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Chapter

Natural Products as an Alternative to Formaldehyde for Disinfection of Fertile Eggs in Commercial Hatcheries

Omar Francisco Prado Rebolledo, Arturo César García Casillas, Isaías Guillermo Téllez and Juan Augusto Hernández Rivera

Abstract

Formaldehyde has been used in commercial hatcheries to cleanse eggs and prevent illness. However, formaldehyde's health risks and customer demand for eco-friendly products have spurred interest in natural egg disinfection. Formaldehyde-free natural materials sterilize viable eggs in commercial hatcheries. Formaldehyde's health and environmental dangers start the chapter. Modern hatcheries need safer and greener options. Natural egg disinfectants are next: plant-based extracts, oils, and acids. These natural chemicals' mechanisms, bactericidal properties, potential commercial hatchery pros, and cons are evaluated. The chapter also examines commercial hatcheries' natural disinfectant limits. Cost-effectiveness, efficacy against common diseases, application simplicity, and hatchery equipment compatibility are discussed. Regulations and uniform egg disinfection using natural agents are covered in the chapter. It emphasizes industry stakeholders, researchers, and regulators working together to promote natural alternatives. Finally, formaldehyde-free natural substances can disinfect viable eggs in industrial hatcheries. Studying natural product-based disinfection methods will increase their efficacy, safety, and feasibility. This book chapter concludes with natural alternatives to formaldehyde for cleaning viable eggs in industrial hatcheries.

Keywords: natural disinfectants, eggs, microbial contamination, hatcheries, formaldehyde

1. Introduction

Population growth varied social conditions, and economic differences in the world have an impact on food supply. Between 1960 and 2020, the world population increased from 3.0 to 7.8 billion, equivalent to 157%. Therefore, it is estimated that between 2020 and 2050, there will be a further increase of 2 billion inhabitants, so the impact on food security will represent a significant challenge. So much so that its

importance is already considered within the 2nd Sustainable Development Goal of the United Nations' (UN) “zero hunger” concept [1]. For its part, the pandemic of Severe Acute Respiratory Syndrome Coronavirus 2, or SARS-CoV2 [2], confirmed the close connection between humans and animals; however, the phylogeny of the virus is still under investigation since the factors involved in its dispersal have not yet been fully resolved. Therefore, this example highlights the importance of the “One Health” concept as a unified and integrated approach that seeks to balance and sustainably optimize the health of people, animals, and ecosystems [3].

Given the global outlook on the deficit of food availability, table eggs represent a source of easily accessible, inexpensive, self-packed protein, which provides a source of highly digestible protein with a homogeneous balance of amino acids; thus, it is considered a food guarantee, since it has no religious barriers in its consumption, and has low production cost due to the high feed efficiency of the hens. Table egg production has increased significantly in recent years, with China contributing 1136.4 million cases of eggs, India 270.2, the United States of America (USA) 263.6, Brazil 146, and Mexico with 132.9 million cases (**Figure 1**), representing the countries with the highest production. It should be noted that each carton of eggs contains 360 units, equivalent to 30 dozen eggs. In 2018, world production was 76.7 million t; therefore, if this value is divided by the 7.6 billion people in the world, the result is a consumption of 161 eggs/person/year. The main consuming countries are Mexico with 23.7 kg *per capita*/year, Japan with 21.3 kg *per capita*/year, and Colombia with 20.3 kg *per capita*/year. Another significant fact is that consumption does not depend on large demographics, as China has a consumption of 255 eggs/person/year, India 76 eggs/person/year and the European Union (EU) with 210 eggs/person/year (**Figure 2**) [4].

