Journal of Dairy Research

cambridge.org/dar

In vitro evaluation of nanocomposites of linseed mucilage and k-carrageenan loaded with *Achyrocline satureioides* nanoemulsion: a gradual-release candidate of antimicrobials for the treatment of bovine mastitis

Gabriela Tasso Pinheiro Machado[1], Roberto Gabriel Ferreira[1], Maria Beatriz Veleirinho[1], Luciana Aparecida Honorato[1], Roberta de Oliveira Ramos[2], Marcos Antônio Segatto Silva[2] and Shirley Kuhnen[1]

[1]Biochemical and Natural Products Laboratory – LABINAT, Federal University of Santa Catarina, Florianópolis 88034-001, Brazil and [2]Quality Control Laboratory- LABCQ, Federal University of Santa Catarina, Florianópolis 88040-900, Brazil

Research Article

Cite this article: Machado GTP, Ferreira RG, Veleirinho MB, Honorato LA, Ramos RdeO, Silva MAS and Kuhnen S (2023). In vitro evaluation of nanocomposites of linseed mucilage and k-carrageenan loaded with *Achyrocline satureioides* nanoemulsion: a gradual-release candidate of antimicrobials for the treatment of bovine mastitis. *Journal of Dairy Research* **90**, 376–381. https://doi.org/10.1017/S002202992300064X

Received: 7 July 2023
Revised: 6 September 2023
Accepted: 13 September 2023
First published online: 8 January 2024

Keywords:
Herbal actives; hydrogel composites; mastitis; nanocomposites; natural polymers

Corresponding author:
Shirley Kuhnen;
Email: shirley.kuhnen@ufsc.br

Abstract

This research paper presents the development and evaluation of pioneering nanocomposites (NCs) based on the combination of k-carrageenan and linseed mucilage. When loaded with macela extract nanoemulsion they present an innovative approach for the sustained release of antimicrobial herbal constituents, specifically tailored for bovine mastitis treatment. The NCs, encompassing various ratios of k-carrageenan and linseed mucilage polymers (8:2, 7:3, and 5:5 w/w) with 1.25 mg of macela extract/g of gel, underwent in vitro assessment, emphasizing viscosity, degradation speed, release of herbal actives from macela nanoemulsion and antimicrobial activity. The NCs exhibited thermoreversible characteristics, transitioning from liquid at 60°C to a gel at 25°C. NCs allowed a gradual release of phenolic compounds, reaching approximately 80% of total phenolics release (w/v) within 72 h. NCs inhibited the growth of MRSA (ATCC 33592) until 8 h of incubation. No toxic effect in vitro of NCs was found on MAC-T cells. Thus, the developed materials are relevant for the treatment of bovine mastitis, especially in the dry period, and the data support future evaluations in vivo.

Natural polymers are biodegradable, biocompatible, renewable, low toxicity and potentially cheap materials that are capable of reducing biological and ecological problems resulting from the use of synthetic polymers (Distantina *et al.*, 2013; Khalil *et al.*, 2017). Among various natural polymers, hydrogel composites, developed with k-carrageenan and flaxseed mucilage, have been investigated owing to their rheological properties and their ability to form a thermoreversible gel (Chen *et al.*, 2006). K-carrageenan is a mucopolysaccharide extracted from the cell walls of tropical algae *Kappaphycus alvarezii* (Campo *et al.*, 2009). It is used in the manufacture of new materials with potential for application in several drug administration systems (Khalil *et al.*, 2017; Muhamad *et al.*, 2019). High antimicrobial activity has been found, for example, when adding plant extracts, seed extracts and essential oils to carrageenan composites (Kanmani and Rhim, 2014; Shojaee *et al.*, 2014). Flaxseed mucilage, in turn, is a hydrocolloid (with neutral polysaccharides) that forms around seeds after imbibition (Naran *et al.*, 2008). It has good water-retention capacity, owing to its marked expansion capacity and high viscosity in an aqueous solution (Chen *et al.*, 2004). Composites can be obtained from the combination of different polymers by simply mixing the materials (Khalil *et al.*, 2017). Thus, there has been increasing research on use of these polymers in pharmaceutical formulations for the development of composites, such as vehicles for release of bioactive compounds. Previous studies have reported a gradual release profile (Hasnain *et al.*, 2018) and high antimicrobial potential (Haseeb *et al.*, 2016).

An innovative study has focused on the development of composites in which at least one of the components has nanometric dimensions, i.e. nanocomposites (Khalil *et al.*, 2017). Nanocomposites (NCs) have some advantages in terms of mechanical and physical barrier properties, controlled drug release and adsorption efficiency (Khalil *et al.*, 2017; Algharib *et al.*, 2020). NCs have been proposed as important intracellular drug transport systems because they show better penetration in diseased sites owing to the reduced particle size of the encapsulated active compounds (Algharib *et al.*, 2020). In this context, the development of nanocomposites for gradual release of nanoemulsified natural antimicrobial active compounds (herbal antimicrobial activities) may be of interest for intramammary application in the treatment of bovine mastitis. Bovine mastitis, a highly prevalent disease in dairy cattle herds (Guimarães *et al.*, 2017), has

shown higher cure rates for staphylococcal infections (25–75%) owing to the use of intramammary antimicrobial therapy in non-lactating, the so-called dry cow therapy (DCT: Langoni *et al.*, 2017). The development of an antimicrobial nanocomposite can be an innovative strategy for intramammary application, especially for organic and agroecological production systems.

