



Research Article

Natamycin-loaded electrospun poly(ϵ -caprolactone) nanofibers as an innovative platform for antifungal applications

Flávio Fonseca Veras¹ · Ana Carolina Ritter¹ · Isabel Roggia² · Patricia Pranke³ · Cláudio Nunes Pereira² · Adriano Brandelli¹ 

Received: 25 March 2020 / Accepted: 15 May 2020 / Published online: 21 May 2020
© Springer Nature Switzerland AG 2020

Abstract

This study aimed to develop and characterize poly- ϵ -caprolactone (PCL) nanofibers containing the natural antifungal natamycin by electrospinning technique using four different formulations. Morphological, structural and thermal properties of PCL nanofibers, with or without natamycin, were investigated by scanning electron microscopy, Fourier-transform and near infrared spectroscopy, thermogravimetric analysis and differential scanning calorimetry. Electrospun nanofibers were tested against filamentous fungi and yeasts. SEM images revealed the formation of nanofibers with typical string-like morphology, with mean diameter ranging from 232 to 363 nm. Results from spectroscopy and thermal analyses indicated that no relevant drug-polymer interaction takes place during nanofiber preparation. Natamycin-loaded nanofibers prepared from the formulations NF1 (PLC dissolved in tetrahydrofuran/dimethylformamide) and NF4 (PLC with polyethylene glycol and medium-chain triglycerides dissolved in tetrahydrofuran/chloroform) presented better antifungal activity. A gradual natamycin migration from the PCL nanofibers was observed. The inhibition zones ranged from 4.3 to 25.6 mm, including when fungi were cultivated in skimmed milk agar as a food model system. The antifungal effect of nanofibers was also confirmed in samples of soft cheese. This work provides electrospinning formulations to prepare natamycin-loaded nanofibers as promising platforms to combat filamentous fungi and yeasts.

Keywords Antimicrobial · Controlled release · Electrospinning · Mycotoxin · Nanofiber

1 Introduction

Fungi are ubiquitous biological agents that are able to colonize different environments. Filamentous fungi are robust organisms capable of producing enzymes that can hydrolyze protective polysaccharides of the plant cell wall and hard proteins of animal skins. They are important pathogenic and spoilage organisms causing significant impact in human and animal health and crop production [1, 2].

In addition, several fungi may produce toxic metabolites under certain environmental conditions. Mycotoxins are fungal secondary metabolites that have been widely associated with intoxications in humans and domestic animals [3] and are not only important by their broad occurrence, but mainly due to their high toxicity [4]. The International Agency for Research on Cancer (IARC) evaluated the carcinogenic potential of these compounds: aflatoxins, broadly produced among some *Aspergillus* species, were recognized as human carcinogenic agents (Group 1),

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s42452-020-2912-z>) contains supplementary material, which is available to authorized users.

✉ Adriano Brandelli, abrand@ufrgs.br | ¹Laboratório de Bioquímica e Microbiologia Aplicada, Instituto de Ciência e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, Porto Alegre 91501-970, Brazil. ²Tecnano Pesquisas e Serviços Ltda, Porto Alegre, Brazil. ³Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.



SN Applied Sciences (2020) 2:1105 | <https://doi.org/10.1007/s42452-020-2912-z>

while ochratoxin A is classified in the Group 2B, as possible human carcinogenic agent [5].

Chemical, physical and biological strategies have been investigated to control mycotoxin contamination. However, decontamination of spoiled crops and food is often very complex and expensive [6, 7]. Thus, the best way to avoid mycotoxin contamination remains the prevention of fungal growth. Despite the use of chemical fungicides is recognized as an efficient strategy to control toxigenic fungi, there is a necessity for safe methods to inhibit these pathogens due to concerns about the toxicity of residues on human health and the environment.

The negative impact of yeast occurrence in different environments also needs proper attention. In addition to the numerous reports of yeasts causing human and animal infections [8, 9], such microorganisms have been associated with spoilage of foods and beverages as well [10, 11]. Chemical, physical and sensory properties of the product can be altered during yeast spoilage, including discoloration, off-flavors formation, softening and gas production, leading to significant economic losses for the food processing and manufacturing industries [11, 12].

Nanotechnology has been used as an interesting strategy for delivery and controlled release of bioactive compounds, including antimicrobial drugs. A promise of the use of nanotechnology in drug delivery is to reduce drug amounts, thus resulting a positive impact on human health and the environment [13]. Diverse nanostructures can be effective as vehicles to transport and controlled release of antimicrobial substances [14, 15]. Among these, electrospun nanofibers have been investigated as delivery platforms with potential applications in food, pharmaceutical and agricultural purposes [16]. Electrospinning is a very versatile and effective technology for obtaining continuous polymer fibers with a diameter ranging in the nanometer scale. The method is ideal for a rapid trial of polymers to be used in drug delivery of different active ingredients. Nanofibers have small diameter sizes and large surface areas, allowing high loadings of antimicrobials or other active molecules [17, 18].

Nanofibers incorporating synthetic antifungal, including conventional azole drugs such as clotrimazole and ketoconazole, have been described to control fungal growth [19, 20]. However, limited information on the encapsulation of natural antifungal molecules into nanofibers is available in the current literature. Natamycin, a natural antifungal produced by *Streptomyces natalensis*, has been employed in food and biomedical applications. The broad activity spectrum of natamycin allows its use as a food preservative, preventing the development of pathogenic and spoilage fungi in food products [21], as well as first choice against fungal keratitis and other eye fungal infections, such as blepharitis, and conjunctivitis

[22]. Thus, the development of nanofibers functionalized with natamycin may represent an interesting alternative to delivery this antimicrobial agent [23]. PCL can be a suitable polymer for the development of natamycin-functionalized nanofibers and different formulations of this polymer could influence the physical and chemical properties of the produced nanofibers. The slow degradation and hydrophobicity of PCL make it interesting for the development of compounds delivery devices that remains active for long periods, in addition to other well-known properties [24]. Thus, the aims of this study were to incorporate natamycin into PCL nanofibers from different formulations and to compare their morphological and physicochemical characteristics in order to determine their effectiveness as a platform to delivery natamycin in practical food packaging applications. These nanostructures were also investigated for the antifungal properties including natamycin release capacity in simulant solutions, milk agar and cheese.

2 Methods

2.1 Chemicals

Poly- ϵ -caprolactone (PCL; average MW 80,000) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Polyethylene glycol (PEG, average MW 4000) was from Synth (São Paulo, Brazil) and medium-chain triglycerides (MCT) was obtained from Croda (Campinas, Brazil). Tetrahydrofuran (THF), *N,N*-dimethyl formamide (DMF), dichloromethane (CH_2Cl_2), chloroform (CHCl_3) and HPLC grade acetonitrile were from Merck (Darmstadt, Germany). Natamycin was purchased from Granotec (Curitiba, Brazil) as a commercial form (Natamilab; 50% natamycin, 42% lactose, 8% moisture), and from Sigma-Aldrich (St. Louis, MO, USA) as standard (purity > 95%). All solutions were prepared using ultrapure water obtained from a Milli Q device (Millipore, Billerica, MA, USA).

2.2 Microorganisms

Aspergillus carbonarius and *Penicillium citrinum* strains were kindly provided by Instituto de Tecnologia de Alimentos (ITAL, Campinas, Brazil). *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus* sp. A29 and *Fusarium oxysporum* strains were from the collection of the Laboratório de Toxicologia de Alimentos, Universidade Federal do Rio Grande do Sul (Porto Alegre, Brazil). *Candida albicans* ATCC 18804 and *C. albicans* DEN13 were kindly provided by Dr. Alexandre Fuentefria (Faculdade de Farmácia, UFRGS). The fungal cultures were maintained on potato dextrose agar (PDA; Merck, Darmstadt, Germany) slants at 4 °C.

2.3 Preparation of PCL/natamycin nanofibers by electrospinning

Electrospinning was essentially carried out as described previously [25]. PCL (100 g/L) was dissolved in solutions of THF:DMF (4:1, v/v), acetone, or CH₂Cl₂:DMF (4:1, v/v) containing 5 g/L commercial natamycin, to produce formulations NF1, NF2 and NF3, respectively. Formulation NF4 was prepared with 50 g/L PCL, 24 g/L PEG and 14.4 g/L MCT dissolved in THF:CHCl₃ (3:1, v/v) containing 5 g/L natamycin. The natamycin concentration was based on previous literature for antifungal film/fiber preparation [23, 26]. Besides the four formulations described above, the same nanofibers without natamycin were produced as controls. The following processing parameters were applied to the electrospinning apparatus: 10 mL of injected polymer solution; voltage of 30 kV; feeding rate of 0.05 mL/min; inner needle diameter of 0.5 mm; distance to the collector 16 cm. The nanofibers were collected on an aluminum plate (15 × 15 cm). The process was developed at 25 °C. The collected nanofibers were dried overnight for elimination of the residual solvent.