After World War II, livestock production systems evolved. Before the war, production was done in the backyard for self-consumption; in the post-war period, agriculture faced a crisis, due to the low number of workers in the primary sector. In response, from the 1980s to 1990s, egg production via cage production systems increased. In that same decade of the 1990s, consumers requested that Livestock Production Units implement the concept of “Animal Welfare”, which is why the poultry industry producing table eggs implemented other production systems, which attempted to satisfy the five

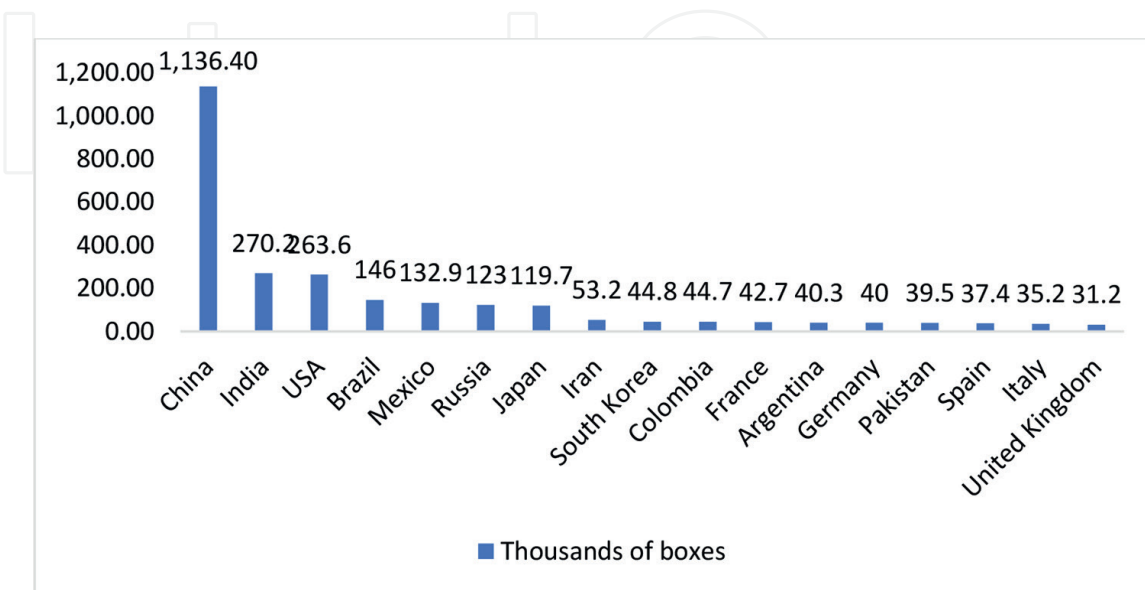


Figure 1.
Main table egg producing countries.

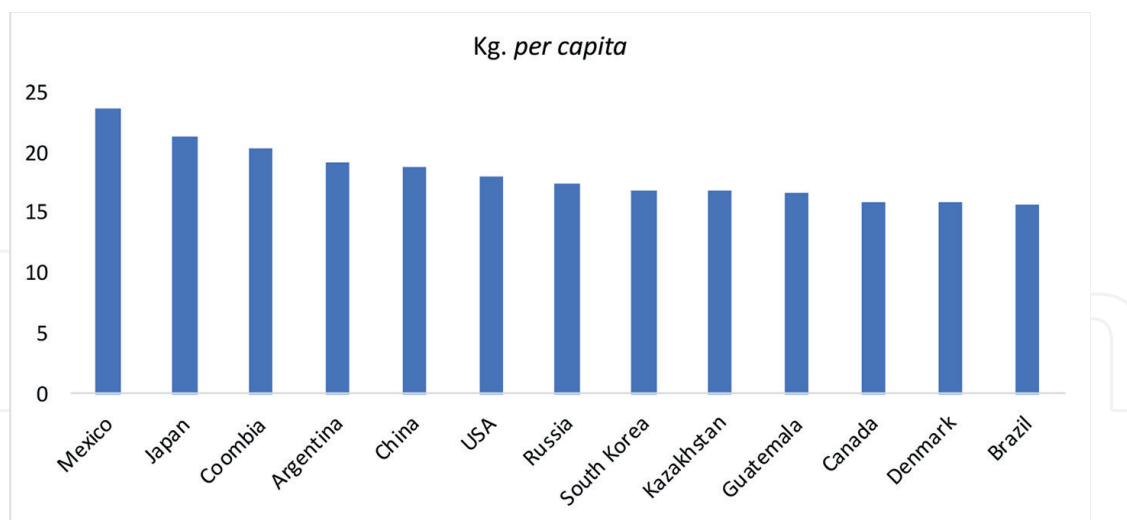


Figure 2.
Main fresh egg consuming countries.

freedoms: (i) absence of hunger, (ii) absence of thirst, (iii) possibility of movement, (iv) absence of fear, and (v) expression of natural animal behavior [5].

