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Application of preservation strategies to improve the shelf life of spreadable cheese

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ABSTRACT

Strategies designed to protract the shelf life of spreadable cheese obtained by acid coagulations are presented. First, the effectiveness of different headspace CO_2 and N_2 mixtures on the microbial and sensorial characteristics of the investigated produce was addressed; afterward, the modified atmosphere showing the best performances was combined with different antimicrobial compounds (lysozyme and Na_2-EDTA; MicroGARD®400; potassium sorbate). Headspace gas concentration, pH, sensory quality, dairy spoilage microorganisms and lactic microflora were monitored. The work results suggest that CO_2 at every tested concentration negatively influence the taste of spreadable cheese, while the best performances were recorded for samples packaged under 100% nitrogen that was able to control yeasts and moulds development. The most effective solution to increase the shelf life of spreadable cheese was obtained by the combination of lysozyme and Na_2-EDTA with the selected MAP, 100% nitrogen, allowing to reach a shelf life of about 26 days confronted to the control samples (6 days).

1. Introduction

Spreadable cheese is a soft produced with cow milk with fine acid flavor and white color. The production process and the acid coagulation by lactic acid bacteria give the peculiar characteristics to the cheese (Rinaldoni, Palatnik, Zaritzky, & Campderrós, 2014). The high moisture content, from 40 to 60%, makes the product susceptible to microbial and sensorial changes (Lee, Anema, & Klostermeyer, 2004). The typical pH range (4.3–4.9) of the product favors molds and yeasts development that affects the product shelf life (Jacobsen & Norvhus, 1996).

Several literature studies described the application of modified atmosphere packaging (MAP) to increase dairy products shelf life (Conte, Gammariello, Di Giulio, Attanasio, & Del Nobile, 2009; Khoshgozaran, Azizi, & Bagheripoor-Fallah, 2012; Mastromatteo et al., 2015). However, the capacity of MAP for increase the shelf life is influenced by numerous variables such as the type of cheese, initial microbial population, use of starter cultures and storage conditions (Floros, Nielsen, & Frankas, 2000; Papaioannou, Chouliara, Karatapanis, Kontominas, & Savvaidis, 2007). Gas mixtures containing CO_2, alone or in combination with N_2, are frequently applied to improve shelf life from a microbial point of view, limiting the advance of spoilage bacteria in fresh cheese (Conte et al., 2009; Gammariello, Conte, Di Giulio, Attanasio, & Del Nobile, 2009). In particular, headspace gas composition characterized by CO_2 concentration from 50 to 95% can retard the development of *Pseudomonas* spp. in soft cheese (Del Nobile, Conte, Incoronato, & Panza, 2009; Gammariello et al., 2009; Khoshgozaran et al., 2012); moreover, yeasts and moulds development can be controlled by concentrations of $CO_2 \geq 20\%$ (Esmer, Balkir, Seckin, & Irkin, 2009; Khoshgozaran et al., 2012). However, the sensory characteristics and their evolution throughout storage for cheese packaged under MAP are also important and should be assessed in order to verify possible organoleptic side-effects: for instance, some authors highlighted the adverse effects of high CO_2 concentrations on cheese flavour, aroma and taste (Costa et al., 2016; Jalilzadeh, Tunçtürk, & Hesari, 2015; Rodriguez-Aguilera & Oliveira, 2009).

The addition of antimicrobial compounds during cheese manufacturing is a valid support to preserve the initial cheese characteristics during the preservation period. In literature, the application of natural compounds or additives able to amplify the

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shelf life of fresh dairy products is well documented (Faccia, Angiolillo, Mastromatteo, Conte, & Del Nobile, 2013; Lucera, Costa, Conte, & Del Nobile, 2012). Lysozyme is a natural compound from animal sources; this lactic enzyme has been found in many natural systems and to limit the microbial spoilage and gas-forming bacteria is added in cheese process (Lucera et al., 2012). In dairy products the lysozyme antibacterial activity can be improved when it is used in combination with other substances, such as ethylene-diamine-tetraacetic disodium salt (Na_2-EDTA) (Conte, Brescia, & Del Nobile, 2011; Conte et al., 2009; Sinigaglia, Bevilacqua, Corbo, Pati, & Del Nobile, 2008). In particular, Sinigaglia et al. (2008) proved that the addition of lysozyme and Na_2-EDTA in brine solution considerably inhibited the development of coliforms and *Pseudomonas* spp. in packaged mozzarella cheese, without affecting lactic acid bacteria. The combination of lysozyme and Na_2-EDTA in burrata cheese stored with 95:5 CO_2:N_2 was a valid strategy to prolong cheese shelf life (Conte et al., 2011). In addition, Conte et al. (2009) successfully tested the effectiveness of an active covering containing lysozyme and Na_2-EDTA combined with MAP at prolonging fiordilatte cheese shelf life.

The most frequently used preservatives to control yeasts and moulds development in dairy products are sorbic acid and its salts (Lucera et al., 2012). In particular, potassium sorbate was wished-for food applications due to the ability to prevent moulds development without changing dairy flavour, odor and color (Lucera et al., 2014; Karabulut, Lurie, & Droby, 2001). Several studies have investigated the effects of potassium sorbate in dairy products including white cheese, mozzarella cheese and yoghurt (Lucera et al., 2014; Öksuztepe, Ilhak, Dikici, Çalicioglu, & Patir, 2010). It was demonstrated that the addition of potassium sorbate in themanufacture of Turkish dairy products decreased the coliform, yeast and mould counts (Öksuztepe et al., 2010; Ozdemer & Demirici, 2006). Moreover, Lucera et al. (2014) showed that potassium sorbate added in a coating system for mozzarella cheese was able to control spoilage microorganism and improve shelf life.

