Food Control 21 (2010) 240–246

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Control of pathogenic and spoilage microorganisms from cheese surface by whey protein films containing malic acid, nisin and natamycin

Cristina M.B.S. Pintado [a,b], Maria A.S.S. Ferreira [b], Isabel Sousa [b,*]

[a] Escola Superior Agrária, Instituto Politécnico de Castelo Branco, Quinta da Senhora de Mércoles, 6001-909 Castelo Branco, Portugal
[b] Instituto Superior de Agronomia, Technical University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal

ARTICLE INFO

Article history:
Received 17 February 2009
Received in revised form 12 May 2009
Accepted 29 May 2009

Keywords:
Antimicrobial films
Cheese safety
Listeria monocytogenes
Rheology of films

ABSTRACT

The inhibitory effects of nisin, natamycin and malic acid, incorporated in whey protein films with pH 3, were investigated alone or with addition of sucrose esters, Tween80 or EDTA. Water vapour permeability measurements and mechanical and rheological tests were also assessed. EDTA and Tween80 did not significantly ($P < 0.05$) influence the inhibitory activity of films against *Pseudomonas aeruginosa* and *Yarrowia lipolytica* in contrast with the improved effect against *Listeria monocytogenes*, *Penicillium commune* and *Penicillium chrysogenum*. Sucrose esters reduced significantly ($P < 0.05$) the inhibitory effect for *Y. lipolytica* and *Penicillium* spp. The present work provides an antimicrobial film formulation with potential to be a hurdle against spoilage and pathogenic microorganisms isolated from cheese surface.

1. Introduction

Cheese is a ready-to-eat food easily contaminated on the surface by undesirable microorganisms. Some are spoilage microorganisms which may produce uncharacteristic visual appearance and diminish the commercial value of the cheeses, such as *Yarrowia lipolytica*, *Pseudomonas aeruginosa* and *Penicillium* spp. but others are pathogenic such as *Listeria monocytogenes*, which have been associated with foodborne listeriosis by consumption of cheese (McLauchlin, Mitchell, Smerdon, & Jewell, 2004). The Gram-negative bacteria *Pseudomanas* spp. are the most important of the psychrotrophs that dominate the microflora of raw milk (Sorhaug & Stepaniak, 1997). Strains of *Ps. aeruginosa* have been associated with undesirable browning reactions on cheese rind (Ogunnariwo & Hamilton-Miller, 1975), and some are phatogenic. The *Y. lipolytica* yeast, frequently found in cheeses, was also reported to be associated with browning phenomenon (Carreira, Paloma, & Loureiro, 1998). *Penicillium* is the genera of moulds most frequently isolated from naturally contaminated cheese rind samples and include mycotoxigenic strains. All these microorganisms comprise strains with psychrotrophic characteristics that could increase in number during cold storage (Sorhaug & Stepaniak, 1997).

Most recently, the food industry showed an increasing interest in antimicrobial edible films to enhance food safety and product shelf life. Different matrix can be used to incorporate antimicrobial agents (Appendini & Hotchkiss, 2002), including whey protein isolate (WPI). In general, the resistance to water vapour transmission of protein films is limited because they are highly polar polymers with a high level of hydrogen bonding and hydroxyl groups (Ko, Janes, Hettiarachchy, & Johnson, 2001). Furthermore, in high humidity environments the water vapour barrier properties are subsequently reduced because of protein filmś susceptibility to moisture absorption and swelling. This attribute could be detrimental when foods coated by these films are submitted to high humidity storage conditions, increasing the diffusion coefficient of antimicrobial agents to the food from the film matrix. Therefore, the incorporation of agents such as fatty acid esters to decrease water vapour permeability (WVP) is necessary. Also, the type and concentration of plasticizer, such as glycerol and sorbitol, influences the ability of films to attract water.