2. Natural egg defenses

Eggshells are the primary packaging and constitute the 1st defense barrier in containing microorganisms; the priority of maintaining their integrity and quality is of great importance for producers [6, 7]. The main component of the hull is calcium carbonate in the form of calcite (94%). Apart from CaCO_3 , there are other inorganic components in the shell: magnesium carbonate (<1%), calcium phosphate (<1%), and silicon oxide (<1%). The approximately 4% of remaining compounds are polysaccharides, various collagens, fatty acids, and water [8]. These components make the eggshell have a unique microstructure, where the CaCO_3 skeleton is characterized by a porous and rough structure with three levels of primary particles with approximately 10 nm. Calcite crystals are arranged in palisades and mammillary layers with different morphology and porosity, in addition to an absence of cell-directed assembly during calcification, compared to bone [9]. The mass of the eggshell is proportional to the egg mass and represents between 10 and 11% of the egg weight. In the eggshell, the cysteine-rich protein membrane, the mineralized layer, and the non-mineralized outer cuticle are deposited as the egg descends through the oviduct of the hens [6]. The eggshell membranes are synthesized during a period of 1.0–2.0 h, when the immature eggs travel through the proximal isthmus. Mineralized multilayers are formed in the distal isthmus and the shell gland over a period of 19–20 h. Finally, the cuticle is deposited on the eggshell in the uterus 1.5–2 h before oviposition [10]; and, at this time, the outer part of the eggshell is exposed to many contaminants that can harbor a wide range of microorganisms [11].

The cuticle covers the pores on the eggshell surface, thus forming a physical barrier against bacteria [12]; the chemical composition of the eggshell plays an important role by limiting bacterial contamination. Some antibacterial proteins (e.g., c-type lysozyme, ovotransferrin, and ovocalyxin-32) have been detected in eggshell; the open pores on the eggshell surface not only serve for gas and water exchange but are also

the route of invasion [13]. Consequently, eggshell thickness is an important factor for the ingress of bacterial contamination [14, 15]. In this regard, it has been shown that good shell cuticle quality can significantly reduce the opportunity for pathogen invasion and that the amount of cuticle as a hereditary trait can be an effective strategy to reduce the transmission of microorganisms in production poultry [6].

In order to reduce Enterobacteriaceae counts on the eggshell, in some countries, such as USA, Australia, Japan, and Sweden, eggs are washed with chemicals (e.g., sodium carbonate and sodium hypochlorite) [16]. This practice may damage or partially remove the cuticle, thus increasing the risk of bacterial ingress. Class A eggs should not be washed, due to potential damage to physical barriers, such as the cuticle. Good cuticle quality is of vital importance, as the safety of table eggs depends, to a large extent, on it. The cuticle and its degree of coverage are affected by many factors, such as the age of the hen, genetic background, rearing system, and egg storage conditions [17, 18].

Eggs can be contaminated at different stages from the production stage, through processing, cooking, and consumption. Transovarial or “vertical” transmission of microorganisms occurs when eggs are infected during their formation in the hen’s ovary. Horizontal transmission occurs when eggs are exposed to an environmental contaminant and microorganisms penetrate through the eggshell [13, 19].

In the past decade, Non-typhoidal *Salmonella* (NTS) caused an estimated 1.028 million cases, >19,000 hospitalizations, and 378 deaths in the USA, at a cost of \$3.3 billion [20]. Although NTS is frequently isolated in different foods of animal origin, poultry is considered an important reservoir, and contaminated poultry products are also a significant vehicle for human infection. There are >2400 recognized serotypes of NTS. However, not all are isolated from poultry; for example, *Salmonella enteritidis*, *Salmonella typhimurium*, and *Salmonella heidelberg* are historically associated with poultry. However, *Salmonella kentucky* has positioned itself as the predominant serotype associated with U.S. poultry. This change in the population dynamics of *Salmonella* in U.S. poultry has a far-reaching implication for food safety [21]. The increase in multi-drug resistance (MOR) in *Salmonella* serotypes of both animal and human origin, and, in particular, resistance to important clinical antimicrobials, is an emerging concern worldwide [22].

