Shelf-life extension of meat products by cellulose acetate antimicrobial film incorporated with oregano's essential oil

Aplicação de filme antimicrobiano de acetato de celulose incorporado com óleo essencial de orégano para aumento da vida útil de produtos cárneos

Prolongación de la vida útil de los productos cárnicos mediante una película antimicrobiana de acetato de celulosa incorporada con aceite esencial de orégano

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Abstract
This study aimed to apply cellulose acetate (CA) films incorporated with oregano essential oil (OEO) to inhibit bacteria growth associated with spoilage of meat products (*Weissella viridescens* (microaerophilic) and *Pseudomonas fluorescens* (aerobic)) and evaluate its effect on the shelf life of vacuum-packed sliced ham (VPSH). CA films were produced using acetone solvent, adding 25, 35, 50, or 75 mg of OEO per film. Antimicrobial activity and mechanical properties of films were determined. CA films in Petri dishes showed a better antimicrobial effect against *W. viridescens* than *P. fluorescens*. As VPSH, presents a microaerophilic environment, product shelf life was determined fitting Baranyi and Roberts' model to *W. viridescens*' growth experimental data, at 8 °C. OEO did not modify films' mechanical properties. Application of the CA film with 75 mg of OEO decreased μ_{max} value of *W. viridescens*, increased its λ value, resulting in a ham` shelf-life increased by eight days, demonstrating excellent application potential.
Keywords: *Weisella viridescens*; *Pseudomonas fluorescens*; Antimicrobial packaging; Spoilage bacteria.

Resumo
O objetivo do trabalho foi aplicar filmes de acetato de celulose (AC) incorporados com óleo essencial de orégano (OEO) para inibir o crescimento de bactérias associadas à deterioração de produtos cárneos (*Weissella viridescens* (microaerofílica) e *Pseudomonas fluorescens* (aeróbia)) e avaliar seu efeito na vida de útil de presunto fatiado embalado a vácuo (PFEV). Os filmes AC foram produzidos usando solvente acetona, adicionando 25, 35, 50 ou 75 mg de OEO por filme. A atividade antimicrobiana em fase vapor e as propriedades mecânicas dos filmes foram determinadas. Os filmes de AC por meio do métodos de análise de vapor de óleo em placas de Petri mostraram melhor efeito antimicrobiano contra *W. viridescens* do que *P. fluorescens*. Como o PFEV apresenta um ambiente microaerofílico, a vida útil do produto foi determinada ajustando o modelo de Baranyi e Roberts aos dados experimentais de crescimento de *W. viridescens*, a 8 °C. O OEO não modificou as propriedades mecânicas dos filmes. A aplicação do filme AC com 75 mg de OEO diminuiu o valor da μ_{max} de *W. viridescens* e aumentou o valor da λ, resultando em um aumento da vida útil do presunto em oito dias, demonstrando excelente potencial de aplicação.
Palavras-chave: *Weisella viridescens*; *Pseudomonas fluorescens*; Embalagem antimicrobiana; Bactérias deteriorantes.

Resumen

El objetivo del trabajo fue aplicar películas de acetato de celulosa (AC) incorporadas con aceite esencial de orégano (OEO) para inhibir el crecimiento de bacterias asociadas al deterioro de productos cárnicos (*Weissella viridescens* (microaerófila) y *Pseudomonas fluorescens* (aeróbica)) y evaluar su efecto sobre la vida útil del jamón lonchado al vacío (JLV). Las películas de AC se produjeron con disolvente de acetona, añadiendo 25, 35, 50 o 75 mg de OEO por película. Se determinó la actividad antimicrobiana en fase de vapor y las propiedades mecánicas de las películas. Las películas de AC por métodos de análisis de vapor en placas de Petri mostraron un mejor efecto antimicrobiano contra *W. viridescens* que contra *P. fluorescens*. Como la JLV presenta un entorno microaerófilo, la vida útil del producto se determinó ajustando el modelo de Baranyi y Roberts a los datos experimentales de crecimiento de *W. viridescens* a 8 °C. La OEO no modificó las propiedades mecánicas de las películas. La aplicación de la película de AC con 75 mg de OEO disminuyó el valor μ_{max} de *W. viridescens*, incrementó su valor λ, resultando en un aumento de la vida útil del jamón de ocho días, demostrando un excelente potencial de aplicación.

