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# Development of active gelatin-based nanocomposite films produced in an automatic spreader

Fernanda Maria Vanin *, Merícia Harumi Hirano, Rosemary Aparecida Carvalho, Isabel Cristina Freitas Moraes, Ana Mônica Quinta Barbosa Bittante, Paulo José do Amaral Sobral

*Food Engineering Department, FZEA, University of São Paulo, P.O. Box 23, 13635-900 Pirassununga, SP, Brazil*

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## ABSTRACT

The objective of this study was to develop an active nanocomposite gelatin-based film in an automatic spreader in order to improve production, which is normally done by casting. The study was divided in two steps: (1) first, the effect of a nanocomposite-forming solution formulation on the rheological properties and on some physical properties of gelatin-based nanocomposite films (GNF) was evaluated using a response surface methodology (RSM); (2) then, the effect of potassium sorbate concentration on some GNF properties, previously chosen by RSM, was assessed. RSM, a $2^3 + \alpha$ factorial design, was used to study and to optimize the effect of three important variables, gelatin concentration ($C_G$), plasticizer concentration ($C_P$) and montmorillonite concentration ($C_N$), on the rheological properties of a nanocomposite-forming solution, and on the properties of the films (mechanical and barrier properties). Films were prepared by casting in an automatic spreader. According to the results of the analysis of variance (ANOVA), in general, nanoparticle incorporation improved the mechanical properties of GNF. However, this improvement was not always proportional to the addition of the nanoparticles, since there is a maximum concentration limit. From the results obtained in the first step of the study, it was possible to optimize a formulation in order to study the effect of potassium sorbate concentration on GNF properties. At the range of concentration studied (5–15%), incorporation of the antimicrobial agent potassium sorbate in the GNF produced significant changes in the mechanical properties and solubility in water of the films. However, despite the hydrophilic characteristic of the antimicrobial compound, moisture content of the films was not affected. It could be concluded that potassium sorbate presented a plasticizer effect in the films, enabling greater molecular chain mobility and, therefore, affecting mechanical properties and solubility in water. The study of the quality of nanoparticle dispersion in the biopolymer matrix is a key strategy that may help researchers to better understand the effect of nanoparticle addition in biopolymer matrices. Spreading technique produced films similar to those produced by casting.

## 1. Introduction

Interest in preventing contamination and microbiological food spoilage is an old issue, and has been one of the major areas of focus of food science research (Chen, Wang, & Weng, 2010). Normally, in order to control microorganism growth in foods during storage, antimicrobial agents can be incorporated in product formulation, or in coated food surface, or even incorporated in food-packaging materials. Thus, packaging is an important barrier to prevent food spoilage, and represents the less invasive alternative for foods.

Antimicrobial edible films and coatings have shown to be efficient alternatives in controlling food surface contamination (Flores, Fama, Rojas, Goyanes, & Gerschenson, 2007; Kraśniewska & Gniewosz, 2012; Kristo, Koutsoumanis, & Biliaderis, 2008). Moreover, the use of active antimicrobial films have some other advantages: (1) this type of film can support additive carriers and, then, release the active compounds on the food surfaces where deterioration by microbial growth often occurs (Chen et al., 2010; Kester & Fennema, 1986), (2) they may replace the direct use of antimicrobial agents in foods (Kraśniewska et al., 2012) and, (3) they may control the release of the active agent, which could be important in processes where their concentration cannot be high, or when the active agent is easily degraded and needs to be protected from external agents. All these advantages support the increasing demand of consumers for food with fewer additives and, therefore, the need for research on antimicrobial-active packaging.

Currently, sorbic acid and its more water-soluble salts, especially potassium sorbate, are widely used throughout the world as antimicrobial preservatives for various foods, including dairy products, bakery items,

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* Corresponding author. Tel.: +55 19 35656866.
*E-mail address:* fernanda.vanin@usp.br (F.M. Vanin).

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fruit and vegetable products, edible fat emulsion products, certain meat and fish products, and sugar and confectionery items, as well as in animal feeds, pharmaceuticals and cosmetics, and in other industrial applications (Stopforth, Sofos, & Busta, 2005). Yeast and mold inhibition, good solubility, stability, and ease of manufacture make potassium sorbate the most widely used agent in food systems (Kraśniewska et al., 2012; Kristo et al., 2008; Stopforth et al., 2005). The maximum potassium sorbate concentration in foods varies according to the specific regulations of each country. An alternative to reducing its concentration in foods, without compromising food conservation, is the use of active packaging containing potassium sorbate. The incorporation of this agent in edible packaging from natural macromolecules, besides reducing the environmental impact of the large-scale use of synthetic packaging, may beneficially impact consumer health, given the current consumer trend for food free of preservatives.