3. Disinfectants based on natural products

Egg disinfection is a process that seeks to minimize the risk of contamination by microorganisms that can compromise both human health and egg quality, as well as the entire production chain of the poultry industry [23]. The disinfection process must ensure a good application of the disinfectant compound on the eggshell, which must be broad spectrum with the lowest toxicity rate. The mechanism of action must also be fast to avoid the dispersion of pathogenic microorganisms without generating high costs in the productive processes [24]. From the fundamental manufacture to the point of consumption, eggs and their markets must be subjected to control procedures aimed at achieving the appropriate level of defense for public health. An important aspect to consider is the marketing chain where egg collection, handling, storage, and transport must be supervised, either manually or automatically, with time and temperature also being taken into account [25, 26].

Studies have been conducted to determine the penetration of *Salmonella enteritidis* in various types of production systems, where *Salmonella* remains an

important transmission pathogen [19]. Therefore, many poultry companies are looking for new alternatives to the use of conventional disinfectants to protect fertile and table eggs from bacterial contamination [16]. In the case of fertile eggs, many hatcheries in different parts of the world have used formaldehyde as part of their disinfection routines; however, this element has genotoxic and cytotoxic properties, which can affect humans and chicken embryos, consequently causing irreversible effects from its inhalation. These effects depend mainly on the dose, exposure time, application method, and egg exposure period [27]. The problem with the use of formaldehyde lies in its concentration as a disinfectant, where at least 600 mg/m³ (489 ppm) is required, which represents a high exposure dose for workers [28], thus presenting the main reason to avoid its use in hatchery disinfection routines [16].

4. Vegetable extracts

Since the origin of civilization, plants have played an essential role in the development and well-being of civilization through their varied uses (e.g., food preservatives, flavorings, and dietary supplements to maintain human health) [29]. Plant extracts have been employed as safe and efficient remedies for ailments and diseases in traditional medicine. The active constituents of many plant extracts have been characterized and are publicly available, although there is little information on their antimicrobial actions [30]. The adoption of natural antimicrobial elements as egg disinfectants opens the door to their use as a safer alternative because they are biodegradable and non-toxic, compared to chemicals that are toxic, non-degradable, and corrosive. There are several methods used for oil extraction, such as the use of liquid CO₂ or microwaves, as well as low pressure distillation with boiling water or hot heat [31]. Among the most significant molecules are phenolic compounds: trans-cinnamaldehyde (an aldehyde found in cinnamon bark extract (*Cinnamomum zeylandicum*)), carvacrol extracted from oregano oil (*Origanum glandulosum*), eugenol (active ingredient of clove (*Eugenia caryophyllis*)), etc. These compounds showed rapid effectiveness in reducing *Salmonella enteritidis* compared to water-washed or chlorine-challenged eggs.

Yamawaki et al. [32] used phytochemicals products of secondary metabolites produced by plants with defensive properties against predators (e.g., caproic acid, caprylic acid, linalool, and pectin-based cuminaldehyde) to reduce *Salmonella heidelberg* on eggshells at a concentration of 1.0% alone or combined at 0.5% v/v with different storage times (0, 1, 3, 5, 7, 7, 14, and 21d) at 4°C. At the end of storage (21d), the lowest *Salmonella* counts were for caproic acid and caprylic acid at 1% pectin combination (2%) from 0d to 14d, and at the end of storage compared to untreated controls [16].

