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# Antimicrobial and aromatic edible coating on fresh-cut pineapple preservation

## Revestimento comestível antimicrobiano e aromático na conservação de abacaxi minimamente processado

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**ABSTRACT**

*The present research aimed to develop an edible coating incorporated with mint essential oil, evaluate its effectiveness in inhibiting* in vitro *microbial development, and improve both quality and shelf-life of fresh-cut pineapple. Mint essential oil-containing edible coatings showed* in vitro *antimicrobial efficiency against **Escherichia coli** and **Salmonella** Enteritidis. Titratable acidity, pH, and texture were not affected (P>0.05) by coating or storage time. Mass loss was not higher than 1.0% after the 6$^{th}$ day of storage. No effect of storage time and coating on total soluble solids was observed. Mint essential oil-containing coatings inhibited the growth of yeasts and molds in fresh-cut pineapple. Compared to uncoated and control-coated samples, mint essential oil-containing coatings lessened psychrotrophic bacteria counts throughout storage. Counts of thermotolerant coliforms were not higher than 3.0MPN·g$^{-1}$ in all treatments, whereas no **Salmonella** sp. was detected during the 6-day storage. Mint essential oil provided a strong flavor to the fruit, as shown by sensory evaluations.*

**Key words**: *minimal processing, edible coating, essential oil, pineapple.*

**RESUMO**

*O objetivo deste trabalho foi desenvolver um revestimento comestível incorporado com óleo essencial de hortelã, bem como avaliar sua eficiência antimicrobiana* in vitro *e em abacaxi minimamente processado. Revestimentos contendo óleo essencial de hortelã mostraram eficiência antimicrobiana* in vitro *contra **Escherichia coli** e **Salmonella** Enteritidis. O pH, a acidez titulável e a textura não foram afetadas (P>0.05) pelos tratamentos durante o armazenamento. A perda de massa dos abacaxis de todos os tratamentos não ultrapassou 1.0% após 6 dias de armazenamento. O tempo e os diferentes revestimentos não afetaram (P>0,05) o teor de sólidos solúveis totais dos abacaxis. Revestimentos contendo óleo essencial de hortelã foram capazes de inibir o crescimento de fungos e leveduras em abacaxi minimamente processado, quando comparado aos frutos sem revestimento e com revestimento controle. Frutos com revestimento contendo óleo essencial de hortelã apresentaram menor contagem de psicrotróficos no final do armazenamento. A contagem de coliformes termotolerantes foi menor que 3.0NMP·g$^{-1}$ para todos os tratamentos e não foi detectada presença de **Salmonella** sp. durante o período de armazenamento. A presença de óleo essencial de hortelã conferiu forte sabor aos abacaxis.*

**Palavras-chave**: *processamento mínimo, revestimento comestível, óleo essencial, abacaxi.*

## INTRODUCTION

Consumer's demands for fresh–cut tropical products are rapidly increasing worldwide. Fresh-cut pineapple (***Ananas comosus*** L. Merril) can be found in many supermarket and foodservice chains (MARRERO & KADER, 2001). However, shelf-life of fresh-cut fruits is short (5 to 7 days undergoing refrigeration) due to injuries from cutting operations, which cause break of the cell membrane . Consequently, respiration rate, ethylene production, and tissue softening are accelerated, making the commercialization of high-quality, fresh-cut fruits difficult. Besides, injured fruits become more susceptible to microbial spoilage, mainly by foodborne pathogens such as ***Escherichia coli***

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O157:H7, ***Salmonella*** sp. and ***Listeria monocytogenes***. This is attributable to vesicular fluid leakage and the absence of protective peel (RAYBAUDI-MASSILIA et al., 2009).

The currently applied sanitizing and washing treatments for industrial applications do not guarantee the entire elimination of microorganisms (ABADIAS et al., 2010). Edible coatings have been developed as an alternative technology that may reduce the gas exchange rates and water loss from fruits and vegetables, as well as incorporate additives to control reactions that are detrimental to their quality (OMS-OLIU et al., 2008). Several classes of antimicrobial compounds have potential application in edible coatings, including organic acids, fatty acid esters, polypeptides, essential oils from plants, nitrites and sulphites. Plant essential oils stand out as an outstanding alternative to replace synthetic preservatives in an effort to overcome health concerns (ROJAS-GRAU et al., 2009).