Palabras clave: *Weisella viridescens*; *Pseudomonas fluorescens*; Envases antimicrobianos; Bacterias de deterioro.

1. Introduction

The control of microbial growth in meat products is crucial to extend products´ shelf life and prevent foodborne diseases (Longhi et al., 2018; Jacob, Mathiasen, & Powell, 2010). Lactic acid bacteria (LAB) is one of the main spoilage microorganisms present in meat products stored under vacuum or modified atmosphere. *Weissella viridescens* is a Gram-positive LAB that can grow in microaerophilic conditions, been strongly stimulated by temperature fluctuation throughout the cold chain (Silva et al., 2017). *Pseudomonas* spp. is Gram-negative, aerophilic, and psychrotrophic bacteria that can grow under refrigeration conditions. These characteristics make them the main spoilage microorganisms in meat products, especially at low temperatures, under aerobic conditions (*Pseudomonas* spp.) and vacuum packaging (*W. viridescens*) (Oh et al., 2014; Sousa et al., 2012).

The antimicrobial packaging is an alternative to delay the growth or inactivate bacterial cells, increasing food safety, and shelf life in a broad range of applications (Jafarzadeh et al., 2020; Kapetanakou & Skandamis, 2016). Active films containing antibacterial essential oils interact with the product surface by direct contact or gradual release of the active molecules previously added to the film (Espitia et al., 2013; Woranuchet al., 2015).

Several essential oils, and some isolated chemical molecules naturally present in them, had their antimicrobial activity tested against a wide range of foodborne microorganisms (Boskovic et al., 2020; Boskovic et al., 2015; Busatta et al., 2007). Essential oils from oregano, thyme, lemon, and lavender are very active against Gram-positive and Gram-negative foodborne bacteria (Correa et al., 2017). Its antimicrobial and antioxidant activity widely recognizes the oregano essential oil (OEO), attributed to phenolic compounds, such as thymol, carvacrol, and eugenol (Burt, 2004; Jafarzadeh et al., 2020). The antimicrobial activity of films added with OEO has been demonstrated in previous studies (Caetano et al., 2017; Munhuweyi et al., 2018), including extending the shelf life of fish fillets using OEO/polybutylene adipate-co-terephthalate films (Cardoso et al., 2017).

One of the biggest challenges in active packaging is to reach a suitable material that meets sustainable and affordable characteristics. Cellulose acetate (CA) is a useful eco-friendly polymer once it is biodegradable, amorphous, non-toxic, and odorless (Rudaz & Budtova, 2013). Films based on CA have shown applications in food packaging, including active films (Pola et al., 2016; Rodríguez et al., 2014).

The use of mathematical models describing microbial growth has become an essential tool in predicting food shelf life, risk assessments, and quality (Koutsoumanis et al., 1999; Mataragas et al., 2006). Mathematical modeling has been widely applied to describe bacteria growth associated with the spoilage of meat products (Longhi et al., 2018; Silva et al., 2017). Previous studies report the use of mathematical modeling to assess the shelf life of vacuum-packed ham, considering the growth of LAB (Menezes et al., 2018; Slongo et al., 2009).

Thus, the aims of this study were: i) to apply CA films incorporated with OEO to inhibit the growth of the bacteria

W.viridescens (microaerophilic) and *Pseudomonas fluorescens* (aerobic) in a culture medium, ii) to characterize the mechanical properties of the resulting films, iii) to model the effect of the active films on the increase of the shelf life of vacuum-packed cooked sliced ham under isothermal conditions (8°C), based on the growth of the *W. viridescens*.

2. Methodology

2.1 Oregano essential oil (OEO)

Oregano essential oil was purchased from Ferquima LTDA (Brazil). Its components were determined by gas chromatography-mass spectrometry (GC-MS, model QP2010 Plus Shimadzu, RTX-5MS column) and gas chromatography with Flame Ionization Detector (GC-FID, model 2010 Shimadzu, OV-5 column) (Almeida, 2017 – personal communication).

