EFFECTS OF PLASMA ACTIVATED WATER ON DISINFECTING *E.COLI* INOCULATED EGGS AND STUDY OF THEIR STORAGE QUALITY ATTRIBUTES.

BY

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ABSTRACT

The study investigates the disinfection efficacy of Plasma-activated water on *E.coli* inoculated shell eggs. This is a critical issue in the food sector because of the potential health hazards linked with food borne illnesses related to eggs. The impact of several PAW treatment durations (5, 10, 15, 20 minutes) and dip durations (30, 90, 150 seconds) on the microbial load of *E. coli* inoculated onto egg surfaces was comprehensively investigated in this work. Furthermore, this study assessed the impact of PAW treatments on important characteristics of egg quality during the duration of the following 0, 7, and 14-day storage intervals. According to the results, PAW treatments significantly reduced the amounts of *E. coli* in all investigated durations and dip times, demonstrating the powerful bactericidal effects of PAW. Longer exposure times significantly improved the disinfection efficacy, demonstrating PAW's promise as a scalable and successful technique for improving the microbiological safety of shell eggs. The non-destructive character of PAW on egg integrity and appearance was highlighted by quality analysis conducted after PAW treatments for 5 and 10 minutes, which showed no significant changes in albumen turbidity, weight loss, color index, or eggshell strength during the 14-day storage period. The yolk index was just one quality factor that changed, and it showed changes after the treatment. More research is necessary to determine how this alteration may affect the sensory characteristics of eggs. This work demonstrates a potential approach to dealing with microbial contamination without losing egg quality, highlighting PAW's feasibility as a secure and efficient substitute for traditional ways of disinfecting eggs.

Résumé

L'étude examine l'efficacité de l'eau activée par le plasma (EAP) sur la désinfection des œufs en coquille frai inoculés par E. coli. Il s'agit d'un problème crucial en raison des risques pour la santé, liés aux maladies d'origine alimentaire liées aux œufs. L'impact de différentes durées d'exposition de l'eau au plasma (5, 10, 15, et 20 minutes) et de périodes d'immersion des œufs dans l'EAP (30, 90, et 150 secondes), sur la charge microbienne d'E. coli inoculées à la surface des œufs, a été mesuré et comparé. Cette étude a aussi permis d'évaluer l'impact des traitements EAP sur des caractéristiques importantes de la qualité des œufs après 0, 7 et 14 jours de conservation réfrigérée. Il a été démontré que les traitements à l'EAP ont réduit de manière significative les quantités d'E. coli sur la surface des œufs traités pour toutes les durées et pour tous les temps d'immersion étudiés, démontrant les puissants effets bactéricides de l'EAP. Des temps d'exposition plus longs ont considérablement amélioré l'efficacité de la désinfection, démontrant ainsi le potentiel de l'EAP en tant que technique évolutive et efficace pour améliorer la sécurité microbiologique des œufs en coquille. Le caractère non destructif de l'EAP sur l'intégrité et l'apparence des œufs a été mis en évidence par une analyse de qualité réalisée sur les œufs qui traités avec de l'eau activé pendant 5 et 10 minutes. Les résultats ont montré aucun changement significatif dans la turbidité de l'albumen, la perte de poids, l'indice de couleur ou la résistance de la coquille de l'œuf après 14 jours de conservation réfrigérée. En revanche, l'indice du jaune des œufs traités a montré des changements après la période de conservation. Des recherches supplémentaires sont nécessaires pour déterminer si ces altérations peuvent affecter les caractéristiques sensorielles des œufs. Ce travail démontre la faisabilité de EAP en tant que substitut sûr et efficace aux méthodes traditionnelles de désinfection des œufs.

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Firstly I want to thank the almighty God, and I am grateful for his presence with me throughout this master's journey. This thesis would not have been possible without the guidance and help from several Individuals who contributed and extended their valuable assistance in the preparation and completion of my study. And the next greatest strength, my utmost gratitude to my supervisor Prof. Vijaya Raghavan for the continuous encouragement and support of my Master's degree study and research. I am very appreciative of my professor for his patience, motivation, and immense knowledge during this period. He spent lots of time, giving me direction and correcting my thesis paper. Besides my supervisor, I am indebted and also would like to thank Mr. Yvan Gariépy, who provided me much needed technical help, research help and ordered the materials for my experiments. I would like to thank Professor Dr Orsat for providing me access to her lab to conduct my experiments. My sincere thanks also goes to Dr. Sellam perinban for her significant guidance and mentorship. She always supported me so carefully in my Master's study period. She was always enthusiastic about guiding me in my studies and research. I also would like to convey my thanks to Sarah, Jolvis, Philip addo and my senior lab mates, including Shuyao Wang, Xin Dong, for their support and motivation for my work. Finally, and most importantly, I would like to thank my parents and my brother and friends. Their support, encouragement, quiet patience and unwavering love are the driving power of my work.

Thesis Format

This thesis is submitted in the format of papers suitable for journal publication. This thesis format has been approved by the Faculty of Graduate and Postdoctoral Studies, McGill University, and follows the conditions outlined in the Guidelines: Concerning Thesis Preparation, which are as follows: “As an alternative to the traditional thesis format, the dissertation can consist of a collection of papers of which the student is an author or co-author. These papers must have a cohesive, Unitary character making them a report of a single program of research. The structure for the Manuscript based thesis must conform to the following:

1. Candidates have the option of including, as part of the thesis, the text of one or more papers submitted, or to be submitted, for publication, or the clearly duplicated text (not the reprints) of one or more published papers. These texts must conform to the "Guidelines for Thesis Preparation" with respect to font size, line spacing and margin sizes and must be bound together a s an integral part of the thesis. (Reprints of published papers can be included in the appendices at the end of the thesis).

2. The thesis must be more than a collection of manuscripts. All components must be integrated into a cohesive unit with a logical progression from one chapter to the next. In order to ensure that the thesis has continuity, connecting texts that provide logical bridges between the different papers are mandatory.

