Short communication: Potential use of passion fruit (*Passiflora cincinnata*) as a biopreservative in the production of coalho cheese, a traditional Brazilian cheese

Camila F. Costa,¹ Andressa Fusieger,² Milimani Andretta,³ Anderson C. Camargo,³ Antonio F. Carvalho,² Daniel R. Menezes,^{1*} and Luís A. Nero^{3*}

¹Departamento Medicina Veterinária, UNIVASF, Petrolina 56304 917 PE Brazil

²Departamento Tecnologia de Alimentos, UFV, Viçosa 36570 900 MG Brazil

³Laboratório de Inspeção de Produtos de Origem Animal, Departamento de Veterinária, Universidade Federal de Viçosa, Viçosa 36570 900 MG Brazil

ABSTRACT

Passion fruit (*Passiflora cincinnata* Mast.) is a native fruit from the Caatinga, a typical ecoregion in northeastern Brazil, and it has potential for use by the food and pharmaceutical industries. In this study, we characterized the antimicrobial activity of *P. cincinnata* and its application in the production of coalho cheese, a traditional Brazilian product. Aqueous extract of *P. cincinnata* exhibited high inhibitory activity against *Listeria* spp. (n = 4, reference strains), *Staphylococcus aureus* (n = 3, reference strains), and multidrug-resistant *Staph. aureus* (n = 8), and low inhibitory activity against lactic acid bacteria (LAB, n = 3, reference strains). Based on these results, we produced coalho cheese using goat milk with and without (control) passion fruit. Cheeses were stored at 10°C for 14 d and populations of mesophilic aerobes, *Staph. aureus*, and presumptive LAB were monitored at d 1, 7 and 14. The passion fruit cheese had lower counts of mesophilic aerobes, *Staph. aureus* (after 7 and 14 d), and presumptive LAB (after 14 d) than the control cheese. Adding ground passion fruit contributed to a reduction of *Staph. aureus* counts in goat cheese, although these differences were not significant. These results indicated the inhibitory potential of passion fruit and its potential use for controlling microbial populations in a cheese model; further studies are needed to characterize the active molecules that are responsible for such activity.

Key words: *Passiflora cincinnata*, passion fruit, antimicrobial activity, goat coalho cheese

Short Communication

Passiflora cincinnata Mast., also known as “passion fruit from Caatinga,” is a native plant species from semi-arid regions mainly in northeastern Brazil, in the states of Bahia, Pernambuco, Alagoas, and Sergipe (Oliveira and Ruggiero, 2005). This plant species is well adapted to local cultivation conditions in northeastern Brazil and highly resistant to pests and diseases (Araujo, 2007). *Passiflora cincinnata* has been found to have aromatic metabolites, substances with antimicrobial activity and antioxidant potential, and is able to reduce nociceptive and inflammatory events in mice (de Lavor et al., 2018; Leal et al., 2018; Siebra et al., 2018). These beneficial properties are important features when evaluating *P. cincinnata* as a potential producer of novel bioactive compounds that could be exploited by the food and pharmaceutical industries (Joana Gil-Chávez et al., 2013).

Natural products have emerged as an alternative option for food preservation. Some plant extracts and essential oils have demonstrated inhibitory activity against pathogens and spoilage bacteria, even in complex food matrixes such as cheeses (Tayel et al., 2015; Khorshidian et al., 2018). Siebra et al. (2018) showed that hydroalcoholic extracts of *P. cincinnata* are potential antimicrobial agents against *Staphylococcus aureus* and *Escherichia coli* when associated with conventional drugs. However, the effects of *P. cincinnata* on the growth of desirable and other contaminant microorganisms has not yet been evaluated in a food matrix.

Coalho cheese is a traditional Brazilian cheese produced mainly by small-scale goat farmers, and thus having social and economic relevance for the region. Despite being considered a local product, coalho cheese is consumed in different Brazilian states, and its identity and quality standards have been established by the Brazilian Ministry of Agriculture. It is characterized

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*Corresponding authors: daniel.menezes@univasf.edu.br and nero@ufv.br

as a medium- to high-moisture cheese that must be consumed within 10 d after production (Brasil, 1996, 2001). This cheese is often associated with poor hygienic conditions that can result in rapid spoilage during storage and reduced shelf life (Azevêdo et al., 2014; Pereira et al., 2018). Previous studies have demonstrated that compounds such as plant extracts or oils can be used as preservatives to enhance cheese quality (Shan et al., 2011; Bukvicki et al., 2018; Khorshidian et al., 2018; Pereira et al., 2018). In this regard, passion fruit could be exploited as an ingredient in coalho cheese production. In this study, we aimed to assess the inhibitory activity of passion fruit against different bacteria and evaluate its potential use as a bioactive ingredient for production of coalho cheese.