Considering the antimicrobial potential of macela in nanoemulsion against *S. aureus* and low cytotoxicity on bovine mammary epithelial cells (Pinheiro Machado *et al.*, 2020; Pinheiro Machado *et al.*, 2022) the present study adressess the hypothesis that nanocomposites (NCs) on the basis of k-carrageenan and linseed mucilage are adequate for prolonged release of herbal actives in a nanoemulsion system. Emphasis was placed on viscosity, degradation speed and release of the herbal actives from macela-nanoemulsion.

Materials and methods

Macela nanoemulsion

Macela extract was prepared by macerating inflorescences with 80% ethanol (v/v) (1:60, w/v). Subsequentely, the extract was vacuum filtered and the organic solvent removed with a rotary evaporator at 60°C. The aqueous phase of the extract was used to prepare the nanoemulsion (NE-ML 1:5) as described by Pinheiro Machado *et al.* (2020)

Linseed mucilage and K-carrageenan

Linseed mucilage was extracted from golden flax seeds (*Linum usitatissimum* L.) according to Ziolkovska (2012). K-carrageenan was purchased from Sigma-Aldrich (K-carrageenan, 22048; Sigma Chemical Co. St. Louis, MO).

Development of nanocomposites

NCs were produced by gelation of different proportions of the K-carrageenan and linseed mucilage. K-carrageenan (2%, w/v) was prepared by dissolving the polymer in distilled water or in the macela-nanoemulsion at 60°C (~ 20 min). For the formation of NCs, the previously prepared liquid carrageenan and linseed mucilage were weighed and immediately mixed in different proportions, namely 9:1, 8:2, 7:3, 6:4 and 5:5 for comparison with pure k-carrageenan and pure linseed mucilage. The composition of the different NCs is shown in online Supplementary Table S1.

Viscosimetric behavior of nanocomposites

NCs were characterized for viscosity using a Brookfield DV-II + Pro rheometer and a LV4 spindle. Immediately after preparation, viscosity measurements were carried out, starting from the preparation temperature (60°C) until the temperatures of interest, i.e., 40, 30 and 25°C, which are close to the animal's body temperature and room temperature. The speed used in all experiments was 60 rpm. After stabilizing temperature, the equipment was programmed to perform five viscosity measurements, with an interval of 10 s between each measurement. The tests were performed in triplicate. The data were presented as mean ± SD for each temperature evaluated.

Release of bioactive compounds from nanocomposites

The release kinetics of the compounds from the macela-nanoemulsion in the NCs were evaluated in vitro in the Dubinhoff system by simulating physiological conditions. Samples from each NC (3 g) and their respective unloaded composites (UNCs) were immersed in 30 ml of PBS (pH 7.0) and kept at 37° C, with horizontal stirring (100 rpm). After time intervals (2, 4, 8, 12, 24, 48 and 72 h), an aliquot (2 ml) was removed and immediately replaced by fresh PBS (2 ml) to maintain the initial conditions. The aliquots were analyzed by UV-VIS, total phenolic content (TPC) and by High Performance Liquid Chromatography (HPLC). Four independent tests were performed. The aliquots were analyzed in triplicate. The data were presented as mean ± SD.

UV–VIS analysis

UV–VIS spectroscopy was used to estimate the degradation of the materials and evaluate the release of the active compounds from the NCs. For that samples were collected at different intervals and scanned over the wavelength range of 200–800 nm. Comparisons were made at the maximum absorption length found, i.e., 300 nm. PBS was used as blank control.

Total phenolic content

TPC was determined based on the Folin–Ciocalteu colorimetric method (Singleton and Rossi, 1965), using a standard curve of gallic acid (Sigma-Aldrich, St. Louis, MO; 10–100 µg/ml; $y = 0.004x$; $R^2 = 0.984$). TPC was expressed as a percentage of the total weight accumulated in the release medium at each time in µg of gallic acid equivalents (GAE) per ml.

HPLC analysis

Analysis of the phenolic composition of the macela extract was performed by HPLC/DAD according to Pinheiro Machado *et al.* (2020) to determine the release of 3-O-methylquercetin (3-O-MQ) and achyrobichalcone (ACB) (Pinheiro Machado *et al.*, 2022). The contents were expressed as a percentage of the total weight accumulated in the release medium at each time in µg of quercetin equivalents (QE) per ml. Details are provided in the online Supplementary File.

