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Proceeding Paper

Evaluation of the Use of Propolis and Sodium Hypochlorite as Methods to Control the Contamination of Free-Range Eggs †

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Abstract: There is a high demand for free-range eggs, although these systems may offer a greater microbiological challenge. Therefore, the aim with this study was to evaluate the effectiveness of 30% propolis extract and 1% sodium hypochlorite in reducing the microbiological contamination of free-range eggs. Eighteen eggs were divided into three groups—treated with propolis, hypochlorite and a control—and tested for mesophilic bacterial count. For the eggshells treated by propolis, the average count was 0.6 log CFU·mL^{-1}, for hypochlorite it was 1.3 log CFU·mL^{-1} and for the control it was 3.26 log CFU·mL^{-1}. It can be concluded that both methods were effective, with propolis being more efficient.

Keywords: shell; mesophiles; layers; microbiology

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1. Introduction

In recent years, free-range egg production has increased due to growing consumer interest in animal welfare [1]. However, this production method introduces new health challenges due to free-range housing, which can increase microbiological risk [2]. Eggshell contamination occurs both in the hen's reproductive organs and after laying. In the reproductive organs, microorganisms such as *Salmonella* spp., *Escherichia coli* and mycoplasmas can infect the ovary [3,4]. In this context, factors such as rearing conditions, the length of time the eggs are stored and the age of the hen have a significant influence on the microbiological quality of the eggs [3].

Farms that do not adopt adequate hygiene measures contribute to the rapid contamination of eggs by various microorganisms. The incomplete formation of the cuticle layer can leave open pores in the shell, allowing microorganisms to penetrate into the egg [3]. Therefore, it is essential to implement appropriate egg sanitization practices to avoid contamination by harmful microorganisms [5,6]. In this sense, propolis extract is a natural beekeeping bioproduct that has aroused the interest of researchers due to its antimicrobial, antifungal and antioxidant properties [3,7,8], and it can be used in the food industry for various purposes, such as controlling pathogenic microorganisms and extending the shelf life of products [3,8]. On the other hand, sodium hypochlorite also has antimicrobial properties [9] and is a more traditional option for controlling contamination in food production systems.

The traditional method (the utilization of chlorine) presents numerous drawbacks, including the generation of carcinogenic substances and a decrease in efficacy due to the presence of organic matter [10]. Consequently, there has been a significant emphasis on

identifying natural antimicrobial agents for food product disinfection. Various natural antimicrobials, such as probiotics, prebiotics and essential oils, have been investigated for their potential to reduce microbial contamination on eggshells [11].

Aligned with that, the aim of this study was to evaluate the effectiveness of 30% propolis extract and 1% sodium hypochlorite as methods for controlling egg contamination on a free-range farm located in southern Brazil.

2. Materials and Methods

The eggs were collected from a cage-free farm in southern Brazil and analyzed at the Quality Control and Food Safety Laboratory (LACQSA) of the Federal University of Paraná (UFPR). The farm has 120 laying hens of the NOVOgen Brown and NOVOgen Tinted breeds, which, due to the avian flu health emergency in Brazil, are kept in confinement, without access to free grazing. Even so, each confined bird has 1 m^2 of space (Figure 1).



Figure 1. Cage-free flock of the NOVOgen Brown and NOVOgen Tinted in southern Brazil, where the eggs were collected for the analysis.

The eggs produced on the farm are deposited in nests with slanted mesh, allowing them to be collected in an area outside the barn. After collection, the eggs undergo an ovoscopy procedure for quality control and are washed with treated water supplied by the public water supply system, in accordance with the rules of the Brazilian regulatory body.

For the study, 18 eggs were randomly collected after candling and washing. These samples were divided into three groups: six eggs were sprayed with 30% propolis extract (Figure 2); six were sprayed with 1% sodium hypochlorite; and six eggs were used as controls, without spraying. The microbiological analysis was carried out on the eggshells. To assess the microbial load on the shell, six eggs from each group were rinsed with a 0.1% buffered peptone water solution (Kasvi, Pinhais, Brazil). Mesophilic aerobes were counted using the standard plate count agar method (Kasvi, Pinhais, Brazil) and colonies were counted after 48 h of incubation (36 °C) [6].

