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Development and Evaluation of Antimicrobial Natamycin-incorporated Film in Gorgonzola Cheese Conservation

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Conservation of food products depends on product quality and packaging suitability. The objective of this work was to develop and evaluate the antimicrobial efficiency of natamycin-incorporated film in the production process of Gorgonzola cheese. It aims to optimize the production process and increase shelf-life and food safety for the consumer. Films with different concentrations of natamycin were produced and tested in Gorgonzola cheeses to evaluate its efficiency against* Penicillium roqueforti *on the cheese surface. Films with 2 and 4% natamycin presented satisfactory results for fungus inhibition and the amount of natamycin released to the cheese was below that allowed by the legislation.

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INTRODUCTION

Cheeses are subject to several types of deterioration during processing and storage. The main cause is the development of micro-organisms, mainly fungi, yeasts and bacteria, which not only can damage the appearance of the product but can cause harm to consumers' health.

Gorgonzola cheese ripening is carried out either by *Penicillium roqueforti* or *Penicillium glaucum*, which grow within its mechanical openings. Due to the strong lipolytic and proteolytic action of the mould, the cheese develops a peculiar and pronounced flavour and aroma.[1]

One of the most common problems affecting the quality of Gorgonzola cheese is the slime formation on the surface resulting from micro-organism growth. The film-forming yeasts belonging to the *Torula* and *Cândida* genera and the mould from the *Penicillum*, *Cladosporium*, *Alternaría*, *Monilia*, *Aspergillus* and *Geotricium* genera can eventually grow on the surface.[1] Contamination by these

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micro-organisms usually comes from the salt solution or from the environment during handling and ripening of cheese. The typically moist atmosphere of cheese ripening chambers can favour the appearance of this contaminating flora.[2]

Gorgonzola cheese ripening takes from 60 to 120 days. At the end of the 30 initial days, the cheese is scraped and then wrapped to complete ripening. Packaging at this stage reduces the internal mould growth and protects the surface against excessive dehydration and potential contaminant growth.[1]

When treating maturated cheeses, a series of measures can be taken to maintain the unfavourable growth of mould under control. The cheese, for example, can be wrapped with an antimicrobial agent (such as natamycin) to inhibit the growth of fungi and yeasts on the surface.[1] Natamycin is applied immediately before cheese perforation, that is, between 7–10 days after starting the ripening, in 0.2–0.4% aqueous solution by immersion.[2] Afterwards, the cheese is perforated and then wrapped. Natamycin, acting only on the cheese surface, does not interfere in the beneficial growth of *Penicillium*, which happens within the product.

Natamycin (or pimaricin) is a polyene antifungal antibiotic produced by *Streptomyces natalensis*. It is used to control fungus growth in the surface of most cheese and is not effective against bacteria or viruses.[3] Natamycin has been used for many years in a large number of countries throughout the world as an authorized preservation treatment for cheeses and certain meat products such as dried sausages. When applied to the surface of the cheese or sausage, natamycin shows very limited diffusion and tends to stay on the surface of the food.[4]

De Boer and Stolk-Horsthuis, cited by Davidson and Harrison,[5] investigated the potential for the development of resistance to natamycin among fungi. They reported no evidence of resistant fungi in cheese warehouses where natamycin was used for periods of up to several years.

The use of natamycin is allowed by the Brazilian legislation, Mercosul (South Economic Community) and the European Community as an additive for food preservation.[6] The maximum natamycin concentration in the final product should be 1 mg/dm^2, and a maximum non-detectable 5 mg/kg at 2 mm depth.[7]

One of the functions of packaging is to preserve maximum product quality, creating conditions that minimize chemical, biochemical and microbiological alterations. However, the traditional concept that this function should be exerted with minimum interaction between the packaging and the product is surpassed by several technologies that have been developed in the last decades. These technologies are based precisely on the packaging/product interaction as a means of preserving food quality and safety.[8]

Therefore, in the last years, different concepts of food packaging have been introduced, such as active packaging, which are technologies that have been growing in the last decades, since traditional packaging has limited capacity to increase the shelf-life of food products.[9]

Suppakul *et al.*[10] said that '*Active Packaging is an innovative concept that can be defined as a mode of packaging in which the package, the product, and the environment interact to prolong shelf life or enhance safety or sensory properties, while maintaining the quality of the product*'.

Antimicrobial packaging is considered as the most promising in the active packaging area (Flores *et al.*, cited by Suppakul *et al.*[10]). It contains antimicrobial agents to delay or inhibit microorganism growth in food in contact with the packaging.