Capsicum essential oil, known as allspice oil, is obtained from the leaves of *Pimenta officinalis* Lindl. The main component is antimicrobial, and its application has proved effective against *Staphylococcus epidermidis*, *Proteus hauseri*, *Micrococcus yunnanensis*, and *Corynebacterium xerosi*. *In vitro*, it acts against *Listeria monocytogenes* and *Salmonella heidelberg* in turkey skin stored over short periods at 4 and 10°C, at a concentration of 0.5 or 1.0% [16, 33]. The compound extracted from clove oil (*Eugenia caryophyllis*), called eugenol, as well as trans-cinnamaldehyde, an aromatic aldehyde extracted from cinnamon bark (*Cinnamomum zeylandicum*), have shown antimicrobial effects on *Salmonella enteritidis* PT8 by interfering with several

genes associated with virulence, colonization, membrane composition, and transport ecosystems.

Ginger, garlic, oregano, and cinnamon extracts, applied in 5% aqueous solutions, showed no differences in fertility, hatchability, embryonic mortality, body weight, or viability of the chicks during 14d of brooding. Regarding the incubation variables, ginger extract was the only one effective in preventing the growth of bacterial colonies [16]. On the other hand, when comparing oregano juice at a concentration of 50% diluted in distilled water at room temperature against fumigation with 100% formaldehyde in white Akbay breeders of 48 weeks of age, no differences were observed between disinfection groups on egg characteristics, eggshell microbial load, hatchability, embryonic death, body weight, weight gain, or feed conversion rate. However, weight loss was lower in formaldehyde fumigation versus oregano juice [34].

In terms of bacterial structure and susceptibility, Positive Gram have a peptidoglycan cell wall bound to other molecules, such as proteins or teichoic acid [35], and Negative Gram have lipopolysaccharide (LPS), which forms a barrier to the hydrophobic compounds that essential oils have in their outer membrane [36]. Therefore, Negative Gram are less susceptible to the effects of essential oils than Positive Gram [37]. However, it is important to note that the hydrophobic structure of essential oils can reach the periplasm of Negative Gram through outer membrane proteins (porins), where it travels slowly, followed by leakage of potassium into the extracellular space and loss of ATP [37–41].

The use of essential oils as preservatives may be limited by changes in the organoleptic characteristics of foods. However, in the disinfection of fertile eggs, their safety has been recognized, and their use is gaining more and more practitioners every day. Therefore, it is important to carry out studies on the minimum inhibitory concentration of essential oils to allow a balance between sensory characteristics and antimicrobial efficacy.

5. Propolis

Propolis is a sticky, gummy, resinous substance harvested by worker bees (*Apis Melifera*) from the buds of certain trees and shrubs. The bees use it to seal parts of the hive. At least 200 compounds have been found in different samples of propolis (e.g., esters, fatty acids, flavonoids, terpenes, β -steroids, aldehydes, aromatic alcohols, sesquiterpenes, naphthalene derivatives, and stilbenes) [42, 43]. For centuries, propolis has been used as a medicinal agent to treat infections and promote wound healing [44]. Due to its broad antimicrobial effect, it has been used as an alternative preservative agent and as a protection for various agricultural products during their storage period [45, 46]. Propolis was used to reduce microbial activity in quail eggs stored for 7 and 14d, but it reduced hatchability and increased embryo mortality between 1 and 9d of incubation.

Oliveira et al. [27] conducted an experiment to evaluate the effectiveness of an alcoholic extract of propolis (15%) as a disinfectant for hatching eggs of Japanese quail (*Coturnix coturnix Japonica*). A low eggshell conductance in the control group (egg weight loss) and a decrease in the microbial load were obtained. Likewise, no differences in hatchability and embryo mortality were observed. Therefore, alcoholic extract of propolis (15%) can be used as a safe disinfectant in fertile quail eggs [16, 47].