Antimicrobial activity: Inhibition of bacterial growth curve

A growth curve was prepared for bacterial strain ATCC 33592. Briefly, 75 ml of TSA broth was inoculated with 0.75 ml of liquid bacterial culture (1.5×10^8 CFU ml^{-1}) and incubated at 37°C with 100 rpm stirring in the *Dubinhoff system* (Lima *et al.*, 2016). Simultaneously, for inhibition of the bacterial growth curve, NCs loaded with macela extract (1.25 mg/g of gel) in nanoemulsion were placed in contact with the bacterial growth curve. Aliquots (3 ml) were taken at different time intervals (1, 2, 3, 4, 5 and 6 h) and optical density was measured at 620 nm, using TSA broth as a reference sample (blank). For comparison, negative controls, i.e., inoculum + broth, NCs + broth and UNCs + broth were included.

Antimicrobial activity: Broth microdilution method for minimum inhibitory concentration (MIC) test

NCs were tested against *S. aureus* ATCC 33592 and four methicillin-resistant *S. aureus* (MRSA) strains from mastitic milk using the broth microdilution method (Clinical and Laboratory Standards Institute, 2013). Previously, the NCs were

https://doi.org/10.1017/S002202992300064X Published online by Cambridge University Press

diluted in sterile PBS for 24 h at 20°C without stirring. After the incubation period, the supernatant was used to perform the microdilution test. The tested concentrations corresponded to the concentration of macela extract in the NCS (1.25–0.01 mg/ml). Details are provided in the online Supplementary File.

Cytotoxicity of unloaded composites (UNCs) on the MAC-T cell line

MAC-T cells suspension was plated in a 96-well microplate (100 µl/well), followed by incubation (24 h) in culture conditions for adherence. Simultaneously, the UNCs were placed in DMEM medium (extraction medium) and kept for 24 h under the same conditions of cell culture. After 24 h (cell adhesion period), the culture medium in the plates was replaced by the extraction medium, and the cells were cultured for further 24 h. Cytotoxicity was determined based on the MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 0.5 mg/ml) method. Details are provided in the online Supplementary File.

Statistical analyses

Statistical analyses were performed using analysis of variance (ANOVA) followed by Tukey's test (GraphPad Software Inc., San Diego, CA). P values < 0.05 were considered as significant. The data were presented as mean ± standard deviation (SD).

Results and discussion

Development of nanocomposites

Initially, seven composites based on k-carrageenan and linseed mucilage were evaluated. All showed thermoreversible characteristics, i.e., they were liquid when heated at temperatures between 40 and 60°C and gelled between 30 and 25°C. Among these, composites 5:5, 7:3 and 8:2 (w/w) of k-carrageenan and linseed mucilage were selected. The selection of these composites was based on their appearance as a gel and their ability to incorporate the desired content of macela extract(1.25 mg/g of gel) *via* nanoemulsion. The addition of the macela-nanoemulsion to the composites did not result in an evident macroscopic modification, except for the characteristic yellowish color of the nanoemulsion (online Supplementary Fig. S1). NC-7:3 and NC-8:2 had a stiffer appearance at room temperature (25°C), possibly owing to the lower concentration of flaxseed mucilage.

Viscosimetric behavior of nanocomposites

Viscosity analysis showed that viscosity of NCs decreases with increasing temperature (Fig. 1). In general, the greatest changes in viscosity values occurred between 30 and 40°C, at which all three materials showed a viscosity state of 1500 cP (Fig. 1). As the normal values of body temperature for dairy cows range between 38 and 39.5°C (Cunninghan and Klein, 2008), it can be suggested that the gel condition of the NCs in this temperature range could also facilitate the initial release of the antimicrobial actives present in the macela-nanoemulsion (Puttipipatkhachorn *et al.*, 2001), as well as allowing greater penetration inside the mammary gland, passing through the cisterns and reaching the mammary ducts and alveoli. Importantly, for a drug to be effective, therapeutic concentrations must be achieved at the site of infection (biophase), taking into account that the pathogens

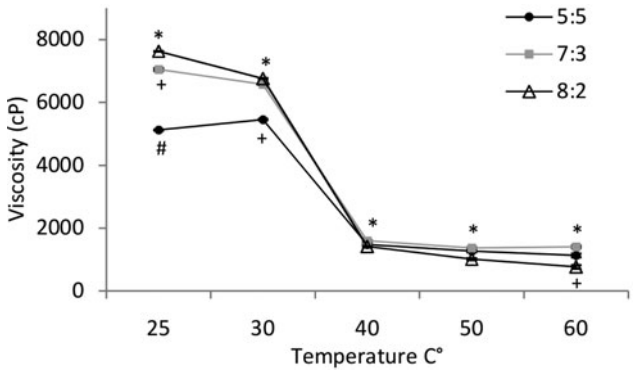

Figure 1. Viscosity in Brookfield DV-II + Pro rheometer using the LV4 spindle of nanocomposites loaded with the macela-nanoemulsion containing the proportions of k-carrageenan and linseed mucilage NC-5:5, NC-7:3 and NC-8:2 (w/w), respectively, on the basis of temperature, at 60 rpm. Five viscosity measurements were made, with an interval of 10 s between each measurement. The tests were performed in triplicate. The data were presented as mean ± SD for each temperature evaluated. Different symbols indicate a statistical difference between nanocomposites at the same temperature.