3. As manuscripts for publication are frequently very concise documents, where appropriate, Additional material must be provided (e.g., in appendices) in sufficient detail to allow a clear and precise judgment to be made of the importance and originality of the research reported in the thesis.

4. In general, when co-authored papers are included in a thesis the candidate must have made a substantial contribution to all papers included in the thesis. In addition, the candidate is

required to make an explicit statement in the thesis as to who contributed to such work and to what extent. This statement should appear in a single section entitled "Contributions of Authors" as a preface to the thesis. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to clearly specify the responsibilities of all the authors of the co-authored papers".

CONTRIBUTION OF AUTHORS

This thesis is submitted in the form of manuscript, prepared according to the guidelines provided by the Faculty of Graduate and Postdoctoral Studies, McGill University. It is comprised of four chapters. Chapter I provides an introduction and the thesis objectives which is followed by Chapter II, in which the literature review on the background of this thesis is presented. This includes the overview on methods of plasma activated water generation, plasma-liquid interaction chemistry, microbial inactivation mechanism by plasma reactive species and the available literature on nonthermal plasma and PAW applications in food. Chapter III presents the preliminary studies conducted in the disinfection of *e.coli* inoculated eggs using the dielectric barrier discharge PAW generation system as well as the storage studies to support the efficiency of PAW washed eggs. Chapters IV includes summary and conclusions which provides the main contributions to scientific knowledge of the research, (1) the confirmation of the bactericidal effect of plasma activated water treatment towards *E. coli*, and (2) the assessment of the chemical and physical characteristics of eggs after the plasma activated water treatment. The generated knowledge will help the development of novel approach to dealing with microbial contamination without losing egg quality. The research work reported here, including review of literature, design of experiments, experimental work, data analysis and manuscript preparation was performed by Mrunalini Hanamkonda under the supervision and guidance Prof. Vijaya Raghavan. Mr. Yvan Gariepy provided the technical support and expert guidance in the design and construction of the PAW system and assisted in the laboratory manipulations for performing the analytical studies. All authors are from the Department of Bioresource Engineering, McGill University, Quebec. The research work was conducted at post-harvest technology lab and Prof. Valérie Orsat's lab.

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CHAPTER 1

INTRODUCTION

Table eggs can be categorized into three groups: processed, unprocessed and free range. Processed eggs are those obtained from commercial or integrated layer farms that are cleaned, washed, sanitized, packed, and marketed under a brand name. Unprocessed eggs are sold as loose eggs without being properly cleaned, sanitized, or packaged. They come from commercial layer farms. Eggs that are collected from free-roaming birds housed in backyards belong to homeowners. However, the commercially available table eggs are cleaned using some of the chemical sanitizers that may harm the cuticle (a biofilm that covers the surface of developing eggs naturally) present on the eggshell, which would allow other kinds of germs to enter, and they may also have no impact against certain diseases (Mine et al., 2003; Wang & Slavik, 1998). The eggshell's permeability is reduced by the cuticle's covering of the pores, which inhibits the microorganisms from penetrating (Wang & Slavik, 1998) into the egg. To lessen or decrease the dirt, debris, and microbial load, table eggs are rinsed with a chlorine solution after being cleaned with detergents in the US, Australia, and Japan (Hutchison et al., 2004; Northcutt et al., 2005). Throughout the world, sanitizers which are chlorine-based are widely used, due to which egg protection could be removed during the washing process, which could have an impact on egg quality. The elimination of the eggshell cuticle and the production of harmful byproducts, such as trihalomethanes, chloramines, halo acetic acids, and chloroform - all of which are carcinogenic and mutagenic that are considered as the two major drawbacks of these disinfectants. According to several studies (Allende et al., 2009; Bull et al., 2011; Legay et al., 2010; Ohtsuka et al., 1997) these by-products may irritate mucosal membranes in workers or cause cancer. According to the 2007 Integrated Pollution Prevention and Control (IPPC) Directive on Industrial Emissions, chlorine is listed as a

corrosive substance. As such, some European nations, including Belgium, Denmark, Germany, and the Netherlands, have outlawed its usage (Bilek & Turantaş, 2013; Fallik et al., 2014; Gopal et al., 2010; Meireles et al., 2015; Ölmez & Kretzschmar, 2009; Ramos et al., 2013). While the fresh-cut, meat, and poultry industries commonly use chlorine for disinfection, there is global interest in creating alternative disinfection techniques to reduce the negative effects on the environment and public health (Gopal et al., 2010; Meireles et al., 2015). Feces, water, caging material, nesting material, insects, hands, broken eggs, blood, soil or dust on the eggs are the most common sources of microbial contamination of the eggshell. Egg washing can reduce the microbial load on the eggshell surface and thus may lower the rate of penetration of microorganisms across the eggshell and decrease the incidence of food poisoning. Important extrinsic factors such as the bacterial strain, temperature differential, moisture on the eggshell, the number of microorganisms in the inoculums and the storage conditions may affect eggshell penetration by *Salmonella* spp (Messens et al., 2005). Intrinsic factors that may affect egg penetration include shell porosity, shell thickness and the extent of cuticle present on the shell. There is also some evidence to suggest that eggshell translucency is associated with greater microbial penetration (Chousalkar et al., 2010). After being laid, the eggs are gathered and may be kept in storage for a few days before being graded and sorted. Eggs are divided into three groups based on the features and flaws in them (Vučemilo et al., 2010). Eggs classified as Category A are considered to be edible (shell eggs) (Kone et al., 2013). They need to be transported and kept in storage at a temperature that best preserves their sanitary properties, ideally at a steady temperature (Kone et al., 2013). Indeed, a disrupted cold chain may cause droplets to form on the surface, altering the cuticle; this alteration may give microorganisms a potential point of entrance. The only eggs that are transported cold are those from French egg production departments overseas (Gautron et al., 2022). Eggs are considered as "extra fresh" for nine days after they are laid. Itemized eggs are