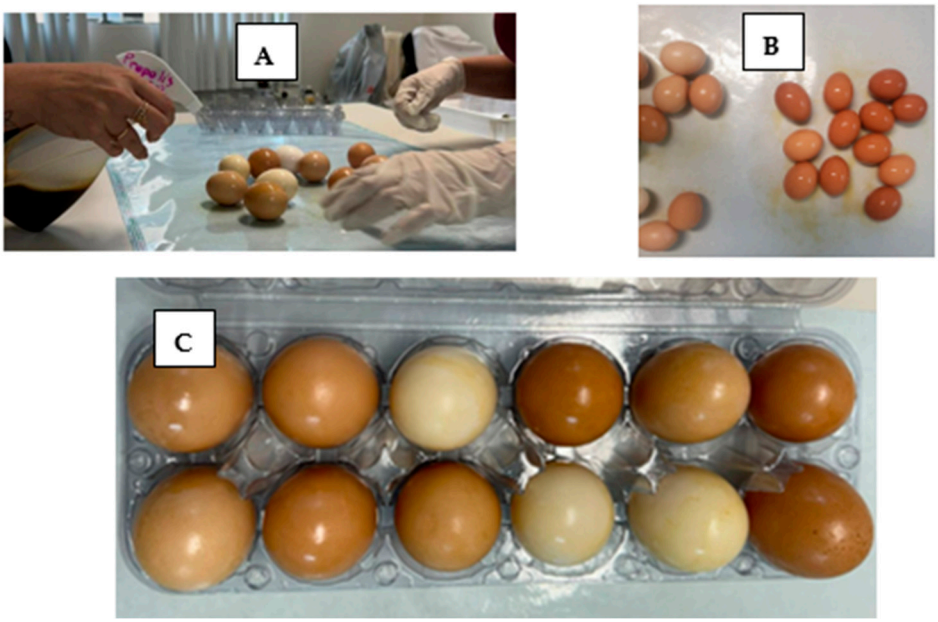


Figure 2. (**A**) Propolis sprayed on the eggs; (**B**) time allowed for the eggs to dry; (**C**) the appearance of the eggs after spaying propolis.

3. Results

The appearance of the eggs was brilliant after spraying propolis, but remained the same after spraying sodium hypochlorite.

After spraying the eggs, they were stored in a domestic refrigerator for 24 h and analyzed for microbial load.

In the eggshell, the results obtained for the count of mesophilic aerobic microorganisms were: propolis group 0.6 log CFU·mL^{-1}, sodium hypochlorite group 1.3 log CFU·mL^{-1} and control group 3.26 log CFU·mL^{-1} (Figure 3).

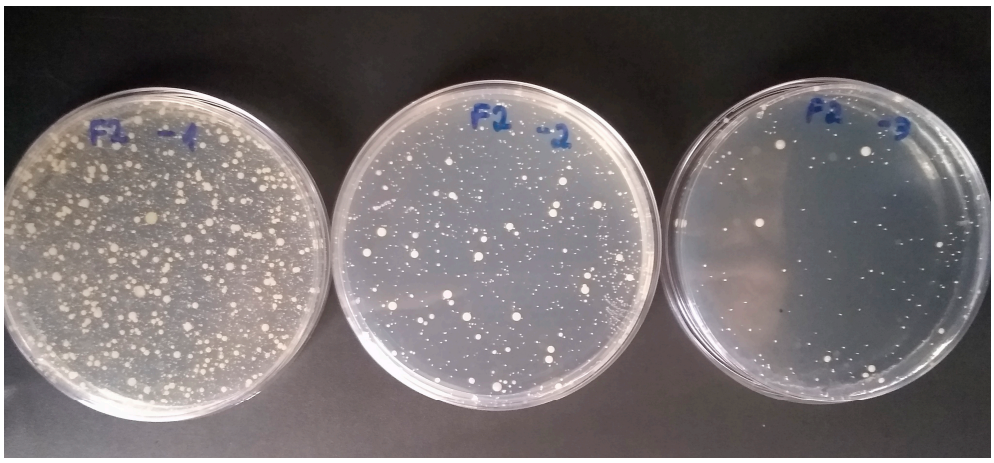


Figure 3. Plate count agar showing the dilution of the samples for counting. Each white point is considered a bacterial colony after 48 h of incubation at 36 °C.