may be free, bind to the surface of the membranes or even live in the intracellular compartment (as is the case for *S. aureus*: Gruet *et al.*, 2001; Erskine *et al.*, 2003; Sandasi *et al.*, 2010). At 25°C, NC-5:5 showed lower viscosity, which can be considered an advantage for potential in vivo intramammary application in the future (Fig. 1). The higher viscosity found for NC-7:3 and NC-8:2 between temperatures of 25 and 30°C may be due to the lower concentrations of linseed mucilage and higher concentrations of k-carrageenan used in the composition of these materials. Importantly, the higher viscosity found for the NCs at room temperature allows greater retention of the macela-nanoemulsion during product storage.

Previous studies have shown that the increase in viscosity of materials developed with k-carrageenan and linseed mucilage could allow greater water retention capacity, and it could also help to reduce the syneresis of this composite, which are important characteristics for conservation and storage (Chen *et al.*, 2006). In that same study, the addition of flaxseed mucilage to k-carrageenan (1% w/w) increased the viscosity of the composite, which presented a gelation temperature around 45°C, ie, a slightly higher value than the one found in the present study (Chen *et al.*, 2006).

UV–VIS and FT-IR analysis

The rates of the degradation of the NCs and UNCs were analyzed by UV–Vis (200–800 nm) (data not shown). The analysis of the scans showed that the maximum absorption wavelength was 300 nm (online Supplementary Fig. S2), which is characteristic for phenolic compounds (Mabry *et al.*, 1970). These are the major compounds in the macela extract (Retta *et al.*, 2012). For UNCs, there was no absorption at 300 nm (data not shown). In general, the three NCs showed an increase in dissolution over time, suggesting the sustained release of the phenolic compounds released from the macela-nanoemulsion for up to 72 h (online Supplementary Fig. S2).

The FTIR of k-carrageenan, linseed mucilage and linseed polysaccharides is shown in online Supplementary Table S2. When evaluating the spectra of both UNCs and NCs, one can detect the various characteristic bands previously reported in the

https://doi.org/10.1017/S002202992300064X Published online by Cambridge University Press

literature. There are no significant differences when comparing the spectra of the UNCs and NCs, so we can conclude that the different concentrations of k-carrageenan and linseed mucilage did not significantly interfere in FTIR characterization.

Total phenolic content

Different phenolic compound releasing profiles were observed for the three NCs. NC 5:5 showed a maximum release peak at 24 h, while for NC 7: 3 and NC 8:2 the peak occurred only at 48 h (Fig. 2a). These differences are related to the variable composition, (polymer content) of the NCs. The three NCs showed a gradual release profile for the phenolic compounds of the macela-nanoemulsion, reaching approximately 80% release (w/v) in 72 h. A higher release of phenolic compounds was observed in the first 4 h for NC-5:5, in comparison to NCs 7:3 and 8:2. These results are in accordance with UV–Vis (online Supplementary Figure S2), which also suggested the slower initial disintegration of NCs 7:3 and 8:2 (Fig. 2a; $P < 0.05$). Similar results regarding the release of bioactive compounds retained in k-carrageenan hydrogel were previously reported (Makino *et al.*, 2001). In view of the potential intramammary application of NCs during the dry period, it is clear that bovine mammary glands are highly susceptible to new intramammary infections during the physiological transition from lactation to the dry period and from the dry period to the beginning of lactation (Sordillo, 2016). This fact can give an advantage to NC 5:5, which showed a greater initial release of antimicrobial phenolic compounds.

HPLC analysis

The phenolic compounds released from the macela-nanoemulsion of the NCs were 3-O-methylquercetin (3-O-MQ) and achyrobichalcone (ACB). NCs 5:5, 7:3 and 8:2 showed a peak release of 3-O-MQ and ACB in 72 h (Figs 2b, 2c). The release pattern of 3-O-MQ over 72 h was slower compared to that of ACB. ACB was quickly released from the three NCs, reaching values close to 50% (w/v) of release in the first hours. For NC-5:5, for example, about 50% (w/v) of the ACB was released within the first 2 h, reaching approximately 65% (w/v) of release within 72 h (Fig. 2c). By comparison, 3-O-MQ from NC-5:5 only reached 50% (w/v) of release within nearly 24 h. None of the compounds reached 100% release in the study period, supporting the gradual release profile of both compounds. The faster release of ACB is related to its greater polarity and solubility in the aqueous medium. Notably, the complex composition of macela-nanoemulsion, ie, compounds with variable