Many essential oils are listed as Generally Recognized as Safe (GRAS) by the United States Food and Drug Administration (FDA), and may be used to increase food safety and improve or diversify its aroma (AVERBECK & SCHIEBERLE, 2011; BRASIL et al., 2012; ROJAS-GRAU et al., 2009; XING et al., 2012). Mint essential oil has been demonstrated to be an efficient antimicrobial against food pathogen and spoilage microorganisms (KARAGÖZLÜ et al., 2011; MOREIRA et al., 2005). The combination of pineapple and mint, which is quite popular in Brazil, is used in formulation of new products (ROSA et al., 2011).

The present research aimed, therefore, to develop an edible coating incorporated with mint essential oil, evaluate its effectiveness in inhibiting the *in vitro* microbial development and in improving both quality and shelf-life of fresh-cut pineapple.

## MATERIALS AND METHODS

The minimum inhibitory concentration (MIC) of mint essential oil (Givaudan, Jaguaré, São Paulo, SP, Brazil) against ***Escherichia coli*** and ***Salmonella*** Enteritidis was determined through the standard broth dilution method using a two-fold dilution of mint essential oil, in accordance to CLSI (2009).

A stock solution was prepared by diluting mint essential oil in dimethyl sulfoxide (DMSO, Sigma-Aldrich Co.). The solution was then added to the culture broth to reach final oil concentrations ranging from 4.096 to 8μg·mL$^{-1}$. Serial dilutions were inoculated into 100μL of 5.0x10$^{5}$CFU·mL$^{-1}$ bacterial suspension. The samples were incubated at 35°C for 16-20h. Control samples consisted of inoculated broth without essential oil and (i) with or (ii) without DMSO. The lowest mint essential oil concentration that completely inhibited microbial growth was recorded as the MIC.

The coating were prepared by heating aqueous solutions containing 5% w/w to 55°C until gelatinization, indicated by increased viscosity and decreased opacity. The solutions were cooled to room temperature, and then mint essential oil was added at concentrations of 0.5, 1.0, or 1.5% w/w. Essential oil-free solutions were prepared likewise and served as control. The essential oil concentrations were determined after the evaluation of its MIC against ***E. coli*** and ***Salmonella*** Enteritidis, bacteria that are representative of the microorganisms used as criteria to the assessment of the microbiological quality of fresh-cut fruits, according to the Technical Regulation on Microbiological Standards for Foods (BRASIL, 2001).

The *in vitro* antimicrobial efficiency of the coatings was evaluated against ***Escherichia coli*** (ATCC 11229) and ***Salmonella*** Enteritidis (ATCC 13076). Suspensions containing 10$^{8}$ CFU·mL$^{-1}$ of either ***E. coli*** or ***Salmonella*** Enteritidis were spread onto solidified Difco™ Mueller Hinton agar (Becton, Dickinson and Co., Sparks, Maryland, USA) in Petri dishes using swabs. Shortly thereafter, the coatings were spread onto the inoculated agar. Plates were then incubated at 35°C for 16-18h. After incubation, the growth was evaluated by CFU counts.

Fresh pineapples were bought at a local supermarket in Viçosa, Brazil and transported to the Laboratory of Food Packaging, Department of Food Technology, Federal University of Viçosa, and their crown leaves were removed. The fruits were washed with water, rinsed with Sumaveg® (Diversey Lever, São Paulo, SP, Brazil) solution (200mg·L$^{-1}$ of total residual chlorine) for 10min, washed in another Sumaveg® solution (3mg·L$^{-1}$ of residual chlorine), and allowed to dry at room temperature. Cleaned pineapple was then peeled and cut into 1-cm-thick slices.

Pineapple slices were surface dehydrated in 1% w/w calcium chloride solution for 3min, dipped in the different coatings for 3min, and dried at 15±2°C for 2h. Treated samples were placed into polypropylene trays (100g each) and stored under refrigeration (7±2°C) for 6d. Trays were randomly taken at 0, 2, 4 and 6 d for physicochemical determinations, texture measurements, and microbiological analysis. Uncoated samples were also evaluated for comparison purposes.

Fresh-cut pineapple pieces were homogenized into a puree using an Ultra Turrax T18

(IKA® WERKE, Germany). The total soluble solid (TSS) content was determined using an Atago RX-1000 refractometer (Atago Company Ltd, Japan). pH was directly measured using the digital pH meter Digimed DM20 (Digimed, Brazil). For titratable acidity (TA), pineapple puree (5g) was added to 50mL of distilled water and titrated with 0.1N NaOH, using phenolphthalein as indicator. TA was expressed as g of anhydrous citric acid per 100g of fresh fruit. Mass loss was determined by weighing the trays with fruits in a semi-analytical scale Gehaka BG 400 (Gehaka, Brazil) daily until 6 d of storage. The results were reported as percentage of mass loss. All measurements were carried out according to ZENEBON & PASCUET (2004).