2.2 Production of CA antimicrobial film incorporated with OEO

Films were produced by the casting method, which consists of pouring and drying a colloidal film-forming solution (FS) on a flat surface (e.g., Petri plate). For the preparation of the FS, CA was solubilized in acetone. Our research group used the acetone solution as solvent-based in previous studies. The films from the acetone solution were less dense, mechanically rigid, and more hydrophobic than the other tested solvent. Besides, it evaporates more quickly, resulting in less time to obtain the films.

The FS was prepared with a CA concentration of 5 g per 100 mL of solvent. The CA was added to the solvent and kept under mechanical stirring at room temperature until the polymer was completely solubilized. Then, 25, 35, 50, or 75 mg of OEO were added to the FS (10 mL per Petri dish) and kept under stirring for 10 min. The OEO quantity was chosen based on previous studies with OEO carried out in our group.

A predetermined volume of 10 mL of the FS was added in the glass Petri dishes (9 cm in diameter) capped, forming a film with an area of approximately 63,6 cm^2. The films were dried in a glass chamber (21 °C and relative humidity around 65 - 70%) containing blue silica with a lower opening to avoid the solvent saturation internally for 48 h. The films were named F25, F35, F50, and F75, to represent the quantities of OEO per film (25, 35, 50, and 75 mg of OEO).

2.3 Bacterial strain and inoculum preparation

Freeze-dried pure cultures of *W. viridescens* (CCT 5843 ATCC 12706, Lot 22.07) and *P. fluoresces* (CCT 7393 ATCC 13525, Lot 25.06) were used. *W. viridescens* inoculum was prepared in Man, Rogosa, and Sharpe broth (MRS) at 30 °C for 18 h, resulting in a cell concentration around 10^8 CFU/mL. *P. fluorescens* inoculum was prepared in Brain Heart Infusion broth (BHI) at 30 °C for 24 h, resulting in a cell concentration close to 10^7 CFU/mL.

2.4 Antimicrobial activity of the CA antimicrobial film incorporated with OEO in the culture medium

The antimicrobial activity of the films F25, F35, F50, and F75 was tested against *W. viridescens*, while films F50, F75 were tested against *P. fluorescens* (OEO concentration was chosen based on previous studies with OEO carried out in our group). Serial dilutions were prepared in 0.1% peptone water and adjusted to 10^4 CFU/mL. Active films were then placed on the lids of Petri dishes previously inoculated with 100 µL of each suspension in five points dropped on agar surface (Petri dish + agar + suspension / Petri dish lid + antimicrobial film). Next, the plates were incubated at 30 °C for 48 h. A control analysis was carried out. The results were compared with the control experiment's growth (without the antimicrobial film) and assessed visually.

2.5 Shelf life determination of vacuum-packed ham with CA antimicrobial film incorporated with OEO in the culture medium

2.5.1 Characterization of ham

Physicochemical composition analysis of ham samples was carried out by measuring pH, water activity (aw), and sodium chloride concentration (NaCl). The pH values were measured using a portable digital pH-meter model 205 (TESTO, Sparta, USA). The aw was performed with a dew-point hygrometer (Aqualab, SERIES 3TE, Pullman, USA). The concentration of NaCl was determined by the analysis of chlorides and conversion in sodium chloride, according to the methodology proposed by Aliño et al. 2011. Samples of ham (2 g) were previously ground with distilled water in Ultra Turrax homogenizer (IKA, model T25 Digital) at 20,000 rpm for 1 min. The solution was made up to 100 mL and then centrifuged (Centrifuge Sigma, model 4k15) for 10 min at 9000 rpm. A previously calibrated automatic chlorine analyzer analyzed an aliquot of 0.5 mL of the supernatant (Cole Parmer, model 926). The results were expressed in milligrams of Chlorine per liter of solution.

