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Natamycin efficiency for controlling yeast growth in models systems and on cheese surfaces

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A B S T R A C T

Consumers demand for more natural ingredients in processed foods is a result of a requirement for more healthy and safe food with also a need for a balanced and adequate diet.

Natamycin is a polyene macrolide antibiotic which is active against yeasts and moulds but not against bacteria, viruses and protozoa.

In this study the effectiveness of natamycin delivered by different methods against *Saccharomyces cerevisiae*, *Zygosaccharomyces rouxii* and *Yarrowia lipolytica* using both food models and cheese with natural antimicrobials.

It was observed that natamycin concentration and yeast type influenced whether the natamycin effect in tapioca starch films was cidal or inhibitory. This was also observed when the antimicrobial was applied directly to a liquid system for comparison purposes. Bioavailability was not compromised by the polymeric supporting matrix and natamycin efficiency against a *S. cerevisiae* contamination that preceded antimicrobial application was superior when film action was compared with spraying.

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1. Introduction

Foods are prone to microbiological deterioration throughout storage and distribution. Addition of antimicrobial agents may enable extension of the shelf-life and safety of fresh and packaged foods, by reducing or even preventing the growth of pathogenic and spoilage microorganisms (Franssen, Rumsey, & Krochta, 2004).

Consumers demand for more natural ingredients in processed foods is a result of a requirement for more healthy and safe foods with also a need for a balanced and adequate diet. As a consequence, the demand for natural food ingredients has increased and the use of natural antimicrobial compounds from a wide variety of sources has been widely investigated (Gould, 1997).

Natamycin is a natural antimycotic polyene characterized by the presence of a large macrocyclic lactone ring containing a series of conjugated double bonds and one or more sugar residues (Hammond & Lambert, 1978). It has a molecular weight of 665.7Da, is produced by *Streptomyces natalensis* and is commonly employed in dairy-based food products to prevent yeasts and moulds contamination (El-Diasty, El-Kaseh, & Salem, 2008; Gallo & Jagus, 2006; Reps, Jedrychowski, Tomasik, & Wisniewska, 2002). It has been approved as a food additive in over 40 countries and has been considered as a GRAS (generally recognized as safe) product by the FDA (Koontz, Marcy, Barbeau, & Duncan, 2003) and also indicated as a natural preservative by the European Union (EEC N° 235).

Natamycin kills yeasts by specifically binding to ergosterol but without permeabilizing the plasma membrane. It inhibits vacuolar fusion through the specific interaction with ergosterol (te Welscher et al., 2008, 2010). Therefore, it is active against yeasts and moulds but not against bacteria, viruses and protozoa. Sterols are known to have an ordering effect on the membrane, it is thought that they reside in specific sterol-rich domains in membranes and they are also known to be involved in endocytosis, exocytosis, vacuolar fusion (Wachtler & Balasubramanian, 2006), pheromone signalling (Jin, McCaffery, & Grote, 2008), membrane compartmentalization (Klose et al., 2010), and the proper functioning of membrane proteins (Zhang et al., 2010). According to Athar and Winner (1971), structural modification and/or decreased expression of ergosterol via mutations in the sterol biosynthesis pathway substantially diminish fungal pathogenicity *in vivo*.

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Natamycin can be applied directly by incorporation in liquid systems (Gallo & Jagus, 2006). In the case of food solid surfaces, it can be applied using different techniques like spraying, dipping or brushing, for the purpose of controlling microbial growth. According to Ture, Eroglu, Ozen and Soyer (2011), direct application of additives can have limited benefits. The antimicrobials can exhibit a loss of activity due to the reduction of their active concentration resulting from interaction or reaction with other additives or components present in the food matrix. Incorporation of antimicrobials in food interfaces by means of the use of edible films where they are entrapped helps to decrease the rate of diffusion from the surface to the bulk of the product, thus assisting in the maintenance of high concentrations of the active ingredient where it is required (Kristo, Koutsoumanis, & Biliaderis, 2008). It can also diminish the interaction with other additives and food components due to its presence being restricted mainly to the surface (Campos, Gerschenson, & Flores, 2011). As a consequence edible matrices with antimicrobial activity constitute a promising form of antimicrobial delivery in the frame of food preservation (Dos Santos Pires et al., 2008; Fajardo et al., 2010; Ollé Resa, Gerschenson, & Jagus, 2012; Ture et al., 2011).

The most commonly known spoilage yeasts are facultative anaerobic fermentative organisms, producing ethanol and carbon dioxide from simple sugars. Some fermentative yeasts are the most osmophilic organisms known, capable of slow growth at water activity (a_w) as low as 0.6 (Martorell, Fernández-Espinar, & Querol, 2005). Representative genera include *Saccharomyces* and *Zygosaccharomyces*. In particular, *Saccharomyces cerevisiae* is especially interesting because it is a yeast for which there is a good understanding of its physiology, biochemistry and genetic responses (Betts, Linton, & Betteridge, 2000; Praphailong & Fleet, 1997).