Fruit resistance to penetration was evaluated through a compression test in a Universal Mechanical Testing Machine, model 3367 (Instron Corp., Canton, MA, USA) fitted with a 5-mm probe and a 1kN load cell, and set to operate at a speed of $1mm \cdot s^{-1}$, according to BENÍTEZ et al. (2012). The results were given by the maximum average compression (N) of two slices.

Counts of yeasts and molds, psychotropic bacteria, thermotolerant coliforms, and ***Salmonella*** sp. were performed. Twenty-five grams of pineapple slices were aseptically sampled, added to 225mL of 0.1% bacteriological peptone (Difco Laboratories, Detroit, Michigan, USA) solution, and homogenized for 1min in a Stomacher 1240 (ITR Ltd., Esteio, RS, Brazil). Serial 10-fold dilutions were prepared, and the microbiological analyses were performed in accordance to the American Public Health Association (DOWNES & ITO, 2001).

The sensorial acceptances of fresh-cut pineapple slices either uncoated or coated with 0 or 1.5% of mint essential oil were evaluated one day after processing. A quarter of slice of each treatment was randomly presented to 50 untrained judges (potential adult consumers), in an appropriate laboratory. The sensory attribute overall impression was rated using the 9-point hedonic scale to assess liking and disliking, with terms varying from *dislike extremely* to *like extremely*. This project was carefully analyzed and approved by the Scientific Graduate Committee of the Department of Food Technology, Federal University of Viçosa, process n. 50718260690/2011, complying, as outlined, with the requirements for its publication.

The experiment was arranged in a completely randomized design, in a factorial experiment with storage time and coating being the studied factors, and repeated three times. The results were submitted to analysis of variance at 5% of probability level and adequately analyzed by Tukey test or regression, as applied. The statistical analyses were carried out using the Statistical Analysis System (SAS) software, version 9.0.

## RESULTS AND DISCUSSION

The MIC of mint essential oil was $1,024\mu g \cdot mL^{-1}$ for both ***E. coli*** and ***Salmonella*** Enteritidis. This analysis played a key role for the determination of the oil concentrations to be incorporated into the edible coatings. Concentrations higher than the determined MIC were picked up to avoid the emergence of resistant strains, since exposure to sub lethal doses could allow the development of resistance mechanisms of the bacteria, according to the action mechanism of mint essential oil.

These results are not in agreement with those found by MOREIRA et al. (2005), since the authors found a MIC value of $2.0mL \cdot 100mL^{-1}$ for mint essential oil against ***E. coli***. The differences might be attributed to inherent factors of the oil such as pre-harvest factors (e.g. variety, ambient conditions, and ecological factors) and differences in oil extraction methods (ELGAYYAR et al., 2001).

Mint essential oil-containing edible coatings showed *in vitro* antimicrobial efficiency as they either reduced or inhibited the growth of ***E. coli*** and ***Salmonella*** Enteritidis. The inhibition effect was improved by increased mint essential oil concentrations (Figure 1). Coatings containing 0.5% or 1.0% of mint essential oil reduced bacterial growth in 0.2 or 1.0log cycles, respectively, when compared to the essential oil-free edible coating, that did not show antimicrobial effect. The coating incorporated with 1.5% of mint essential oil completely inhibited the growth of the tested microorganisms.

ERNANDES & GARCIA-CRUZ (2007) and SILVA et al. (2009) also reported antimicrobial effect of mint essential oil against Gram-positive and Gram-negative bacteria.

Neither pH nor TA was affected ($P>0.05$) by the treatment or storage time. The mean values for pH and TA were 3.36±0.41 and 0.58±0.10g of citric acid/100g of fresh-cut pineapple, respectively. The average pH of fresh-cut pineapple was reported to be within the range of 3.0 to 3.3 (USDA, 2011), which is similar to the pH found in this research.