deemed "fresh" within the 10-28 days window. After 28 days, the eggs are no longer fit for sale. Eggs classified as category B are either stored or are second-grade eggs that no longer meet quality A standards. They may be dirty or cracked a little, they are not completely broken or incubated; rather, they are meant to be utilized in pasteurized egg products and for both food and non-food uses. The bacteria *Escherichia coli*, commonly referred to as *E. coli*, are frequently found in the intestines of both people and animals, where it typically lives unnoticed. Some strains, on the other hand, have the potential to be harmful, which could result in food-borne infections ranging from moderate gastroenteritis to severe bloody diarrhea and even potentially fatal consequences like hemolytic uremic syndrome. Food safety is put at risk by *E. coli*, especially when it contaminates foods like eggs. Eggshells with *E. coli* carry a serious risk, especially if the eggs are cracked or handled carelessly, which allows the bacteria to move from the outside of the shell into the inside. Bacteria can proliferate once they are inside, particularly if the eggs are kept at room temperature or used in recipes where they aren't fully cooked or uncooked. The presence of *E.coli* on eggs emphasizes the necessity of caution when producing, handling, and processing eggs. Novel disinfection technologies have the potential to significantly improve food safety by limiting the risk of food-borne illness linked to contaminated eggs. Alternatives to conventional chemical sanitizers are being investigated, considering the use of plasma activated water aim to eradicate infectious agents with efficacy while preserving the safety and quality of the eggs.

1.1 Problem statement

The challenge is that pathogenic germs found on the surface of eggshells might move to the egg contents during handling, transit, and eating, making contaminated eggshells a potential

source of food-borne diseases. Current egg processing practices may not properly eradicate these pollutants, putting consumers' health at risk. As a result, a dependable and effective method for disinfecting eggshells and reducing the risk of microbiological contamination is required.

1.2 Hypothesis

Using a novel eggshell disinfection method will significantly reduce the microbial load on eggshell surfaces, reducing the potential transmission of pathogens to egg contents and, as a result, the incidence of food borne illnesses associated with egg consumption. This project is based on the concept that plasma-activated water is a promising technique for ensuring food safety in the food sector while avoiding the use of chemical additives. The findings of this study will aid in understanding the impact of PAW reactive species on food components and quality.

1.3 Objectives

1. To evaluate the antimicrobial properties of Plasma activated water as a potential disinfectant for reducing *E.coli* contamination on inoculated egg surfaces.
2. To study the effects of Plasma activated water on certain quality attributes of eggs during storage.

1.4 Connecting text

Chapter 1 gives a thorough overview of the role that disinfection plays and its importance to provide safe eggs for consumers. It also highlights the necessity of taking appropriate steps to reduce the danger of contamination during the manufacturing and distribution processes. It also lays the groundwork by analyzing the significance of disinfecting table eggs. Bridging the gap between theoretical understanding and practical implementation chapter 1 is followed by chapter 2, which explores traditional and novel disinfection methods. In order to ensure the safety and quality of table eggs. The second chapter turns the attention to a thorough analysis of the conventional and cutting-edge disinfection techniques used in the context of handling and producing eggs, firmly embedded in past practices, traditional methods are examined for their applicability to current industry requirements as well as their limitations and effectiveness. In the meantime, the investigation of innovative disinfection techniques demonstrates breakthroughs in technology and innovation, providing viable substitutes that seek to resolve current issues and improve the general integrity and safety of table eggs. Through a systematic investigation of classic and novel disinfection procedures, the thesis aims to provide a comprehensive understanding of the shifting landscape of egg sanitation practices, ultimately contributing to the progress of food safety regulations and the promotion of public health.

Chapter 2

REVIEW OF LITERATURE

Egg consumption, Outbreaks, Traditional disinfection methods and Plasma activated water as Disinfectant.

2.1 Abstract

Along with traditional commercial methods including chemical sanitization, UV irradiation, thermal treatment, and natural disinfectants the practicality and effectiveness of Plasma activated water (PAW) as an alternative disinfection approach for table eggs are critically assessed in this literature review. Innovative techniques like PAW provide potentially safer and more environmentally friendly options as worries about chemical residues and environmental sustainability grow. The study summarizes research findings from multiple investigations into the antibacterial qualities of PAW, emphasizing the substance's capacity to inactivate a wide range of pathogens frequently linked to eggs, such as *E. coli* and *Salmonella spp*. In the context of their processes of microbial inactivation, reactive species such as reactive oxygen and nitrogen generated in PAW are described, offering effective disinfection without the hazardous residues associated with chemical methods. Additionally, without compromising safety, the ability of PAW to extend shelf life and preserve egg quality is investigated. The results indicate that although PAW exhibits great potential, more investigation and advancement of technology are required to incorporate this technology completely into commercial egg disinfection procedures.

2.2 Introduction

Eggs are a source of affordable and easily obtainable food since they are an ideal supply of important vitamins and minerals and high-quality protein. Across the world, from major cities to tiny rural villages, eggs are an essential component in diets because they provide an adaptable, nutrient-dense alternative for people of all ages. Eggs also include important amino acids, vitamins A and B_3, and folate (Dev et al., 2008). Beyond just their nutritional value, eggs are essential for combating malnutrition and enhancing food security, particularly in developing nations. Between 1960 and 1995, the number of table eggs consumed per person in Canada decreased from 22.97 dozen to 14.42 dozen. Consumption has been consistent since 1995, with a minor rise to 14.60 dozen in 2008 (Bejaei et al., 2011). During that period (1960-1995), there was a decrease in the consumption of table eggs primarily due to changes in consumer lifestyles and growing concerns about heart disease and dietary cholesterol (Kennedy, 1999) as well as the increased availability of novel and ready-to-eat foods, particularly for higher income families (Fearne & Lavelle, 1996). The preference for conventional white eggs was higher (55.1%) than the choice for other egg varieties (Bejaei et al., 2011). *Salmonella spp*. and other infections are best transmitted through eggs. The singular, intricate, and varied porosity structure of the eggshell provides the perfect hiding place for germs from traditional disinfection techniques. The yolk in particular is a rich medium that promotes microbial growth within the egg (Bermudez-Aguirre & Niemira, 2023). Because *salmonella* has been linked to outbreaks of food poisoning spanning many decades, it is perhaps the food borne pathogen that has been studied the most. Few foods have not been linked to *salmonellosis* outbreaks, and investigating these outbreaks can yield critical insights into the variables crucial to the organism's control. Usually, an infection that causes an outbreak of illness enters the food production or manufacturing process due to a

