenoid compounds showed inhibition of *Listeria innocua* growth, and reactive oxygen species reduction, with significantly lower extract concentration than required by the free extract, although equivalent or higher concentrations for free radical scavenging were required (Pereira et al., 2015). Further studies are needed to fully understand the effect of encapsulation on the functional properties of fruit extracts, especially, phenolic compounds.

Naturally occurring phenolic compounds such as phenolic acids, anthocyanins, flavonols and flavan-3-ols have gained great interest due to their functional properties, such as antioxidant, anti-inflammatory, anticarcinogenic and antimicrobial effects (Friedman, 2007). However, their application has been limited due to their instability during food and pharmaceutical processing, storage (temperature, light, pH, oxygen and interaction with other food components and ingredients), and during digestion (pH, enzymes, interaction with of other nutrients) (Fang & Bhandari, 2010). Encapsulation of these compounds using nanotechnology approaches could address some of these problems and enhance their functional properties (Lu, Kelly, & Miao, 2016). Biodegradable polymeric nanoparticles have been extensively used as

* Corresponding author at: 303B Scoates Hall, Biological & Agricultural Engineering, Texas A & M University, College Station, TX 77843-2117, United States.
E-mail address: carmen@tamu.edu (C.L. Gomes).

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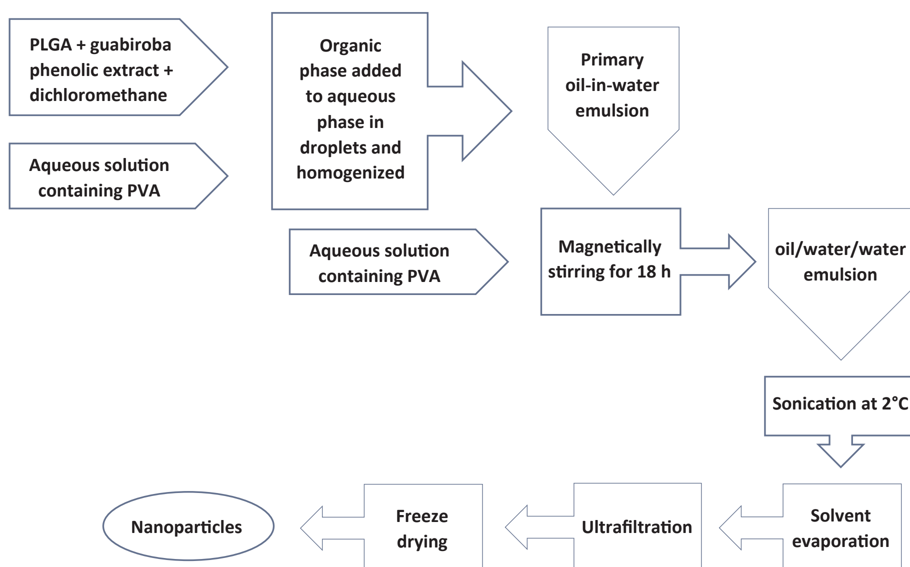

Fig. 1. Flow diagram of PLGA nanoparticle synthesis with entrapped guabiroba fruit phenolic extract by the modified emulsification-evaporation method.

delivery systems of different active components such as anticancer drugs, vitamins, proteins, peptides and others. The advantages are numerous, since polymeric nanoparticles protect active compounds from degradation, improves their solubility, and promotes controlled drug release and targeting (Kumari, Yadav, & Yadav, 2010). Synthetic polymers have an advantage of higher purity and reproducibility over natural polymers. In particular, polylactic-co-glycolic acid (PLGA), has been approved by the US Food and Drug Administration for 14 pharmaceutical and biomedical applications (Wang, Qu, & Choi, 2016), meaning it is safe under the conditions of its intended use. It is of great interest to the biomedical field because of its biocompatibility, biodegradability and controlled delivery properties (Stevanović & Uskoković, 2009).

Studies have previously shown that nanoparticle synthesis parameters including polymer composition and weight, synthesis method, surfactant, surface charge, hydrophobicity and particle size, directly affect nanoparticle physicochemical properties and active compound release (Stevanović & Uskoković, 2009). These properties will ultimately affect the active compound's functionality and their use in food and pharmaceutical products. As a drug carrier, PLGA nanoparticle-based delivery systems have been broadly studied for controlled delivery applications; however, little information is available on the use of PLGA nanoparticles to enhance hydrophilic phytochemical compound functionalities such as their antioxidant, anti-inflammatory and antimicrobial activities, particularly those of phenolic compounds.

This study aimed to synthesize PLGA nanoparticles for delivery of phenolic extracts with enhanced functional properties. Hydrophilic extract of guabiroba fruit was used as a model plant extract as a continuation to the previous study of Pereira et al. (2015) that used the hydrophobic extract (rich in carotenoids) of the same fruit. The objective of the present study was to apply a modified emulsion-evaporation encapsulation method, different than the single-emulsion method applied on the previous study (Pereira et al., 2015), to synthesize PLGA (same monomer ratios as the previous study, PLGA 50:50 and PLGA 65:35) nanoparticles loaded with the phenolic extract and analyze its physico-chemical and functional properties (antimicrobial and antioxidant activities) to determine its efficiency to encapsulate the hydrophilic guabiroba fruit extract.

2. Materials and methods

2.1. Plant material

Guabiroba fruits (*Campomanesia xanthocarpa* O. Berg) were obtained from a local farm in the state of Rio Grande do Sul, Brazil. Fully ripe fruits were preselected for size and color uniformity and the absence of visible injury and infection. Seeds were discarded and the fruits were freeze-dried (−50 °C) under 1.09 Pa at the Federal University of Pelotas, Pelotas, RS, Brazil. Samples were shipped to Texas A & M University, College Station, TX, USA, where they were kept at −20 °C until extraction assays were conducted.

2.2. Phenolic extract of guabiroba fruit

The phenolic extract of guabiroba fruit (GPE) was obtained according to the method described by Wrosltad (2005) for phenolic compound extraction. Briefly, 200 mg of the freeze-dried fruit and 4 mg of ascorbic acid (to prevent oxidation of polyphenolics during extraction) were suspended in methanol (10 ml) and thoroughly mixed using an Ultra-Turrax homogenizer for 5 min. The receptacle with the sample was placed inside a beaker with water at 4 °C to avoid heating during homogenization. Then, the extract was filtered through qualitative paper (Whatman 4, GE Healthcare Life Sciences, Piscataway, NJ, USA) and concentrated under vacuum (6.7 kPa) at room temperature in a rotary evaporator (Buchi R-210 Rotavapor, BuchiCo., New Castle, DE, USA) until a final volume of 5 ml was obtained, corresponding to 160 mg of dried methanol extract. The extract was prepared immediately before use.