2.5.2 Sample preparation

Cooked ham pieces (about 3 kg) (Seara®, São Paulo, Brazil), purchased from a local supermarket and stored at 4 °C, were cut aseptically in a slicer (Metvisa, model CFIE 250, Brusque, Brazil), with thickset to 1.5 mm, resulting in 5.32 g ± 0.73 g per slice. Aliquots with 100 µL bacterial suspension of W. viridescens (around 10^5 CFU/mL) were inoculated and spread on the surface of the ham slices. After inoculation, the film was placed on the surface of the sample and folded. The control samples were inoculated and folded. The samples were put into a sterile mixer bag, packaged in a plastic vacuum bag, and stored in a temperature-controlled incubator (Dist, Florianopolis, Brazil) at 8 °C. The temperature of the samples was measured by data-loggers (Testo 174, Lenzkirch, Germany) every 10 min. For simplicity, samples of cooked, sliced, and vacuum-packed ham will sometimes be called ham samples.

2.5.3 Microbial analyses

Samples were taken at selected times to measure the *W. viridescens* cells concentration. Each vacuum-packaged sample was aseptically homogenized with peptone water (1% w/v) in the ratio 9:1 [volume peptone water (mL): ham mass (g)] for 60 s in a stomacher (ITR model 1204) to carry out the first dilution. Then, series dilutions were prepared, and 1 mL of each dilution was transferred to sterile Petri dishes with a double layer of agar MRS (Difco Laboratories, Detroit, USA). All the procedures were carried out in a laminar flow chamber. Finally, the plates were incubated at 30 °C for 48 h. The *W. viridescens* concentration was expressed in CFU/g of ham.

2.6 Mathematical modeling

2.6.1 Primary model

The primary model proposed by Baranyi and Roberts (1994) was fitted to the experimental data of *W. viridescens* growth on the samples of vacuum packaged ham, stored under isothermal condition (8 °C). The curves describe the logarithm of the microbial concentration y = log (N) during the time (t). The parameters of equation 1 are i) y_0 that is the logarithm of the initial microbial concentration, ii) μ_{max} that is the specific growth rate (h^{-1}), y_{max} that is the maximum population (CFU/g), and λ that is the lag phase duration (h). The whole mathematical modeling is described by Equations 1, 2 and, 3, which $F(t)$ is related to the physiological state of the cells (Equation 2), and h_0 is the parameter concerning the initial physiological state of the cell that is related to the duration of the lag phase (λ) (Equation 3). The fitting procedure was performed in the MATLAB

R2013a (The MathWorks Inc®, Natick, USA) using the algorithm developed by Longhi et al. (2013)

$$y = y_0 + \mu_{max}F(t) - log\left(1 + \frac{exp(\mu_{max}F(t)) - 1}{exp(y_{max} - y_0)}\right) \tag{1}$$

$$F(t) = t + \frac{1}{\mu_{max}} log\left(exp(-\mu_{max}t) + exp(-h_0) - exp(-\mu_{max}t - h_0)\right) \tag{2}$$

$$\lambda = \frac{h_0}{\mu_{max}} \tag{3}$$

2.6.2 Statistical analysis

The statistical indices used to evaluate primary model ability of fitting to experimental data were the Coefficient of Determination (R^2 (Equation 4)), the Root-mean-square Error ($RMSE$ (Equation 5)), and the Bias (BF (Equation 6)) and Accuracy factors (AF (Equation 7)) (Ross, 1996). The $value_{predicted}$ are the values predicted by the model, $value_{observed}$ are the experimental growth data, and n is the number of experimental data, y is the value of microbial cell concentration, $\bar{y}$ is the arithmetic mean of all values of y.

$$R^2 = \frac{\Sigma(y_{predicted} - \bar{y})^2}{\Sigma(y_{observed} - \bar{y})^2} \tag{4}$$

$$RMSE = \sqrt{\frac{\Sigma(value_{observed} - value_{predicted})^2}{n}} \tag{5}$$

$$BF = 10^{\left(\Sigma\frac{log(value_{predicted}/value_{observed})}{n}\right)} \tag{6}$$

$$AF = 10^{\left(\Sigma\frac{|log(value_{predicted}/value_{observed})|}{n}\right)} \tag{7}$$

2.7 Mechanical properties

The film samples that showed better efficiency and a control film (CA film without the addition of OEO) were analyzed immediately after their production. Film thicknesses were determined with a digital micrometer (Digimatic MDC-Lite, Japão). Tensile strength (TS), Elongation at break (EB), and Young's modulus (YM) were measured using a texturometer (TA.HD.plus Texture Analyse) assisted by the Stable Micro Systems program. Film samples were cut into rectangular strips (25x60 mm) and tested with an initial claw distance of 40 mm and a test velocity of 0.8 mm/s. The tests were performed in triplicate.