breakdown in the control mechanisms. A lot of these mistakes are made when it comes to basic areas of control including personal cleanliness, culinary procedures, cross-contamination, and food storage temperature (Bell & Kyriakides, 2002). In the United States and other industrialized nations, *C. jejuni* is the primary cause of bacterial food borne infections. The majority of people who suffer from such bacterial infections are rare and are mainly caused by eating undercooked poultry or other items that have come into contact with raw poultry meat while being prepared (Friedman et al., 2000). *Staphylococcus spp* is one of the most common gram positive infections on the surface of eggshells (Ansah et al., 2009). Yeast and mould are ubiquitous in nature. Inappropriate storage and environmental conditions lead to the contamination of the eggs (Arthur & Osei-Somuah, 2001). Eggshell bacterial counts range greatly, from hundreds to millions of bacteria/eggshells, according to (Board & Tranter, 2017). Dust, trash, and excreta are the primary causes of contamination. It may be possible to lessen bacterial infection in developing embryos and neonatal chicks by reducing microbial contamination on eggshells (Fasenko et al., 2009). According to (Rai et al., 2005), the common bacteria involved in this process are *Escherichia coli*, *Pseudomonas*, and *Proteus*. Vertical and horizontal infections with bacteria can be the cause of serious infectious poultry diseases which are frequently accompanied by heavy economic losses for the poultry industry (Atanassova & Ring, 1999). Thus, effective cleaning and disinfection procedures are essential for successful operations in the poultry sector.

2.3 Microbiology of eggs

Eggs have frequently been linked to food-borne outbreaks. Salmonella spp. is the most common microorganism found in these outbreaks. This particular bacterium has the ability to create biofilms on the eggshell and penetrate the pores (Lin et al., 2020). However, the

microbial ecology of eggshells is rich in many bacteria, including *Pseudomonas, Escherichia, Staphylococcus, and Bacillus,* to name a few. Table 2.1 contains a thorough list of microorganisms associated with eggs. According to the Centers for Disease Control and Prevention (https://www.cdc.gov, 2021), multiple food-borne outbreaks have been linked to contaminated eggshells with *Salmonella spp*. in the last 5 years. *S. enteritidis* is the primary species identified in eggs. Salmonella-contaminated eggs often have 10 or fewer cells per infected egg due to the hen illness, and the microbe will be in the albumen. On the other hand, Salmonella can get through the vitelline membrane and contaminate the yolk of the egg if it stays at room temperature (Guan et al., 2006). In addition, this microorganism tends to concentrate and reproduce more in the yolk of contaminated eggs (Stadelman et al., 1996) due to its rich composition (Wilkin & Winter, 1947).

Table 2.1 Microorganisms associated with eggshells.

Genera of common contaminants	**Pathogens linked to Food-borneoutbreaks**
Pseudomonas	*Salmonella spp.*
Alcaligenes	*Campylobacter spp.*
Proteus	*Listeria spp.*
Arthrobacter	*Staphylococcus spp.*
Escherichia	*Escherichia spp.*

2.4 Egg contaminants

From the time of production until the processing, cooking, and eating stages, eggs can get contaminated at various points. Microorganisms can spread transovarial, or "vertically" when eggs become contaminated while still in the hen's ovary. When eggs are exposed to an

environmental contamination and bacteria get through the eggshell, this is known as horizontal transmission (De Reu et al., 2006). Laying hens can ingest contaminants from the environment, via water and feed, and those contaminants can be transferred to the eggs. Eggs have higher fat content and have potential enough to accumulate persistent organic pollutants (POPs) such as Polychlorinated biphenyls (PCBs) and dioxins. Such substances can pose a potential threat to human health. Also, in later phases of the egg production chain, possible contamination of eggs and egg by-products may occur. There are many kinds of pollutants that can contaminate eggs, including physical, biological, and chemical ones. It is important that producers and consumers are aware of these hazards in order to guarantee the safety of eggs prior to consumer ingestion.

