Food Control 64 (2016) 10–16

Contents lists available at ScienceDirect

Food Control

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

Active coating for storage of *Mozzarella* cheese packaged under thermal abuse

Giuliana Gorrasi [a, *], Valeria Bugatti [a], Loredana Tammaro [a], Luigi Vertuccio [a], Giovanni Vigliotta [b], Vittoria Vittoria [a]

[a] Department of Industrial Engineering, University of Salerno, Via Giovanni Paolo II 132, 84084-Fisciano, SA, Italy
[b] Department of Chemistry and Biology, University of Salerno, Via Giovanni Paolo II 132, 84084-Fisciano, SA, Italy

ARTICLE INFO

Article history:
Received 5 June 2015
Received in revised form
25 November 2015
Accepted 1 December 2015
Available online 14 December 2015

Keywords:
PET
Active packaging
Food preservation
Microbial shelf life

ABSTRACT

A novel antimicrobial packaging system, as active coating on a commercial Poly(ethylene terephthalate) (PET) film, was formulated and tested in vitro against the spoilage bacteria of *Mozzarella* cheese. It is based on layered double hydroxide (LDHs) intercalated with salicylate and carbonate anions dispersed in a solvent-based heat seal. The population of spoilage microorganisms (total coliforms, *Pseudomonas*, fungi), along with the functional microbiota of *Mozzarella* cheese (lactic acid bacteria) was characterized. Microbial shelf life was evaluated at 18 °C, to simulate thermal abuse. Experimental results show an increase in the microbial shelf life of the packaged *Mozzarella* cheese of about 20 days, confirming that the investigated active coating may exert an inhibitory effect on the microorganisms responsible for spoilage phenomena, without affecting the functional microbiota of the product.

1. Introduction

The research on the formulation and production of innovative materials for food applications, such as packaging and other food contact surfaces, is rapidly increasing in recent years thanks to the growing field of preparation of advanced structural and functional composites and nano-composites. A considerable effort to extend the shelf-life to retain the food quality has encouraged the exploration of new packaging materials, as well as innovative coatings (Ahmed and Alam, 2012; Azzi, Battini, Persona, & Sgarbossa, 2012; Rhima, Park, & Ha, 2013; Silvestre, Duraccio, & Cimmino, 2011). Among the challenging solutions in this field, antimicrobial packaging is gaining interest from researchers and industries due to its potential to provide quality and safety benefits (Appendini & Hotchkiss, 2002; Joerger, 2007; Suppakul, Miltz, Sonneveld, & Bigger, 2003). The reason of antimicrobials incorporation into a food package is to prevent surface microbial growth. Great attention has recently emerged around the hybrid organic-inorganic systems and, in particular to those in which layered fillers are dispersed in a polymeric matrix. Such hybrid composites possess very unusual properties, very different from their microscale counterparts. In particular Layered Double Hydroxide (LDHs), or hydrotalcite like compounds, have received considerable attention as active molecular ions delivery vehicles due to their anion exchange properties. These compounds, also known as "anionic clays" have general formula $[M(II)_{(1-x)}M(III)_x(OH)_2](A_{x/n})^- \cdot mH_2O$ where M(II) is a divalent cation such as Mg^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+} or Co^{2+} and M(III) is a trivalent cation such as Al^{3+}, Cr^{3+}, Fe^{3+}, or Ga^{3+} with A^{n-} an anion of charge n such as CO_3^{2-}, Cl^-, NO_3^- or an organic anion. (Costantino, Marmottini, Nocchetti, & Vivani, 1998; Tammaro, Tortora, Vittoria, Costantino, & Marmottini, 2005). The new trend of the research is based on the fact that the active molecules, fixed by ionic bonds to the inorganic lamellae, not only can improve the compatibility with the polymer matrix but can exhibit the antimicrobial activity being anchored to the lamellae, or being slowly released in particular environments. The release occurs via a de-intercalation process, which consists in anion exchange displacement reactions. As a consequence, the release rate is also dependent on the rate of the de-intercalation process. This in turn, depends on the electronic and spatial structure of the guest species. In all these processes is of fundamental importance the transport of water to bring the counter ion and to allow the exchange and the release of the active molecule (Costantino et al., 2009). Fresh *Mozzarella* cheese is a typical "*pasta filata*" cheese from Southern Italy, with high moisture and a high fat content. It could be cut and manufactured in various shapes, and usually brined. It is

