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Effect of pH on the effectiveness of whey protein/glycerol edible films containing potassium sorbate to control non-O157 shiga toxin-producing *Escherichia coli* in ready-to-eat foods

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ABSTRACT

Potassium sorbate (PS) (0.5%, 1.0% and 1.5% w/w) was included into whey protein concentrate (WPC)/glycerol (Gly) edible films at pH 5.2 and 6.0. The films inhibited or retarded the growth of Shiga toxin-producing *Escherichia coli* (STEC) pathogens in both diffusion and barrier tests. Bacterial growth inhibition was dependent on PS content at both pH values. PS release was not affected by pH. Scanning electron microscopy (SEM) was used to analyze the microstructure of the films and gain a better understanding of their optical parameters. Acidic control films (pH 5.2) prepared without PS were the least transparent. SEM micrographs confirmed the greater structural heterogeneity of these films, coinciding with opacity. The incorporation of PS into WPC/Gly films improved transparency and produced a smoother surface than acidic control ones. The utilization of active packaging based on whey proteins and organic acids to control and prevent the dissemination of STEC pathogens may be an effective, safe, ecological and relatively inexpensive alternative to be used in the food packaging industry.

1. Introduction

Active packaging is defined as packaging in which subsidiary constituents are deliberately included in or on either the packaging material or the package headspace to enhance the performance of the package system (Robertson, 2006). In order to control undesirable microorganisms on food surfaces, natural or synthetic antimicrobial agents can be incorporated into polymer coatings (Appendini & Hotchkiss, 2002; Kuorwel, Cran, Sonneveld, Miltz, & Bigger, 2011). Several compounds have been proposed as antimicrobial agents in food packaging, including organic acids, enzymes, fungicides and natural compounds such as spices and essential oils (Kuorwel et al., 2011; Seydim & Sarikus, 2006; Tharanathan, 2003). Sorbic acid, p-aminobenzoic acid, lactic acid, and acetic acid have a long history as generally recognized as safe (GRAS) food preservatives that have been extensively used as fungistatic and bacteriostatic agents for foods. Several studies have proved the effectiveness of some food preservative addition into edible films to control microbial growth (Cagri, Ustunol, & Ryser, 2001, 2004; Vásconez, Flores, Campos, Alvarado, & Gerschenson, 2009; Ye, Neetoo, & Chen, 2008). However, the effect of edible film matrix pH on the antimicrobial activity of GRAS organic acids has not examined.

Potassium sorbate (PS) is the potassium salt of sorbic acid that can effectively restrain the activity of mold, yeast and areophilic bacteria. The use of PS is effective up to pH 6.5 but effectiveness increases as pH decreases, consequently the pH of the film and the food to which the film is applied are important parameters.

The proteins in cheese whey, a by-product in the cheese making process, have excellent nutritional and functional properties in addition to their capacity to form films (Chen, 1995; Javanmard, 2009; Pérez-Gago & Krochta, 2002). Use of whey proteins to manufacture films has received a great deal of attention since they are edible and biodegradable (Krochta, & de Mulder-Johnston, 1997; Ramos, Fernandes, Silva, Pintado, & Malcata, 2012).

Cagri et al. (2001) reported that incorporating 0.5%–1.5% of sorbic acid or p-aminobenzoic acid into acidic whey protein isolate

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films (pH 5.2) can inhibit the growth of *Listeria monocytogenes*, *S. typhimurium* and *Escherichia coli* O157:H7 in trypticase soy agar supplemented with yeast extract (TSAYE) acidified to pH 5.2 but no inhibition was observed in TSAYE adjusted to pH 6.5. These results reveal the importance of the medium pH for a superior effectiveness of the organic acids. Moreover, some restrictions in the pH limit for the elaboration of whey protein edible films are related to the isoelectric point of whey proteins.

Casting films at pH values lower than the isoelectric point proved to be inefficient since the reactivity of SH groups decreases significantly (Ferreira, Nunes, Delgadillo, & Lopes-da-Silva, 2009). In addition, at acidic pH values whey proteins aggregate and films properties could decrease (Pérez-Gago & Krochta, 1999). Hence, changes in the pH of the whey protein/Gly system may affect solution stability, and thus, the antimicrobial characteristics of the films containing PS. To our knowledge, no comparative studies regarding the differences in the pH of the edible films based on whey protein and sorbate have been reported.