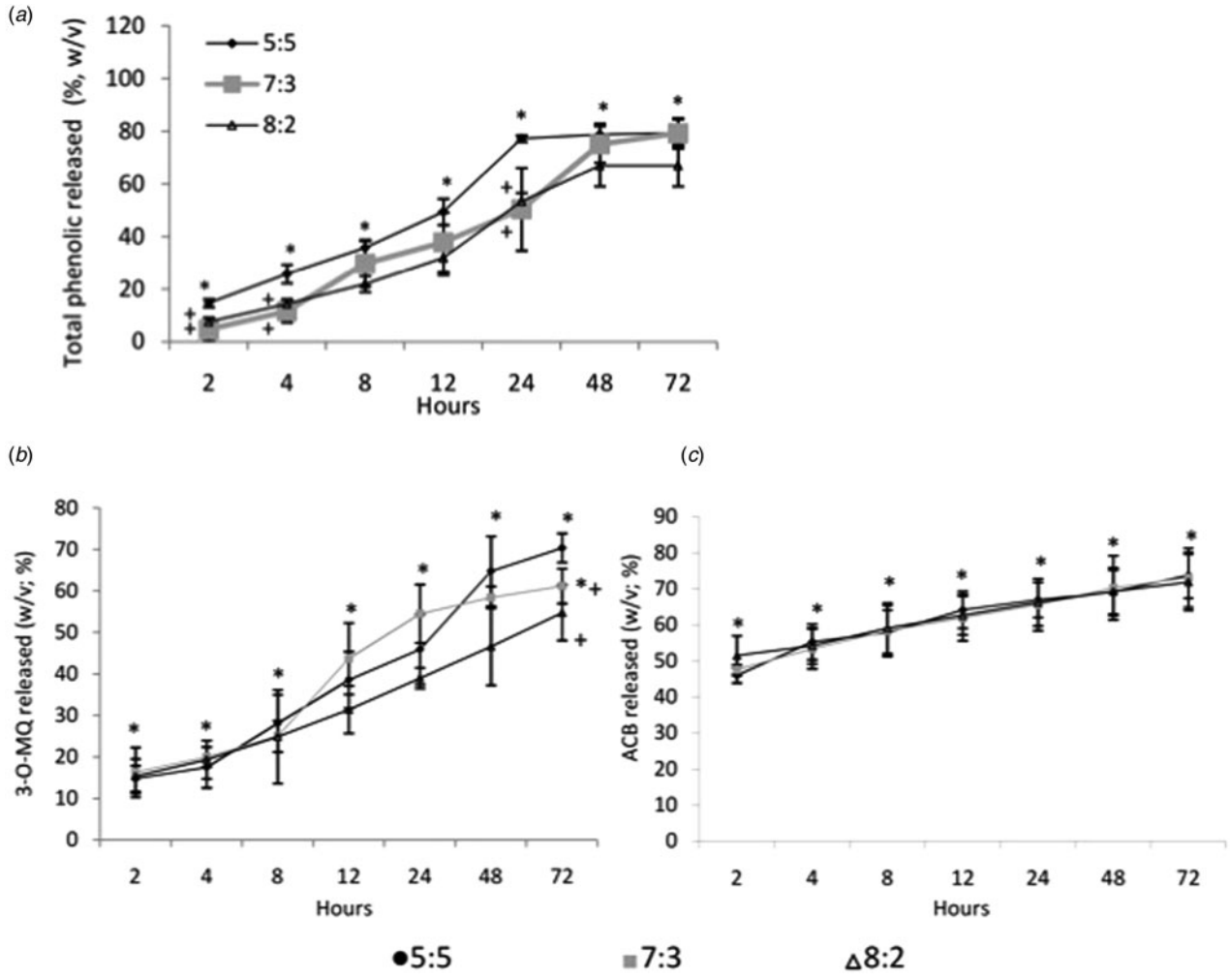

Figure 2. Percentage of the total accumulated and released phenolic mass (a), 3-O-MQ (b) and ACB (c) of the macela-nanoemulsion from the nanocomposites (NCs) containing the proportions of k-carrageenan and linseed mucilage of 8:2, 7:3 and 5:5 (w/w), respectively, over 72 h. The results are expressed as mean ± SD ($P < 0.05$). Different symbols indicate a statistical difference between the NCs at the same collection time.

https://doi.org/10.1017/S002202992300064X Published online by Cambridge University Press

polarity, allows different releasing profiles for the compounds which represents an advantage regarding the microbial control. Thus, several compounds could be released at different times, and they could perform microbial control over time.