2.3. Nanoparticle synthesis

Nanoparticles were synthesized by an adapted emulsion-evaporation method (Fig. 1) from Zigoneanu, Astete, and Sabliov (2008) and Pereira et al. (2015). An organic phase was prepared containing 50 mg of PLGA with a ratio of lactic to glycolic acid of 65:35 or 50:50 (MW 40,000 to 75,000 g/mol, Sigma-Aldrich, St. Louis, MO, USA) in 2 ml of dichloromethane (CTL Scientific Supply Co., Deer Park, NY, USA) and 5 ml of the methanolic GPE. The aqueous phase (20 ml) was prepared with 0.3 g/100 ml PVA (Poly(vinyl alcohol), MW 30,000 to 50,000 g/mol, Sigma-Aldrich) in nanopure water (Macron Chemicals, Charlotte, NC, USA). A primary oil-in-water emulsion was formed by the dropwise addition of the organic phase to the aqueous phase while homogenizing at 13,000 rpm (Ultra-Turrax T25 basic Ika, Works, Wilmington, NC, USA) for 2 min. Next, this emulsion was added to 20 ml of a second PVA solution (0.3 g/100 ml) and magnetically stirred at room temperature and protected from light for 18 h. After that, this oil-in-water-in-water emulsion (o/w/w) was sonicated for 30 min in an ice bath (2 °C) at 70 W (Cole Parmer 8890 ultrasonic cleaner, Vernon Hills, IL, USA). The

organic phase (methanol and dichloromethane) was evaporated at 25 °C under vacuum using a rotary evaporator. The same procedure without the addition of GPE in the organic phase was used to produce unloaded nanoparticles to be used as control during further analysis. After synthesis, nanoparticles were purified by ultrafiltration using a Millipore-Labscale™ TFF system (10 kDa cutoff Pellicon XL-Millipore, Millipore Co., Kankakee, IL, USA). D(+) trehalose (cryoprotectant, EMD Chemicals, Philadelphia, PA, USA) was added at a 1:1 (w/w) ratio relative to nanoparticles. The samples were frozen (−80 °C) and freeze-dried (−50 °C) under 1.09 Pa for at least 48 h. Lyophilized samples were stored at -20 °C and used within one week.

2.4. Entrapment efficiency (EE)

The EE of GPE in the PLGA nanoparticles was determined by total phenolic content analysis using Folin-Ciocalteu assay (Wrosltad, 2005). GPE-loaded nanoparticles (10 mg) were suspended in 5 ml of 95 ml/100 ml methanol in water, mixed well, and left in the dark for 1 h at room temperature. Each sample was filtered with a 0.2-μm filter (Acrodisc, Pall Life Sciences, Port Washington, NY, USA) and 0.25 ml of sample was mixed with 0.25 ml Folin-Ciocalteau reagent (Fisher Scientific, Pittsburgh, PA, USA), 4 ml of water, and 0.5 ml of 20 g/100 ml sodium carbonate (Sigma-Aldrich). Next, samples were allowed to stand for 2 h at room temperature protected from light, and the absorbance was measured at 765 nm (Shimadzu UV-1601 spectrophotometer). A standard curve was prepared using gallic acid as the standard to quantify the total phenolic compound concentration (expressed as gallic acid equivalents/100 g GPE dry basis). For all further analyses, the values for GPE equivalent content in loaded nanoparticles were calculated based on EE. EE was calculated according to Eq. (1) (Teixeira, Ozdemir, Hill, & Gomes, 2013):

$$EE = \frac{amount\ of\ active\ compound\ entrapped}{initial\ active\ compound\ amount} \times 100 \tag{1}$$

2.5. Nanoparticle morphology, size and size distribution

Transmission Electron Microscopy (TEM) (FEI Morgagni, FEI Co., Hillsboro, OR, USA) was used to evaluate nanoparticle morphology. A droplet of particles (1 mg/ml in water) was placed on 0.037 mm copper grids and stained with a 2 g/100 ml uranyl acetate aqueous stain (Electron Microscopy Sciences, Hatfield, PA, USA) for contrast under magnification. The sample was allowed to air dry before viewing under 36,000 to 89,000 times magnification. Observations were performed at 80 kV.

A Delsa TM Nano C Particle Analyzer (Beckman Coulter, Brea, CA, USA) was used to measure nanoparticle size and distribution (polydispersity). Nanoparticles were suspended in water (10 mg/ml) at 25 °C and sonicated for 15 min before analysis at a 165° scattering angle, with a pinhole set to 20 μm, and a refractive index of 1.3328 for 120 continuous accumulation times (Hill, Taylor, & Gomes, 2013).

2.6. In vitro release study

Lyophilized nanoparticles were suspended in a release medium, phosphate buffered saline (PBS, pH 7.2) at a 10 mg/ml concentration, which was sufficient to establish sink conditions, and divided into 2-ml Eppendorf microcentrifuge tubes. Tests were performed at 37 °C and 100 rpm (Orbit Shaker, Lab Line Instruments, Melrose Park, IL, USA) with samples taken at predetermined time intervals, filtered with a 0.2-μm Acrodisc filter (Hill et al., 2013), and 0.25 ml was collected to carry out the total phenolic content analysis as described in Section 2.4.

2.7. Antioxidant activity

Oxygen radical absorbance capacity (ORAC) assay: Free GPE, unloaded and GPE-loaded nanoparticles were monitored for their ability to inhibit oxidation in the presence of the peroxyl radicals generator 2,2′-azobis(2-amidinopropane) dihydrochloride (Sigma-Aldrich). A standard curve of Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid, Tokyo Chemical, Tokyo, Japan), a water soluble analogue of vitamin E, with concentrations ranging from 3 to 50 μmol/l, was used to calculate the results, expressed in μmol/l Trolox Equivalent/g of GPE. The samples were diluted in pH 7.2 phosphate buffer prior to pipetting into a 96-well microplate for analysis, as previously described by Ou, Hampsch-Woodill, and Prior (2001), with fluorescein-sodium salt (Sigma-Aldrich) as a fluorescent probe. The fluorescence loss was monitored on a FLUOstar Omega plate reader (BMG LabtechInc, Durhan, NC) at 485 nm excitation and 520 nm emission.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging: unloaded and GPE-loaded nanoparticles (100 mg) were suspended in water (1 ml). Free GPE was suspended in methanol (40 mg/ml) as previously described (Section 2.2). These solutions were thoroughly mixed and 6 different dilutions (ranging from 1500 to 7500 μg/ml of GPE for each treatment with corresponding dilutions for unloaded nanoparticles) were prepared. Each sample dilution (0.1 ml) was added to 3.9 ml of DPPH radical solution (0.1 mmol in methanol, Sigma-Aldrich), vigorously mixed, and, after a 30 min incubation in the dark at room temperature, their absorbance values were measured at 517 nm (Brand-Williams, Cuvelier, & Berset, 1995). The results were expressed as antioxidant concentration required to reduce the original amount of free radicals by 50% (IC50), and the values were expressed as grams of GPE/gram of DPPH.

2.8. Reactive oxygen species (ROS) generation assay

Cell culture: human colon adenocarcinoma HT-29 cancer and non-cancer CCD-18Co colon fibroblast cells were purchased from ATCC (Manassas, VA, USA). HT-29 cells were cultured using McCoy's-5a modified medium with 1 g/100 ml penicillin/streptomycin and 10 g/100 ml fetal bovine serum (FBS). CCD-18Co cells were cultured using high glucose Dulbecco's Modified Eagle Medium with 1 g/100 ml penicillin/streptomycin, 1 g/100 ml non-essential amino acids (10 mmol/l), 1 g/100 ml sodium pyruvate (100 mmol/l) and 20 g/100 ml FBS (Invitrogen, Carlsbad, CA, USA). Cells were kept at 37 °C in a humidified incubator with 5% CO_2 atmosphere and seeded in 96-well plates (10,000 cells/well); then grown for 24 h to allow cell attachment before exposure to different GPE equivalent concentrations for each treatment (202.2 μg/ml, 24.0 μg/ml and 20.2 μg/ml GPE for free extract, loaded-PLGA 50:50 and PLGA 65:35, respectively). Nanoparticle and free extract concentrations were chosen based on preliminary tests over a range of concentrations that showed initial protective effect against ROS generation for both cell types. Both cells were also treated with 0.2 g/100 ml of methanol and 5 g/100 ml of unloaded PLGA nanoparticles in water to ensure nanoparticle encapsulant materials and solvent had no inhibitory effect or cell toxicity. ROS production was determined according to Meng, Velalar, and Ruan (2008). ROS generation was induced in CCD-18Co cells with 100 μmol/l of H_2O_2 for each treatment for 2 h, but not in HT-29 cells, since cancer cells present higher levels of intracellular oxidative stress than normal cells, usually producing more ROS than normal cells (Schumacker, 2006). Cells were washed with PBS and ROS generation was measured after incubation with 100 μmol/l of 2′,7′-dichlorofluorescein diacetate (Sigma-Aldrich) at 37 °C for 30 min. Fluorescence intensity was used as an indicator for ROS generation and it was measured using a FLUOstar Omega plate reader (520 nm emission and 480 nm excitation).