Nisin is a hydrophobic and cationic polypeptide, a food-grade preservative that exhibits antimicrobial activity towards a wide range of Gram-positive bacteria but shows little or no activity against Gram-negative bacteria, yeasts, and moulds (Delves-Broughton, 2005). It has been observed that Gram-negative cells, normally insensitive to the action of nisin, can be sensitized by the addition of chelating agents, such as EDTA, which disrupt the integrity of the outer membrane and allow the bacteriocin to access the cytoplasmic membrane (Boziaris & Adams, 1999; Stevens, Sheldon, Klapes, & Klaenhammer, 1992). Sucrose esters of fatty acids and Tween80, used commonly as food emulsifiers, are non-

* Corresponding author. Address: Secção de Ciência e Tecnologia dos Alimentos, Departamento de Agro Indústrias e Agronomia Tropical, Instituto Superior de Agronomia, Technical University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal. Tel.: +351 213 653 246; fax: +351 213 653 200.
E-mail address: isabelsousa@isa.utl.pt (I. Sousa).

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doi:10.1016/j.foodcont.2009.05.017

ionic emulsifiers known to inhibit the growth of a wide range of microorganisms, primarily against Gram-positive bacteria and fungi, when used alone or in combination with an antimicrobial agent as nisin (Thomas, Davies, Delves-Broughton, & Wimpenny, 1998) or organic acids (Monk, Beuchat, & Hathcox, 1996). Natamycin is a polyene natural antimycotic with a wide range of antimicrobial spectrum against yeasts and moulds (Welscher, Napel, Balagué, Souza, Riezman, et al., 2008). Natamycin incorporating coat solutions are used by cheese industry but the commercial available products are in general based on polyvinyl acetate or polyvinyl alcohol, which food safety iniquity has been discussed (EFSA., 2005). This is of great concern because there are cheese consumers that appreciate cheese as a whole, consuming the inner and the rind of the cheeses.

The objectives of this work were to study how the incorporation of different emulsifier, plasticizer and chelator agents into WPI-based films with malic acid, nisin and natamycin interfere with the antimicrobial activity against *L. monocytogenes*, *Ps. aeruginosa*, *Penicillium* spp. and *Y. lipolytica* and the mechanical and WVP properties of the films.

2. Materials and methods

2.1. Materials used to produce films and specifications

Whey protein isolate (with a protein content of 92% minimum, dry basis) was kindly supplied by Carbery Food Ingredients, Ballineen, Co. Cork, Ireland. The sucrose esters of fatty acids were a gift from Mitsubishi-Kagaku Foods Corporation, Tokyo, Japan (Ryoto Sugar Ester S970 with 31–33% sucrose monostearate and 20–22% sucrose distearate, HLB-value 9) and from Sisterna B.V., BH Roosendaal, The Netherlands (SP30, nonionic emulsifier, sucrose distearate, HLB-value approx. 6, 30% monoester content; and SP50, nonionic emulsifier, sucrose stearate, 50% monoester content, HLB-value approx. 11). Nisin NP (5×10^6 IU/g potency), Nisaplin (minimum 10^3 IU/mg of Nisin A and minimum 50% NaCl), and Natamax Salt were a gift from Danisco Beaminster Ltd., Beaminster, UK Glycerol and D-Sorbitol were purchased from Sigma Chemical Co., St. Louis (MO), USA; Hydrochloric acid from Riedel-de-Haën, Seelz, Germany; DL-Malic acid from BDH Chemicals Ltd.; EDTA from Aldrich, St. Louis (MO), USA; and Tween80 from Acroyali, Quingdao, China.