MicroGARD®400 is a patented natural compound used for the shelf life extension of milk derivates products consisting on fermentation metabolites of starter cultures. MicroGARD®400 may retard the development of Gram-positive and Gram-negative bacteria and yeasts and moulds in dairy products when used in combination with heat processing, pH and other preservation treatments. Hussain, Garg, and Pal (2014) tested the antimicrobial activity of MicroGARD on fermented milk beverage showing that the natural compounds preserve the initial product characteristics for about 3 weeks at refrigeration temperature.

The intention of this study was to assess the influence of different headspace gas composition and the effectiveness of a combination of antimicrobial compounds (lysozyme/Na2-EDTA; MicroGARD®400; Potassium sorbate) and selected MAP on the shelf life of spreadable cheese. To this aim, microbiological, chemical, and sensory alterations of cheese stored at 8 ± 1 °C were monitored for about one month.

2. Materials and methods

2.1. Sample preparation

2.1.1. Step-1

Spreadable cheese was kindly provided by Artigiana (Putignano, Italy). For the cheese production, a mix of selected strains (*Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis, Leuconstoc* sp. *Lactococcus lactis* subsp. *lactis biovar diacetylactis*) provided by CHR HANSEN (Hoersholm, Danimarca) were added in pasteurized cow milk heated at 25 °C. More or less after 10 h, when milk pH reached 4.6 the whey was drained. Subsequently, the soft cheese was put into nylon towels for about 12 h at 4 °C. After production, cheese was transported to the laboratory in refrigerated conditions and packaged at different headspace conditions. Nylon/polyethylene bags (thickness of 95 µm) purchased by Miliotti & C srl (Bari, Italy), characterized by the following permeability values: oxygen transmission rate (OTR): 49.39 ± 1.77 cc/(m^2·day); CO_2 transmission rate (CDTR): 162.83 ± 1.46 cc/(m^2·day); water vapour transmission rate (WVTR): 1.64 ± 0.05 cc/(m^2·day) was used for packaging 150 g of spreadable cheese. The film characterization was conduced at 23 °C with 0% of relative humidity for OTR and CDTR and 23 °C with 85% of relative humidity for WVTR test. Samples were packaged under air (CntAir-1); 60:40 CO_2:N_2 (MAP-1); 40:60 CO_2:N_2 (MAP-2); 20:80 CO_2:N_2 (MAP-3); 0:100 CO_2:N_2 (MAP-4). All samples were placed at 8 ± 1 °C.

2.1.2. Step-2

In the second step different antimicrobial compounds were added to the spreadable cheese and mixed to obtain a homogeneous distribution. In particular, 0.5 g Kg^{-1} of potassium sorbate (PS), 0.5 g Kg^{-1} of lysozyme + 50 m*M* Na_2-EDTA salt (LY) and 10 g Kg^{-1} of MicroGARD®400 (MG) were tested. Potassium sorbate, lysozyme and Na_2-EDTA were purchased from Perrin's Chemicals (Triggiano, Bari); MicroGARD®400 from Danisco (Milano, Italy). Samples were packaged under 100% N_2 headspace conditions as previously described. As control, cheese samples without antimicrobial compounds packaged under air (CntAir-2) and 100% N_2 (CntMAP) were tested.

2.2. Headspace gas composition

According to Costa et al. (2016) a gas-metre (PBI Dansensor, Checkmate 9900, Ringsted, Denmark) was used to oxygen and carbon dioxide determination.

2.3. Microbial and sensory analyses

Spoilage microorganisms (*Pseudomonas* spp., Enterobacteriaceae and yeasts) and lactic acid bacilli, lactococci and mesophyilic bacteria were determined as suggest by Lucera et al. (2014).

The sensorial analysis was set as suggest by Faccia et al. (2013). Precisely, firmness, colour, odour, flavour and overall quality of spreadable cheese samples were evaluated by a 7-value scale, where 4 was considered the threshold for cheese acceptability (Gammariello et al., 2009). Moreover, the presence of moulds on cheese surface was researched by panellist. The visual mould time (VMT) was calculated as reported by Costa et al. (2016).

2.4. Shelf-life calculation

For shelf life determination the VMT, the microbial acceptability limit (MAL) and the sensory acceptability limit (SAL) values were considered (Conte et al., 2009). For spreadable cheese MAL was calculated with the re-parameterized Gompertz equation and is the day at which the yeasts concentration reached the threshold of 10^6 CFU g^{-1} as suggest by Jacobsen and Norvhus (1996). To determine the SAL, the same modified equation was applied and score of 4 was imposed for the threshold (Conte et al., 2009).

2.5. Statistical analysis

SAL and shelf life data of the investigated spreadable cheese samples were compared by one-way Anova (Statistica 7.1 for Windows). To determine significance among differences a Duncan's multiple range test, with the option of uniform groups ($p < 0.05$), was applied.