Additives released from active packaging increase the consumers' safety since these compounds, instead of being directly added to the food, are released in a controlled way, in smaller amounts and only where they are necessary, such as on the product surface, in which most of the deterioration takes place with the occurrence of larger microbial contamination.[11,12]

Plackett *et al.*[13] used a l-polylactide and l-polylactide–polycaprolactone co-polymer films and evaluated their suitability as materials for cheese packaging. Tests indicated that a cyclodextrin-encapsulated antimicrobial (allyl isothiocyanate) incorporated in l-polylactide–polycaprolactone copolymer films would be effective in controlling fungi on packaged cheeses.

Scannell *et al.*[14] studied the immobilization of the bacteriocins nisin and lacticin to packaging materials and observed their activity against *Lactococcus lactis* subsp. *lactis*, in addition to *Listeria innocua* and *Staphylococcus aureus.* The resulting film main-

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tained its activity for a 3-month period, both at room temperature and under refrigeration. When applied to food systems, the antimicrobial packaging reduced the population of lactic acid bacteria in sliced cheese and ham stored in modified-atmosphere packaging at refrigeration temperatures, thus extending product shelf-life.

Buonocore *et al.*[15] proposed the use of a monolayer cross-linked PVOH Poly (Vinyl Alcohol) film and a multilayer structure made of cross-linked PVOH layers to control the release of an active compound, lysozyme, and investigated its antimicrobial activity against *Micrococcus lysodeikticus.* Results showed that the incorporation of lysozyme into PVOH does not lead to a loss of enzyme activity.

Krejcova *et al.*[16] tested the effect of polyethylene (LDPE) Low Density Poly (echylene) packaging film incorporated with nisin on lactic acid bacteria, aerobic spore-forming bacteria and *Bacillus cereus* growth in packed meat products and processed cheese. The results confirmed the significant inhibitory effect of such packaging system.

Var *et al.*[17] studied the effect of natamycin and PVC Poly (Vinyl Chloride) on the microbiological control of Kashar cheese during the ripening period. Natamycin and packaging materials had no effect on the total aerobic mesophilic bacteria, yeast and lipolytic micro-organism counts. However, natamycin had showed an inhibitory effect on the proteolytic micro-organisms by itself and combined with packaging material. No mould growth was detected in the Kashar cheese samples produced by combined application of natamycin and packaging materials during the 5-month ripening period.

The objective of this work was to develop a natamycin-incorporated film (NIF) and to evaluate its antimicrobial efficiency on Gorgonzola cheese preservation, seeking to increase product shelf-life and higher food safety for the consumer.

MATERIAL AND METHODS

Cheese production

Gorgonzola cheese was produced at the Dairy Product Laboratory, Department of Food Technology – Federal University of Viçosa, according to Furtado and Neto.[18] Cheese was produced with 3.8% fat milk, placed in molds (12 cm height, 10 cm diameter) and taken to a cold chamber (10 ± 2°C; 85 ± 5% RU (Relative Humidity)).

Film production

The films (patent requested) were produced with a cellulose polymeric base incorporated with natamycin (Natamax), kindly provided by Dansico, Sao Paulo, Brazil. The films were produced by casting process, with 33 μm and 95 μm thickness and an average coating weight of 43.7 and 126.1 g/m^2, respectively. The natamycin concentrations tested were 0, 0.2, 0.5, 1, 2 and 4% in relation to cellulose flake weight.

The same solution used to produce the film was also spread by casting process on an aluminum foil to form a laminated film (LAF). The LAF was produced using 2 and 4% of natamycin and with 33 μm thickness.

The NIF and the LAF were used to involve Gorgonzola cheese and to verify their efficiency against *P. roqueforti* growth.

Microbiological analyses

In vitro. The evaluation of film antimicrobial activity and determination of optimum natamycin concentration was carried out *in vitro* in Petri dishes containing potato dextrose agar (PDA – Merck Rio de Janeiro, Brazil). The plate was divided in six areas, 20 μl (one drop) of the solution containing *P. roqueforti* (7.8×10^4 UFC/ml) was put in each area. A piece of film (1.8 cm in diameter) with natamycin concentrations of 0, 0.2, 0.5, 1, 2 and 4% was laid over each drop. Both NIF and LAF were tested. The plates were incubated at 25°C for 48 h and the efficiency was evaluated by the micro-organism growth around and under the film.

In the cheese. After salting and drying, the control cheeses were sprayed with an aqueous solution of 0.2% natamycin. After being dried, they were profusely perforated with a metal stick to guarantee internal oxygenation for fungus growth and were left to ripen for 25–30 days, with turnings every 2 days. After this period they were cleaned, scraped

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and wrapped in aluminum foil to complete the ripening for an additional period of 45 days in the ripening chamber.