Shiga toxin-producing *E. coli* (STEC) is recognized worldwide as one of the most important causes of food-borne infections (Armstrong, Hollingsworth, & Morris, 1996). Clinical presentation of STEC infection varies from an asymptomatic state to bloody diarrhea and life-threatening complications such as hemolytic uremic syndrome (Johnson et al., 1996; Williams et al., 1999). STEC posses the Shiga toxin 1 and/or Shiga toxin 2 (*stx1* and *stx2*) genes considered the critical virulence factors in disease. Environmental conditions present in foods (nutrients, pH, humidity, etc.) are suitable for a rapid colonization by STEC strains to harmful levels for human health. Non-O157 STEC is now recognized as an important group of bacterial enteropathogens (Gilmour et al., 2009; Gyles, 2007). Several outbreaks caused by non-O157 STEC were described (Johnson et al., 1996; Williams et al., 1999), although data implicating these STEC in some outbreaks were scanty and the source of infection was not always known. In the United States, most STEC outbreaks were traced to beef containing *E. coli* O157:H7 and for that reason most epidemiological studies have focused on the prevalence of this serotype in beef and beef cattle (Gill & Gill, 2010; Hussein, 2007; Hussein & Sakuma, 2005). Interestingly, undercooked ground beef and other beef products are now considered reservoirs of O157 and non-O157 STEC (Hussein, 2007). However, worldwide, additional STEC serotypes, including members of the recently named big six serogroups (O26, O45, O103, O111 and O121), have been isolated from foods other than beef and caused human illnesses (Mathusa, Chen, Enache, & Hontz, 2010). Balagué et al. (2006) reported the phenotypic and genotypic characteristics and virulence properties of non-O157 strains isolated from ready-to-eat food samples obtained from supermarkets and shop selling in Argentina were hemolytic uremic syndrome is endemic (Ibarra et al., 2008; Reilly, 1998).

Because of the global nature of the food supply, safety concerns and new challenges facing the food industry are increasing both at the production and processing levels. Considering that antimicrobial edible packaging is a novel technology with the potential to help food preservation, the purpose of the present study was to evaluate the inhibitory effects of WPC/Gly edible films incorporated with PS against non-O157 STEC strains isolated from ready-to-eat food samples and its relationship with the pH of the film. However, since pH modifications can negatively alter other relevant properties of protein-based films, influence of both pH and organic acid presence on the optical properties of WPC/Gly films was also analyzed. One goal of this work was to provide an array of data to support comparative studies on the molecular structure, optical and biocide properties of acidic WPC/Gly films for rational improvement of such films toward their eventual application as edible packaging.

2. Materials and methods

2.1. Materials

WPC 80% (Arla Food Ingredients S.A., Buenos Aires, Argentina) was used to prepare the film forming solutions. Gly (Cicarelli, Santa Fe, Argentina) was added to all film forming solutions as a plasticizer; PS (Sigma Chemical Co., St. Louis, Mo., U.S.A.) was included in the formulations at different concentrations to evaluate the biocide properties of the WPC/Gly films. Trypticase Soy Broth (TSB), Cystine Lactose-Electrolyte-Deficient (CLDE) and Mueller–Hinton culture mediums were purchased from Britania (Buenos Aires, Argentina). 2,3,5-triphenyltetrazolium chloride (TTC) was obtained from Merck (Darmstadt, Germany).

2.2. Film preparation

Edible films (casting solution 11.5% total solid) were obtained with a modification of the method described by Soazo, Rubiolo, and Verdini (2010). Briefly, WPC and Gly (in proportion WPC/Gly 3:1 w/w dry solid basis) were dissolved in distilled water. After mixing, the solution was heated at 90 °C for 30 min in a water bath (TDS-40, Tecno Dalvo, Santa Fe, Argentina). Finally, the solution was homogenized (4 min; 20,000 rpm) with an Omni GLH homogenizer (Omni International Inc., Warrenton, Virginia, U.S.A.). After homogenization, the solutions were placed in an ice bath to prevent further denaturation of the whey proteins and rapidly cooled to room temperature. After incorporating 0.5%, 1.0% or 1.5% (w/w) of PS, the pH was adjusted to 5.2 or 6.0 with 1.0 N HCl using a Metrohm 713 pH-meter (Metrohm Ltd., Herisau, Switzerland). The pH-adjusted film-forming solutions were degassed at room temperature with a vacuum pump. Following degassing, the film forming solutions (8 g/plate) were casted by pipetting the solution into sterile 90 mm diameter plastic plates. The plates were dried for 2 h at 45 °C plus 24 h at 25 °C and 48 ± 4% relative humidity, after which the films were peeled from the plates and stabilized during 24 h at 25 °C and 48 ± 4% relative humidity. The films used in the different tests were selected based on the lack of physical defects such as cracks, bubbles and holes.

2.3. Film thickness

The thicknesses of three replicates of each film formulation were measured with an electronic digital disk micrometer (Schwyz®, China) at nine locations on the film to the nearest 0.001 mm. Average film thickness was 0.137 ± 0.030 mm.

2.4. Transparency

Film transparency was determined according to ASTM D1746 (ASTM, 1997) with modifications of the method described by Ozdemir and Floros (2008). The films were cut into rectangular pieces (10 mm × 30 mm) and placed on the internal side of a spectrophotometer cell. Transparency of films was measured using a spectrophotometer (Model V-530, Jasco International, Tokyo, Japan) at 560 nm. Five replicates of each film were tested. The transparency (% Transparency) was calculated as the percentual relationship between the light intensity with the specimen in the beam and the light intensity with no specimen in the beam.

2.5. Scanning electron microscopy

In order to study the structure of WPC/Gly films and to asses their homogeneity, SEM experiments were carried out. Film samples were cryo-fractured by immersion in liquid nitrogen and