4. Discussion

In this study, the 30% propolis extract proved to be more effective than 1% sodium hypochlorite at reducing the microorganisms present in the eggshells. The propolis extract resulted in an 81.60% decrease in the total mesophile count, reducing the concentration from 3.26 log CFU·mL^{-1} to 0.6 log CFU·mL^{-1}. In comparison, 1% sodium hypochlorite

led to a reduction of 60.12%, reducing the concentration from 3.26 log CFU·mL^{-1} to 1.3 log CFU·mL^{-1}.

The antimicrobial potential of propolis extract is mainly attributed to the presence of phenolic compounds and flavonoids, which have the ability to damage the cell structure of these microorganisms, preventing them from multiplying or even leading to their elimination [3]. In addition, propolis extract can extend the storage life of eggs [3,12]. A study conducted by Alkan et al. [3] showed that treating eggshells with 30% propolis extract was effective in preserving the internal quality of eggs for up to 21 days at 25 °C, with a lower level of microbiological contamination compared to eggs that had not been washed or sanitized with chlorine or peracetic acid. This effect is due to the waxy and resinous composition of propolis, which forms a protective barrier around the egg, reducing gas exchange and moisture loss through the pores of the shell [3,4,12].

Similar results were found in the study by Gomes et al. [12] who used green propolis in their experiment. The study showed that eggs stored at room temperature, when treated with a green propolis coating, had better qualities, including lower weight loss, a higher yolk index and better physicochemical characteristics. The author concluded that refrigerated storage is the most suitable way to preserve egg quality. However, at room temperature, coating eggs with green propolis contributes significantly to maintaining egg quality for up to 28 days.

Another relevant factor to mention in relation to propolis is its concentration. In this study, a concentration of 30% proved to be effective at reducing the microorganisms present on eggshells, outperforming sodium hypochlorite. On the other hand, in a study carried out by Alves et al. [4], which used propolis spray at a concentration of 15%, it was observed that although propolis reduced the initial microbial growth on the shell, this effect was not sustained until the end of the storage period. Thus, the 15% concentration proved to be less effective in preserving the microbiological quality of the shell over 28 days of storage.

In addition, the formation of a physical barrier by propolis extract can be beneficial for embryonated eggs by helping to protect against microbiological contamination and maintaining suitable conditions for embryo development. A study conducted by Genc et al. [13] on Japanese quail eggs showed that treatment with propolis extract significantly reduced the microbial load on the shell, with no negative effects on hatchability, maintaining safety and quality during storage. Compared to formalin and distilled water, propolis showed better control of mesophilic aerobic bacteria, making it an effective, non-toxic and sustainable alternative for disinfection in hatcheries. Another experiment evaluated the spraying of fertile eggs with propolis, analyzing hatching characteristics and bacterial counts on the shell. The results showed the potential of propolis extract, especially at a concentration of 14%, as an efficient disinfectant for hatching eggs, promoting chick quality and reducing losses during incubation [7].

In this sense, propolis extract can be an interesting alternative to chemical sanitizers, mainly because it is effective in reducing microbiological contamination of eggshells. As well as making food safer, propolis extract attracts attention because it is a natural product [8], a characteristic that is increasingly valued by consumers, adding value to the product on the market. However, the higher cost of propolis can limit its use, especially among small farmers. On the other hand, sodium hypochlorite, although less efficient, has shown satisfactory results in microbiological reduction and is more affordable, making it a viable alternative for these producers [9].

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