2.4 Nanofiber characterization

The morphology of nanofibers was observed by scanning electron microscopy (SEM) using a JEOL JSM 5800 microscope (Jeol, Tokyo, Japan) at an accelerating voltage of 10 kV. Images were taken after coating the samples with a 5 nm Au/Pd layer by sputtering. The average fiber diameter (AFD) was determined from the SEM images, and around 100 fibers were analyzed for each treatment [27].

Fourier transform infrared (FTIR) spectra were recorded using a FTIR Varian 640-IR spectrometer (Varian Inc., Palo Alto, CA, USA) in attenuated total reflectance (ATR) mode with a diamond crystal. The scans were collected between 600 and 4000 cm⁻¹ at a 4 cm⁻¹ resolution. Near infrared (NIR) spectra were obtained using a Spectrum 100 N spectrometer equipped with an ATR device (Perkin Elmer, Waltham, MA, USA). The samples were scanned from 4000 to 10,000 cm⁻¹ at a 4 cm⁻¹ resolution.

A thermogravimetric analyzer model TGA Discovery (TA Instruments, New Castle, DE, USA) was used for the thermal stability evaluation of PCL nanofibers. The samples were heated in platinum pans from 25 to 800 °C at the rate 10 °C/min under nitrogen atmosphere (flow rate 25 mL/min). Differential scanning calorimetry (DSC) studies were performed using a DSC Q2000 apparatus (TA Instruments). Samples equivalent to approximately 11 mg were placed in aluminum pans and heated from -80 to 200 °C, with a heating rate of 20 °C/min under nitrogen atmosphere. An empty pan sealed with a cover pan was used as a reference sample [28]. Crystallinity for PCL nanofibers was calculated

as $\chi_c = \Delta H_m / \Delta H_c^0$, considering the melting enthalpy of 100% crystalline PCL is 81.6 J/g [29].

Drug load was determined for each of the four formulations of PCL nanofibers. Samples of 4 mg were added to a solution of 0.2 mL ultrapure water and 1.8 mL acetonitrile. After 30 min at 37 °C, an aliquot was removed and the natamycin concentration was determined by HPLC as detailed below. The drug load was calculated as DL = (released drug amount/sample weight).

2.5 Fungal inhibition assays

For antifungal activity tests, the methodology employed for filamentous fungi was described elsewhere [20]. Nanofiber samples were cut into squares (approximately 1 × 1 cm), sterilized under UV light for 60 min (30 min each side), and placed onto the surface of PDA plates containing 10⁶ spores/mL for each evaluated microorganism. Assays against yeast were performed using cells suspension (10⁸ CFU/mL) in saline solution (9 g/L NaCl) followed inoculation with a swab onto PDA plates [30]. Zone diameters were measured after 5 days incubation at 25 ± 2 °C for filamentous fungi, and 48 h at 30 ± 2 °C for yeasts. A paper disk containing 20 µg natamycin was used as positive control. As negative control, PCL nanofibers prepared from polymer solution without the antifungal were also tested.

Samples were also evaluated in milk agar as a food model system [31]. Briefly, skimmed milk agar (5.0 g/L peptone; 3.0 g/L yeast extract, 1.0 g/L powder skim milk, and 12 g/L agar) was prepared and mixed with the spore suspension (10⁶ spores/mL). Nanofibers were overlaid onto the inoculated medium and incubated at 20 °C for 5 days.

Finally, the nanofibers were tested against fungal growth by direct application onto cheese samples, using two previously described experimental approaches: (1) during intentional contamination with filamentous fungi and (2) in the control of natural cheese mycobiota [32]. For the first test, slices (2 mm thick × 5.5 cm diameter) of Minas Frescal soft cheese were sterilized under UV light for 30 min and kept in Petri plates. Then, 10 µL of the spore suspension (10⁶ spores/mL) were spread onto the cheese surface. Samples were coated with sterile nanofiber discs (16 mm diameter) and incubated at 20 °C for 7 days. Fungal growth was checked after the incubation period. Nanofibers were also used to control the growth of fungi naturally present in cheese [32]. In this case, pieces of cheese (1.6 cm diameter) were cut under aseptic conditions, but without exposure to UV light and no fungal inoculation. Samples were introduced into Petri plates and completely covered with nanofiber discs of the same diameter. After 14 days incubation at 20 °C, discs were removed from the cheese pieces for visual inspection of fungal growth. Subsequently, the same samples (now without the nanofiber

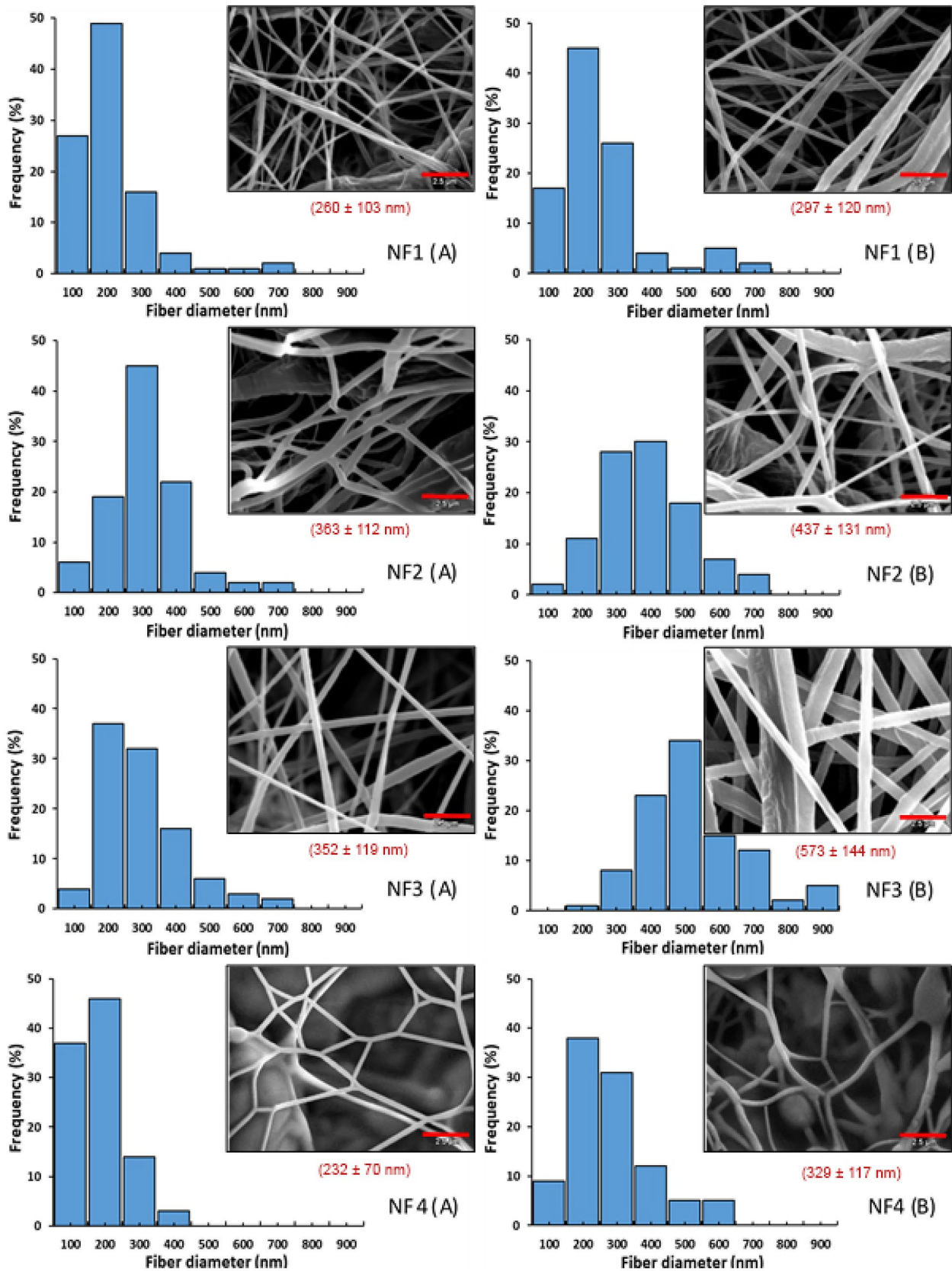


Fig. 1 Diameter distribution and scanning electron microscopy images (inset) of electrospun PCL nanofibers obtained from different solutions: (NF1) THF:DMF, (NF2) acetone, (NF3) CH₂Cl₂:DMF, and (NF4) PEG + MCT in THF:CHCl₃ solution. Letters A and B indicate control and natamycin-functionalized nanofibers, respectively. Red bar in SEM images represents the scale = 2.5 μm. Values immediately below the microscopy images are the mean ± standard deviation of nanofiber diameter

coating) were incubated again at 20 °C for 3 days or placed on PDA plates followed by incubation at 30 °C for 3 days. The presence or absence of fungal growth was determined in both conditions.