3. Results and Discussion

3.1 Oregano essential oil

The results of CG-MS and CG-FID analysis in the OEO are shown in Table 1. The presence of 21 major compounds in OEO was observed. Both analyses showed a high concentration of phenolic compounds, especially carvacrol (76.1%/GC-MS and 53.76%/GC-FID), considered an active compound against microorganisms (Burt, 2004). The profile of OEO obtained in the present study was very similar to the previous results reported by Boskovic et al. (2020), who also found carvacrol as a major compound.

Table 1 - Composition of OEO (*Origanum vulgare*), retention times (min) and relative concentration (%).

Compound	Retention times (min)	Relative concentration (%) GC-MS*	Relative concentration (%) GC-FID**
α-Thujene	6.67	0.40	0.19
α-pinene	6.93	0.69	0.33
Camphene	7.49	0.33	0.17
β-pinene	8.62	1.73	1.00
Myrcene	9.25	0.70	0.42
α-Phellandrene	9.81	0.38	0.14
α-Terpinene	10.36	1.28	0.47
o-Cymene	10.73	5.55	3.82
Limonene	10.91	1.75	0.78
1,8-Cineole	11.01	1.84	0.85
γ-Terpinene	12.33	5.25	3.60
Linalool	14.31	3.21	1.93
Camphor	16.34	1.53	0.38
Borneol	17.40	2.13	0.88
4-Terpinenol	17.96	1.68	0.67
α-Terpineol	18.63	0.51	0.15
Thymol	23.50	5.66	2.32
Carvacrol	24.22	53.76	76.10
β-Caryophyllene	28.83	6.32	3.83
β-Bisabolene	32.53	0.24	0.10
Oxide Caryophyllene	35.43	0.85	0.35

*GC-MS: gas chromatography-mass spectrometry.   **GC-FID: gas chromatography with Flame Ionization Detector.
Source: Authors.

3.2 Antimicrobial effect of with CA antimicrobial film incorporated with OEO against *W. viridescens* and *P.fluorescens*

The visual analysis of applications of the films F25, F35, F50, and F75 and control on *W. viridescens* and *P. fluorescens* growth are presented in Table 2. The films incorporated with OEO resulted in a growth reduction of *W. viridescens* and *P. fluorescens*, which decreased with the increase of OEO quantity. Different responses were obtained for each bacterium. The active films F50 and F75 led to complete inhibition for *W. viridescens*. The films F25 and F35 led to results that were not different from the control experiment. The film F50 also resulted in the same pattern observed for the control experiment for *P. fluorescens*, while the film F75 showed a growth reduction when compared to the control.

Table 2 - Visual analysis of the films incorporating 25 (F25), 35 (F35), 50 (F50), and 75 (F75) mg of OEO and Control on *W.viridescens and P.fluorescens* growth.

Film	*W.viridescens*	Control (*W.viridescens*)	*P.fluorescens*	Control (*P.fluorescens*)
F25	**	**	-	-
F35	**	**	-	-
F50	×	**	**	**
F75	×	**	*	**

(-) No evaluated, (**) growth (*) Moderate growth and (×) Growth inhibition. Source: Authors.

The results showed that the Gram-negative bacteria, *P. fluorescens*, was more resistant to the OEO CA-film than the Gram-positive bacteria, *W. viridescens*. Marino et al. (2001) reported that EO from sage, mint, hyssop, camomile, and oregano

were more effective against Gram-positive bacteria. *Escherichia coli* O157:H7 was the most sensitive species of Gram-negative bacteria, while *Listeria innocua* and the *Bacillaceae* were the most sensitive among the Gram-positive species. However, the bactericidal activity was more pronounced against the Gram-negative bacteria. Ouattara et al. (1997) observed that several EOs, including OEO, caused similar effects in Gram-negative and Gram-positive bacteria after a 24-hour treatment. Although, after 48 hours of treatment, the inhibition effect appeared to be higher against Gram-negative bacteria.