6. Probiotics

Metchnikoff recommended, since the early twentieth century, the intake of beneficial microbes for health, particularly in the treatment of pathologies of the gastrointestinal tract. In 2001, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) officially defined probiotics as those live microorganisms that can confer a health benefit to the host, when consumed in adequate amounts [48]. Probiotics have had a considerable increase as an alternative over antibiotics used as growth promoters and pathogen control. This phenomenon has motivated the development of effective probiotic products for use in animal production [49, 50]. Prado et al. [50, 51] conducted an experiment where they evaluated an aerosolized probiotic formulation as a bactericidal method during incubation, compared against formaldehyde fumigation, where the results showed that the number of recovered non-selective aerobic bacteria and lactic acid bacteria increased in the incubation environment, thus suggesting the application of lactic acid bacteria in setters and hatchers. Likewise, lactic acid bacteria (*Lactobacillus acidophilus* and *Bacillus animalis*) administered *in ovo*, with the use of a commercial automated multiple egg injection system, have been used without affecting the hatch of fertile eggs. Although it recommends against administering *Bacillus animalis* at high concentrations of 105 and 106 CFU/mL, because they increase the number of chicks that bite and die, as well as contaminated eggs, the *Bacillus subtilis* strain is not recommended because it affects all stages of embryonic development, due to competition for nutrients or secretion of byproducts, such as bacteriocins, enzymes, and 2,3 butanediol, which is toxic to biological systems and damages the defense system and central nervous system [32, 52].

7. Chitosan

Chitosan is a modified natural carbohydrate polymer derived from the deacetylation of chitin; it is insoluble in water but soluble in weak, organic acid solutions [53]; it is part of the exoskeleton of crustaceans, cuticle of insects, algae, and fungal cell walls [54]; it has physical and chemical properties, including antibacterial activity; and it has a high degree of biocompatibility [55]. Chitosan is primarily used as a reinforcement in vegetation development, due to its anti-fungal properties. Chitosan is a biomaterial that can be used as a biofilm with a selective permeability effect for O₂ and CO₂ with good properties to effectively control pathogenic microbial growth. Its antimicrobial activity is dose-dependent, and it exhibits simultaneous cell membrane permeability to small components [56–58].

Prado et al. [51] developed a chitosan biofilm to preserve table and fertile eggs; chitosan concentrations were 0.1, 5, and 10%; table and fertile eggs were impregnated with chitosan and subsequently challenged with *Salmonella enteritidis*, then stored for 1, 24, 96, and 168 h at 4°C. The lowest concentration of *Salmonella enteritidis* was for the 5 and 10% concentrations in the table egg. For the fertile egg, incubation variables showed no differences for the different concentrations of chitosan [51].

From the most recent studies, chitosan has been used in combination with essential oils across a wide application in the food industry, although for applications in table and fertile eggs, there are no reports of its effectiveness [59]. Another combination has been with slightly acidic, electrolyzed water, as a protective alternative against bacteria present in the eggshell. However, this process damages the cuticle, so

after disinfection with slightly acidic, electrolyzed water, a chitosan-based coating was used to form a new, artificial cuticle to prevent loss of humidity and CO₂ from the damaged cuticle, which had positive effects on eggs stored at 25°C for 42d, without loss of internal egg quality [14, 26].

8. Organic acids

Organic acids, being natural products, have emerged as viable alternatives to formaldehyde for disinfection of fertile eggs in commercial hatcheries. These acids exist in a non-dissociated form and exhibit a measure of their dissociation through the Ka (acid dissociation constant) value. Organic acids are commonly found in nature and can be derived from various sources such as fruits, vegetables, and fermentation processes. Examples of organic acids include acetic acid (found in vinegar), citric acid (found in citrus fruits), lactic acid (found in dairy products), and formic acid (found in ants).

In their non-dissociated form, organic acids remain intact, allowing them to effectively penetrate the eggshell and target potential pathogens without harming the developing embryo inside. This characteristic makes them suitable for disinfecting fertile eggs in commercial hatcheries, where maintaining a sterile environment is crucial for successful incubation. The Ka value, also known as the acid dissociation constant, measures the extent to which an organic acid dissociates into its constituent ions in an aqueous solution. It provides an indication of the acid's strength and its ability to release hydrogen ions (H⁺) when in contact with water. The higher the Ka value, the greater the extent of dissociation and the stronger the acid.

By considering the Ka value of organic acids, hatchery operators can select appropriate disinfectants that effectively combat pathogens while minimizing any potential adverse effects on the developing embryos. The choice of organic acid for disinfection can be based on factors such as its antimicrobial efficacy, safety, and compatibility with the hatchery environment. Overall, organic acids offer a natural and sustainable alternative to formaldehyde for disinfection of fertile eggs in commercial hatcheries. Their non-dissociated form allows for effective penetration of the eggshell, while the Ka value helps determine the acid's dissociation extent and strength, aiding in the selection of appropriate disinfectants for optimal hatchery operations.