* Corresponding author.
E-mail address: ggorrasi@unisa.it (G. Gorrasi).

http://dx.doi.org/10.1016/j.foodcont.2015.12.002
0956-7135/

characterized by a notable economic relevance because of the steady rise in its production and consumption. Traditional *Mozzarella* is packaged in a dilute solution of salts (NaCl and/or $CaCl_2$) called conditioning brine. The determining factors affecting preservation of the freshness of the *Mozzarella* are different: pH; hydrolysis of casein; calcium; ratio of calcium/sodium; acidity; presence of microorganisms. This last factor is crucial to maintain the freshness and the microbial shelf life of the *Mozzarella* (Baruzzi, Lagonigro, Quintieri, Morea, & Caputo, 2012; Quintieri et al., 2012). Soft cheese products are excellent growth media for a wide range of microorganisms and, thus, display a short shelf life (Ruegg, 2003). Several studies have characterized the *Mozzarella* from a microbiological point of view (Belli, Cantafora, Stella, Barbieri, & Crimella, 2013; Caputo et al., 2015; Costa Dias et al., 2012; Faccia et al., 2014;), recovering several microbial species: Lactic Acid bacteria (LAB) group, such as *Lactobacillus lactis* subsp. *lactis*, *L. lactis* subsp. *diacetylactis*, *L. lactis* subsp. *cremoris*, *Streptococcus thermophilus*, enterococci such as *Enterococcus faecium* and *E. faecalis*, *Enterobacteriaceae* such as *Escherichia coli*, yeasts such as *Debaryomyces hansenii* and *Kluyveromyces marxianus*, and various spoilage psychrophilic microflora (Cantoni, Iacumin, & Comi, 2003; Cantoni, Stella, Cozzi, Iacumin, Comi, 2003; Parisi, 2003a,b). Lactic acid bacteria constitute indigenous microflora of raw milk and represent the major component of starter cultures used for the production of dairy products. These microorganisms contribute to the quality by improving the taste and texture and inhibiting food spoilage bacteria by producing growth-inhibiting substances bacteriocins and large amounts of lactic acid (Jana & Mandal, 2011). Thus the evaluation of lactic acid bacteria concentration during production and microbial shelf life should be considered as an indicator of quality. Faecal coliforms live in the intestine of mammals and their presence in foods is an index of faecal contamination (Sinigaglia, Bevilacqua, Corbo, Pati, & Del Nobile, 2008). According to the Italian legislation (Gazzetta Ufficiale della Republica Italiana, D.P.R. 14/1/1997), coliforms are the test microorganisms for evaluating the microbial shelf life of *Mozzarella* cheese. Another microbiological parameter for safety control is the Total Viable Count (TVC) that gives a quantitative idea of the presence of mesophilic aerobic microorganisms of animal origin and of the global contamination. The growth of *Pseudomonas* spp. on the cheese surface has also to be considered a consequence of contaminated water using during manufacture (Cantoni et al., 2003; Cantoni et al., 2003). Presence of coliforms in cheese is an indication of poor sanitation. Coliforms grow rapidly during the first days of storage, producing lactic acid, acetic acid, formic acid, succinic acid, ethanol, CO_2 and causing decrease in pH. The pH is also an important factor. It affects the casein proteolysis, the amount of calcium, the ratio Ca–Na-Casein and sensory characteristics of *Mozzarella* cheese, also during thermal abuse at 15 °C and 18°C (Conte, Scrocco, Sinigaglia, Del Nobile, 2007; Laurienzo et al., 2007). Extending the microbial shelf life of *Mozzarella* cheese is an important issue to the dairy industry due to the high interest in extending the distribution of the traditional product beyond the market borders. Currently, *Mozzarella* is packaged in rigid containers or flexible films of multilayer material, packages made of polyethylene/paper laminated films or tetrapack-type packages. None of these packages solves the problems of the limited shelf life of fresh *Mozzarella* cheese. Generally, at 4 °C, the shelf life of *Mozzarella* is about 2–3 days. One approach for extending the microbial shelf life of *Mozzarella* cheese is to introduce antimicrobials. (Gill & Holley, 2000). The aim of our work was to develop an innovative packaging system for *Mozzarella* cheese that would be able to guarantee prolonged microbial shelf life, with no influence on the production methodology and no influence on the taste, mechanical properties, and nutritional properties of the product. We verified the effectiveness of a controlled-release active system containing an antimicrobial molecule incorporated in a LDH within the packaging material of fresh *Mozzarella*. We report on the main results of our research, which include evaluation of pH, microbiological analysis and microbial shelf life evaluation, as function of storage time. It is worth noting that the temperature used in this study (i.e. 18 °C) is not the storage temperature of *Mozzarella*. We operated in this extreme conditions in order to accelerate spoilage phenomena.