The antibacterial effect of OEO against bacteria can be attributed to its hydrophobicity that damages the membrane cell, increasing their permeability and nutrient losses (Burt, 2004). Most studies agree that EOs are slightly more active against Gram-positive than Gram-negative bacteria. Gram-negative microorganisms have an outer membrane surrounding the cell wall that restricts the diffusion of hydrophobic compounds through their lipopolysaccharide cover. This fact can make these organisms less susceptible to the action of Eos (Burt, 2004). Dorman & Deans (2000) reported that individual components of EOs exhibit different degrees of activity against Gram-positive and Gram-negative bacteria. The chemical composition of EOs from a particular plant can vary according to the geographical origin and harvesting period. Thus, variation in the composition among EOs from different batches can be sufficient to cause variability of their action against Gram-negative and Gram-positive bacteria (Burt,2004).

Lee et al. (2019) reported the antimicrobial activity of hydroxypropyl methylcellulose-based films incorporated with OEO nanoemulsions by the disc diffusion method. The authors also observed that the inhibition increased significantly with increasing OEO concentration. Films containing over 5.0% (v/v) of OEO nanoemulsions showed antibacterial activity against all the tested strains (*Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *E. coli*, *Salmonella Typhimurium*, *Pseudomonas aeruginosa*, and *Vibrio parahaemolyticus*).

The results obtained in the present study showed that the OEO had good antimicrobial activity. Incorporating EOs in packaging films is of great interest to increase shelf life and food safety (Laird & Phillips, 2012; Pola et al., 2016). A case study with vacuum-packaged ham is presented in the following, considering *W. viridescens* as the target microorganism.

3.3 Determination of the shelf life of vacuum-packaged ham with CA antimicrobial film incorporated with OEO

3.3.1 Characterization of ham

The samples of cooked ham used in this work present water activity 0.975 (sd=±0.004, cv=0.4%), pH 6.267 (sd=±0.049, cv=0.7%), and sodium chloride 2.126 (sd=±0.048, cv=2.25%) (% in mass). The results showed low standard deviations (sd) and coefficients of variation (cv), indicating homogeneous samples. Those found agree with Bressan et al. (2007) that reported sliced meat products to have salt contents of 2 to 4%. Kalschne et al. (2014) reported a ham aw of 0.970, while Garrido et al. (2010) reported that the pH range appropriate to cooked ham is 6.00 to 6.73. These results agree with the previous find by Menezes et al. (2018) that indicates pH 6.22 (sd=± 0.04). Therefore, the samples of ham used in the present study had the expected characteristics for this product and are adequate to study the action of antimicrobial films on its shelf life increase.

3.3.2 Antimicrobial activity of the CA antimicrobial film incorporated with OEO in vacuum-packed slices of ham

Based on previous results, the antimicrobial effect of CA active film F50 was assessed in samples of sliced ham, inoculated with *W. viridescens*, vacuum-packed, and stored at 8 °C. According to the literature (Kreyenschmidt et al., 2010; Slongo et al., 2009), a LAB concentration of 10^7 CFU/g (7 (log CFU/g)) can be the criterion to determine the end of the ham shelf life. Growth evaluation of *W. viridescens* in ham using F50 showed no significant difference in time to reach the concentration of 10^7 CFU/g (7 (log CFU/g)) when compared to the control (results not shown). Then, the growth of *W. viridescens* in vacuum-packed cooked sliced ham using F75, stored at 8 °C, was investigated. In Figure 1, it is possible to

observe that the film F75 inhibited the growth of *W. viridescens*.