2.9. Antimicrobial activity

Bacterial Culture: Listeria innocua culture (NRRL B-33076) was maintained on tryptic soy agar (TSA, Becton, Dickinson and Co. (BD),

Franklin Lakes, NJ, USA) containing 0.6 g/100 ml of yeast extract (TSAYE) slants at 4 °C. Working cultures were produced daily by transferring a loop of culture from TSAYE slants to 10 ml tryptose phosphate broth (TPB, BD) and incubating for 24 h at 35 °C. *L. innocua* was selected as a non-pathogenic surrogate for *L. monocytogenes* due to its importance to the food industry (Gravani, 1999).

Minimum inhibitory and bactericidal concentrations (MICs and MBCs): MIC and MBC values were determined using the broth dilution assay according to Brandt et al. (2010). *L. innocua* inoculum (3.0 $\log_{10}$ CFU/ml) was prepared in double strength TPB to enable the correct nutrients concentration in the final sample cuvette upon treatment addition. Free GPE and GPE-loaded nanoparticles were suspended in water and then serial dilutions were prepared to reach final concentrations ranging from 8000 to 50,000 μg/ml and 2000 to 10,000 μg/ml, respectively. Controls were also prepared containing inoculum and methanol or unloaded nanoparticles at test concentrations to ensure additives and encapsulant materials had no inhibitory effect on bacterial growth. Optical density (OD) readings of the samples at 630 nm were taken at 0 h and after incubation at 35 °C for 24 h to observe bacterial growth. Treatment solutions that showed ≤0.05 change in OD (after baseline adjustments) at 630 nm were considered inhibited. The MIC was the lowest treatment concentration that inhibited growth for all replicates. Treatments that displayed microorganism inhibition were then tested for bactericidal activity by spreading a 100 μl aliquot onto TSAYE plates and incubating for 24 h at 35 °C. The lowest treatment concentration showing no colony formation after incubation across all replicates was considered the MBC.

2.10. Statistical analysis

Determinations were made at least in triplicate as independent experiments based on a completely randomized design for all analyses. Statistical analysis was performed with a one-way analysis of variance (ANOVA) with Tukey test for significantly different means ($P < 0.05$) with JMP v.9 software (SAS Institute, Cary, NC, USA).

3. Results and discussion

3.1. Entrapment efficiency (EE)

Both PLGA nanoparticles EE values were within the same range of results previously reported for PLGA nanoparticles with entrapped phenolic compounds; for instance, EE values of 18 and 26% were reported for the entrapment of black (theaflavin) and green (epigallocatechin-3-gallate) tea compounds, respectively (Srivastava et al., 2013). The EE values for PLGA 50:50 were higher ($P < 0.05$) than PLGA 65:35 for the entrapment of GPE (Table 1). This is probably related to the difference in hydrophobicity between PLGA ratios. As PLGA 50:50 is more hydrophilic than PLGA 65:35 due to its higher glycolic acid content, and the phenolic extract of guabiroba fruit is also hydrophilic, consequently, the first seems to be more propitious for entrapping hydrophilic compounds (Budhian, Siegel, & Winey, 2005). The previous study of Pereira et al. (2015) supports this finding, as the entrapment efficiency of the hydrophobic extract of this same fruit (guabiroba) was significantly higher for PLGA 65:35 (more hydrophobic). The EE values for PLGA nanoparticles are also related to the solubility of the entrapped material in water (Pereira et al., 2012) as the hydrophilic compounds tend to move from the organic phase to the outer aqueous phase during nanoparticle synthesis and present weaker interactions with the polymer, resulting in lower EE compared to hydrophobic compounds (Astete & Sabliov, 2006; Stevanović & Uskoković, 2009). This can be further corroborated by the higher EE values reported by Pereira et al. (2015) for the hydrophobic extract of guabiroba fruit (83.7 and 98.5%, for PLGA 50:50 and 65:35, respectively), when compared to the phenolic extract of the same fruit (hydrophilic) in the present work (39.2 and 32.0%, respectively, for the same polymers).

Table 1
Entrapment efficiency (EE), average particle diameter, polydispersity index of poly-D,L-lactic-co-glycolic acid (PLGA) nanoparticles unloaded and loaded with guabiroba phenolic extract (GPE).

Nanoparticles	EE (%)[1]	Size (nm)	Polydispersity Index
Unloaded PLGA 65:35	–	144.8 ± 1.3[b]	0.24 ± 0.01[b]
PLGA 65:35 GPE	32.0 ± 1.7[b]	243.8 ± 79.7[a]	0.43 ± 0.09[a]
Unloaded PLGA 50:50	–	162.1 ± 0.6[a]	0.24 ± 0.01[b]
PLGA 50:50 GPE	39.2 ± 1.0[a]	202.5 ± 50.8[a]	0.37 ± 0.04[a]

PLGA with two lactic to glycolic acid ratios of 65:35 (PLGA 65:35) and 50:50 (PLGA 50:50) were used. Values are shown as the mean ± standard deviation of three independent repetitions.

[1] Values were obtained using the Equation of Teixeira et al. (2013).

[a,b] Means within a column which are not followed by a common superscript letter are significantly different ($P < 0.05$).

3.2. Nanoparticle morphology, size and size distribution

The effect of polymer type on size does not seem to be significant, except for the unloaded PLGA 65:35 particles, which were significantly smaller (Table 1). The higher hydrophobicity of PLGA 65:35 could explain the smaller size of the unloaded particles since they are less likely to aggregate (Astete & Sabliov, 2006). Silva, Hill, Figueiredo, and Gomes (2014) reported particle size values for PLGA 65:35 and 50:50 nanoparticles loaded with phenolic compounds and extracts to be between 140 and 250 nm, similar to the results obtained in the present work.

The polydispersity index (PDI) is a dimensionless measure of the particle's size distribution. The PDI values presented in Table 1 are considered high (above 0.10), ranging from 0.24 to 0.43, indicating polydisperse systems for all nanoparticles studied (Zigoneanu et al., 2008). PDI values of loaded particles were significantly higher than unloaded particles. Similar results were reported previously for studies with entrapped phenolic compounds and extracts that utilized similar synthesis methods and emulsifiers with polydispersity values significantly lower (PDI = 0.173) for unloaded PLGA 50:50 particles than the ones loaded with quercetin and catechin (PDI = 0.262 and 0.390, respectively) (Pool et al., 2012). The unloaded particles tended to be slightly smaller in size than those with entrapped GPE, similar to results found in other studies and attributed to the increased viscosity of the organic phase in the presence of the compounds to be encapsulated. This makes it more difficult to disperse the phases (i.e., diffusion of methanol to the aqueous phase) during the emulsification process and leads to larger particles (Mainardes & Evangelista, 2005). Moreover, micelles formed with GPE and PVA (surfactant) during the emulsification process contributed significantly to increase the PDI values of loaded particles.