2.6 Drug release assay

In order to describe the release of natamycin from the nanofibers, the migration assay was performed using different simulant solutions [33, 34]. These included distilled water (simulant A), 5% (v/v) Tween 20 in 9 g/L NaCl solution (simulant B), 3% (v/v) acetic acid (simulant C), 15% (v/v) ethanol (simulant D). These solutions have been used as mimetics of different food categories to investigate the migration of antimicrobial or antioxidant compounds from polymeric materials [31, 35, 36]. An amount of 20 mg of nanofiber sample was immersed into 20 mL of each simulant solution and shaken at 125 rpm at 25 °C. After 1, 2, 4, 8, 24, 48, and 72 h, aliquots were removed for natamycin quantification and determination of antifungal activity as described above. A recovery experiment was carried out by incubating standard natamycin into simulant solutions under the same conditions.

2.7 Natamycin quantification

The natamycin quantification was performed by high performance liquid chromatography (HPLC) according to the instructions of the standard antifungal manufacturer (Sigma-Aldrich, article 32417) with minor modifications. Analyses were performed using an HPLC equipment (Waters, model E2695) equipped with a diode array detector (PAD-2998) and C18 reverse-phase column (XBridge™ Shield RP18; 5 μm; 4.6 mm × 150 mm, Waters, Dublin, Ireland). Samples were eluted in gradient mode as detailed in Table S1. The mobile phase consisted of acetonitrile:acidified water with 0.1% (v/v) phosphoric acid. The sample injection volume was 10 μL at a flow rate of 1.23 mL/min. The column temperature was adjusted to 30 ± 1 °C and the detection wavelength was 220 nm. Mobile phase was filtered through 0.45 μm nylon filters and degassed by sonication prior to use. A standard calibration curve was prepared in the mobile phase with 1, 5, 10, 25, 50 μg/mL natamycin (Sigma-Aldrich). Regression analysis for HPLC provided a linear relationship of

concentration to absorbance with a R^2 value of 0.9997. The values of detection and quantification limits under these conditions were determined as approximately 0.01 and 0.04 μg/mL, respectively.

2.8 Hemolysis assay

Hemolysis assay was performed using 1 mL heparinized sheep blood (Laborclin, Pinhais, Brazil), mixed with 4 mg nanofibers suspended in 1 mL PBS. Samples were incubated for 60 min at 37 °C, centrifuged at 3000g for 10 min, and the supernatant (1 mL) was collected. The amount of released hemoglobin was determined by reading the absorbance at 540 nm. Natamycin at 60 μg/mL was used as control [23]. Erythrocytes were lysed with 0.1% (v/v) Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) to determine the absorbance of 100% hemolysis. The negative control was PBS. The hemolytic activities were calculated relative to the Triton X-100-treated samples. Experiments were performed in triplicate.

2.9 Statistical analysis

All the tests were performed in triplicate and data were shown as mean ± standard deviation (SD) values. The results were subjected to analysis of variance (ANOVA) using SAS for Windows ver. 9.0 (SAS Institute Inc., Cary, NC). Differences were considered significant at a 95% confidence level by using the Tukey test.

3 Results and discussion

3.1 Nanofiber morphology

Four electrospinning formulations based on poly-ε-caprolactone (PCL) were used to prepare nanofibers functionalized with natamycin, in order to characterize and investigate their efficacy as antifungal nanomaterials.

SEM images revealed that electrospun PCL nanofibers presented uniform appearance, with well-formed structures and a typical string-like morphology (Fig. 1). It is generally assumed that smooth nanofibers are formed when all the ingredients of the formulation have good miscibility, although other factors like viscosity and conductivity can interfere in morphology and diameter of nanofibers obtained by electrospinning [37, 38]. Exceptionally, there was evidence of beads formation in NF4 nanofibers, mainly after natamycin incorporation (Fig. 1). The formation of beads has been attributed to the elasticity of the solution. Fluids with low relaxation time or low extensional viscosity tend to result in beads formation. The mechanism is due to Rayleigh instability driven by surface tension, which

can be suppressed by viscoelastic behavior of the fluid jet [39]. In this regard, the MCT introduced in the polymeric system should be encapsulated inside the polymeric shell, composed of PEG/PCL, due to surface tension differences.

The distribution charts of nanofiber diameter are also presented in Fig. 1. The control PCL nanofibers showed mean diameters of 260 ± 103 , 363 ± 112 , 352 ± 119 and 232 ± 70 nm for NF1, NF2, NF3 and NF4 formulations, respectively. The mean fiber diameter increased when natamycin was included in the electrospinning solutions, especially for NF3 nanofibers in which the diameter has increased 64%. Nanofibers NF1 (THF:DMF) functionalized with natamycin showed the smallest increase of mean diameter (14%). The increase in nanofiber diameter after addition of certain compounds, including antimicrobials, in the polymer matrix has been reported [40, 41]. In a previous study, the incorporation of ketoconazole enlarged the diameter range of PCL nanofibers [20]. A study on electrospun gelatin fiber mats functionalized with different antifungals (amphotericin B, fluconazole, itraconazole, natamycin and terbinafine) revealed that among the five compounds tested, natamycin had a greater influence on the nanofiber diameter after its addition in the formulation (from 800 to 1800 nm) [23]. On contrast, the addition of silver nanoparticles up to 1 wt% reduced the diameter of poly(vinyl chloride)/cellulose acetate nanofibers, which was attributed to increased charge density subjecting the jet to stronger stretching forces resulting in smooth and thinner fibers [17].

One of the most important parameters in the characterization of nanostructures is the evaluation of their size. Although there are different methods to prove the nanometric size, such as dynamic light scattering, laser diffraction and particle tracking [42], these techniques could not be appropriate for the characterization of nanofibers mats produced by electrospinning process. To employ such methodologies the samples must be in the powder or suspension form [43, 44]. For these reasons, electron microscopy has still been the main approach adopted in the current literature for diameter evaluation of nanofibers mats produced by electrospinning [45–47].

The electro-spinnability of polymers depends on several factors, such as polymer solubility, surface tension/viscosity, conductivity, dielectric constant (or dipole moment) and evaporation ratio of the solution [18]. PCL dissolves equally well in acetone, THF, CH_2Cl_2 , CHCl_3 and DMF, which were the solvents used in the present study. One important approach to evaluate the solubility is the evaluation of the Hansen solubility parameters of the polymer [48, 49]. It is common to use a solvent mixture to achieve the correct evaporation ratio (in order to avoid wet spinning or clogging of the needle tip), a

sufficient electronic charge transfer to the nanofiber collector (dependent on the polymer and solvent dielectric constants) or to reduce the needle tip drip (dependent on voltage applied, viscosity and surface tension).

It seems therefore advantageous to dissolve the polymer in binary solvent mixes. The solvents should be sufficiently good to avoid phase separation during evaporation when producing the nanofibers. It is also advisable to use a rapid evaporating solvent mixed with a low evaporating solvent (such as DMF) in order to avoid needle drip or clogging of the needle tip. Different binary solvent mixes along with a single solvent (acetone, a preferable solvent for PCL) were tested to evaluate possible differences in electrospinning process and nanofiber properties. In sample NF4, besides the use of a binary solvent mix, a polymeric blend system (PEG/PCL) was tested. A hydrophilic polymer, such as PEG, is commonly added to increase dissolution in polar media, such as biological systems, stabilizing the formulation in polar solvents or increasing the transfer of active ingredients (natamycin in this study) to the dissolution medium.

3.2 Infrared spectroscopy

Infrared spectroscopy studies were carried out to determine possible drug/polymer interactions. FTIR results indicate that the presence of natamycin does not alter the spectra of PCL nanofibers (Fig. 2). Control and natamycin-functionalized nanofibers presented similar FTIR spectra, with the typical peaks of PCL at 2927 cm^{-1} (asymmetric CH_2 stretching), 2864 cm^{-1} (symmetric CH_2 stretching), 1727 cm^{-1} (carbonyl stretching), 1294 cm^{-1} (C=O and C–C stretching), 1242 cm^{-1} (asymmetric C–O–C stretching) and 1173 cm^{-1} (symmetric C–O–C stretching) [50]. On the other hand, the presence of absorption peaks between 1270 and 1180 cm^{-1} also corresponds to characteristic C=O groups of natamycin [51] and could justify the discrete increase of these peaks in the spectra of nanofibers incorporating the antifungal.

The NIR spectra of control and functionalized nanofibers for all formulations were similar (Fig. S1) and also presented typical peaks of PCL: bands at 5174 cm^{-1} (C=O ester) and 5820 cm^{-1} (asymmetric CH_2 stretching), and broad bands at 6990 cm^{-1} and 8360 cm^{-1} assigned to C–H combination [52]. As suggested in a previous study, the absence of significant differences in the spectra of PCL nanofibers when incorporated with ketoconazole may indicate the lack of relevant chemical interaction between the antifungal and the polymer [20]. This fact could also be observed in nanofibers functionalized with natamycin.