Acetic, ascorbic, citric, formic, lactic, propionic, and peracetic organic acids are regularly used in food disinfection processes at concentrations of 0.05–2.5%, with no toxic residues [60]. Some organic acids, such as lactic, acetic, citric, and peracetic acids, are weak acids in solution, since one part of their molecule is dissociated [H⁺] [A⁻] and the other is not [A]. The ratio between the dissociated and non-dissociated part is expressed by the dissociation constant pKa. By determining the acid concentration, pH and pKa, the concentration of the non-dissociated acid present in the solution is established [61].

Lactic acid or its ionized form, lactate, known by the official nomenclature 2-hydroxypropanoic acid, is a carboxylic acid, with a hydroxyl group on the carbon adjacent to the carboxyl group. There are two optical isomers: D (–) lactic and L (+) lactic, as well as a racemic form consisting of equimolar fractions of the L (+) and D (–) forms. Unlike the D (–) isomer, the L (+) configuration is metabolized by the human organism [62]. It is a slightly brown liquid; it is the natural component of meat produced by post-mortem glycolysis; and it is used in carcass washing with doses of 2.5–5.0% at temperatures not exceeding 55°C with application before or after the carcass cooling stage [63].

Acetic or ethanoic acid of natural origin is present in most fruits. It is produced by bacterial fermentation, is present in all fermented products, and its commercial form (vinegar) has been used as a disinfectant since the beginning of civilization. Doses used range from 1.5 to 14.4% or 52°C in spray for 10s. Negative Gram bacteria are more susceptible to acids than Positive Gram bacteria [64].

Citric acid is the main organic acid in fruits, such as lemons, which contain between 7 and 9% citric acid on a dry weight basis. The three carboxylate groups of citric acid mono-hydrate have different pKa values ranging from 3.15, 4.78, and 6. At doses of 2–5%, it reduces the count of pathogenic bacteria [65]. The antimicrobial action is due to the dissociated form; being an anion, it is highly polar, so it does not cross the plasma membrane of microorganisms easily, but its non-dissociated form does cross the membrane [66]. The references found on the use of organic acids as antimicrobials only refer to their use in carcasses and parts of raw poultry, where they measured the effectiveness in reducing the native flora or inoculated bacteria that were mostly *Salmonella* or *Campylobacter*; in the case of the use of organic acids in the disinfection of the eggshell, they can demineralize the eggshell and eliminate the cuticle [67], which is why it is important to conduct experiments that consider the form of preparation, concentration, and measurement of cuticle integrity and calcium carbonate levels.

9. β -Glucans

Components of the cell wall of the yeast *Saccharomyces cerevisiae* have drawn interest in recent years, since their inclusion has had a positive impact on production parameters, due to their physiological effects on the intestinal digestive mucosa, by increasing the height of the jejunal villi [68]. The β -glucans are carbohydrates made of glucose polymers which provide the primary structure that is located in the wall of yeasts, fungi, algae, and cereal grains, such as oats and barley. Their structure can vary depending on the source and type of bonds present in the glucose polymers [15]. The backbone of β -glucans is formed via glucose molecules linked at carbon atoms 1 and 3 [69]. The six-sided glucose rings are connected to each other in linear or branched forms with glycosidic bonds, so the structure of these glycosidic bonds will affect the functionality of β -glucan molecules [70]. There are three structural types of these molecules: α -glucans, β -glucans, and mixed α,β -glucans. The configuration of glycosidic bonds and molecular mass are important for their characterization [71]. Fungal cell walls, which are mostly structural polysaccharides and glycoproteins, are the main source of various structural types of glucans [72].