The TEM images revealed that spherical-shaped nanoparticles were formed with a dark ring at the surface associated with the PVA (Fig. 2) similar to previous studies (Hill et al., 2013; Zigoneanu et al., 2008). The PVA chain alternates hydrophilic and hydrophobic segments. The affinity of PVA with water pulls its hydrophilic regions to the surface of micelles, exposing them to the water phase; whereas hydrophobic segments interlink with the PLGA chains to create a matrix and shift to the inner core of the micelles (Gomes, Moreira, & Castell-Perez, 2011; Zigoneanu et al., 2008). The size of the nanoparticles estimated from TEM images is in agreement with the particle sizes measured by the particle analyzer, also showing a broad range of particle sizes. Furthermore, TEM images for loaded particles (Fig. 2C, D, E and F) showed a tendency to aggregate forming clusters, which explains the broad range of particle sizes and the high polydispersity results observed in Table 1. This agglomeration tendency was also observed by Silva et al. (2014) and Gomes et al. (2011), and is likely due to insufficient steric stabilization by the PVA, which is a non-ionic surfactant.

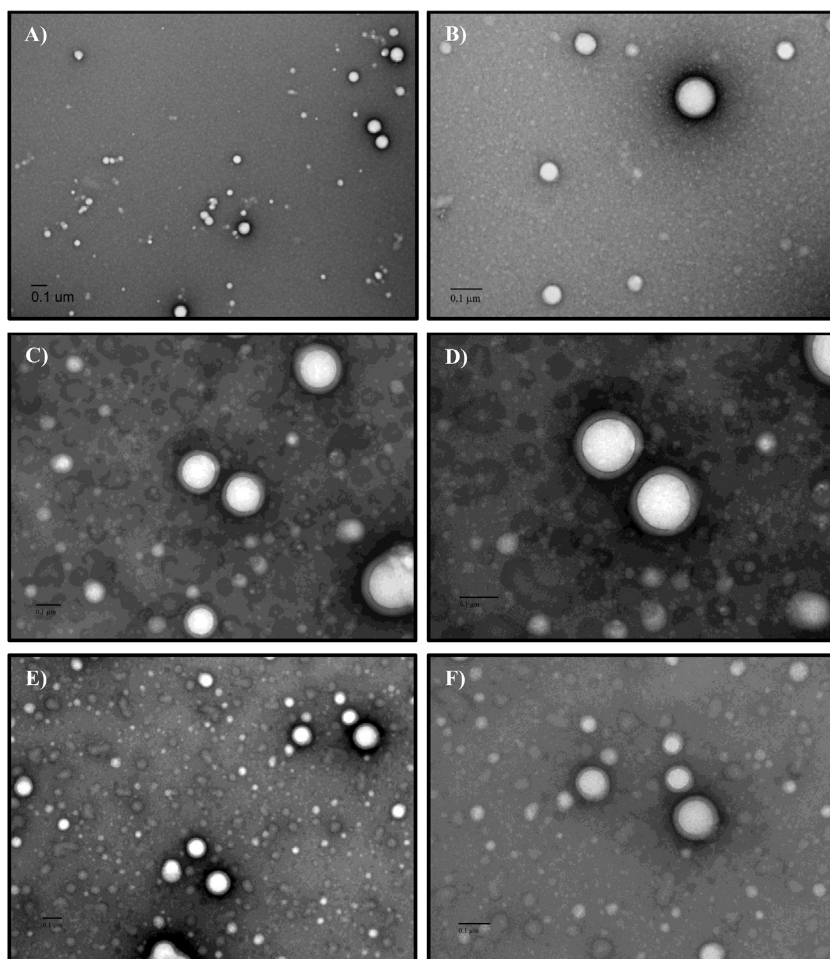

Fig. 2. Transmission electron microscope (TEM) images of PLGA nanoparticles loaded and unloaded with guabiroba fruit phenolic extract (GPE). Images are representative of samples and depict A) unloaded PLGA 65:35, B) unloaded PLGA 50:50; (C) and (D) GPE-loaded PLGA 65:35; (E) and (F) GPE-loaded PLGA 50:50. Images were taken at 80 kV with (a) 36,000, (b) 71,000, (c) 56,000, (d) 89,000, (e) 44,000, and (f) 71,000 magnification times. Scale bars are shown by horizontal lines.

3.3. Controlled release study

The phenolic compound release profiles, from both PLGA nanoparticles, followed a similar trend with an initial burst effect in the first hour, followed by a gradual decline in cumulative release over time (Fig. 3). PLGA 65:35 presented a more pronounced initial burst effect, reaching approximately 73% of phenolic compounds released in 15 min, while PLGA 50:50 reached 57%, and both nanoparticles showed similar release after 30 min, gradually declining to 42% and 32% phenolic compounds released by the end of 12 h, respectively. After 12 h, the release reached steady state (data not shown) over the remaining experiment period of 72 h. In this study, we worked with an extract and the release from nanoparticles was monitored through total phenolic content; therefore, the cumulative release profiles depended on each phenolic compound's polarity, where some of them can have a higher affinity for the polymeric matrix and others for the release medium. For reference, the free guabiroba fruit extract presented total phenolic content of 18.6 ± 0.9 g of gallic acid equivalents/100 g GPE dry basis. GPE phenolic compounds are mostly hydrophilic in nature. Thus, the burst effect could be explained by the fast release of hydrophilic compounds found close to or at the surface of the nanoparticles (Gomes et al., 2011; Zigoneanu et al., 2008). The higher initial burst from PLGA 65:35 would be attributed to the weaker interaction among hydrophilic compounds and polymer, since it has a lower content of glycolic acid, and consequently it is more hydrophobic. The opposite would be observed for PLGA 50:50 due to its higher hydrophilicity (Wischke & Schwendeman, 2008).

The decrease in cumulative release over time could be explained by the phenolic compounds present in the GPE being labile under the *in vitro* release conditions; indicating their degradation. The major phenolic compounds present in GPE consisting of epicatechin, gallic acid, ellagic acid, ferulic acid and p-coumaric acid (Hass, 2011) have been shown to be sensitive to oxidation, light and pH and to easily be oxidized by free radicals at a pH higher than 6.5 (Fang & Bhandari, 2010). Free GPE were suspended in the release medium under the same conditions to verify its stability and within the first few hours the level of total phenolic content was below the detection limit (data not shown). Therefore, the encapsulation allowed the preservation of 30 and 40% of the phenolic compounds up to 12 h for PLGA 65:35 and 50:50, respectively.