The other cheeses were covered with NIF and LAF in natamycin concentrations of 2 and 4%, with three repetitions per concentration, assuring the maximum adhesion of the film to the cheese surface. These were kept in the same storage and ripening conditions given to the control cheeses. The films were added after the cheeses have been salted, dried and perforated.

Quantification of natamycin level on cheese surface

The analysis to verify natamycin migration from the film to the cheese surface was carried out according to Fletouris *et al.*[19] The cheese rind were removed at approximately 2 mm in depth after 45 days of ripening. The rind removed from each cheese (approximately 10 g) were mixed to 25 ml of extraction solvent, containing four parts of acetonitrile and one part of phosphoric acid 1M. The homogenized material was filtered and analysed in a spectrophotometer (GBC Model UV/VIS918, GBC Scientific equipment, Melbourne, Australia). The obtained curves were subjected to the third-derivative spectrum and the natamycin values in the cheeses were obtained by measuring the distance between the peak at 317 nm and the base line (Figure 1a). The curves obtained for the control cheeses and the cheeses with active films were compared with a standard curve obtained from the natamycin solutions of known concentrations to determine the amount of natamycin in the cheese's surface.

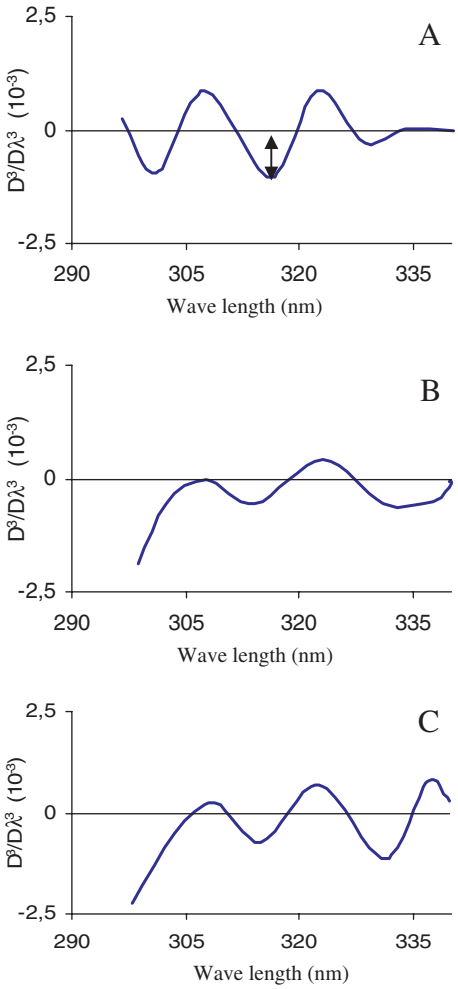

Figure 1. Curves obtained by the spectrophotometer method (third derived) for the (a) control cheese, (b) LAF 2% and (c) LAF 4%.

RESULTS AND DISCUSSION

Effect of the natamycin concentration and film thickness

The thicker films (95 μm) of NIF showed higher antimicrobial efficiency, and from 1% concentration no fungus growth was observed around and under the film laid in Petri dishes (Figure 2b). The thinner films, 33 μm, showed effective control from 2% (Figure 2a). However, the thicker films had lower flexibility and presented less adhesion to the agar, which can be observed in Figure 2b where the film caused some scratches on agar. So, films with approximately 30 μm thickness were selected to wrap the cheeses. Concentrations of 2 and 4% were used to increase film effectiveness.

Evaluation of the efficiency of the NIF

The cheeses wrapped with NIF showed growth inhibition of *P. roqueforti*. It was verified that in the cheese parts directly in contact with the film there was no significant growth of fungi in the concen-

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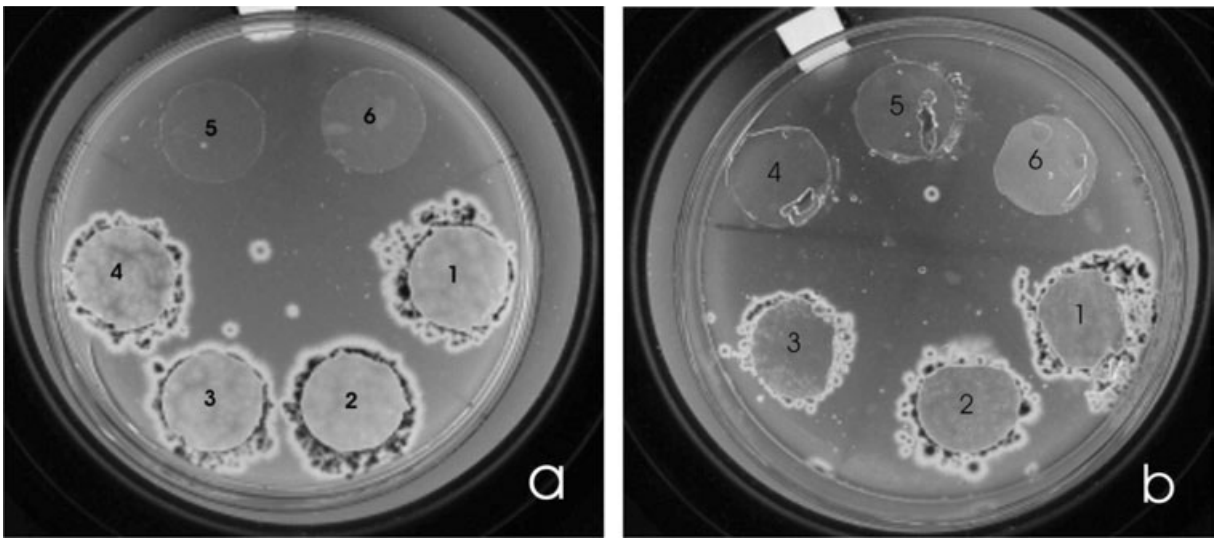