Fig. 2 FTIR–ATR spectra of electrospun PCL nanofibers. **a** Full spectra of nanofibers obtained from different electrospinning solutions: (NF1) THF:DMF, (NF2) acetone, (NF3) CH₂Cl₂:DMF, and (NF4) PEG + MCT. Spectra of natamycin-functionalized nanofibers from these formulations are also presented as NF/NAT. Inside box **b** shows the magnification of the FTIR spectra between 1600 and 800 cm⁻¹

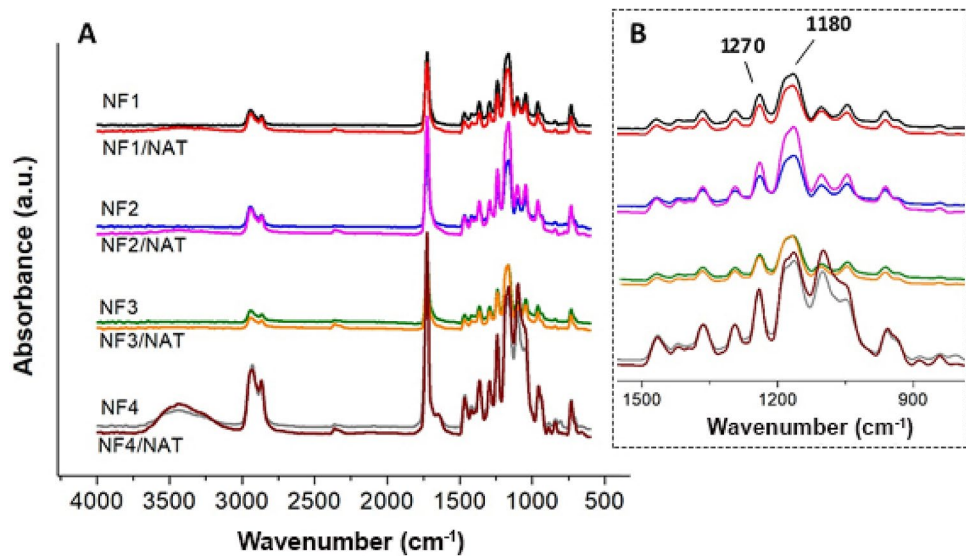


Table 1 Thermal parameters of different poly-ε-caprolactone (PCL) nanofibers derived from DSC thermograms

Samples ^a	Thermal parameter ^b				
	T_{onset} (°C)	T_m (°C)	ΔH_m (J/g)	T_g (°C)	χ_c
NF1	50.0	55.0	50.1	-64.5	0.61
NF1/NAT	48.2	56.5	52.1	-70.5	0.64
NF2	49.3	54.1	59.5	-64.8	0.73
NF2/NAT	50.3	55.2	52.1	-65.0	0.64
NF3	49.3	54.5	58.6	-64.5	0.72
NF3/NAT	50.7	55.5	49.4	-64.0	0.60
NF4	45.5	49.8	26.5	-49.5	0.32
NF4/NAT	48.8	55.5	20.7	-32.7	0.25

Nanofibers functionalized with natamycin (NF/NAT) were also evaluated

^aNF1, NF2, NF3 and NF4 indicate nanofibers produced by different formulations (for further clarification of these formulations, see item 2.3 of this article)

^b T_{onset} =onset of melting temperature; T_m =melting temperature; ΔH_m =enthalpy of fusion; T_g =glass transition temperature; χ_c =crystallinity, calculated as $\chi_c = \Delta H_m / \Delta H_c^0$, considering the melting enthalpy of 100% crystalline PCL is 81.6 J/g

3.3 Thermal properties

DSC studies were performed to obtain information about the physical state of the natamycin in the electrospun nanofibers. The DSC thermograms and main thermal parameters evaluated are presented in Fig. S2 and Table 1, respectively. A typical endothermic melting peak for PCL at around 54 °C was observed in the thermograms of both control PCL nanofibers and natamycin-functionalized nanofibers, indicating that obtained PCL fibers had a crystalline structure. The thermograms for

the natamycin-loaded nanofibers showed only a peak for the presence of PCL. The absence of any detectable crystalline peak for natamycin within the electrospun nanofibers suggests that the substance was molecularly dispersed within the polymer matrix or was present within the fibers in an amorphous state [53, 54].

The onset of melting temperature (T_{onset}) ranged from 45 to 50 °C. Values of enthalpy of fusion (ΔH_m) were between 49.4 and 59.5 J/g. The glass transition temperature (T_g) was measured around -65.5 °C. The degree of crystallinity (χ_c) were found ranging from 0.60 to 0.73 (Table 1). Independent of natamycin addition, the thermal parameters presented in this study are in agreement with the literature [28, 29, 55]. However, a slight variation in melting peaks (intensity and position of these endotherms) indicate that the type of solvent used in formulations might affect the thermal behavior of PCL. These effects may be related to solvent properties, including different interactions with certain polymers and the degree of volatility. A rapid solvent evaporation is required for nanofiber formation during electrospinning, and this rate can influence polymer crystallization [28, 56, 57].

Furthermore, when PEG and MCT were added to the formulation (NF4), important differences were observed in DSC thermogram. In this case, the values for parameters ΔH_m , T_g and χ_c were 26.5 J/g and 20.7 J/g, -49.5 °C and -32.7 °C, and 0.32 and 0.25 for the control and functionalized nanofibers, respectively. Depression of glass transition temperature (T_g) was observed with the introduction of PEG, as effective plasticization induces the depression of T_g in polymer/plasticizer system. Thus, PEG was expected to be compatible with PCL, similarly as described for PLA/PEG blends [58, 59]. In addition,

an exothermic peak at 158 °C was observed for NF4 nanofibers when incorporated with natamycin (Fig. S2B).

DSC analysis of the components (natamycin, PEG, MCT and PEG/MCT mixture) showed an endothermic peak around 213.7 °C for natamycin sample (data not shown). This peak corresponds to natamycin melting [60] and its absence in the nanofiber thermograms suggests the drug is in amorphous state within the polymeric matrix. Both PEG sample and the mixture PEG/MCT showed a PEG-related melting peak at 59.7 °C [61].

TGA results showed that NF2 and NF3 nanofibers underwent single stage thermal degradation. Weight losses started from around 350 °C and almost completed at 460 °C (Fig. S3). The decomposition of NF1 and NF4 nanofibers occurred in two stages. The minor step of NF1 nanofibers corresponds to water loss while second weight loss starting at about 360 °C was attributed to decomposition of PCL polymer. The first stage of thermal degradation of NF4 nanofibers was found between 160 and 310 °C and due to its considerable weight decrease (28%), it could be associated to water and MCT loss. Studies on the thermal stability of PEG show that polymer degradation start at around 320 °C and completely decompose before 440 °C [62, 63]. This temperature range is within the values found only at the second degradation peak of NF4 nanofibers.

In derivative thermogravimetric (DTG) analysis, the maximum degradation rate was similar for all control nanofibers at around 410 °C (Fig. S3). Natamycin-functionalized nanofibers presented similar values for this parameter when compared to control nanofibers. Therefore, the natamycin incorporation into PCL nanofibers did not affect their thermal stability, excepting NF4

nanofibers, which showed decreased maximum degradation rate to 378 °C.

3.4 Drug load

The drug load was determined for each natamycin-functionalized nanofiber as 8.2 ± 0.8 , 2.5 ± 0.3 , 3.6 ± 0.7 and 5.0 ± 1.8 µg natamycin per mg of nanofiber for NF1, NF2, NF3 and NF4 formulations, respectively. These results suggest a better compatibility of natamycin with the formulations NF1 and NF4, and could be associated with the good drug loading capacity of PCL and the lipophilic character of natamycin [64]. The addition of MCT in formulations increases the entrapment of compounds like penicillin and colchicine in polymeric nanoparticles [65, 66], and could benefit the natamycin load in NF4 as well. The lower drug load observed in NF2 and NF3 may be related with their higher crystallinity (Table 1), thus hindering interactions between the hydrophobic polymer core and natamycin.

3.5 Antifungal activity

The antifungal properties of PCL nanofibers were initially tested against eight isolates including both yeasts and filamentous fungi. Control nanofibers caused no inhibition of fungal growth, while natamycin-functionalized nanofibers regularly inhibited all the tested fungi (Fig. S4). The results showed that NF1 and NF4 nanofibers were more efficient as antifungal materials since the values of inhibition diameters were significantly higher than those observed for NF2 and NF3 (Table 2). *P. citrinum* and *F. oxysporum* were the most sensitive to functionalized nanofibers, with inhibition diameters of 25.6 and 23.3 mm, respectively. As expected, the disks containing natamycin (positive control) showed

Table 2 Antifungal activity of poly-ε-caprolactone nanofibers against several fungal isolates