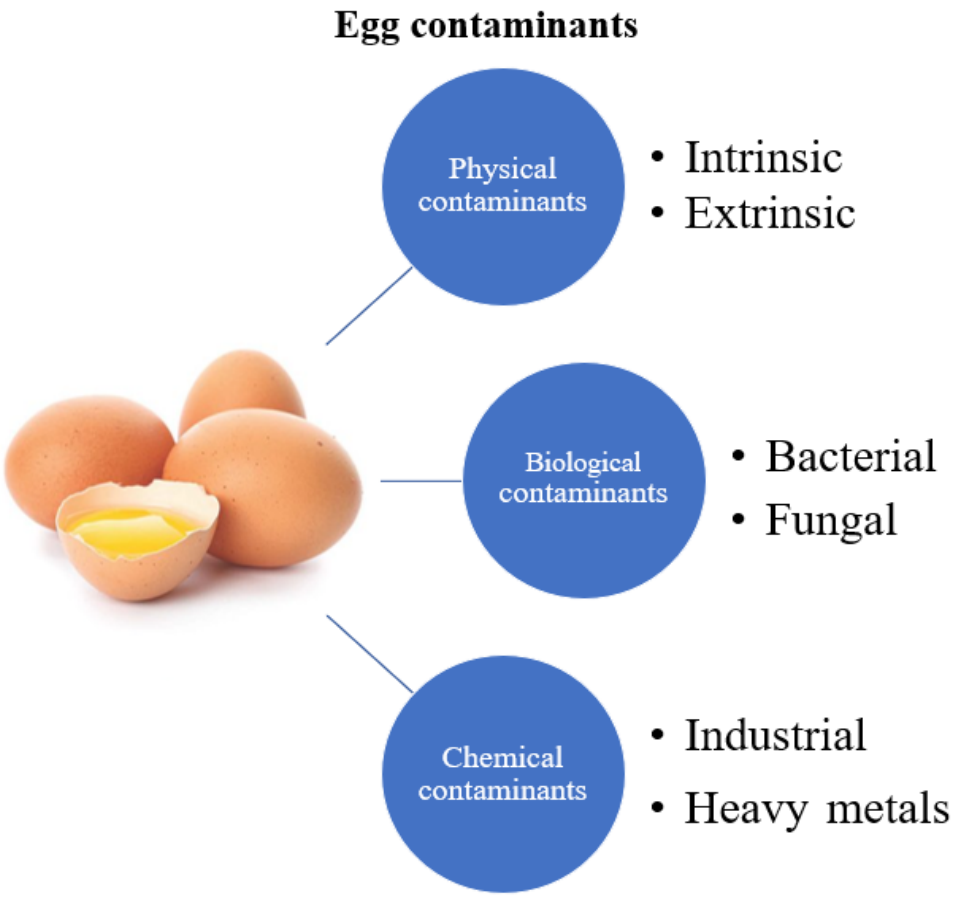

Figure 2.1 Various types in egg contaminants

2.4.1 Physical contaminants

Physical contaminants associated with eggs include intrinsic contaminants and extrinsic contaminants. On-farm (intrinsic) contaminants are those introduced through broken/dirty eggshells, and improper candling and grading activities. While extrinsic contaminants are introduced through improper processing activities, the probability of physical contaminants affecting eggs at the layer farm stage is limited due to the protection of the egg by shell, except for blood spots. Foreign bodies such as feathers and droppings can enter the egg, when the eggshell is broken, during the primary processing. Extrinsic contamination may occur at any stage of the processing chain via raw materials, poorly maintained facilities and equipment, packaging materials, and poor food safety practices. Measures to prevent physical hazards include good processing practices such as frequent control and timely maintenance of equipment, and visual examinations of products. Specific measures using filters, metal detector or magnet and strainers to monitor physical hazards during processing should be applied as part of GMP (Good Manufacturing practices) (H.J Van der Fels-Klerx, 2017).

2.4.2 Biological contaminants

Egg can be contaminated at both egg shell and egg contents through a variety of microbes with a wide range of pathogens especially *Escherichia coli*, *salmonellae,* fungi, and *Staphylococci* which are considered as the most common bacteria contaminating egg shells (Ricke, 2017). The various fungi which cause contamination include a large group of microorganisms which are ubiquitous in nature. Food-borne mycotoxicosis is thought to be caused by the presence of mycotoxins, which are toxic metabolites of fungi. This is indicated by the growth of fungi in food (Hassan et al., 2014). The entry of fungi into eggs causes them

to deteriorate, and certain species have been linked to health risks for the general public (Neamatallah, 2009). After the egg is laid, it is usually moistened and becomes soiled at the same time. The presence of dirt in the surrounding environment increases the number of contaminating organisms (Board et al., 1964). The amount of egg contamination appears to be the function of cleanliness of the surface onto which they are laid, and the manner in which the eggs are handled after laying, which shows greater effect when the eggs are cracked. The eggs collected with gloved hands show lesser contamination than compared to the eggs handled normally (D'Aoust et al., 1980). External environmental conditions such as temperature and length of storage as well as any treatment they receive such as washing has an impact on the contamination.

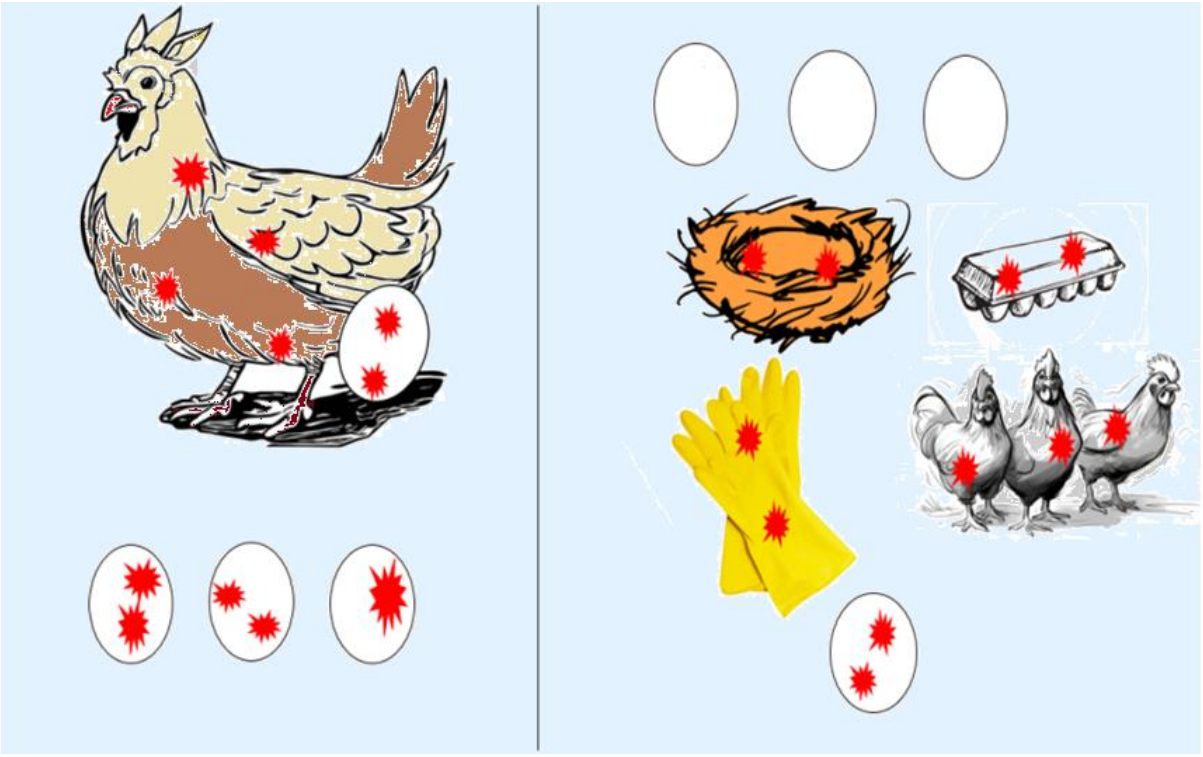

Figure 2.2 Schematic representation of egg contamination with pathogens: (a) vertical Transmission; (b) horizontal transmission