3.4. Antioxidant activity

GPE antioxidant activity in this study (Table 2) was higher ($P < 0.05$) than other native Brazilian fruits such as yellow mombim (1064 g/g DPPH, *Spondias mombin*), cashew apple (906 g/g DPPH, *Anacardium occidentale*), açai (598 g/g DPPH, *Euterpe oleracea*), gurguri (360 g/g DPPH, *Mouriri guianensis*), murta (363 g/g DPPH, *Blepharocalyx salicifolius*) and uvaia (276 g/g DPPH, *Eugenia pyriformis*) (Rufino et al., 2010). Rufino et al. (2011) reported a superior antioxidant capacity of the extractable polyphenols fraction of defatted Açaí 'BRS-Pará' (*Euterpe oleraceae*) pulp, 10.20 g/g DPPH and 379.97 µmol Trolox/g. Results show that GPE-loaded PLGA 50:50 nanoparticles presented higher ($P < 0.05$) antioxidant activity than free GPE and GPE-loaded PLGA 65:35, for the ORAC method (Table 2). Generally, a high Trolox equivalent number indicates a high antioxidant activity as compared to a standard antioxidant (Trolox) (Re et al., 1999). While for the DPPH method, the opposite was observed, free GPE and GPE-loaded

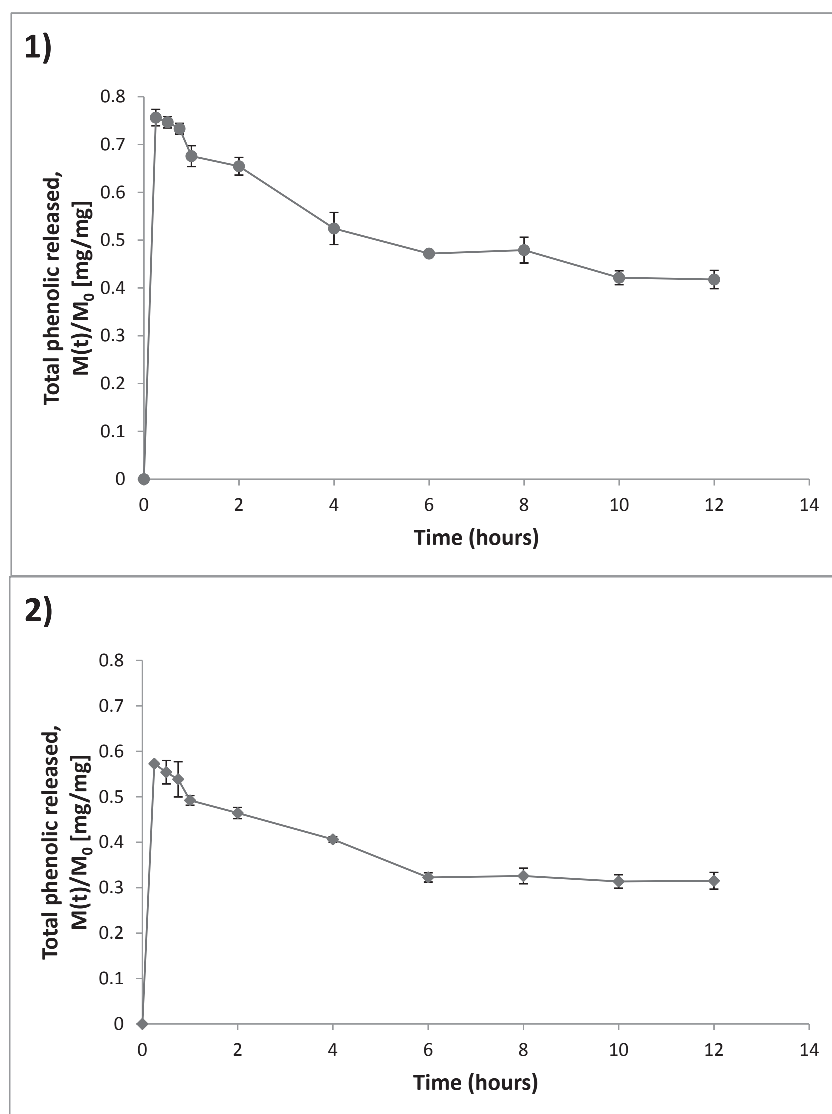

Fig. 3. Guabiroba fruit phenolic extract (expressed as total phenolic content) release kinetics from (1) PLGA 65:35 and (2) PLGA 50:50 nanoparticles in phosphate buffered saline (0.15 mol/l, pH 7.4) at 35 °C. Symbols are means of 3 replicate measurements and error bars represent standard deviations. M(t) is the phenolic content in the nanoparticles (in mg gallic acid equivalents) at time t (hours), and M_0 is the initial phenolic content (in mg gallic acid equivalents). M(t)/M_0 is the phenolic compounds release.

PLGA 65:35 showed higher ($P < 0.05$) antioxidant activity than GPE-loaded PLGA 50:50, where a high scavenging activity is indicated by a small IC50 value (Brand-Williams et al., 1995). Unloaded PLGA 50:50 and 65:35 nanoparticles showed no antioxidant effect (data not shown) for both methods. These contrasting results between the two antioxidant activity methods might be explained by the different compounds present in the extract that will have different release rates from the PLGA nanoparticles, and consequently affect the antioxidant activity response. These results indicate that GPE individual compounds have different antioxidant action mechanisms, and consequently will react differently with different free radicals (i.e.; peroxyl radicals and DPPH). Similar results have been reported previously, Laine, Kylli, Heinonen, and Jouppila (2008) encapsulated phenolic-rich cloudberry extract by freeze drying, using maltodextrins as wall materials. The microencapsulated cloudberry extract offered better protection for phenolic compounds during storage, while the antioxidant activity remained the same or even improved slightly. Moreover, several studies in the literature have reported that the antioxidant activity of the compounds of interest was preserved or enhanced after these compounds were encapsulated in PLGA, such as thymoquinone-loaded PLGA nanoparticles (Ganea et al., 2010) and curcumin-loaded PLGA nanoparticles (Mathew et al., 2012), similarly to the present study's results.

3.5. *Reactive oxygen species (ROS) generation assay*

Oxidative damage by ROS seems to be a crucial event in the initiation of cancer (Schumacker, 2006); therefore, the potential of free GPE extract and its loaded PLGA nanoparticles to reduce ROS generation was investigated. For non-cancer cells (CCD-18Co), results indicated that free GPE and GPE-loaded PLGA nanoparticles were effective in significantly decreasing the ROS generation (Fig. 4.1). Concentrations around 10 times lower ($P < 0.05$) than free GPE (202 µg extract/ml) were needed when the extract was entrapped in PLGA 50:50 (24 µg extract/ml) and PLGA 65:35 (20 µg extract/ml) to obtain the same ROS generation inhibition effect (approximately 6% inhibition compared to positive control cells). Thus, encapsulation using PLGA enhanced the ROS generation inhibition effect of GPE by reducing the extract amount needed to obtain the same effect. Unloaded PLGA 50:50 and 65:35 nanoparticles showed no ROS generation inhibition effect (data not shown). Similar results by Das, Das, Samadder, Bhadra, and Khuda-bukhsh (2012) also reported a concentration 10 times lower of *Phytolacca decandra* extract encapsulated in PLGA than the free extract to be effective against chemopreventive effects in regard to toxicity biomarkers like ROS generation. Studying the hydrophobic extract of the guabiroba fruit, Pereira et al. (2015) reported a higher concentration (275 µg extract/ml) of that extract to generate approximately 7% inhibition compared to positive control

Table 2
Antioxidant activity by the ORAC and DPPH free radical methods, and minimum inhibitory and bactericidal concentration (MIC and MBC) values for *Listeria innocua* of free guabiroba phenolic extract (GPE) and its loaded poly-D,L-lactic-co-glycolic acid (PLGA) nanoparticles.