MANTILLA et al. (2013) found similar TA values in fresh-cut pineapple treated with sodium-alginate-based edible coatings incorporated with cinnamaldehyde. According to the authors, higher TA values are preferred during storage because they

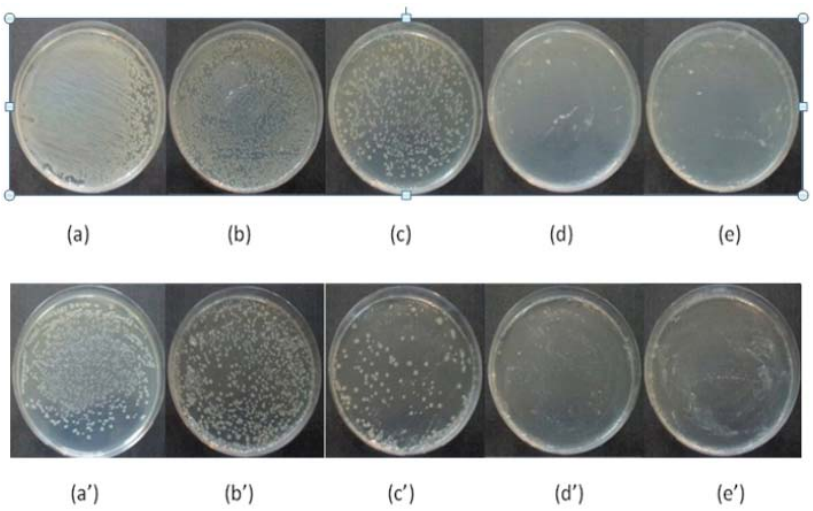


Figure 1 - Antimicrobial effects against ***Escherichia coli*** and ***Salmonella*** Enteritidis, respectively, of: no coating (a and a'); essential oil-free coating (b and b'); coating with 0.5% (c and c'), 1.0% (d and d'), or 1.5% of mint essential oil (e and e').

correlate well with low pH values, thereby preventing the untimely microbial growth in fresh-cut fruits.

Mass losses of the fresh-cut pineapple were not higher than 1.0% after the 6$^{th}$ day of storage. The edible coatings did not affect ($P>0.05$) mass loss, presumably due to the low water permeability of the polypropylene trays used to contain the fruit.

There was no effect ($P>0.05$) of storage time in the TSS content, reported as °Brix, whereas the different coatings did affect ($P<0.05$) this parameter. The TSS content of the uncoated fresh-cut pineapple was smaller ($P<0.05$) than the coated ones, though there was no difference ($P>0.05$) between the mean values found in the coated fruits. This outcome is attributable to the starch from the edible coatings, since gelatinization can break the starch granules, releasing amylose and amylopectin chains and increasing the TSS contents of the coated samples. MANTILLA et al. (2013) tested multilayered antimicrobial edible coatings in fresh-cut pineapple, and found TSS to vary as a function of treatments and storage time, ranging from 10.4 to 13.13°Brix.

Neither coatings nor storage time affected ($P>0.05$) the maximum load compression of the fresh-cut pineapples. The mean value was 25.7±5.9N. Pineapple softening was not observed during the storage in other researches (PRADO et al., 2003; SARZI & DURIGAN, 2002). This behavior has been attributed to enzymatic modifications of cellular wall structure and composition (CHITARRA, 1999).

The antimicrobial properties of the edible coatings were evaluated by comparing the counts of yeasts and molds, psychrotrophic bacteria, thermotolerant coliforms, and ***Salmonella*** sp. in uncoated and control-coated fresh-cut pineapple with those treated with mint essential oil, throughout storage.

The counts of yeast and molds were affected ($P<0.05$) by the different coatings. Mint essential oil-containing coatings were able to inhibit ($P<0.05$) the growth of yeast and molds in fresh-cut pineapple, when compared to uncoated and control-coated fruits (Figure 2A). Fruits treated with coatings containing 1.0% or 1.5% of mint essential oil had substantially lower counts of yeasts and molds by the end of storage (with reductions of 1.11 and 1.23log cycles, respectively) in comparison with the uncoated samples. The National Health Surveillance Agency does not specify the microbiological standards for yeast and molds in fresh-cut fruits. However, it is a limiting factor to the shelf-life of such products because therey are spoilage microorganisms whose visual observation affects the sensorial characteristic and may lead to consumer repelling.

The counts of psychrotrophic bacteria increased ($P<0.05$) in all treatments with storage time (Figure 2B). This behavior was expected due to the refrigeration temperature used for storing the samples (ZAGORY, 1999). All samples coated with mint essential oil-containing coatings had lower counts of psychrotrophic bacteria by the end of storage, and fruits treated with the highest concentration had the lowest count. However, the reduction was lower than 1 log cycle in comparison with the uncoated and control-coated fresh-cut pineapples.