Different macromolecules have been widely used in the production of edible films and, in general, gelatin was one of the first materials employed in biomaterial development. It continues to be used in studies on biodegradable packaging due to its excellent filmogenic properties (Lim, Mine, & Tung, 1999; Moraes et al., 2008; Sobral, Menegalli, Hubinger, & Roques, 2001; Thomazine, Carvalho, & Sobral, 2005; Vanin, Sobral, Menegalli, Carvalho, & Habitante, 2005), and because it is an abundant raw material produced at low cost all over the world. Despite these excellent characteristics, in general, gelatin-based films exhibit characteristics typical of biopolymer-based films with hygroscopic plasticizers: good mechanical strength and high elasticity, but sensitivity to environmental conditions, especially relative humidity, and therefore low barrier for water vapor. In order to improve these weaknesses, several alternatives have been studied (Carvalho & Grosso, 2004, 2006; Thomazine et al., 2005; Vanin et al., 2005). An alternative still not fully applied to improve the gelatin-based film properties is the incorporation of nanoparticles as a strengthening material, producing a type of material called nanocomposites (Lagarón et al., 2005; Sorrentino, Gorrasi, & Vittoria, 2007). Different experimental studies have shown that polymer/layered silicate nanocomposite films can improve the mechanical, barrier, thermal, and moisture adsorption properties of packaging material (Arora & Padua, 2010; Krook, Alberritsson, Gedde, & Hedenqvist, 2002; Ray & Okamoto, 2003; Ray, Quek, Easteal, Chen, & Campus, 2006; Rhim, Hong, Park, & Perry, 2006; Zhao, Torley, & Halley, 2008). It is worth eliciting that all these improvements were obtained at very low filler contents, generally lower than 5% (Sorrentino et al., 2007). Moreover, nanoparticles can be also used as carriers of antimicrobials and additive compounds (Rhim et al., 2006; Sorrentino et al., 2007). Some studies have demonstrated their ability to stabilize additives and efficiently control their diffusion in foods. Among nanoparticles, montmorillonite (MMT) is one of the most widely used clays in medicine and materials science, among others. Studies have shown that MMT is atoxic and, thus, has no side effects (Arora & Padua, 2010; Lee et al., 2005).

Another limitation to development of biomaterials and their use in large scale is the technique employed in their production. The majority of studies in the literature use the “casting” technique for film production, where a filmogenic solution is dispersed in plates and spreading is done by gravity. This process makes large-scale film development difficult. A still little explored alternative is film production by the automatic “spreading”.

Therefore, the development of gelatin-based nanocomposite films (GNF), potential carriers of additives, such as potassium sorbate via “spreading” technique represents a potential alternative in the development of an active edible packaging. Thus, the objective of this study was to evaluate the effect of gelatin concentration ($C_G$), plasticizer concentration ($C_P$), and montmorillonite concentration ($C_N$) on the rheological properties of the nanocomposite-forming solution using response surface methodology (RSM) and on the mechanical and barrier properties of the films. After optimizing the GNF properties, the effect of potassium sorbate concentration on some GNF properties was also investigated.

## 2. Materials and methods

### 2.1. Materials

Pigskin gelatin (Bloom 260/Mesh 40), kindly provided by Gelita South America (São Paulo, Brazil), was used for film production. Glycerol (Synth) was used as the plasticizer, montmorillonite as the nanoparticle (hydrophilic bentonite nanoclay, Sigma-Aldrich), and potassium sorbate (Synth), as the antimicrobial agent.

### 2.2. Preparation of the nanocomposite-forming solution

The nanocomposite-forming solutions (NFS) were produced from a mixture of gelatin (solution A), nanoparticle and glycerol dispersion (solution B). Solution A was prepared as follows: gelatin was hydrated for 30 min, and then dissolved at 70 °C (30 min) using a thermostatic bath (Marconi, Model TE 184). At the same time, solution B was prepared. In order to prepare solution B, the nanoparticle was first hydrated in distilled water and glycerol. Then, solutions A and B were mixed and homogenized during 10 min at 60 °C with a magnetic stirrer (Tecnal — TE085). These solutions underwent rheological analysis immediately after they were prepared.

### 2.3. Film production

For the production of the films, NFS were dispersed on acrylic plates using an automatic spreader film (Model Speed II, TKB Erichsen) under the following conditions: spreader speed = 35 mm/s and spreader height = 1.5 mm; support temperature = 25 °C. This temperature, as well the NFS temperatures, was defined according to results of rheological essays (Jorge et al., in press). The films were obtained by drying NFS for 24 h at 30 °C (MA 037 — TECNAL). Before any characterization, all the films were conditioned in desiccators containing NaBr saturated solutions (58% relative humidity) at 25 °C for seven days. After conditioning, thickness of the films was measured by a digital micrometer (Mytutoyo ±0.001 mm) with a 6.4 mm diameter probe. All the tests were undertaken in rooms with air-conditioning (T = 22 °C, and relative humidity between 55 and 65%). Water content (WC) of the films was determined by drying in an oven at 105 °C for 24 h at the end of the conditioning period.

### 2.4. Experimental design

The response surface methodology (RSM) was used to study the effect of gelatin concentration ($C_G$) (4.3–7.7 g/ 100 g nanocomposite forming solution), plasticizer concentration ($C_P$) (16.6–33.4 g/ 100 g gelatin), and montmorillonite concentration ($C_N$) (0–10 g/ 100 g gelatin), on dependent variables (rheological properties of nanocomposite-forming solution, mechanical properties and water vapor permeability of the films) in the first step of the study. The levels of the independent variable were established according to preliminary tests, and then defined according to a $2^{3\,+\,\alpha}$ factorial design (Table 1). Runs 15–19 at the center points were used to determine experimental error and reproducibility of the data. Table 1 shows the complete design matrix of the experiments. The experimental sequence was randomized in order to minimize the effects of the uncontrolled factors. Statistical analysis (95% confidence level) was performed using Statistica 9.1 software. Data were fitted to a second-order equation as a function of dependent variables (Eq. (1)):

$$y = \beta_0 + \sum_i \beta_i x_i + \sum_i \beta_{ii} x_i^2 + \sum_{i<j} \sum_j \beta_{ij} x_i x_j + \varepsilon \tag{1}$$

where $y$ is the predicted response, $\beta_0$, the constant coefficient, $\beta_i$, the linear coefficients, $\beta_{ij}$, the interaction coefficients, $\beta_{ii}$, the quadratic coefficients, $\chi_i$ and $\chi_j$, independent variables, and ε represents the error associated with the predicted response.