Treatment	ORAC[1] (μmol/l TE/g)	DPPH[2] (g/g DPPH)	MIC[3] * (μg/ml)	MBC[4] * (μg/ml)
Free GPE	174.7 ± 20.0[b]	254.5 ± 19.6[b]	8,107[c]	> 50,000
PLGA 65:35 GPE	181.8 ± 5.4[b]	304.8 ± 23.5[b]	2,251[a]	> 10,000
PLGA 50:50 GPE	229.0 ± 15.6[a]	378.3 ± 39.5[a]	2,670[b]	> 10,000

PLGA with two lactic to glycolic acid ratios of 65:35 (PLGA 65:35) and 50:50 (PLGA 50:50) were used. Values are shown as the mean ± standard deviation of three independent repetitions.

[a,b] Means within a column which are not followed by a common superscript letter are significantly different ($P < 0.05$).

[1] ORAC results are expressed as μmol/l Trolox Equivalent (TE) per g of dry extract. A high Trolox equivalent number indicates a high antioxidant activity.

[2] DPPH results are expressed as IC50 (concentration required to reduce the original amount of free radical by 50%) in g of dry extract per g of DPPH. A high scavenging activity is indicated by a small IC50 value.

[3] Values are the lowest concentration of guabiroba fruit phenolic extract for which a ≤ 0.05 OD at 630 nm change was observed after 24 h incubation at 35° C in tryptose phosphate broth.

[4] Values are the lowest concentration for which a 3.0 $\log_{10}$ CFU/ml inactivation in cells was determined by plating. Values preceded by a higher than (>) means that tested concentrations were not sufficient to determine the MBC values.

* For the antimicrobial analysis, controls consisting of inoculum exposed to unloaded nanoparticles and solvents at the tested concentrations showed no inhibitory action against *L. innocua*.

cells; however, the reduction on the concentration necessary to obtain the same ROS generation inhibition effect was about 30 times lower when the extract was entrapped in PLGA.

Previous findings suggest that cancer cells use ROS signals to drive proliferation and other events required for tumor progression (Schumacker, 2006). Thus, ROS generation inhibition may contribute to slowing down tumor progression. For cancer cells (HT-29), the treatment with 202 μg extract/ml of free GPE was capable of significantly decreasing the ROS generation by 2.3% compared to negative control (untreated). These results indicate that the free GPE concentration (202 μg extract/ml) tested protected the non-cancer cells against ROS generation, as well as the cancer cells. However, encapsulation with PLGA was able to effectively inhibit ROS generation at lower GPE concentrations (20 to 24 μg extract/ml) only for non-cancer cells, as the same concentration was not enough to show any significant inhibition effect on ROS generation for cancer cells (Fig. 4.2). On the study of Pereira et al. (2015) with the hydrophobic extract of the guabiroba fruit, the encapsulation of the extract yielded a higher inhibition of the ROS generation (13.4% and 14.9%, for PLGA 50:50 and 65:35, respectively) than the free extract (2%), indicating that the encapsulation of the hydrophobic extract of this fruit is more efficient.

3.6. Antimicrobial activity

Free GPE and its loaded-PLGA nanoparticles showed inhibitory action against *Listeria innocua* (Table 2). Controls (methanol and unloaded nanoparticles) showed no inhibitory action against *L. innocua* (data not shown). Once GPE was nanoencapsulated with PLGA, it showed improved antimicrobial activity, with a concentration around 3 times lower than the free GPE required for inhibition. These results are consistent with previous studies on encapsulation of antimicrobial extracts or compounds with PLGA against foodborne pathogens (Gomes et al., 2011; Hill et al., 2013; Oliveira, Angonese, Ferreira, & Gomes, 2017; Silva et al., 2014) indicating the benefits of encapsulation with PLGA, as a lower antimicrobial concentration is necessary for inhibition. The presence of PVA on the particle coating, a bioadhesive material, may also play a role on the enhancement of the antimicrobial activity as it improves the particles adhesion and absorption into cells (Stevanovic & Uskokovic, 2009). The GPE-loaded in PLGA 65:35 showed lower MIC values than PLGA 50:50, which could be explained by the differences in GPE release from PLGA. Specifically, more antimicrobial compounds were released initially by PLGA 65:35 than PLGA 50:50 (see Section 3.3). Consequently, bacteria were exposed to a higher concentration of antimicrobial compounds, which ended up damaging more bacteria during the initial burst. The antimicrobial activity of GPE was likely due to its major phenolic compounds (i.e., phenolic acids and catechins), which have been previously reported to exhibit antimicrobial effects (Ravichandran, Hettiarachcy, Ganesh, Ricke, & Singh, 2011). For all treatments, none of the concentrations tested were sufficient to kill all the bacteria present; higher concentrations would likely result in bactericidal activity (Ramos-Nino, Clifford, & Adams, 1996). MIC and MBC values vary depending on the microorganism, raw materials, and extraction methods. Souza-Moreira et al. (2011) have reported MIC for free guabiroba fruit extract in 70 ml/100 ml ethanol in water at 5000 μg/ml for *Salmonella sebutal*. The MIC value of the free GPE is in the range presented by Martin et al. (2012) for ethanolic extracts of different agro industrial wastes against *Listeria monocytogenes*, between 1.56 and 12.5 mg/ml. The MIC values obtained in this study for GPE-loaded nanoparticles, 2251 and 2670 μg/ml for PLGA 65:35 and 50:50, respectively, can be compared to previously reported values for eugenol-loaded PLGA and trans-cinnamaldehyde-loaded PLGA nanoparticles (hydrophobic phenolic compounds), which presented a MIC of 1600 μg/ml against *Listeria* spp. (Gomes et al., 2011). In a previous study with the hydrophobic extract of the guabiroba fruit, Pereira et al. (2015) presented MIC values of 1124 and 954 μg/ml for PLGA 65:35 and 50:50, respectively.

4. Conclusions

The encapsulation of guabiroba fruit phenolic extract in PLGA nanoparticles, by the adapted emulsion-evaporation method, was effective in preserving the extract's phenolic content and its bioactivity until its application, and for a prolonged time during release. There were significant differences among the physico-chemical and functional properties of nanoencapsulated GPE for the two PLGA types studied. GPE release profiles had similar behaviour with differences in magnitude between PLGA types, which was shown to have a significant effect on the nanoparticles functional properties. Overall, GPE-loaded PLGA 65:35 showed the best results for nanoencapsulation, in terms of release profile, antioxidant and antimicrobial activities and ROS inhibition, and it should be the preferred polymer ratio used for hydrophilic extract delivery applications. These results suggest that PLGA nanoparticles can be used as a delivery system for phenolic compounds at levels lower than originally required for enhanced functional properties. Therefore, the encapsulation of the guabiroba fruit phenolic extract in PLGA nanoparticles offers a viable alternative to add value to an economically underexplored fruit in Brazil, by protecting its active components and enhancing its functional properties.

Conflict of interest

The authors declare no competing financial interest.