2. Materials and methods

2.1. Materials

Poly(ethylene terephthalate) (PET) (CLEARTUF 8006, intrinsic viscosity 0.80 dL/g), was supplied by 2R Packaging Ltd, Naples, Italy, in film form 12 μm thick.

The active filler, based on a Layered Double Hydroxide intercalated with antimicrobial salicylate anions (listed in EC-Directive 10/2011/EC of 14 January 2011) and carbonate anion, was produced by Nicefiller Ltd, an start up of the University of Salerno (Italy), accordingly to a previously reported procedure (Frunza, Lisa, Popa, Miron, & Nistor, 2008). The glue used was a solvent-based paint normally used for packaging of dairy products (Novacote HS-8256, solid content 38 ± 2%, viscosity 300–600 mPa s at 25 °C) purchased from COIM S.p.A. (Italy). Its constituents are in accordance with the EC-Directive 2002/72/EC of 6 August 2002, including amendments.

Ethyl acetate (99% pure) was purchased from Sigma–Aldrich (Italy) and used as received.

2.2. Release kinetics

The release kinetic of the salicylate molecule in a fixed volume of a saline solution (0.9% w/v) was obtained by ultraviolet spectrometric measurement at ambient temperature, using a Spectrometer UV-2401 PC SHIMADZU.

2.3. Coatings preparation and processing

The active filler was dispersed in the solvent-based heat paint using ethyl acetate as solvent to obtain a relatively smooth and uniform coating (Vittoria, Tammaro, Bugatti, & Bianchi, 2011). After the application of coating on PET film the solvent was evaporated at room temperature. The obtained active film, named PET_active, has 3.8 g/m^2 surface density and a coating thickness of 8–10 μm, measured respectively by weight difference on a known area, and a micrometer.

2.4. Samples packaging

Mozzarella cheese, made from cow milk, were purchased from a cheese factory located in Campania (De Caro Factory, Fisciano (SA)), as soon as prepared, and brought to our laboratory under refrigeration (4 °C). In Italy, *Mozzarella* is sold in small size pieces (cylindrical cheese samples of 50 g called *bocconcino*), usually 20 pieces for 1 kg of mozzarella and packaged in bags containing 1 L of diluted brine solution (preservation solution).

For our experiment a single cylindrical cheese samples (*bocconcino*) (50 g of weight and 5–7 cm of diameter) were removed from their commercial packages and packaged in individual pouches (20 cm × 20 cm) of PET_active containing 50 mL of pristine preservation solution, in the same ratio product/preservation solution of commercial mozzarella. *Mozzarella* cheeses packaged in PET bags were used as control. The obtained packaged samples (in total 17 single *bocconcini*) were stored at 18 °C to simulate thermal abuse during storage and analyzed at different storage times. All