Certain molds, particularly those belonging to the Fusarium and Penicillium species, have the ability to infiltrate eggs at varying temperatures and release toxins that intensify throughout time of storage (El-Torkey, 1982). While Mucor and Rhizopus species are often found in food contamination, Aspergillus species can cause pulmonary aspergillosis, pulmonary allergic skin infections, nasal infections, nail infections, and external ear infections. These individuals can cause deep wound infection, orbital cellulitis, intraocular infection, external otomycosis, and skin infections. They can also affect the rhino facial region, the cranial area, the lungs, the gastrointestinal tract, and possibly other organ systems (Jones et al., 1981).

2.4.3 Chemical contaminants

Chemical pollutants found in eggs can come from a variety of sources and, if left unchecked, can be harmful to human health. Eggs may come into contact with these toxins through food, exposure to the environment, or handling and processing. It is essential to comprehend the kinds and sources of possible pollutants in order to protect public health and food safety. The following are some common categories of chemical pollutants discovered in eggs. Polybrominated diphenyl ethers (PBDEs), polychlorinated diphenyl ethers (PCDEs), per fluorinated compounds (PFCs), metals, and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) or polycyclic aromatic hydrocarbons (PAHs) are some of the notable pollutants among these pollutants. Information on exposure and adverse effects for most of these organic pollutants in humans is still relatively limited.

A compromised health might also result from certain dietary practices that expose one to environmental toxins. It is well known that poultry treated with pharmaceutical products can produce eggs contaminated with drug residues. Since many of these potentially harmful pollutants dissolve in fat, fatty foods, like eggs, frequently have high concentrations of

persistent organic pollutants (POPs). Several studies have demonstrated that eggs, along with many other foods, particularly those high in fat content, may potentially expose people to environmental pollutants, the potential toxicity of which is well established. The Japan Nutrition Survey provided the data on food intake in the Tokyo region, and the study was conducted using the complete diet-market basket approach based on food classification (14 groups). Fish and shellfish, meat and eggs, and milk and dairy products were shown to account for almost 90% of the daily consumption of dioxins. Piskorska-Pliszczynska et al. (2014) had evaluated the dioxin transfer from polluted soils to eggs in Poland. The main causes of dioxins in soil are erosion from adjacent polluted areas, sewage sludge or compost applications, air deposition, and spills.

2.5 Cleaning and disinfection methods of Eggs

Many nations, including the US, Australia, and Japan, use egg washing as a way to lower the contamination of eggshells (Hutchison et al., 2004). Egg washing chemicals, according to some experts, may harm the eggshell's cuticle layer (Wang & Slavik, 1998), which could lead to moisture loss and a decline in the egg's internal quality. Additionally, washing eggs could encourage the spread of microorganisms throughout the eggshell, especially if the storage and drying conditions that follow washing are inadequate. This has led to a discussion over the advantages of egg washing nowadays. Different egg washing procedures may cause damage to the cuticle or alter the surface of the eggshell (Wang & Slavik, 1998), which may change the penetration of bacteria across the eggshell. Table eggs may become externally contaminated when they pass through the cloacae of diseased hens or when they come into contact with contaminated areas after they have been laid (De Reu et al., 2008). Certain

Salmonella serovars, such as typhimurium and enteritidis, can also contaminate the egg by traveling via the ovary (Schoeni et al., 1995). During egg packing operation and egg washing, Salmonella's penetration of egg contents and storage time are correlated, according to Wang and Slavik (1998); the longer the period before washing, the greater the pathogen's penetration levels. Nonetheless, this aligns with contemporary business practices that seek to reduce the duration between egg laying, grading, and packing, and delivery to the point of sale. In nations where egg washing is approved, corporations strive to clean the most eggs within 24 hours of laying. However, the quick egg turnaround time could lead to issues of its own. One specific issue is that moving eggs in and out of cold storage facilities may result in the formation of condensation on the shell, a process known as "sweating." which in turn can move bacteria through the shell and beyond the reach of sanitizers (Hutchison et al., 2003). For cleaning eggs, only sanitizers that have been approved are to be used on food. Typical options include hydrogen peroxide, quaternary ammonium compounds, and sanitizers with a chlorine base. Some of the other disinfection methods include the following methods: Thermal and Nonthermal.

2.5.1 Thermal

Thermal egg disinfection helps get rid of pathogens and lessen microbial contamination on the surface of eggs. A study conducted on external disinfection of shell eggs using steam in a thermal tray showed that steam is applied to the eggs while they move through a Thermal Trap (TT) - a partially enclosed chamber filled with steam. The short treatment of a few seconds in a TT protype completely inactivated Salmonella (>7.8 log CFU reduction) on artificially inoculated fresh shell eggs. The treatment did not have an adverse effect on Haugh units, albumen and yolk pH, and albumen whip (Zion et al., 2021). In many countries,

washing table eggs is forbidden because of worries about pathogens penetrating the shell and to avoid cross-contamination. Eggs are immersed in hot water for over 40 minutes as part of the pasteurization process (Lara, 2016), and then they are allowed to cool for a considerable amount of time. When a poultry house found positive for *Salmonella Enteritidis*, the egg rule of the FDA requires either egg testing or diverting eggs to a treatment resulting in at least a 5-log reduction in *Salmonella* count (Fda, 2011). The proposed thermal treatment does not attempt to disinfect the contents of the shell eggs. However, external disinfection of shell eggs is of crucial importance to health in reducing egg-borne salmonellosis, since *Salmonella* on the shells can be easily transmitted to other food products, resulting in cross-contamination, as well as infection of food handlers (Zion et al., 2021). To reduce the danger of contamination, thermal disinfection is frequently used in conjunction with other strategies like cleaning, cooling, and excellent manufacturing processes. Impact of thermal processing on the structural and allergenic properties of egg white showed when compared to the untreated control, ovalbumin's (one of the major egg white proteins) antigenicity decreased by 82% following thermal treatment at above 80°C and pH 9.5. The fact that only 5.5% reduction was observed when the same thermal treatment was carried out at pH 7.0 further demonstrated in this study the critical function pH played in denaturation. The findings demonstrated the impact of pH and temperature on a protein's structure and antigenic qualities (Stănciuc et al., 2016). Heat treatment is a conventional food processing technique used to improve food texture and palatability, remove toxins, and boost microbiological safety. Thermal processing can reduce allergenicity because the structures of allergic components tend to change after heat treatment. About 50–60°C is when the tertiary structures of proteins start to alter due to variations in molecular weight, stability, and size. As the temperature rises above 65°C, secondary structure changes occur (Vanga et al., 2017).