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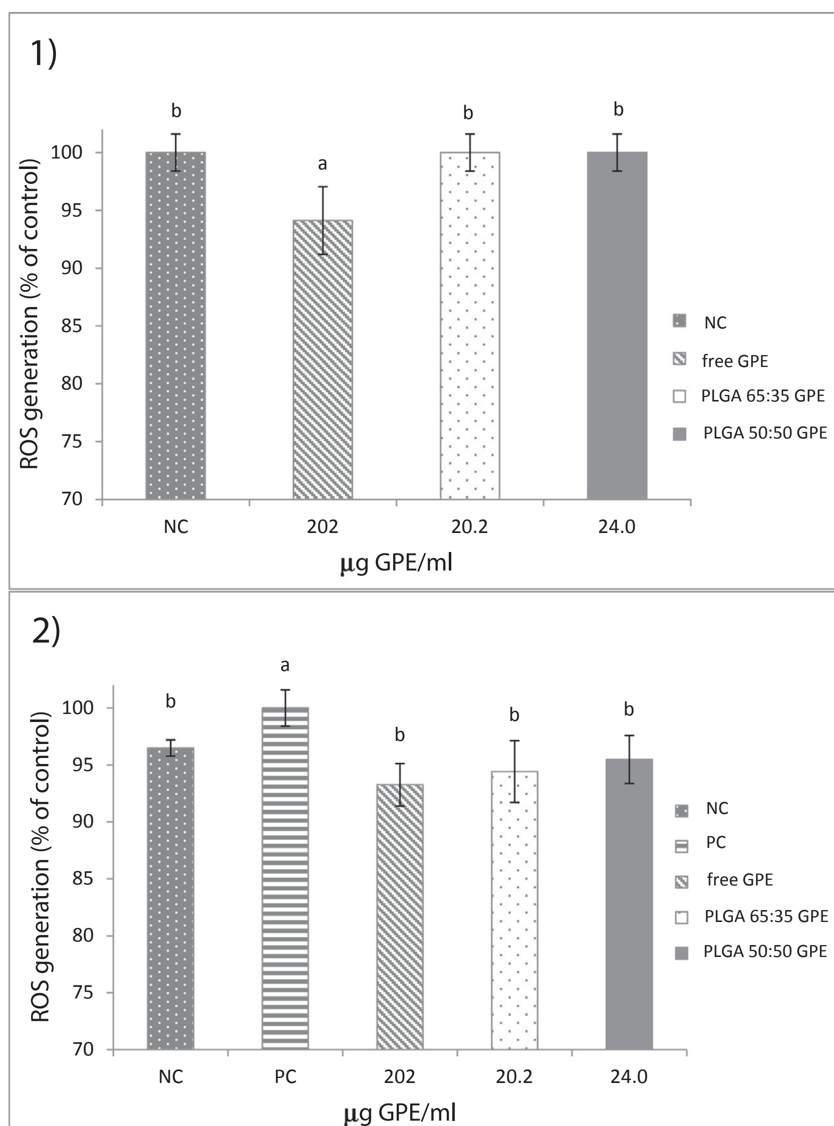

Fig. 4. Generation of reactive oxygen species (ROS) in (1) in human colon adenocarcinoma HT-29 cells and (2) noncancer colonic myofibroblasts CCD-18Co cells treated with free guabiroba fruit phenolic extract (GPE) and its loaded poly-D,L-lactic-co-glycolic acid (PLGA 65:35 and PLGA 50:50) nanoparticles. PLGA with two lactic to glycolic acid ratios of 65:35 (PLGA 65:35) and 50:50 (PLGA 50:50) were used. HT-29 cells (1) were not stimulated with H_2O_2 because they are constitutively under higher than normal oxidative stress. CCD-18Co cells (2) were stimulated with H_2O_2. NC = negative control cells were not H_2O_2 induced and PC = positive control cells were H_2O_2 induced. Values are means of 5 replicate measurements and error bars represent standard deviations; different letters indicate significance at $P < 0.05$.

References

Astete, C. E., & Sabliov, C. M. (2006). Synthesis and characterization of PLGA nanoparticles. *Journal of Biomaterials Science. Polymer Edition*, *17*(3), 247–289. http://dx.doi.org/10.1163/156856206775997322.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, *28*(1), 25–30.

Brandt, A. L., Castilho, A., Harris, K. B., Keeton, J. T., Hardin, M. D., & Taylor, T. M. (2010). Inhibition of Listeria monocytogenes by food antibacterial applied singly and in combination. *Journal of Food Science*, *75*(9), 557–563.

Budhian, A., Siegel, S. J., & Winey, K. I. (2005). Production of haloperidol-loaded PLGA nanoparticles for extended controlled drug release of haloperidol. *Journal of Microencapsulation*, *22*, 773–785.

Das, J., Das, S., Samadder, A., Bhadra, K., & Khuda-bukhsh, A. R. (2012). Poly (lactide-co-glycolide) encapsulated extract of *Phytolacca decandra* demonstrates better intervention against induced lung adenocarcinoma in mice and on A549 cells. *European Journal of Pharmaceutical Sciences*, *47*(2), 313–324. http://dx.doi.org/10.1016/j.ejps.2012.06.018.

Fang, Z., & Bhandari, B. (2010). Encapsulation of polyphenols: A review. *Trends in Food Science & Technology*, *21*, 510–523.

Friedman, M. (2007). Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. *Molecular Nutrition and Food Research*, *51*(1), 116–134.

Ganea, G. M., Fakayode, S. O., Losso, J. N., Nostrum, C. F. V., Sabliov, C. M., & Warner, I. M. (2010). Delivery of phytochemical thymoquinone using molecular micelle modified poly (D,L lactide-co-glycolide) (PLGA) nanoparticles. *Nanotechnology*, *21*(28), 1–10.

Gomes, C., Moreira, R. G., & Castell-Perez, E. (2011). Poly (DL-lactide-co-glycolide) (PLGA) nanoparticles with entrapped trans-cinnamaldehyde and eugenol for antimicrobial delivery applications. *Journal of Food Science*, *76*, 16–24. http://dx.doi.org/10.1111/j.1750-3841.2010.01985.x.

Gravani, R. (1999). Incidence and control of Listeria in food-processing facilities. In E. T. Ryser, & E. H. Marth (Eds.), *Listeria, listeriosis and food safety* (pp. 657–709). New York: Marcel Dekker.

Hass, L. I. R. (2011). Physico-chemical and phytochemical characterization and antioxidant activity in vitro and in vivo and anti-proliferative effects of araca (Psidium cattleianum Sabine) and guabiroba (Campomanesia xanthocarpa O. Berg) fruit extracts. Food and Agroindustrial Science. Federal University of Pelotas, Pelotas.

Hill, L. E., Taylor, T. M., & Gomes, C. (2013). Antimicrobial efficacy of poly(DL-lactide-co-glycolide) (PLGA) nanoparticles with entrapped cinnamon bark extract against *Listeria monocytogenes* and *Salmonella typhimurium*. *Journal of Food Science*, *78*(4), http://dx.doi.org/10.1111/1750-3841.12069.

Kumari, A., Yadav, S. K., & Yadav, S. C. (2010). Biodegradable polymeric nanoparticles based drug delivery systems. *Colloids Surf. B. Biointerfaces*, *75*(1), 1–18.

Laine, P., Kylli, P., Heinonen, M., & Jouppila, K. (2008). Storage Stability of Microencapsulated Cloudberry (*Rubus chamaemorus*) Phenolics. *Journal of Agriculture and Food Chemistry*, *56*, 11251–11261. http://dx.doi.org/10.1021/jf801868h.

Lu, W., Kelly, A. L., & Miao, S. (2016). Emulsion-based encapsulation and delivery systems for polyphenols. *Trends in Food Science and Technology*, *47*, 1–9. http://dx.doi.org/10.1016/j.tifs.2015.10.015.

Mainardes, R. M., & Evangelista, R. C. (2005). PLGA nanoparticles containing praziquantel: Effect of formulation variables on size distribution. *International Journal of Pharmaceutics*, *290*, 137–144. http://dx.doi.org/10.1016/j.ijpharm.2004.11.027.