Fungi	Diameter of inhibitory halo (mm)								
	Natamycin-functionalized nanofiber				Control nanofiber				Control Natamycin
	NF1	NF2	NF3	NF4	NF1	NF2	NF3	NF4	
<i>Aspergillus flavus</i>	4.3 ± 0.5 ^{ab}	0 ± 0 ^c	1.3 ± 0.5 ^b	8.3 ± 0.5 ^a	0 ± 0 ^c	0 ± 0 ^c	0 ± 0 ^c	0 ± 0 ^c	10.0 ± 0.1 ^a
<i>A. carbonarius</i>	20.6 ± 0.8 ^a	9.0 ± 0.4 ^d	13.0 ± 0.6 ^c	18.3 ± 0.8 ^{ab}	0 ± 0 ^e	0 ± 0 ^e	0 ± 0 ^e	0 ± 0 ^e	17.0 ± 1.0 ^b
<i>Aspergillus</i> sp. A29	7.0 ± 0.3 ^a	0 ± 0 ^b	0 ± 0 ^b	4.3 ± 0.3 ^a	0 ± 0 ^b	0 ± 0 ^b	0 ± 0 ^b	0 ± 0 ^b	6.5 ± 0.9 ^a
<i>A. niger</i>	19.3 ± 1.0 ^a	9.3 ± 0.8 ^c	14.0 ± 0.7 ^b	22.0 ± 1.1 ^a	0 ± 0 ^d	0 ± 0 ^d	0 ± 0 ^d	0 ± 0 ^d	9.3 ± 0.2 ^c
<i>Fusarium oxysporum</i>	23.3 ± 1.2 ^a	8.3 ± 1.0 ^d	13.0 ± 0.7 ^{cd}	21.0 ± 1.5 ^{ab}	0 ± 0 ^e	0 ± 0 ^e	0 ± 0 ^e	0 ± 0 ^e	17.7 ± 1.8 ^{bc}
<i>Penicillium citrinum</i>	25.6 ± 1.0 ^a	14.6 ± 0.7 ^c	14.0 ± 0.8 ^c	19.3 ± 0.8 ^b	0 ± 0 ^d	0 ± 0 ^d	0 ± 0 ^d	0 ± 0 ^d	15.7 ± 0.6 ^c
<i>Candida albicans</i> DEN13	12.6 ± 0.6 ^{ab}	5.6 ± 0.4 ^b	10.6 ± 0.5 ^b	11.0 ± 0.6 ^{ab}	0 ± 0 ^c	0 ± 0 ^c	0 ± 0 ^c	0 ± 0 ^c	18.8 ± 2.2 ^a
<i>C. albicans</i> ATCC 18804	14.6 ± 0.7 ^b	12.6 ± 0.6 ^c	11.6 ± 0.5 ^b	15.3 ± 0.7 ^b	0 ± 0 ^d	0 ± 0 ^d	0 ± 0 ^d	0 ± 0 ^d	18.9 ± 0.7 ^a

Paper disks containing natamycin were used as positive control. Results are the means ± standard deviations; PCL was dissolved in solutions of THF:DMF (4:1), acetone, CH₂Cl₂:DMF (4:1) to produce formulations NF1, NF2 and NF3, respectively. The polymer was also mixed with 40% PEG and middle chain triglycerides (MCT) in THF:CHCl₃ (3:1) for NF4 formulation; ^{a,b,c}Values followed by different superscript letters in each line are significantly different ($p < 0.05$)

a fungicidal effect against all tested fungi, being possible to visualize inhibition halos ranging from 6.5 to 17.7 mm. Antimicrobial activity was also observed against yeasts (Fig. S4). Once again, NF1 and NF4 nanofibers presented better results.

These results indicate that the antifungal activity of natamycin was preserved during the electrospinning procedure. The best results observed for NF1 and NF4 nanofibers could be justified by the higher drug load in these nanostructures, as previously described. The maintenance of active antifungal compounds after incorporation into electrospun nanofibers has been described [19, 20]. Inhibition halos below 15 mm were observed for electrospun gelatin fiber mats containing natamycin [23]. Those authors also developed nanofibers functionalized with itraconazole and fluconazole, but no effective inhibitory effect was observed for most fungi tested.

The absence of inhibition haloes against *A. flavus* and *Aspergillus* sp. A29 was observed for NF2 and NF3 nanofibers. However, there was no fungal growth under the surface of functionalized material, which was not observed in the control nanofibers (Fig. S4). The same behavior was verified for nylon nanofibers containing phloxine B against *A. fumigatus* [67]. It has been suggested that high water content in culture media can alter the release properties of natamycin in controlled distribution systems [68]. Thus, besides the lower loading of natamycin in NF2 and NF3 nanofibers, the antifungal concentration released to the medium may not have been sufficient to inhibit the growth of these fungi.

Based on these results, nanofibers NF1 and NF4 showing better antimicrobial properties were selected to confirm their effect in food systems, as well as to verify the potential cytotoxicity on erythrocytes and natamycin migration into simulant solutions for drug release.

3.6 Hemolysis

Control PCL nanofibers caused no important hemolysis while free natamycin resulted in lysis of erythrocytes. The nanofibers incorporated with natamycin resulted in similarly low hemolytic degree than observed for bare nanofibers (Fig. S5). The hemocompatibility of PCL and PCL/PLA nanofibers was previously investigated and hemolysis values ranging from 1.2 to 6.5% were described [69], indicating that PCL nanofibers present little hemolytic activity. Vancomycin-loaded chitosan/poly(ethylene oxide) nanofibers intended for wound healing were also evaluated for hemolysis, showing values below 2% [70]. In addition, cytotoxicity studies of gelatin fiber mats revealed that the hemolytic activity of natamycin was significantly reduced by interaction with gelatin [23]. Considering that up to 5% hemolysis is tolerable for biomaterials, these

results suggest that the association of natamycin with biocompatible polymers such as PCL or gelatin can lead to decreased cytotoxicity, as the drug is not freely available in sufficient amount to damage the cells.

3.7 Drug release assays

The nanofibers were subjected to drug release assays by incubation in four different simulant solutions. Initially, natamycin recovery was tested using a final concentration of 25 and 50 µg/mL. After 72 h, natamycin concentration in each solution was determined by HPLC. The results of the recovery tests are shown in Table S2. In the four simulant solutions evaluated natamycin recovery ranged from 95.0 to 100%.

A gradual natamycin migration from the PCL nanofibers was observed during 72 h incubation in simulant solutions, excepting for ethanol solution where a burst release was seen (Fig. 3). Although ethanol causes no apparent changes in PCL nanofiber morphology, the mean diameter slightly increases suggesting swelling, which could facilitate natamycin release. Ornidazole-loaded electrospun PCL showed a similar burst release with almost 80% drug release in first 3 h and complete release in about 18 h. In the other simulants tested, antifungal release was slower for NF1 nanofibers, in which about 30% natamycin content was released at 48 h. Natamycin release reached maximum values for NF4 nanofibers after 48 h incubation with both 5% Tween 20 and 3% acetic acid. This behavior was consistent with the antifungal properties since aliquots showing highest natamycin release showed activity against the two fungi preselected for this assay (Table S3). In the case of NF4 nanofibers, the use of the amphiphilic PEG/PCL blend would give an advantage concerning natamycin release from the nanofiber matrix. In addition, MCT have been used as an additive to increase the porosity of polymeric nanoparticles rendering them susceptible to water penetration, which results in improved drug release [65].

3.8 Antifungal activity in milk agar and cheese

The antifungal activity of NF1 and NF4 nanofibers in skimmed milk agar is shown in Fig. 4. Inhibition zones ranging from 6.6 to 24 mm were obtained against *A. flavus* and *P. citrinum* for both functionalized nanofibers (Table S4) indicating natamycin diffusion from the nanofibers. The rich constitution of this culture medium allows the growth of numerous microorganisms commonly found in milk and dairy products being used as a model system in studies about antimicrobial food packaging [31, 71].

Natamycin-functionalized nanofibers were also effective in inhibiting fungal growth when tested in cheese as a real food system (Fig. 5). NF4 nanofibers showed better results

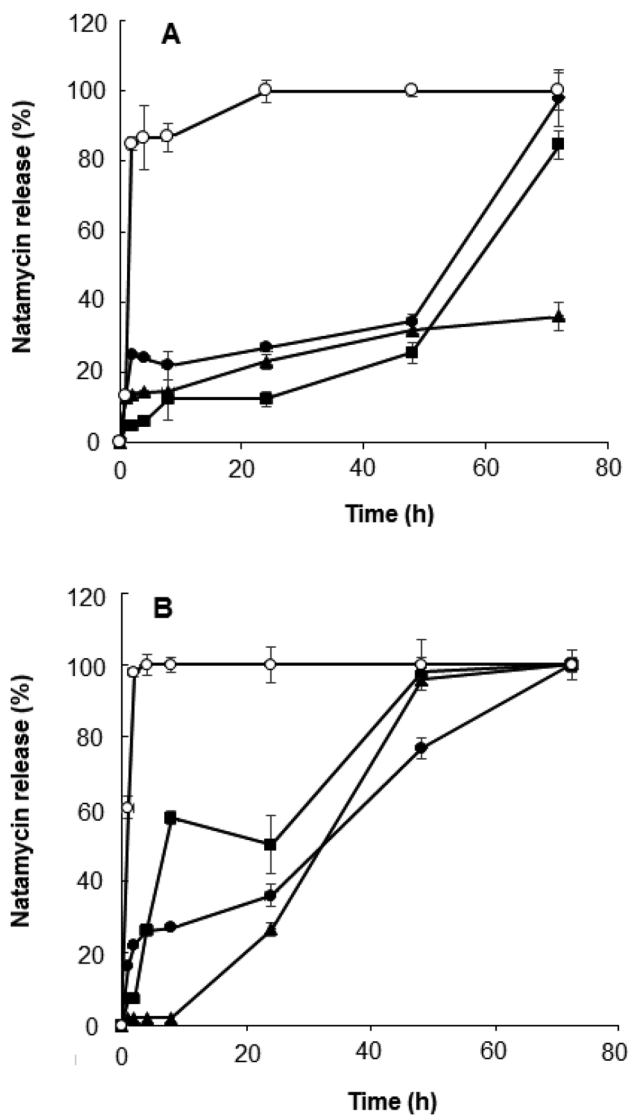


Fig. 3 Release profile of natamycin from poly- ϵ -caprolactone nanofibers into food simulant solutions over 72 h incubation at 25 °C and 125 rpm. Nanofibers NF1 (a) and NF4 (b) were incubated in different solutions, as detailed in Sect. 2. Simulants correspond to water (filled circle), 5% Tween 20 (filled square), 3% acetic acid (filled triangle) and 15% ethanol (opened circle), respectively. The experiment was conducted under sink conditions, considered as the ability of the media to dissolve at least 3–10 times the amount of drug that is in the dosage form, and natamycin solubility in water as 4.1 mg/mL (PubChem CID 5284447). Results are the means \pm standard deviations of three independent experiments