2.2. Microbial cultures and media

Twelve different microorganisms (Table 1) were used, which included six isolated from the rind of Castelo Branco Cheese, a semi-soft ripened ewe's cheese from the central part of Portugal (Pintado, Oliveira, Pampulha, & Ferreira, 2005). The P2 and P6 strains of *Ps. aeruginosa*, and the moulds *Penicillium chrysogenum* and *Penicillium commune*, were isolated using selective agar media: Pseudomonas Aeromonas Selective Agar Base acc. to Kielwein (GSP Agar; Merck KGaA, Darmstadt, Germany) and Potato Dextrose Agar medium (PDA; from Oxoid, UK), respectively. *Pseudomonas* spp. strains were purified and identified to the species level using API® 20NE (bioMérieux® SA, France). *Ps. aeruginosa* ATCC 15692 was kindly supplied by Instituto Superior Técnico, Technical University of Lisbon, and was used as reference strain. All the *Pseudomonas* strains used in this study were maintained at 4 °C on slants of TSYEGA medium (l^{-1}): 30 g Tryptone Soy Broth (Biokar Diagnostics, Beauvais, France), 6 g Yeast Extract (Oxoid), 10 g Glucose (COPAM, Portugal), and 18 g Agar (Dário Correia, Portugal), with the pH adjusted to 6.2 with HCl. The *L. monocytogenes* strains were maintained at −80 °C in Trypticase™ Soy Broth (TSB; Becton, Dickinson and Company) containing 10% (v/v) Glycerol, and subcultured twice in TSYEGB medium (containing the same components as TSYEGA, without Agar), at 37 °C through 24 h and 18 h before use. Strains of *Y. lipolytica* were maintained at 4 °C on slants of GYPA medium which comprised 5.0 g l^{-1} Peptone (Oxoid), 5.0 g l^{-1} Yeast Extract (Oxoid), 2.0 g l^{-1} Glucose (COPAM), and 20.0 g l^{-1} Agar (Dário Correia), with the pH adjusted to 6.2 with HCl. *Ps. aeruginosa* and *Y. lipolytica* strains were selected after assessing for the production of brown pigments on a Cheese-Tyrosine Agar medium (Carreira et al., 1998) containing (l^{-1}): 200 g soft cheese, 40 g NaCl (Merck), 10 g L-tyrosine (Sigma), 12 g agar, and pH adjusted to 7.0. All six strains produced brown pigmentation in this medium. Strains of *Penicillium* spp. were isolated from rind of ripened cheeses and identified to the genera level using the following media: Czapek Yeast Autolysate Agar (CYA), Creatine Sucrose Agar (CREA), Yeast Extract Sucrose Agar (YES) and Blakeslee Malt Extract Autolysate Agar (MEA), as recommended by Samson and Frisvad (2005). The strains were then sent to Centraalbureau voor Schimmelcultures (CBS, Fungal Biodiversity Centre, The Netherlands) for further identification. They were identified as *P. chrysogenum* and *P. commune*. *Penicillium roqueforti*, PRB6 HYP5D, CHOOZIT, is a cheese culture used as reference, and was kindly supplied by Danisco (Copenhagen, Denmark).

2.3. Preparation of film solutions and films

Seven grams of whey protein isolate were completely dissolved in distilled water (100 ml of total volume) by stirring for 15 min. Glycerol or sorbitol (1.5%, 2.25% and 3.0% w/v) were then incorporated and stirred for a further 15 min followed by the addition of

Table 1
Identification and source of microorganisms used in this study.

Microorganisms and references	Source of isolation
L. monocytogenes NCTC[a] 11994	–
L. monocytogenes CP6	Rind of ewe's cheese
L. monocytogenes M12	Ewés cheese (Pintado et al., 2005)
Ps. aeruginosa ATCC[b] 15692	–
Ps. aeruginosa P2	Rind of ewe's cheese
Ps. aeruginosa P6	Ewés raw milk
Y. lipolytica CBS[c] 6659	–
Y. lipolytica ISA 1668	Rind of ewe's cheese (Carreira et al., 1998)
Y. lipolytica ISA 1708	Rind of ewe's cheese (Carreira et al., 1998)
P. roqueforti (CHOOZIT™ PRB6 HYP5D)[d]	–
P. chrysogenum	Rind of ewe's cheese
P. commune	Rind of ewe's cheese

[a] National collection of type cultures, London, United Kingdom.
[b] American type culture collection, Manassas (VA), USA.
[c] Centraalbureau voor Schimmelcultures, Delft, the Netherlands.
[d] Danisco, Copenhagen, Denmark.