Martin, J. G. P., Porto, E., Corrêa, C. B., De Alencar, S. M., Da Gloria, E. M., Ribeiro, I. S., et al. (2012). Antimicrobial potential and chemical composition of agro-industrial wastes. *Journal of Natural Products*, *5*, 27–36.

Mathew, A., Fukuda, T., Nagaoka, Y., Hasumura, T., Morimoto, H., Yoshida, Y., ... Kumar, D. S. (2012). Curcumin loaded-PLGA nanoparticles conjugated with Tet-1 peptide for potential use in Alzheimer's disease. *Plos One*, *7*(3), 1–10.

Meng, Q. Y., Velalar, C. N., & Ruan, R. S. (2008). Effects of epigallocatechin-3-gallate on mitochondrial integrity and antioxidative enzyme activity in the aging processo fo

human fibroblast. *Free Radical Biology & Medicine*, *44*(6), 1032–1041.

Oliveira, D. A., Angonese, M., Ferreira, S. R. S., & Gomes, C. L. (2017). Nanoencapsulation of passion fruit by-products extracts for enhanced antimicrobial activity. *Food and Bioproducts Processing*, *104*, 137–146. http://dx.doi.org/10.1016/j.fbp.2017.05.009.

Ou, B. X., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry*, *49*(10), 4619–4626. Retrieved from http://pubs.acs.org/doi/abs/10.1021/jf010586o.

Pereira, M. C., Hill, L. E., Zambiazi, R. C., Mertens-Talcott, S., Talcott, S., & Gomes, C. L. (2015). Nanoencapsulation of hydrophobic phytochemicals using poly (DL-lactide-co-glycolide) (PLGA) for antioxidant and antimicrobial delivery applications: Guabiroba fruit (Campomanesia xanthocarpa O. Berg) study. *LWT – Food Science and Technology*, *63*, 100–107.

Pereira, M. C., Steffens, R. S., Jablonski, A., Hertz, P. F., & Rios, A. D. O. (2012). Characterization and antioxidant potential of Brazilian fruits from the Myrtaceae family. *Journal of Agricultural and Food Chemistry*, *60*, 3061–3067.

Pool, H., Quintanar, D., Figueroa, J. D., Mano, C. M., Bechara, J. E. H., Godinez, L. A., et al. (2012). Antioxidant effects of quercetin and catechin encapsulated into PLGA nanoparticles. *Journal of Nanomaterials*, *2012*, 1–12.

Ramos-Nino, M. E., Clifford, M. N., & Adams, M. R. (1996). Quantitative structure activity relationship for the effect of benzoic acids, cinnamic acids and benzaldehydes on Listeria monocytogenes. *Journal of Applied Bacteriology*, *80*, 303–310.

Ravichandran, M., Hettiarachcy, N. S., Ganesh, V., Ricke, S. C., & Singh, S. (2011). Enhancement of antimicrobial activities of naturally occuring phenolic compounds by nanoscale delivery against Listeria monocytogenes, Escherichia coli O157:H7 and Salmonella Typhimurium in broth and chicken meat system. *Journal of Food Safety*, *31*, 462–471.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9–10), 1231–1237. http://dx.doi.org/10.1016/S0891-5849(98)00315-3.

Rufino, M. do S. M., Pérez-jiménez, J., Arranz, S., Alves, R. E., Brito, E. S. De, Oliveira, M. S. P., & Saura-Calixto, F. (2011). Açaí (Euterpe oleraceae) " BRS Pará ": A tropical fruit source of antioxidant dietary fiber and high antioxidant capacity oil. Food Research International Journal, 44, 2100–2106. https://doi.org/10.1016/j.foodres.2010.09.011.

Rufino, M. S. M., Alves, R. E., Brito, E. S., Perez-Jimenez, J., Saura-Calixto, F., & Mancini-Filho, J. (2010). Biocative compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chemistry*, *121*(4), 996–1002.

Schumacker, P. T. (2006). Reactive oxygen species in cancer cells: Live by the sword, die by the sword. *Cancer Cell*, *10*(3), 175–176.

Silva, L. M., Hill, L. E., Figueiredo, E., & Gomes, C. L. (2014). Delivery of phytochemicals of tropical fruit by-products using poly (dl-lactide-co-glycolide) (PLGA) nanoparticles: Synthesis, characterization, and antimicrobial activity. *Food Chemistry*, *165*, 362–370. http://dx.doi.org/10.1016/j.foodchem.2014.05.118.

Souza-Moreira, T. M., Salvagnini, L. E., Santos, E., Silva, V. Y. A., Moreira, R. R. D., Salgado, H. R. N., et al. (2011). Antidiarrheal activity of Camponesia xanthocarpa fruit. *Journal of Medicinal Food*, *14*(5), 528–531. Retrieved from http://online.liebertpub.com/doi/abs/10.1089/jmf.2009.0278.

Srivastava, A. K., Bhatnagar, P., Singh, M., Mishra, S., Kumar, P., Shukla, Y., et al. (2013). Synthesis of PLGA nanoparticles of tea polyphenols and their strong in vivo protective effect against chemically induced DNA damage. *International Journal of Nanomedicine*, *8*, 1451–1462.

Stevanović, M. M., & Uskoković, D. P. (2009). Poly(lactide-co-glycolide)-based micro and nanoparticles for the controlled drug delivery of vitamins. *Current Nanoscience*, *5*(1), 1–14.

Stevanovic, M., & Uskokovic, D. (2009). Poly(lactide-co-glycolide)-based micro and nanoparticles for the controlled drug delivery of vitamins. *Current Nanoscience*, *5*, 1–14. http://dx.doi.org/10.2174/157341309787314566.

Teixeira, B. N., Ozdemir, N., Hill, L. E., & Gomes, C. L. (2013). Synthesis and characterization of nano-encapsulated black pepper oleoresin using hydroxypropyl beta-cyclodextrin for antioxidant and antimicrobial applications. *Journal of Food Science*, *78*(12), http://dx.doi.org/10.1111/1750-3841.12312.

Wang, Y., Qu, W., & Choi, S. H. (2016). FDA's regulatory science program for generic PLA/PLGA – Based drug products. *American Pharmaceutical Review*, *19*(4), 5–9. Retrieved from http://www.americanpharmaceuticalreview.com/Featured-Articles/188841-FDA-s-Regulatory-Science-Program-for-Generic-PLA-PLGA-Based-Drug-Products/.

Wischke, C., & Schwendeman, S. P. (2008). Principles of encapsulating hydrophobic drugs in PLA/PLGA microparticles. *International Journal of Pharmaceutics*, *364*, 298–327. http://dx.doi.org/10.1016/j.ijpharm.2008.04.042.

Wrosltad, R. E. (2005). Polyphenolics. In R. E. Wrosltad, T. E. Acree, E. A. Decker, M. H. Penner, D. S. Reid, S. J. Schwartz, … P. Sporns (Eds.), Handbook of Food Analytical Chemistry (Vol. 2, pp. 461–535). Hoboken, N. J.: John Wiley & Sons.

Zigoneanu, I. G., Astete, C. E., & Sabliov, C. M. (2008). Nanoparticles with entrapped α-tocopherol: Synthesis, characterization, and controlled release. *Nanotechnology*, *19*, 105606. http://dx.doi.org/10.1088/0957-4484/19/10/105606.