since they provided growth inhibition of both *A. flavus* and *P. citrinum* at the cheese/nanofiber mat interface. However, this effect was not observed at the cheese regions uncoated with nanofibers suggesting that for this type of cheese the antifungal activity is restricted to the contact area. NF1 sample was effective only against *P. citrinum*. The absence of antifungal activity against *A. flavus* is consistent with the previous results, in which this fungus showed greater resistance

to the nanofibers action. In the control PCL nanofibers, the fungi grew even over the polymer disk.

In addition, natamycin-functionalized nanofibers were able to control the growth of fungi naturally present in cheese (Fig. S6). It was possible to observe the absence of fungi in the cheese coated with NF1 and NF4 nanofibers during 14 days incubation at 20 °C. After removal of nanofiber discs, the antifungal effect remained when the cheese samples were incubated for additional 3 days at 20 °C, but not for those incubated at 30 °C in PDA medium. According to the proposed methodology [32], this experiment can indicate the fungicidal or fungistatic effect of antimicrobial coatings. Therefore, the results suggest that nanofibers have a fungistatic effect on the mycobiota of the cheese under investigation. The effectiveness of NF1 nanofiber in inhibiting the cheese mycobiota, when compared to its failure to control *A. flavus* growth (Fig. 5), can be justified because the microbial load naturally present in cheese would be less than the spore concentration intentionally added to the samples. Moreover, the fungi commonly present in cheese could have different degrees of sensitivity to natamycin.

Dairy products favor the undesirable development of both spoilage and toxigenic fungi [72]. Species of the genera *Penicillium* and *Aspergillus* are often isolated from cheese and their presence is usually associated with inadequate standards of hygiene and resistance to antimicrobials or heat treatment [72, 73]. Although natamycin is commonly used as a food preservative, some factors such as poor solubility in water, inactivation by components of the food matrix and rapid migration, may compromise its application effectiveness on the food surface [74, 75]. The incorporation of natamycin in nanofibers appeared to favor its antifungal action when applied as coatings on cheese slices, probably due a better distribution and sustained release on the surface of this product. The results obtained on milk agar and soft cheese suggest the use of natamycin-functionalized nanofibers as possible antimicrobial material. In this case, the development of an active packaging containing these nanofibers mats could contribute to the prevention and control of fungal growth in dairy products, especially in cheese. Future studies on the effectiveness of these PCL nanofibers can be performed with other dairy products, including different soft, semi-soft and semi-hard cheese, since their different compositions could influence the antifungal activity of nanofibers.

4 Conclusion

In this work, four formulations based on PCL were successfully electrospun as natamycin-functionalized nanofibers. The antifungal addition in the polymeric

Fig. 4 Antifungal activity of poly- ϵ -caprolactone nanofibers against *Aspergillus flavus* (a) and *Penicillium citrinum* (b) when cultivated in skimmed milk agar. Numbers 1 and 3 correspond to the nanofibers functionalized with natamycin obtained from formulations NF1 and NF4, respectively. Numbers 2 and 4 indicate control nanofibers NF1 and NF4, respectively (detailed formulations are provided in Sect. 2)

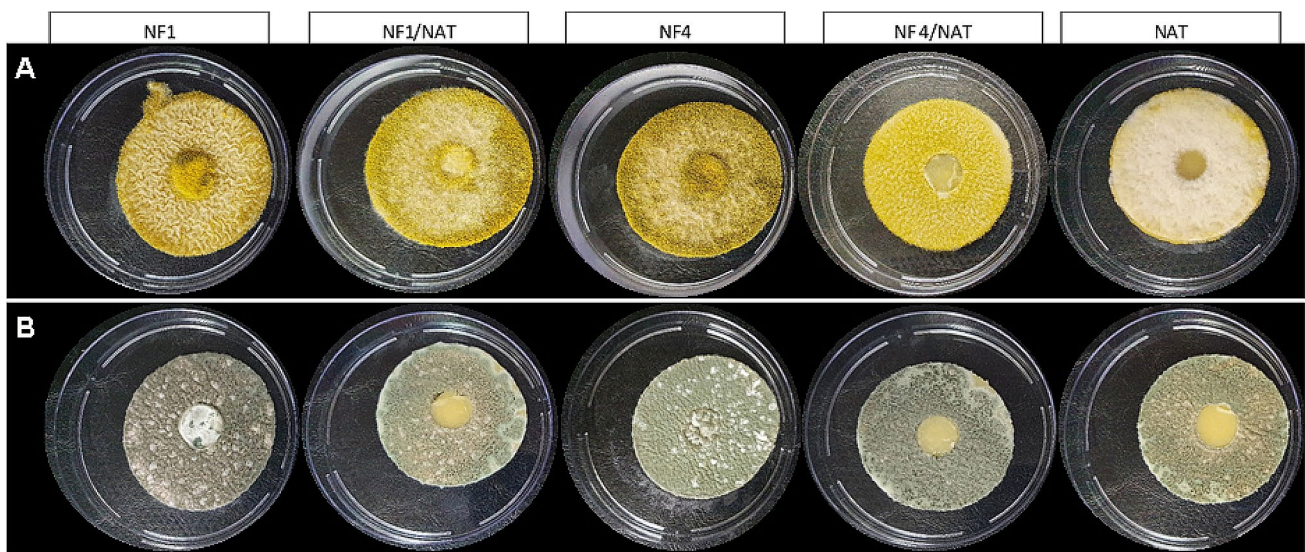
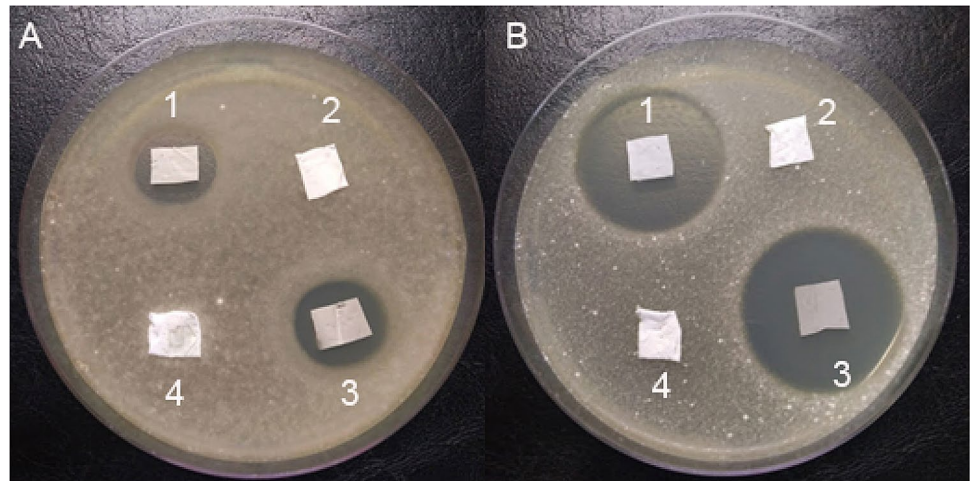


Fig. 5 Inhibition of *Aspergillus flavus* (a) and *Penicillium citrinum* (b) inoculated on the surface of soft-cheese in contact with PCL nanofibers (NF1 and NF4), including natamycin-functionalized nanofibers (NF/NAT). Cheese samples were incubated at 20 °C during 7 days

solutions did not cause marked alterations in the morphological and physicochemical parameters of the nanofibers. Antifungal properties of the PCL nanofibers were confirmed against all indicator fungi. In addition, it has been proven that natamycin could be released from PCL nanofibers in food simulant solutions. This is the first report incorporating natamycin into PCL nanofibers in order to investigate their efficacy as antifungal nanomaterials, and highlights the importance of PCL electrospun nanofibers as an interesting platform for natamycin delivery. Whereas most studies have been focused on development of nanofibers functionalized with conventional azole drugs, the use of natamycin for functionalization of nanofibers has a relevance due to the low toxicity and broad spectrum of action on fungi. Therefore, obtaining such nanostructures encourages

future studies for the development of antifungal packaging as a promising strategy for controlling the presence of undesirable microorganisms in food systems.