Antimicrobial activity: Inhibition of bacterial growth curve

NCs controlled the growth of *S. aureus* with OD620 nm <0.2 up to 8 h of incubation (Fig. 3). There was no difference in the absorbance values between the UNCs and the control with inoculum (broth + inoculum). This result is in accordance with the release profile of total phenolic compounds and major compounds (3-O-MQ and ACB) of the NCs (Figs 2b, 2c), showing release of active compounds within the first 8 h for the three materials. Total phenolics, for example, were released in concentrations close to 40 µg/ml after 8 h, equivalent to 30% (w/v), which supports the antimicrobial potential found. It was also found that the release of 3-O-MQ and ACB was around 30 and 60% (w/v), respectively, for the three NCs after 8 h of incubation (Figs 2b, 2c; $P < 0.05$). The findings are relevant for the treatment of bovine mastitis, since *S. aureus* is a bacterium frequently isolated in difficult-to-treat infections (Gruet *et al.*, 2001; Erskine *et al.*, 2003; Locatelli *et al.*, 2017). The intramammary application of antimicrobial bioactive compounds formulated in vehicles of gradual and prolonged release with a longer time of persistence in the mammary gland has been related to the success of the treatments of staphylococcal infections (Radostits *et al.*, 2000; Cunninghan and Klein, 2008). This success depends on release time, carrier vehicle being used, particle size of the active compounds and diffusion capacity of the antimicrobials (Radostits *et al.*, 2000). In a similar context, chitosan-based hydrogels were investigated for intramammary application. They were associated with the ability to activate the immune response, acceleration of gland involution and the antimicrobial potential against *Staphylococcus* sp. (Lanctôt *et al.*, 2017).

Antimicrobial activity: Minimum inhibitory concentration (MIC)

NCs reduced the microbial growth of all MRSA samples (isolated from mastitic milk and ATCC 33592) after 24 h of exposure.

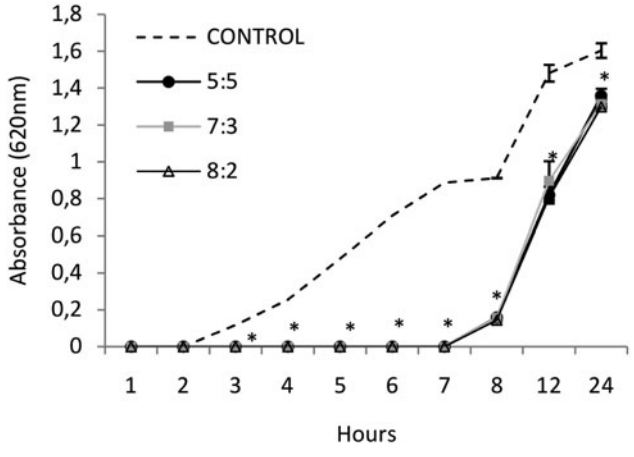

Figure 3. Antimicrobial activity of nanocomposites (NCs) loaded with the macela-nanoemulsion containing the proportions of k-carrageenan and linseed mucilage of 5:5, 7:3 and 8:2 (w/w) respectively and control (broth + inoculum) on growth curves of *S. aureus* ATCC 33592. Error bars represents the means (±SD) of three independent experiments performed in duplicate. ($P < 0.05$). Equal symbols indicate that there was no statistical difference among the nanocomposites at the same collection time.

Different MIC values were found among the materials developed. For NC-5:5, MIC was 312 µg/ml while for NCs 7:3 and NC-8:2, it was 625 µg/ml (online Supplementary Fig. S4). The higher microbial reduction found for NC-5:5 may be due to the greater release of phenolic compounds by this material within the first 4 h (Fig. 2a). The lower microbial reduction found for NCs 7:3 and 8:2 occurred for three of the four isolates analyzed and, therefore, this was considered the minimum inhibitory concentration (MIC). Table 1 shows the reduction in microbial growth of the NCs.

NCs showed a capacity to reduce microbial activity close to 100% at a concentration of 625 µg/ml. This result indicates that the release found for the antimicrobial active compounds of the NCs within 24 h was sufficient to inhibit microbial activity in that period. The presence of the macela-nanoemulsion in the materials was responsible for the antimicrobial properties, as there was no bacterial inhibition by the UNCs (data not shown). In a previous study, the MIC found for the macela-nanoemulsion was equivalent to 30 µg/ml of extractive content incorporated in the nanoemulsion, which contained around 185 µg/ml of 3-O-MQ (Pinheiro Machado *et al.*, 2020). These results show the high antimicrobial potential of macela in different forms, namely crude extract, aqueous nanoemulsion and nanoemulsion gel.