The main biological activities attributed to medicinal mushrooms are due to the β -glucans present in their wall and in some plants. These substances are antitumor, immunomodulatory, antimicrobial, contraceptive, anti-inflammatory, prebiotic, and antioxidant [73, 74]. Supplemental β -glucans in poultry diet can enhance their innate defense by inducing intestinal colonization and invasion of internal organs by *Salmonella* [75]. The main biological properties of β -glucan (1,3/1,6) are the ability to form viscous solutions in contact with water and to form hydrogen bonds at different binding sites [76]. The β -glucan is soluble in water, although its solubility decreases with time, temperature, and pH. The highest solubility is reached at a temperature of 55°C [77]. The β -glucan has been evaluated to increase humoral response, productive performance, and viability, where an increase in serum IgA and IgG was observed [72].

10. Fructans

Inulin is a natural storage polysaccharide with many applications in food and pharmacology. It can be a low-calorie substitute for sugar or fats. It is widely distributed in plants and is present in the reserve carbohydrates of just over 30,000 plant products [78]. Inulin is not a simple molecule—it is a fructan which fructose units are connected by β -bonds (1, 2). The chain lengths of these fructans range from 2 to 60 units [79]. Inulin is a storage carbohydrate in many plants. It is found in fruits and vegetables (e.g., chicory, Jerusalem artichoke, artichoke, onion, leek, garlic, asparagus, banana) and in the stem of some cereals, such as wheat, as well as agave, which has been used for the production of distilled and undistilled alcoholic beverages [80]. Many biological properties have been found in *in vitro* and *in vivo* tests with antimicrobial, antifungal, antioxidant, anti-inflammatory, antihypertensive, immunomodulatory, antiparasitic, and anticancer activity [81, 82]. An important aspect to consider is that being a material rich in different compounds of interest for agroindustry, future research aimed at the isolation, purification, and protection of agave's secondary metabolites with environmentally-friendly processes is required, in addition to thoroughly investigating the development of products based on the use of pure metabolites or their extracts, evaluation of their activity and bioactivity, as well as experiments that allow determining applications to different areas of operation [83]. Regarding the application of agave fructans in the poultry industry, so far, they have only been used as prebiotics in broiler diets to improve performance and intestinal health [15, 84]. The use of natural alternatives as antimicrobials and disinfectants is increasingly arousing interest in the consumption of safe products, as well as the interest of scientists in offering natural alternatives to prevent the transmission of pathogens through food, such as those referred to here.

11. Conclusions

In conclusion, the utilization of natural products as an alternative to formaldehyde for disinfection of fertile eggs in commercial hatcheries offers a promising avenue for achieving effective and environmentally sustainable egg sanitation. This book chapter has highlighted the growing concerns surrounding the use of formaldehyde due to its potential health hazards, environmental impact, and regulatory restrictions. The exploration of natural alternatives has provided valuable insights into the efficacy and safety of various compounds derived from plant extracts, essential oils, and bioactive substances.

The research presented in this chapter has demonstrated that natural products possess remarkable antimicrobial properties, capable of effectively eliminating pathogenic microorganisms from fertile eggs. Furthermore, these alternatives have exhibited favorable characteristics such as biodegradability, low toxicity, and minimal residue accumulation, making them attractive options for commercial hatcheries seeking to adhere to stringent environmental regulations and consumer demands for sustainable practices.

While natural products offer numerous advantages, it is essential to acknowledge the challenges associated with their implementation. Factors such as product consistency, standardization, and cost-effectiveness must be carefully considered to ensure practicality and viability on a larger scale. Additionally, further research and development are required to optimize formulations, dosages, and application methods to maximize their efficacy and minimize any potential negative impacts.

Nevertheless, the potential benefits of using natural products as a substitute for formaldehyde in the disinfection of fertile eggs are substantial. By adopting these alternative approaches, commercial hatcheries can enhance their biosecurity protocols, improve animal welfare, and reduce the ecological footprint of their operations. Furthermore, the adoption of sustainable and environmentally friendly practices can foster positive public perception and contribute to the overall sustainability goals of the poultry industry.

In conclusion, this book chapter has shed light on the potential of natural products as a viable alternative to formaldehyde for disinfection of fertile eggs in commercial hatcheries. While there are challenges to overcome, the positive attributes of these alternatives make them worthy of further exploration and development. The incorporation of natural products into hatchery practices has the potential to revolutionize the industry by providing effective, safe, and sustainable disinfection solutions.

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