Authors' contributions FFV and performed the experiments and wrote the manuscript, ACR and IR performed experiments and data analysis, PP participated in experimental design and data analysis, CNP and AB conceived the idea, participated in design and coordination, and secured funding. All authors reviewed the final manuscript.

Funding This work received financial support of FAPERGS (Grant 18/2551-000497-9) and CNPq (Grant 306936/2017-8).

Compliance with ethical standards

Conflicts of interest The authors declare that they had no conflict of interest.

Ethics approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

- Spadaro D, Gullino ML (2014) State of art and future prospects of the biological control of postharvest fruit diseases. *J Med Microbiol* 91:185–194
- Magro A, Carolino M, Bastos M, Mexia A (2006) Efficacy of plant extracts against stored products fungi. *Rev Iberoam Micol* 23:176–178
- Zain ME (2011) Impact of mycotoxins on humans and animals. *J Saudi Chem Soc* 15:129–144
- Alshannaq A, Yu JH (2017) Occurrence, toxicity, and analysis of major mycotoxins in food. *Int J Environ Res Public Health* 14:632
- IARC (2012) Chemical agents and related occupations. Volume 100F: a review of human carcinogens. In: IARC Monographs on the evaluation of carcinogenic risks to humans. International Agency for Research on Cancer, Lyon, pp 225–248
- Awad WA, Ghareeb K, Böhm J, Zentek J (2010) Decontamination and detoxification strategies for the *Fusarium* mycotoxin deoxynivalenol in animal feed and the effectiveness of microbial biodegradation. *Food Addit Contam A* 27:510–520
- Hathout AS, Aly SE (2014) Biological detoxification of mycotoxins: a review. *Ann Microbiol* 64:905–919
- Miceli MH, Díaz JA, Lee SA (2011) Emerging opportunistic yeast infections. *Lancet Infect Dis* 11:142–151
- Moubasher AH, Abdel-Sater MA, Soliman Z (2017) Incidence and biodiversity of yeasts, dermatophytes and non-dermatophytes in superficial skin infections in Assiut, Egypt. *J Mycol Méd* 27:166–179
- Howell K (2016) Spoilage: yeast spoilage of food and beverages. In: Caballero B, Finglas P, Toldrá R (eds) *Encyclopedia of food and health*. Academic Press, New York, pp 113–117
- Hernández A, Pérez-Nevado F, Ruiz-Moyano S, Serradilla MJ, Vilalobos MC, Martín A, Córdoba MG (2018) Spoilage yeasts: what are the sources of contamination of foods and beverages? *Int J Food Microbiol* 286:98–110
- Kabisch J, Erl-Höning C, Wenning M, Böhnlein C, Gareis M, Pichner R (2016) Spoilage of vacuum-packed beef by the yeast *Kazachstania psychrophile*. *Food Microbiol* 53:15–23
- Brandelli A (2015) Nanobiotechnology strategies for delivery of antimicrobials in agriculture and food. In: Rai M, Ribeiro C, Mattoso L, Duran N (eds) *Nanobiotechnologies in food and agriculture*. Springer International Publishing, Cham, pp 119–139
- Álvarez-Paino M, Muñoz-Bonilla A, Fernández-García M (2017) Antimicrobial polymers in the nano-world. *Nanomaterials* 7:48
- Lopes NA, Brandelli A (2018) Nanostructures for delivery of natural antimicrobials in food. *Crit Rev Food Sci Nutr* 58:2202–2212
- Muñoz-Bonilla A, Fernández-García M (2015) The roadmap of antimicrobial polymeric materials in macromolecular nanotechnology. *Eur Polym J* 65:46–62
- Tarus BK, Mwasiagi JI, Fadel N, Al-Oufy A, Elmessiry M (2019) Electrospun cellulose acetate and poly(vinyl chloride) nanofiber mats containing silver nanoparticles for antifungal packaging. *SN Appl Sci* 1:245
- Wei Q (2012) *Functional nanofibers and their applications*. Woodhead Publishing, Philadelphia
- Mir AS, Garg T, Vaidya B, Goyal AK, Rath G (2014) Development and characterization of clotrimazole loaded electrospun nanofibers for the management of oral candidiasis. *J Nanoparm Drug Deliv* 2:192–198
- Veras FF, Roggia I, Pranke P, Pereira CN, Brandelli A (2016) Inhibition of filamentous fungi by ketoconazole-functionalized electrospun nanofibers. *Eur J Pharm Sci* 84:70–76
- Pisoschi AM, Pop A, Georgescu C, Tuscus V, Olah NK, Mathe E (2018) An overview of natural antimicrobials role in food. *Eur J Med Chem* 143:922–935
- Patil A, Lakhani P, Majumdar S (2017) Current perspectives on natamycin in ocular fungal infections. *J Drug Deliv Sci Technol* 41:206–221
- Lakshminarayanan R, Sridhar R, Loh XL, Nandhakumar M, Barathi VA, KalaiPriya M, Kwan JL, Liu PS, Beuerman RW, Ramakrishna S (2014) Interaction of gelatin with polyenes modulates antifungal activity and biocompatibility of electrospun fiber mats. *Int J Nanomed* 9:2439–2458
- Woodruff MA, Hutmacher DW (2010) The return of a forgotten polymer—polycaprolactone in the 21st century. *Prog Polym Sci* 35:1217–1256
- Castañeda L, Genro C, Roggia I, Bender S, Bender R, Pereira CN (2014) Innovative rice seed coating (*Oryza sativa*) with polymer nanofibres and microparticles using the electrospinning method. *J Res Update Polym Sci* 3:33–39
- Silva MA, Iamanaka BT, Taniwaki MH, Kieckbusch TG (2013) Evaluation of the antimicrobial potential of alginate and alginate/chitosan films containing potassium sorbate and natamycin. *Packag Technol Sci* 26:479–492
- Canbolat MF, Celebioglu A, Uyara T (2014) Drug delivery system based on cyclodextrin–naproxen inclusion complex incorporated in electrospun polycaprolactone nanofibers. *Colloids Surf B* 115:15–21
- Ji Y, Liang K, Shen X, Bowlin GL (2014) Electrospinning and characterization of chitin nanofibril/polycaprolactone nanocomposite fiber mats. *Carbohydr Polym* 101:68–74
- Danesin R, Brun P, Roso M, Delaunay F, Samouillan V, Brunelli K, Lucci G, Ghezzi F, Modesti M, Castagliuolo I, Dettin M (2012) Self-assembling peptide-enriched electrospun polycaprolactone scaffolds promote the h-osteoblast adhesion and modulate differentiation-associated gene expression. *Bone* 51:851–859
- Motta AS, Brandelli A (2002) Characterization of an antibacterial peptide produced by *Brevibacterium linens*. *J Appl Microbiol* 92:63–71
- Meira SMM, Zehetmeyer G, Jardim AI, Scheibel JM, Oliveira RVB, Brandelli A (2014) Polypropylene/montmorillonite nanocomposites containing nisin as antimicrobial food packaging. *Food Bioprocess Technol* 7:3349–3357
- Balaguer MP, Fajardo P, Gartner H, Gómez-Estaca J, Gavara R, Almenar E, Hernández-Muñoz P (2014) Functional properties and antifungal activity of films based on gliadins containing cinnamaldehyde and natamycin. *Int J Food Microbiol* 173:62–71
- Simoneau C (2009) Guidelines on testing conditions for articles in contact with food-stuffs. Office for Official Publications of the European Communities, Luxembourg
- ANVISA (2010) Regulamento técnico Mercosul sobre migração em materiais, embalagens e equipamentos plásticos destinados a entrar em contato com alimentos, RDC 51. *Diário Oficial da União* 244:75
- Ribeiro-Santos R, Sanches-Silva A, Motta JFG, Andrade M, Neves IA, Teófilo RF, Carvalhi MG, Melo NR (2017) Combined use of essential oils applied to protein base active food packaging: study in vitro and in a food simulant. *Eur Polym J* 93:75–86