Figure 2. Plate inoculated with Penicillium roqueforti *fungi and covered with (a) 33-µm- and (b) 95-µm-thick films. (1) 0% natamycin film; (2) 0.2% natamycin film; (3) 0.5% natamycin film; (4) 1% natamycin film; (5) 2% natamycin film; (6) 4% natamycin film.*

trations of 2 and 4%, compared with the control cheese covered in natamycin solution. Due to the wrinkles on the cheese surface the fungus growth was only observed in the parts with less film contact (Figure 3).

Evaluation of the efficiency of the NIF laminated to aluminum foil (LAF)

The results with the LAF were similar to the NIF, hindering fungus growth from the concentrations of 2%. Cheeses presented a whiter colouration with no rind formation. This was probably due to the fact that the double layer of the packaging avoided excessive moisture loss which inhibited development of a hard surface (Figure 3).

Figure 3. Comparison between the cheeses after 45 days of ripening (control cheese on the left; on the top, cheeses wrapped with 2 and 4% NIF, respectively; at the bottom, cheeses wrapped with 2 and 4% LAF).

Evaluation of natamycin migration from the film to the cheese surface

The natamycin levels in the cheeses comply with the legislation, which allows a maximum of 5 mg/kg not detectable at 2 mm depth, according to the Mercosul Resolution no. 134/96[7] (Table 1).

The highest natamycin levels found in the cheeses wrapped with LAF may be due to higher moisture, which facilitated the additive migration (Table 1). The cheeses covered with the active packaging showed lower natamycin level in the rind when compared with the control, which is very satisfactory since they achieved greater

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Table 1. Amount of natamycin (mg/kg) found on the cheese surface after 45 days of ripening

Cheese	Mean (mg/kg)
Control	2.58 ± 0.21
With NIF 2%	Not detectable
With NIF 4%	Not detectable
With LAF 2%	1.67 ± 0.26
With LAF 4%	1.99 ± 0.25

microbial control, guaranteeing to the consumer more quality and a healthier product, with a lower ingestion of additive (Table 1).

The curves obtained in the spectrophotometer can be seen in Figure 1, where the control cheese and the cheese wrapped with 2 and 4% LAF are compared. One-layer films (NIF) are not shown since they obtained very small peaks, making their visualization difficult.

Reps *et al.*[20] also obtained reduction on natamycin concentration in cheeses' surface involved with polyvinyl acetate incorporated with natamycin, comparing with cheeses that had been immersed in an aqueous solution of natamycin, using a spectrophotometric method. Polyvinyl acetate containing 0.05% natamycin effectively protected the surface of cheeses against the development of undesirable moulds.

The LAF has great potential when compared to the one-layer film (NIF) since, besides presenting good results in the control of superficial fungus growth, it is more convenient, considering that the aluminum foil is generally used to wrap Gorgonzola cheese. Also, using the antimicrobial film, it is not necessary to scrap and immerse cheeses in natamycin solution many times, reducing some stages during the ripening process.

CONCLUSION

The need for packaging with flexible conditions for transport and storage associated with the growing demand for healthy, convenient and safe food assure a brilliant future for active packaging, which is considered as an emerging technology.

In this work, the effectiveness of active packaging in providing product conservation is confirmed by the inhibition of the growth of *P. roqueforti*, allied to the reduction of stages in the ripening process of Gorgonzola cheese, and therefore contributing to decrease possible undesirable contamination during these stages. Moreover, the level of natamycin released to cheese was lower than that found in the control cheese (conventional process of immersion in natamycin solution), decreasing, in this way, the levels of additives ingested by the consumer.

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