Cytotoxicity of unloaded composites (UNCs) on the MAC-T cell line

Unloaded composites (UNCs) underwent a cytotoxicity test *via* MTT in bovine mammary epithelial cells (MAC-T). The evaluation of possible cytotoxic effects of the macela-nanoemulsion used in the present study had been carried out in a previous study in which the authors underscored the reduced toxicity and the cytoprotective potential of this nanoemulsion (Pinheiro Machado *et al.*, 2022). In the present study, the viability of bovine MAC-T mammary epithelial cells was not affected by exposure to the UNCs. This trial showed absence of toxicity, as it resulted in cell viability close to 100%. This result is significant, as it supports the use of the composite developed with k-carrageenan and linseed mucilage for the release of herbal activities in pharmaceutical formulations. For bovine mastitis therapy, the absence of toxicity in this material infers a reduction in the risks associated with the pro-inflammatory effect, ensuring a recommendation for safe intramammary use during the dry period. Moreover, an adequate dry period is necessary to guarantee sustainable milk production, and any situations that interfere with the renewal of epithelial cells during this period can have detrimental effects on the subsequent performance of

Table 1. Reduction in growth of *S. aureus* at different concentrations of nanocomposites NC-5:5, NC-7:3 and NC-8:2 (w/w)

Macela extract (µg ml^{-1})	NC-5:5	NC-7:3	NC-8:2
1250	100	100	100
625	98	97	99
312	92	50	41
156	49	0	34
80	6	0	22
40	1.6	0	2
20	0	0	0
10	0	0	0

https://doi.org/10.1017/S002202992300064X Published online by Cambridge University Press

lactation (Sordillo, 2016). It is important to highlight that an important advantage of the use of composites developed with mucilage extracted from plant seeds has been related to the biocompatibility of this material, which has even shown potential to contribute to cell proliferation (Urena-Saborio *et al.*, 2018).

To the best of our knowledge the combination of k-carrageenan, linseed mucilage and macela-nanoemulsion has not been described in the literature. We can conclude that the innovative development of these NCs by combining three natural materials can represent sustainable pharmaceutical formulation with potential for use in the gradual release of antimicrobial activities, with application in bovine mastitis and mainly in, but not limited to, sustainable milk production systems. These results support future evaluations in vivo of the NCs developed. The NCs also have other advantages: developed exclusively with natural widely available materials, less expensive and biodegradable, which can impact the health, animal welfare and sustainability of dairy production.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S002202992300064X

Acknowledgements. The authors are grateful to Dr Valquiria L. Bassani and Dr Sara E. Bianchi from the Galenic Development Laboratory (UFRGS) for the identification of achyrobichalcone in the macela extract. This work was supported by the CNPq (Notice 39/2013, 403415/2013-6).

References

Algharib SA, Dawood A and Xie S (2020) Nanoparticles for treatment of bovine *Staphylococcus aureus* mastitis. *Drug Delivery* **27**, 292–308.

Campo VL, Kawano DF, da Silva Jr DB and Carvalho I (2009) Carrageenans: biological properties, chemical modifications and structural analysis – a review. *Carbohydrate Polymers* **77**, 167–180.

Chen HH, Xu SY and Wang Z (2004) Rheological properties of flaxseed gum [J]. *Journal of Wuxi University of Light Industry* **1**, 30–35.

Chen HH, Xu SY and Wang Z (2006) Gelation properties of flaxseed gum. *Journal of Food Engineering* **77**, 295–303.

Clinical and Laboratory Standards Institute (CLSI) (2013) Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard – Eighth Edition. NCCLS document M2-A8 [ISBN 1-56238-485-6]. USA.

Cunninghan JG and Klein BG (2008) *Tratado de Fisiologia Veterinária*, 4th Edn. Rio de Janeiro: Elsevier Saunders.

Distantina S, Rochmadi R, Fahrurrozi M and Wiratni W (2013) Preparation and characterization of glutaraldehyde-crosslinked kappa carrageenan hydrogel. *Engineering Journal* **17**, 57–66.

Erskine RJ, Wagner S and DeGraves FJ (2003) Mastitis therapy and pharmacology. The veterinary clinics of North America. *Veterinary Clinics* **19**, 109–138.

Gruet P, Maincent P, Berthelot X and Kaltsatos V (2001) Bovine mastitis and intramammary drug delivery: review and perspectives. *Advanced Drug Delivery Reviews* **50**, 245–259.

Guimarães JL, Brito MA, Lange CC, Silva MR, Ribeiro JB, Mendonça LC, Mendonça JF and Souza GN (2017) Estimate of the economic impact of mastitis: a case study in a Holstein dairy herd under tropical conditions. *Preventive Veterinary Medicine* **142**, 46–50.

Haseeb MT, Hussain MA, Yuk SH, Bashir S and Nauman M (2016) Polysaccharides based superabsorbent hydrogel from linseed: dynamic swelling, stimuli responsive on–off switching and drug release. *Carbohydrate Polymers* **136**, 750–756.

Hasnain MS, Rishishwar P, Rishishwar S, Ali S and Nayak AK (2018) Isolation and characterization of *Linum usitatisimum* polysaccharide to prepare mucoadhesive beads of diclofenac sodium. *International Journal of Biological Macromolecules* **116**, 162–172.

Kanmani P and Rhim JW (2014) Development and characterization of carrageenan/grapefruit seed extract composite films for active packaging. *International Journal of Biological Macromolecules* **68**, 258–266.