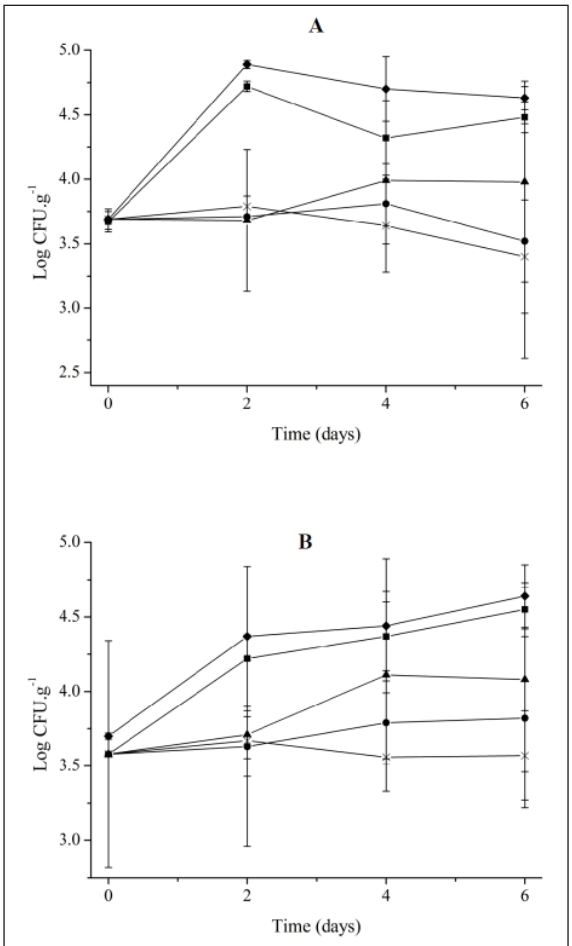


Figure 2 - Counts of yeast and molds (A) and psychrotrophic bacteria (B) in fresh-cut pineapple throughout storage: ♦ uncoated; ■ control coating; ▲coating with 0.5%, ● 1.0%, or × 1.5% of mint essential oil.

MANTILLA et al. (2013) found better antimicrobial effects of sodium alginate-based coatings incorporated with cinnamaldehyde in fresh-cut pineapple compared to these results. The authors evaluated the shelf-life of fresh-cut pineapple during 15 days, and obtained reductions of 2.7log cycles for psychrotrophic bacteria and 3.0log cycles for yeast and molds. The antimicrobial agent tested by the authors appears to be more efficient than mint essential oil.

Counts of thermotolerant coliforms were not higher than 3.0MPN·g$^{-1}$ for all treatments, whereas no ***Salmonella*** sp. was detected during the 6 days of storage. These results meet the microbiological standard for fresh-cut fruits (BRASIL, 2001; DOWNES AND ITO, 2001) so that the use of the developed antimicrobial edible coatings ensures food safety.

The acceptances of fresh-cut pineapples were different ($P<0.05$) among the studied treatments. Uncoated pineapples had the same acceptance ($P>0.05$) as control-coated samples, indicating that the coating did not change the appearance of the fresh-cut fruit. Pineapple samples treated with the antimicrobial coatings had the lowest score, since mint essential oil provided a strong flavor to the product. The score means of sensory acceptance were 7.38±1.55, 7.27± 1.33, and 5.22±2.10 for uncoated, control-coated samples and samples coated with 1.5% of mint essential oil, respectively. Therefore, further studies are needed to improve the acceptance of mint essential oil-treated products, oil which provides a good antimicrobial activity and contributes for the microbiological quality and safety of fresh-cut pineapple.

## CONCLUSION

The evaluation of MIC and *in vitro* antimicrobial effect of mint essential oil allowed the

determination of its concentration to be applied. Also, both techniques corroborated the potential use of the edible coating as a carrier for releasing antimicrobial compounds to a food matrix.

The starch-based antimicrobial coatings did not affect texture and physicochemical characteristics of fresh-cut pineapple. The coatings were efficient against psychrotrophic bacteria and yeast and molds, ensuring the agreement of the treated pineapples with the microbiological standard for fresh-cut fruits.

The sensorial acceptance should be improved by further studies regarding both different concentrations of the antimicrobial compound and other essential oils, since 1.5% of mint essential oil led to the perception of a strong flavor in the treated pineapple by the potential consumers.

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