Figure 1. Growth data of *W. viridescens* on vacuum-packed cooked sliced ham stored at 8 °C. Control (●) and with AC antimicrobial film incorporated with 75 mg of OEO (○). Continuous lines indicate Baranyi and Roberts model fitted to the experimental data. The dashed line indicates the final count that defines ham`s shelf life (7 log CFU/g).
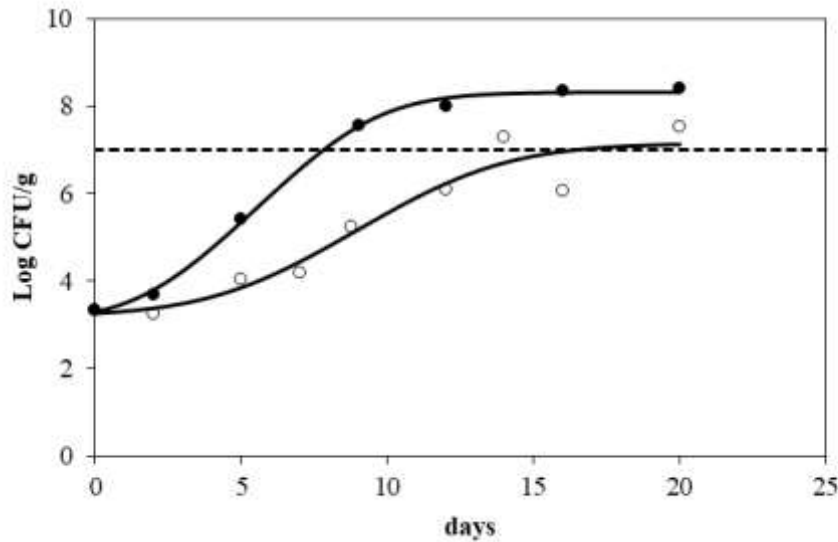
Source: Authors.

The Baranyi and Roberts model was fitted to the experimental data of microbial growth in the samples of ham packed with the active film (F75) and in the control samples (Figure 1). The predictive ability of the model was assessed through statistical indices (R^2, $RMSE$, BF, AF). The control samples presented R^2, BF, and AF of 0.998, 1.002, and 1.010, respectively. The statistical indices R^2, BF, and AF for samples with F75 were 0.929, 1.002, and 1.052, respectively. Slongo et al. (2009) studied the growth of LAB in pressurized hams obtaining an average R^2 value of 0.80. Menezes et al. (2018) found an average R^2 value of 0.941 for the growth of LAB natural microbiota in ham samples with 0.4% OEO, using the Baranyi and Roberts model. The $RMSE$ value for the control samples was 0.092 and for samples with F75 was 0.410. Menezes et al. (2018) obtained an average $RMSE$ value for ham samples with 0.4% OEO of 0.593 for the Baranyi and Roberts model. Baranyi and Roberts' model fitted well to the experimental data, mainly for the control sample. The values of R^2 and $RMSE$ obtained are acceptable, taking into account that the microbial concentrations come from solid foods, which can lead to changes in the scores. The BF and AF values were slightly over 1, providing safe predictions of the growth of *W. viridescens*. Table 3 shows the values of the primary model parameters (λ, μ_{max}, and y_{max}) and the shelf life at 8 °C for the samples using F75 and control. The use of the F75 increased λ, decreased μ_{max}, and y_{max} resulted in a shelf life eight days longer compared to the control. Thus, OEO active CA films had an effective antimicrobial action against *W. viridescens* in ham, with an inhibitory effect since the beginning of the storage.

Table 3 - Growth parameters obtained by fitting Baranyi and Roberts model to the experimental data obtained in the growth of *W. viridescens* in ham at 8 °C, with film incorporating 75 mg of OEO (F75) and Control.

Sample	μ_{max} (h^{-1})	λ (days)	y_{max} (log CFU/g)	Shelf life (days)
Control	0.029	2.2	8.3	8.8
F75	0.021	5.2	7.2	16.7

Source: Authors.

The literature reported the effect of oregano's essential oil on the shelf life of vacuum-packed cooked sliced ham, based on the growth of LAB natural microbiota under isothermal conditions. The use of this essential oil led to an increase of λ, a decrease of μ_{max}, and a consequent increase in the ham shelf life compared to the control samples (Menezes et al., 2018).

The precise parameter estimation is essential to develop kinetic models, characterize the antimicrobial effects of commercial and new natural compounds, predict the microorganism-count-based shelf life, and even in fermentation technology (Longhi et al., 2017; Silva et al., 2017). Previous studies have stated that the lag and exponential phases during microbial growth are of most significant interest in food preservation because the spoilage occurs before the stationary phase (Augustin & Carlier, 2000; Baranyi & Roberts, 1994).