Passion fruits were harvested in the municipality of Mundo Novo (11°51'32" S; 40°28'21" W), a semi-arid region of Bahia, Brazil. The fruits were washed, cut, and placed in a forced circulation oven at 45°C for 72 h, ground aseptically, and stored at room temperature. Preparation of the aqueous extract was carried out as recommended by Mohanta et al. (2017), with modifications. Briefly, the ground passion fruit was macerated with distilled water (1:1) for 20 min and centrifuged for 15 min (3,260 × g) at 10°C; then, the supernatant was aseptically filtered (0.22 µm, syringe filter K18-230, KASVI, São José dos Pinhais, PR, Brazil). The semi-purified extract was used for microbiological assays immediately after preparation.

The antimicrobial activity of semi-purified extract was determined against bacteria usually associated with dairy products: lactic acid bacteria (**LAB**; *Lactobacillus sakei* ATCC 15521, *Lactococcus lactis* ssp. *cremoris* ATCC 19257, *Lactococcus lactis* ssp. *lactis* ATCC 13675), *Listeria* spp. (*Listeria monocytogenes* Scott A, ATCC 15313, and ATCC 19112, and *Listeria innocua* ATCC 33090), and *Staph. aureus* [ATCC 6538, ATCC 14458, ATCC 12598, and 8 multi-drug resistant isolates, obtained from goat milk (Lima et al., 2018) and from goat milk cheese (isolates obtained in our laboratory, unpublished)]. The isolates were stored at -80°C in de Man, Rogosa, and Sharpe broth (**MRS**, Becton, Dickinson and Co., Franklin Lakes, NJ) or brain heart infusion (**BHI**, Oxoid Ltd., Basingstoke, UK) in 20% (vol/vol) glycerol; before use, isolates were transferred to BHI or MRS, incubated at 37°C overnight, and checked for purity in trypticase soy agar (TSA, Oxoid) or MRS agar after incubation at 37°C. Then, a single colony from each strain was transferred to BHI (Oxoid), incubated at 37°C overnight, and diluted with BHI to a turbidity similar to tube 1 of the MacFarland scale, equivalent to approximately 3×10^8 cfu/mL; these cultures were used for the in vitro assessment of inhibitory activity of *P. cincinnata* fruits.

Inhibitory activity of semi-purified extract of *P. cincinnata* was assessed by using 96-well dilution plates. To each well was added 150 µL of semi-purified extract and 150 µL of target culture (approximately 10^4 cfu/mL) and plates were incubated at 37°C for 24 h in a Multiskan GO Microplate Spectrophotometer (Thermo Fisher Scientific, Madison, WI). The optical densities of the cultures were measured at 600 nm every 30 min (Vijayakumar and Muriana, 2015). Wells without the semi-purified extract were used as controls for growth of microbial reference target, with 150 µL of sterile water added to make the final volume. Two independent experiments were carried out in triplicate.

Based on the obtained results, ground *P. cincinnata* fruit was used as an ingredient for the production of coalho cheese, as described by Rincón et al. (2017), with modifications. Two treatments were considered: control coalho cheese (**CCC**) and passion fruit coalho cheese (**PFCC**; Figure 1). The CCC was produced with 2 L of goat milk mixed with 0.025 mL of coagulant (bovine chymosin, Maxiren XDS, DSM Food Specialties, Delft, the Netherlands); coagulation was conducted at 35°C for 20 min, after which the curd was cut and mixed for 40 min to obtain grains 5 mm in diameter and then mixed with 15 g of salt. The whey was drained by pressure and 200- to 250-g cheeses were molded (24 h), packed in plastic packages, and kept at 10°C in a ripening chamber with 85 to 86% relative humidity. The PFCC was produced following the same procedure, except that 60 g of ground passion fruit was added directly to the 2 L of goat milk in the first production step. Cheeses were produced in 3 independent repetitions.

Cheeses were subjected to microbial analysis during storage. After 1, 7, and 14 d, cheese units were collected, and portions of 25 g were aseptically obtained, transferred to sterile bags with 225 mL of citrate solution (0.85%, wt/vol), homogenized, and 10-fold diluted in NaCl 0.85% (wt/vol). Selected dilutions were plated onto Petrifilm STX, Petrifilm AC (3M Microbiology, St. Paul, MN), and acidified MRS agar (pH 5.7; Njongmeta et al., 2005), for enumeration of *Staph. aureus*, mesophilic aerobes, and presumptive LAB, respectively. Plates were incubated at 35°C for 24 h (Petrifilm AC), 48 h (Petrifilm STX), and 72 h (LAB), after which the typical colonies were enumerated and the results expressed as colony-forming units per gram. Counts were converted to log₁₀ and results were compared considering the treatments and storage days by ANOVA and Tukey honestly significant difference (HSD) test ($P < 0.05$), using XLSTAT software (Addinsoft, New York, NY).