Thermal disinfection is often utilized in conjunction with quality manufacturing methods, cleaning, and cooling techniques to minimize the risk of contamination. In general, thermal egg disinfection is a crucial step in making sure that eggs are safe to eat, but it should be a part of a thorough strategy for food safety that incorporates good sanitation, hygiene, and quality control methods all the way through the production and distribution process.

2.5.1.1 Pasteurisation

It has been demonstrated that pasteurizing eggs in hot air, water, and infrared light is efficient against Salmonella sp., however it also damages eggshells, leading to fissures in the shells (Himathongkham et al., 1999). Furthermore, it has been demonstrated that thermal egg-washing methods impair the sensory qualities of eggs and interfere with the rheological aspects of the egg albumen (Kiosseoglou & Paraskevopoulou, 2005). The functional quality of eggs is substantially impacted by conventional heat pasteurization procedures that use hot air and hot water baths. As a practical substitute for pasteurizing in-shell eggs, microwaves (Dev et al., 2008) electromagnetic radiations with a frequency range of 300 MHz to 300 GHz are known as microwaves (MW). On the other hand, industrial microwave systems run at 915 MHz, whereas household microwave gadgets run at 2.45 GHz. The microwaves operating at 2.45 GHz and 915 MHz have a penetration depth of approximately 12 mm and 32 mm, respectively, which is dependent on the temperature of the meal. Particularly in industrial applications, the restricted penetration depth of microwaves results in uneven heat distribution in meals (Herve et al., 1998).

Microwaves have been used to pasteurize pastries, confections, and packaged bread. When the use of chemicals to suppress mold growth is prohibited or when the use of chemicals materially alters the volume and aroma of the products, microwave pasteurization may be

used. In a similar vein, microwaves have been used to sterilize packed and precooked foods. For industrial use, microwave pasteurization and sterilization of fluids and semi-fluids have been developed. Heat is applied to the pumpable foods while they are being transported through the tube to achieve continuous processing. Using microwaves, foods like milk, soups, sauces, and purees have been pasteurized or sterilized (Raaholt et al., 2014).

2.5.2 Non thermal

Non-thermal plasma (NTP), which is both chemical-free and environmentally safe, has shown a strong potential for reducing microbial burdens on eggs while maintaining good product quality features (Zhou et al., 2018). Because of this, it is perfect for use in applications related to food safety and agricultural production (Butscher et al., 2016). Reactive oxygen species (ROS) (singlet oxygen, superoxide anion, ozone), reactive nitrogen species (RNS) (atomic nitrogen, excited nitrogen, nitric oxide), charged particles, neutral particles, and UV photons are the main reactive agents that contribute to the inactivation of microbial targets, either individually or in combination (Deng et al., 2006; Scholtz et al., 2015). Techniques used in order to eliminate or minimize germs on the surface of eggs without using heat are referred to as non-thermal disinfection procedures for table eggs. Due to concern about possible adverse effects of standard thermal pasteurization on egg quality, including flavor and texture changes, these techniques are becoming more and more common. Table egg non-thermal disinfection techniques include high pressure processing, pulsed light, ultrasound, irradiation, neutral electrolyzed water.

2.5.2.1 High pressure processing (HPP)

The initial use of high-pressure processing was to get rid of microbes to extend the shelf life of food items (Hite & Giddings, 1914). It is also known as high hydrostatic pressure processing. Its regular working pressure varies from 100 to 800 MPa (Farkas & Hoover, 2000). It has been demonstrated to be useful in changing the conformation of food ingredients, changing the structural and functional characteristics of proteins, including their allergenicity (Rivalain et al., 2010). Pressure's effect on regions stabilized by hydrophobic and electrostatic interactions—both of which are susceptible to pressure—causes conformational changes in proteins. Hydrogen bonds are nearly insensitive to pressure, but they nevertheless serve a significant role in maintaining protein structures (Ledward, 2000). Disrupting the cellular structures of bacteria, viruses, and other pathogens is the main way that HPP disinfects food. Elevated pressure has the potential to inactivate microorganisms by rupturing cell membranes, denaturing proteins, and changing their biochemistry. Crucially, covalent bonds are not considerably impacted by the process, meaning that vital nutrients and flavorings are mostly retained. To assess microbial development in post-processed (Hard cooked eggs (HCEs) under refrigeration, a storage study was carried out. Day 0 non post processed HCEs had a microbial population of 2.4 logs CFU/g. Following growth, the level increased dramatically to a maximum of 8.0 logs CFU/g in less than 10 days. After steam heat post-processing, no microbial growth was seen in HCEs till day 24, but after 45 days of chilled storage, microbial counts gradually rose to a level of 3.04 log CFU/g (Shahbaz et al., 2018). Juices, smoothies, deli meats, seafood, dips, and sauces are all processed with HPP. More and more research is being done on its potential uses in dairy products, prepared foods, and even processed fruits and vegetables.