Zygosaccharomyces rouxii has unusual physiological characteristics which are largely responsible for their ability to cause spoilage, including resistance to weak-acid preservatives, extreme osmotolerance and ability to ferment hexose sugars (James & Stratford, 2003). *Z. rouxii* is characterized by its ability to tolerate low a_w environments, being one of the most xerophilic organisms known. It possesses two plasma-membrane antiporters with different substrate specificities which confer this property (Martorell, Stratford, Steels, Fernández-Espinar, & Querol, 2007; Pribylova, Papouskova, & Sychrova, 2008).

Yarrowia lipolytica strains are readily isolated from dairy products, and also from salads containing meat or shrimps. This yeast, frequently found in cheeses, was also reported to be associated with browning phenomenon (Carreira, Paloma, & Loureiro, 1998). The inability of this yeast to survive under anaerobic conditions permits its easy elimination from dairy products. *Y. lipolytica* does not require nitrogen limitation for induction of sporulation in contrast to *S. cerevisiae* and some other yeasts. Diploid strains sporulate on solid or in liquid complete medium between 20 °C and 30 °C, when glucose is exhausted (Barth & Gaillardin, 1997).

All these microorganisms comprise strains that are capable of growth during cold storage (Sorhaug & Stepaniak, 1997).

According to Ollé Resa et al. (2012) and Krause Bierhalz, da Silva and Kieckbusch (2012) the incorporation of natamycin on edible films constituted by tapioca starch and alginate and pectin, affect the physical properties of the films.

Although extensive information on the antimicrobial properties of natamycin is available in the literature, scarce data exist about the activity of natamycin against different yeasts when incorporated alone or supported in edible films.

Therefore, the objective of this study was to evaluate the effectiveness of natamycin against *S. cerevisiae*, *Z. rouxii* and *Y. lipolytica*, when different application techniques are assayed in model systems and in a food matrix.

2. Materials and methods

2.1. Materials

Tapioca starch was provided by Industrias del Maíz S.A. (Argentina) and glycerol by Mallickrodt (Argentina). Commercial natamycin (Delvocid® Salt) containing 50% NaCl and 50% natamycin was kindly supplied by DSM (Argentine). Whey protein concentrate (WPC35) with 35% w/w protein content was kindly supplied by Arla S.A. (Argentina). Its composition was: lactose 48.8% w/w; protein 38.3% w/w; ash 7.5% w/w; moisture 3.2% w/w and fat 2.2% w/w. Port Salut cheese (La Serenisima, Argentina), purchased in a local supermarket was used for the evaluation of the antimycotic activity of the films in a real food.

2.1.1. Film preparation

Mixtures of starch, glycerol and water (2:1:37, in weight) with and without commercial natamycin added were prepared. In this last case, 10 ml of the water were replaced with a solution of natamycin of adequate concentration for obtaining different final concentrations in the films, such as: film I with 1.85 mg natamycin/dm^2, film II with 3.70 mg natamycin/dm^2, or film III with 9.25 mg natamycin/dm^2. Starch gelatinization was performed at a constant rate of ~1.5 °C/min attaining a final temperature of 82 °C. Vacuum was applied to remove air bubbles from the gel. The slurry was dispensed in aliquots of 12 g in silicone plates of 7 cm diameter. The drying of the films was performed at 37 °C during 48 h in a convection chamber. Films without natamycin are named as films C. Once constituted, films were peeled off from plates and, before evaluating their properties, were conditioned at 28 °C, over saturated solution of NaBr (water activity, $a_w \cong 0.575$) during 7 d to assure equilibration.

2.1.2. Liquid WPC preparation

WPC35 was dissolved in sterile distilled water at the desired concentration (16% w/v) and the pH was adjusted to 5.5 by 0.5 N HCl addition (von Staszewski & Jagus, 2008).

2.2. Microbiological assay

2.2.1. Strains and growth conditions

S. cerevisiae (CBS 1171, strain collection SC), *Z. rouxii* (ATCC 28.253) and *Y. lipolytica* (National Institute of Infectious Diseases "Dr. Carlos G. Malbrán", CABA, Argentina) were grown in 150 ml Sabouraud broth (Biokar, France) at 28 °C in a continuously agitated temperature-controlled shaker until early stationary phase was achieved.

2.2.2. Antimicrobial activity in a model liquid food (liquid WPC)

This test was used to determine the antimicrobial effectiveness of natamycin applied directly to a liquid system. The systems evaluated were: WPC (system containing WPC and without natamycin), WPC/N1 (system containing WPC and with 20 ppm of natamycin or 0.02 mg natamycin/g system) and WPC/N2 (system with WPC and containing 50 ppm of natamycin or 0.05 mg natamycin/g system). These systems were inoculated with the different microorganisms and incubated at 25 °C. To determine the viable population of microorganisms, samples were serially diluted with peptone water. Microbial counts were then made on plates of chloramphenicol glucose agar (YGC, Biokar Diagnostics, France). The number of CFU/ml was determined after incubation at 28 °C for 48 h. Determinations were carried out in duplicate.

2.2.3. Barrier to microbial contamination

Petri dishes containing YGC agar with pH adjusted to a value of 5.2 (citric acid 50% w/w), were used to resemble a food product.

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