In the present study, the bacterial concentration in ham, using F75, stayed below 7 log CFU/g (limit count to the end of the shelf life for ham) until the 17th day of storage, while the control reached 7 log CFU/g after 9 days of storage (Table 3). The lag values were extended from 2.2 days to 5.2 days by using F75.

Correa et al. (2017) reported that polyhydroxybutyrate/polycaprolactone biodegradable films activated with nisin present a bacteriostatic inhibition effect on *Lactobacillus plantarum* inoculated on ham. The results showed 3.4 log cycles reduction on the 21st day of storage at 5 °C, and 2.6 log cycles reduction at the end of the experiment (28th day). The authors reported an extended lag phase from 2.17 to 5.18 days due to active film with OEO.

Our results showed that the active films could be an excellent alternative to increase meat product shelf life. Ouattara et al. (2000) reported that antimicrobial films might be more efficient than the direct use of antimicrobial compounds in foods. The slow and gradual migration of the active compound from the packaging to the surface of the food could maintain the necessary concentration to inhibit the development of microorganisms (Jafarzadeh et al., 2020). In most fresh or processed foods, microbial contamination occurs at higher levels on the surface, requiring effective control of microbial growth at this local.

3.4 Mechanical properties

The mechanical properties of the films without (control) and containing 50 and 75 mg of OEO were determined. Table 4 shows the thickness, tensile strength (TS), elongation at break (EB), and Young modulus (YM) of these three films. From a visual analysis, both films formed with OEO were transparent, uniform, and continuous, without ruptures. The films presented constant thickness, which is essential to their functionality. Pola et al. (2016) reported that the film thickness influences its mechanical properties and that a constant thickness indicates an appropriate production process, which can form uniform films.

Table 4 - Measures of thickness and mechanical properties tensile strength (TS), Elongation at break (EB), and Young's modulus (YM) of cellulose acetate active films with 50 (F50) and 75 mg of OEO (F75) and control (CA film without the addition of OEO).

Sample	Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)	Young's modulus (MPa/%)
Control	0.04 (±0.008)	37.34[a] (±5.17)	2.84[b] (±1.02)	33.64[a] (±1.96)
F50	0.04 (±0.008)	42.39[a] (±5.12)	7.50[a] (±0.89)	30.24[a] (±7.72)
F75	0.05 (±0.005)	38.36[a] (±1.05)	5.58[a] (±0.81)	30.12[a] (±4.43)

Reported values are the means (± standard deviation). Different letters (a, b) in the same column indicate significant differences ($p < 0.05$). Source: Authors.

The TS, EB, and YM results did not show significant differences between the control and the two formulated films ($p < 0.05$). However, it was possible to observe that the EB increased with increasing OEO concentration in the films. Llana-Ruiz-Cabello et al. (2018) reported that the EB was affected by the incorporation of OEO. Lee et al. (2019) also noted that incorporating OEO nanoemulsions (5% v/v) in hydroxypropyl methylcellulose-based films increased EB compared to the control films.

Our results agree with those reported by Aguirre et al. (2013) and Jouki et al. (2014). The increase in the films' EB showed that the OEO had a typical plasticizing effect. The plasticizing effect of OEO in CA-based films is due to the low molecular weight of essential oils when compared to the macromolecules of AC. The OEO molecules entered between the polymer chains, weakening the intermolecular interactions (Pola et al., 2016).

4. Conclusion

The CA films incorporated with OEO showed antimicrobial effects against the microorganisms. The F75 films partially reduced the growth of *P. fluorescens*. On the other hand, F50 and F75 films completely inhibit *W. viridescens* growth in the culture medium.

Baranyi and Roberts' model can describe the growth of *W. viridescens* in ham with the application of the OEO active CA film. The CA films incorporated with OEO present mechanical properties close to the control film, which is essential for its practical application. In ham, F75 films decrease the value of μ_{max}, increases the value of λ, which increases the ham's shelf life from 9 to 17 days. Thus, OEO active CA films demonstrate excellent potential and could be used to prolong the shelf life of vacuum-packed ham. The use of oregano essential oil microencapsulation in active packaging is suggested for potential future research.

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