36. Tampau A, González-Martínez C, Chiralt A (2018) Release kinetics and antimicrobial properties of carvacrol encapsulated in electrospun poly(ϵ -caprolactone) nanofibres. Application in starch multilayer films. *Food Hydrocolloids* 79:158–169
37. Zahedi P, Rezaeian I, Jafari SH, Karami Z (2013) Preparation and release properties of electrospun poly(vinyl alcohol)/poly(ϵ -caprolactone) hybrid nanofibers: optimization of process parameters via D-optimal design method. *Macromol Res* 21:649–659
38. Tonglairoum P, Ngawhirunpat T, Rojanarat T, Kaomongkolgit R, Opanasopit P (2015) Fabrication of a novel scaffold of clotrimazole-microemulsion-containing nanofibers using an electrospinning process for oral candidiasis applications. *Colloids Surf B* 126:18–25
39. Islam MS, Ang BC, Andriyana A, Affi AM (2019) A review on fabrication of nanofibers via electrospinning and their applications. *SN Appl Sci* 1:1248
40. Lian Y, Zhan JC, Zhang KH, Mo XM (2014) Fabrication and characterization of curcumin-loaded silk fibroin/P(LLA-CL) nanofibrous scaffold. *Front Mater Sci* 8:354–362
41. Park JA, Kim SB (2015) Preparation and characterization of antimicrobial electrospun poly(vinyl alcohol) nanofibers containing benzyl triethylammonium chloride. *React Funct Polym* 93:30–37
42. Stetefeld J, McKenna SA, Patel TR (2016) Dynamic light scattering: a practical guide and applications in biomedical sciences. *Biophys Rev* 8:409–427
43. Fahmi MZ, Prasetya RA, Dzikri MF, Sakti SCW, Yuliarto B, Irzaman F (2020) MnFe₂O₄ nanoparticles/cellulose acetate composite nanofiber for controllable release of naproxen. *Mater Chem Phys* 250:123055
44. Wijesena RN, Tissera ND, Rathnayaka VWG, de Silva RM, Nalin de Silva KM (2020) Colloidal stability of chitin nanofibers in aqueous systems: effect of pH, ionic strength, temperature and concentration. *Carbohydr Polym* 235:116024
45. Khoshbakht S, Asghari-Sana F, Fathi-Azarbayjani A, Sharifi Y (2020) Fabrication and characterization of tretinoin-loaded nanofiber for topical skin delivery. *Biomater Res* 24:8
46. Mehrali F, Ziyadi H, Hekmati M, Faridi-Majidi R, Qomi M (2020) Kefiran/poly(vinyl alcohol)/poly(vinyl pyrrolidone) composite nanofibers: fabrication, characterization and consideration of effective parameters in electrospinning. *SN Appl Sci* 2:895
47. Ng IS, Ooi CW, Liu BL, Peng CT, Chiu CY, Chang YK (2020) Antibacterial efficacy of chitosan- and poly(hexamethylene biguanide)-immobilized nanofiber membrane. *Int J Biol Macromol* 154:844–854
48. Bordes C, Fréville V, Ruffin E, Marote P, Gauvrit JY, Briançon S, Lantéri PP (2010) Determination of poly(ϵ -caprolactone) solubility parameters: application to solvent substitution in a microencapsulation process. *Int J Pharm* 383:236–243
49. Vebber GC, Pranke P, Pereira CN (2014) Calculating Hansen solubility parameters of polymers with genetic algorithms. *J Appl Polym Sci* 131:39696
50. Benkaddour A, Jradi K, Robert S, Daneault C (2013) Grafting of polycaprolactone on oxidized nanocellulose by click chemistry. *Nanomaterials* 3:141–157
51. Shin J, Liu X, Chikthimmah N, Lee YS (2016) Polymer surface modification using UV treatment for attachment of natamycin and the potential applications for conventional food cling wrap (LDPE). *Appl Surf Sci* 386:276–284
52. Workman J, Weyer L (2012) Practical guide and spectral atlas for interpretative near infrared spectroscopy. CRC Press, Boca Raton
53. Xin H, Chen L, Gu J, Ren X, Wei Z, Luo J, Chen Y, Jiang X, Sha X, Fang X (2010) Enhanced anti-glioblastoma efficacy by PTX-loaded PEGylated poly(ϵ -caprolactone) nanoparticles: in vitro and in vivo evaluation. *Int J Pharm* 402:238–247
54. Rychter M, Baronowska-Korczyk A, Milanowski B, Jarek M, Maciejewska BM, Coy EL, Lulek J (2018) Cilostazol-loaded poly(ϵ -caprolactone) electrospun drug delivery system for cardiovascular applications. *Pharm Res* 35:1–20
55. Dulnik J, Denis P, Sajkiewicz P, Kołbuk D, Choińska E (2016) Biodegradation of bicomponent PCL/gelatin and PCL/collagen nanofibers electrospun from alternative solvent system. *Polym Degrad Stabil* 130:10–21
56. Oliveira JE, Mattoso LHC, Orts WJ, Medeiros ES (2013) Structural and morphological characterization of micro and nanofibers produced by electrospinning and solution blow spinning: a comparative study. *Adv Mater Sci Eng* 2013:409572
57. Kołbuk D, Sajkiewicz P, Maniura-Weber K, Fortunato G (2013) Structure and morphology of electrospun polycaprolactone/gelatine nanofibers. *Eur Polym J* 49:2052–2061
58. Yoon YI, Park KE, Lee SJ, Park WH (2013) Fabrication of microfibrous and nano-/microfibrous scaffolds: melt and hybrid electrospinning and surface modification of poly(L-lactic acid) with plasticizer. *Biomed Res Int* 6:309048
59. Athanasoulia IG, Tarantili LA (2016) Preparation and characterization of polyethylene glycol/poly(L-lactic acid) blends. *Pure Appl Chem* 89:141–152
60. Khames A, Khaleel MA, El-Badawy MF, El-Nezhawy AOH (2019) Natamycin solid lipid nanoparticles—sustained ocular delivery system of higher corneal penetration against deep fungal keratitis: preparation and optimization. *Int J Nanomed* 14:2515–2531
61. Golitsyn Y, Pulst M, Samiullah MH, Busse K, Kressler J, Reichter D (2019) Crystallization in PEG networks: the importance of network topology and chain tilt in crystals. *Polymer* 165:72–82
62. Sari A, Bicer A, Al-Sulaiman FA, Karaipekli A, Tyagi VV (2018) Diatomite/CNTs/PEG composite PCMs with shape-stabilized and improved thermal conductivity: preparation and thermal energy storage properties. *Energy Build* 164:166–175
63. Zhao Y, Min X, Huang Z, Liu Y, Wu X, Fang M (2018) Honeycomb-like structured biological porous carbon encapsulating PEG: a shape-stable phase change material with enhanced thermal conductivity for thermal energy storage. *Energy Build* 158:1049–1062
64. Chandasana H, Prasad YD, Chhonker YS, Chaitanya TK, Mishra NN, Mitra K, Shukla PK, Bhatta RS (2014) Corneal targeted nanoparticles for sustained natamycin delivery and their PK/PD indices: an approach to reduce dose and dosing frequency. *Int J Pharm* 477:317–325
65. Kohee S, Yaghoobian M (2009) An investigation into the role of surfactants in controlling particle size of polymeric nanocapsules containing penicillin-G in double emulsion. *Eur J Med Chem* 44:2392–2399
66. Khalil R, Hashem F, Zaki H, El-Arini S (2014) Polymeric nanoparticles as potential carriers for topical delivery of colchicine: development and in vitro characterization. *Int J Pharm Sci Res* 17:1746–1756
67. Kim JR, Michielsen S (2015) Photodynamic antifungal activities of nanostructured fabrics grafted with rose bengal and phloxine B against *Aspergillus fumigatus*. *J Appl Polym Sci* 132:42114
68. Fajardo P, Martins JT, Fuciños C, Pastrana L, Teixeira JA, Vicente AA (2010) Evaluation of a chitosan-based edible film as carrier of natamycin to improve the storability of Saloio cheese. *J Food Eng* 101:349–356
69. Horakova J, Mikes P, Saman A, Svarcova T, Jencova V, Suchy T, Heczkova B, Jakubkova S, Jirousova J, Prochaskova R (2018) Comprehensive assessment of electrospun scaffolds hemocompatibility. *Mater Sci Eng C* 82:330–335
70. Samadieh S, Dehnad AR, Naghili B, Sadri M, Bazmani A (2019) Hemocompatibility assessment and drug release kinetics

- investigation from materials based on electrospun nanofibers. *Nanomed Res J* 4:10–15
71. Meira SMM, Zehetmeyer G, Scheibel JM, Werner JO, Brandelli A (2016) Starch-halloysite nanocomposites containing nisin: characterization and inhibition of *Listeria monocytogenes* in soft cheese. *LWT Food Sci Technol* 68:226–234
 72. Garnier L, Valence F, Mounier J (2017) Diversity and control of spoilage fungi in dairy products: an update. *Microorganisms* 5:42
 73. Anelli P, Haidukowski M, Epifani F, Cimmarusti MT, Moretti A, Logrieco A, Susca A (2019) Fungal mycobiota and mycotoxin risk for traditional artisan Italian cave cheese. *Food Microbiol* 78:62–72
 74. Fucinos C, Guerra NP, Teijon JM, Pastrana LM, Rúa ML, Katime I (2012) Use of poly(N-isopropylacrylamide) nanohydrogels for the controlled release of pimaricin in active packaging. *J Food Sci* 77:N21–N28
 75. Lantano C, Alfieri I, Cavazza A, Corradini C, Lorenzi A, Zucchetto N, Montenero A (2014) Natamycin based sol–gel antimicrobial coatings on polylactic acid films for food packaging. *Food Chem* 165:342–347

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.