Khalil HA, Saurabh CK, Tye YY, Lai TK, Easa AM, Rosamah E, Fazita MRN, Syakir MI, Adnan AS, Fizree HM and Aprilia NAS (2017) Seaweed based sustainable films and composites for food and pharmaceutical applications: a review. *Renewable and Sustainable Energy Reviews* **77**, 353–362.

Lanctôt S, Fustier P, Taherian AR, Bisakowski B, Zhao X and Lacasse P (2017) Effect of intramammary infusion of chitosan hydrogels at drying-off on bovine mammary gland involution. *Journal of Dairy Science* **100**, 2269–2281.

Langoni H, Salina A, Oliveira GC, Junqueira NB, Menozzi BD and Joaquim SF (2017) Considerations on the treatment of mastitis. *Pesquisa Veterinária Brasileira* **37**, 1261–1269.

Lima GG, de Souza RO, Bozzi AD, Poplawska MA, Devine DM and Nugent MJ (2016) Extraction method plays critical role in antibacterial activity of propolis-loaded hydrogels. *Journal of Pharmaceutical Sciences* **105**, 1248–1257.

Locatelli C, Cremonesi P, Caprioli A, Carfora V, Ianzano A, Barberio A, Morandi S, Casula A, Castiglioni B, Bronzo V and Moroni P (2017) Occurrence of methicillin-resistant *Staphylococcus aureus* in dairy cattle herds, related swine farms, and humans in contact with herds. *Journal of Dairy Science* **100**, 608–619.

Mabry TJ, Markham KR and Thomas MB (1970) The ultraviolet spectra of flavones and flavonols. In Mabry TJ, Markham KR and Thomas, MB (eds), *The Systematic Identification of Flavonoids*. Heidelberg (Berlin): Springer, pp. 41–164.

Makino K, Idenuma R, Murakami T and Ohshima H (2001) Design of a rate-and time-programming drug release device using a hydrogel: pulsatile drug release from κ-carrageenan hydrogel device by surface erosion of the hydrogel. *Colloids and Surfaces B: Biointerfaces* **20**, 355–359.

Muhamad II, Lazim NAM and Selvakumaran S (2019) Natural polysaccharide-based composites for drug delivery and biomedical applications. In Hasnain MdS and Nayak AK (eds), *Natural Polysaccharides in Drug Delivery and Biomedical Applications*. Cambridge, MA: Academic Press, pp. 419–440.

Naran R, Chen G and Carpita NC (2008) Novel rhamnogalacturonan I and arabinoxylan polysaccharides of flax seed mucilage. *Plant Physiology* **148**, 132–141.

Pinheiro Machado GTP, Veleirinho MB, Honorato LA and Kuhnen S (2020) Formulation and evaluation of anti-MRSA nanoemulsion loaded with *Achyrocline satureioides*: a new sustainable strategy for the bovine mastitis. *Nano Express* **1**, 030004.

Pinheiro Machado GTP, Veleirinho MB, Ferreira RG, Zuglianello C, Lemos-Senna E and Kuhnen S (2022) Protection of bovine mammary epithelial cells by *Achyroline satur*eioides (Lam.) DC. Nanoemulsion and its capacity of permeation through mammary epithelium. *Journal of Dairy Research* **28**, 1–6.

Puttipipatkhachorn S, Nunthanid J, Yamamoto K and Peck GE (2001) Drug physical state and drug–polymer interaction on drug release from chitosan matrix films. *Journal of Controlled Release* **75**, 143–153.

Radostits OM, Mayhew IG and Houston DM (2000) *Veterinary Clinical Examination and Diagnosis*, 2nd Edn. London, UK: W.B. Saunders, 771 p.

Retta D, Dellacassa E, Villamil J, Suárez SA and Bandoni AL (2012) Marcela, a promising medicinal and aromatic plant from Latin America: a review. *Industrial Crops and Products* **38**, 27–38.

Sandasi M, Leonard CM and Viljoen AM (2010) The in vitro antibiofilm activity of selected culinary herbs and medicinal plants against *Listeria monocytogenes*. *Letters in Applied Microbiology* **50**, 30–35.

Shojaee-Aliabadi S, Mohammadifar MA, Hosseini H, Mohammadi A, Ghasemlou M, Hosseini SM, Haghshenas M and Khaksar R (2014) Characterization of nanobiocomposite kappa-carrageenan film with *Zataria multiflora* essential oil and nanoclay. *International Journal of Biological Macromolecules* **69**, 282–289.

Singleton VL and Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* **16**, 144–158.

Sordillo LM (2016) Nutritional strategies to optimize dairy cattle immunity. *Journal of Dairy Science* **99**, 4967–4982.

Urena-Saborio H, Alfaro-Viquez E, Esquivel-Alvarado D, Madrigal-Carballo S and Gunasekaran S (2018) Electrospun plant mucilage nanofibers as biocompatible scaffolds for cell proliferation. *International Journal of Biological Macromolecules* **115**, 1218–1224.

Ziolkovska A (2012) Laws of flaxseed mucilage extraction. *Food Hydrocolloids* **26**, 197–204.