Passion fruit is widely used in Brazil, especially in the northeastern region, for preparing beverages and in popular medicine, because of its potential applica-

tion as a sedative, antioxidant, and anxiolytic (Dhawan et al., 2004; Leal et al., 2018). It is well known that extracts of certain plants can increase the quality and sensory attributes of foods because of their antimicrobial and antioxidant activity and the presence of pleasant aromatic compounds and coloring characteristics; however, these extracts must be characterized before proper application (Tayel et al., 2013). All microbial targets included in this study were inhibited *in vitro* by the semi-purified extract of *P. cincinnata* but growth of LAB was less inhibited than growth of *Listeria* spp.

or *Staph. aureus* (Figure 2). The high inhibitory effect against multidrug-resistant *Staph. aureus* is particularly relevant in milk and cheeses made with raw goat milk, niches that are the focus of the application of *P. cincinnata* as a biopreservative.

The antimicrobial activity observed against *Staph. aureus* is relevant because this species is the most frequent cause of mastitis in dairy-producing animals and often associated with foodborne outbreaks (Smith, 2015; Kong et al., 2016; Lima et al., 2018). Also, *Staph. aureus* and *Listeria monocytogenes* are often linked to contaminated dairy products (Smith, 2015; Kong et al., 2016; Maury et al., 2019). Siebra et al. (2018) showed that conjugation of hydroalcoholic extract of *P. cincinnata* with antibiotics resulted in synergistic activity against multidrug-resistant *Staph. aureus* and *E. coli*. Further studies are warranted to assess the association of *P. cincinnata* bioactive compounds with conventional drugs, such as aminoglycosides and β -lactams, which alone are not very effective against multidrug-resistant strains (de Carvalho et al., 2019).

Terpenoids and phenolic compounds are the usual substances obtained from plants with antimicrobial activity, and these substances are reportedly produced by *P. cincinnata* (Costa et al., 2016; Leal et al., 2018). Phenolic substances, in particular, have potential application in food systems as antioxidants: low concentrations of phenolic acids act negatively on microbial enzymatic activity and high concentrations cause protein denaturation (Hassanien et al., 2014). Flavonoids have a strong effect on biological systems, demonstrating antimicrobial, antiviral, antioxidant, antihepatotoxic, antiulcerogenic, antihypertensive, and anti-inflammatory effects (Dhawan et al., 2004). According to Tajkarimi et al. (2010), other mechanisms of these compounds relate to their ability to alter the permeability of the microbial cell or the function of the cell membrane, or from effects on nutrient absorption, electron transport, and enzymatic activity to synthesize nucleic acids.

The inhibitory effect of *P. cincinnata* on growth of selected microbial groups in the goat milk coalho cheese model are presented in Figure 3. Growth of *Staph. aureus* was inhibited by the presence of the ground dried passion fruit, although no significant differences were observed between CCC and PFCC ($P > 0.05$). We did observe significant differences in growth of LAB and mesophilic aerobes in PFCC (Figure 3). These differences were clearly observed after 7 d of storage, which would be expected due to the potential release of bioactive compounds by the dried passion fruit in the PFCC. However, the antimicrobial effects of ground fruit in cheese were much lower compared with effects reported in *in vitro* studies (Figure 2). According to

Figure 1. Goat milk coalho cheese production, demonstrating the differences for control coalho cheese (CCC) and passion fruit coalho cheese (PFCC).

Figure 2. Growth curves (optical density at 600 nm) of selected strains of lactic acid bacteria (A), *Listeria* spp. (B), *Staphylococcus aureus* (C), and multidrug-resistant *Staph. aureus* (D) in brain heart infusion (control, ●) and in the presence of a semipurified extract of passion fruit (○), incubated at 37°C for up to 24 h.

Gutierrez et al. (2008), the lipid and protein contents of dairy products are intrinsic factors that can impair the effectiveness of natural antimicrobial substances. Fat and protein can cover the bacterial surface, forming a protective capsule that prevents contact with the bioactive compound. This effect was confirmed by Cava et al. (2007), who observed reduced antimicrobial activity of cinnamon and cloves in high-fat milk samples compared with skim milk samples. Because cheese has high levels of fat and protein, the concentrations of natural compounds that must be added to achieve microbiological inhibition must be higher than those tested in vitro (Shan et al., 2011; Amatiste et al., 2014). These findings are in agreement with our observations, perhaps because goat coalho cheese has a high lipid content (Rincón et al., 2017).

In the present study, we demonstrated potential use of *P. cinnamata* in controlling the growth of pathogenic bacteria often associated with dairy products, including multidrug-resistant bacteria. We propose its use as a bioactive ingredient in the production of coalho cheese. Further studies will characterize the bioactive compounds in *P. cinnamata* and their potential use for application in food systems.

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Figure 3. Growth of presumptive lactic acid bacteria (A), mesophilic aerobes (B), and *Staphylococcus aureus* (C) in control (●) and passion fruit (○) coalho cheese models stored at 10°C for up to 14 d. * $P < 0.05$; ** $P < 0.005$, Tukey honestly significant difference test.

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