2.5.2.2 Pulsed light

Eggs are subjected to brief, powerful light bursts from equipment that pulses light. The light often consists of UV-C wavelengths, which have germicidal qualities that can harm bacteria' DNA or RNA and prevent them from replicating. Within the area of light technologies, several approaches to pasteurization and disinfection of products and model systems have been investigated. Ultraviolet (UV), pulsed light, high-intensity light pulses, blue light, and light emitting diodes (LED) are examples of these technologies. Pulsed light (PL) effectively inactivates a broad range of bacteria implicated in food deterioration; it may be a viable substitute technique to increase the safety of egg products (Dunn, 1996; Gómez-López et al., 2005; Lasagabaster & de Maranón, 2006). With this innovative technology, short-duration, high-power flashes of broadband emission light (200-1100 nm) are repeatedly produced, with around 40% of the light released falling into the ultraviolet spectrum (Wekhof, 2000). They investigated the potential migration of germs through the pores as well as the transmission of albumen and yolk. Nonetheless, no salmonella cells were found inside the eggs under the testing conditions. The specifics of pulsed light treatment, such as the length of exposure and the strength of the light pulses, are carefully regulated to guarantee efficient disinfection and reduce the possibility of harming the eggs or degrading their quality. The type and condition of the eggs, together with specific features of the pulsed light equipment and process, may affect how successful the pulsed light treatment is.

2.5.2.3 Ultrasound

Ultrasound is another novel non-thermal food processing method that employs sound waves with a wide frequency range (Feng et al., 2011). High-frequency sound waves are used in

ultrasound disinfection, a physical technique, to inactivate microorganisms such as bacteria, viruses, and fungi. Three types of ultrasound are commonly used, depending on the frequency: power ultrasound (20–100 kHz), high frequency ultrasound (100 kHz–1 MHz), and diagnostic ultrasound (1–500 MHz) (Wu et al., 2012). This method provides an alternative to chemical disinfectants and heat treatments and is used in a variety of industries, including the food, healthcare, and water treatment sectors. By producing mechanical vibrations, ultrasound disinfection operates and is useful both on its own and in conjunction with other techniques. The use of ultrasonic treatment has been shown to lower microbial activity on hatched eggs while having no detrimental effects on hatchability, embryonic mortality, chick performance, or chick survival of Japanese quail (Coturnix coturnix japonica) eggs. When ultrasonography was used on shell eggs, the quantity of mesophilic bacteria in the yolk and albumen decreased while the eggs' sensory acceptability-which includes their stickiness, smell, smoothness, and general acceptability of eggs (Sert et al., 2013). Ultrasound is a disinfection technology that shows promise as it is non-chemical and may even be more sustainable. It can be used in a variety of settings and works well with other disinfection techniques, while its efficacy varies and depends on the situation.

2.5.2.4 Irradiation

Irradiation has been extensively researched for its ability to inactivate pathogens in a variety of items (Bisht et al., 2021).Song et al. (2009) investigated how gamma irradiation affected the functional characteristics of egg white powder and liquid egg white. The foaming capability of both products increased with increasing irradiation dose (0, 1, 2, and 5 kGy), according to the authors' findings. They investigated this impact using angel cake and baked goods. The end cake produced by processing irradiated eggs had a higher height and volume,

as well as an improved quality. The baked good's chewiness, gumminess, and hardness all decreased. Min et al. (2012) noted oxidative alterations in egg white proteins and a decline in the foaming capability and foaming stability of LEW when the irradiation was carried out at ≥ 5 kGy. Packaging sterilization already uses UV irradiation, and it has been shown that using it has no negative effects like cytotoxicity (Bintsis et al., 2000; Gopisetty et al., 2018). Moreover, short UV-C wavelengths have been certified by the Food and Drug Administration for the inactivation of harmful microorganisms in a variety of liquid meals, including juices and water (Fda, 2001). Similarly, in the US, ozone is permitted for use as a sanitizer or disinfectant in food and food processing (Guzel-Seydim et al., 2004; Smilanick), such as in the chicken industry for the disinfection of cold water and carcasses. The following are the primary advantages of these food processing treatments: They employ nonchemical chemicals to treat food, leaving no residues in the food or water, are nonthermal methods of controlling pathogens and microorganisms, and are compatible with organic production systems (Doyran et al., 2002). Kataoka et al. (2023)shown the inactivation of Salmonella cells within the egg with an electron beam dose as low as 100 mGy. By using this source of irradiation, the overall process expenses can be significantly decreased while maintaining the product's microbiological purity.

2.5.2.5 Neutral electrolyzed water

The use of neutral electrolyzed water has shown positive outcomes for food's physicochemical and functional qualities, as well as for bacterial reduction. Neutral electrolyzed water (NEW) is a desirable substitute because of these ideal qualities: antimicrobial properties, ease of use, and environmental friendliness (Ramírez Orejel & Cano-Buendía, 2020) and null effects on egg cuticle (Medina-Gudiño et al., 2020). On the

surface of table eggs, neutral electrolyzed water (NEW) was evaluated as a disinfectant, one flock of bovans (Hen breed) white eggs were collected and used for exposure. Three distinct treatment groups were created using artificially infected eggs: 2% CAS (citric acid solution), saline solution (SS), and NEW. The Mexican norm for antimicrobial activity determination protocol was followed in order to assess the bactericidal effect. Comparisons were made between the bactericidal effects observed and those derived from CAS and SS. The number of bacterial cells on the eggshells was measured. When examined on the surfaces of chicken eggshells, NEW showed a considerably stronger bactericidal action than CAS (6.11 logs CFU/ml reduction in vitro and a 2.18 log CFU/egg decrease on eggs vs. 1.06 logs CFU/ml in vitro reduction and 1.74 logs CFU/egg). Furthermore, it was discovered that CAS reacted with the carbonate egg shield, causing the cuticle's integrity to be lost. The mineral content of eggshells treated with NEW was comparable to that of eggshells treated with SS; in contrast, the phosphorous concentration of CAS-treated eggshells was significantly lower than that of NEW-treated eggshells (Rivera-Garcia et al., 2019). Eggshell cleaning has been done using both basic electrolyzed water (BEW) and acid electrolyzed water (AEW) (Medina-Gudiño et al., 2020; Park et al., 2005). But compared to traditional chlorine-based sanitizers, these cleaning and sanitizing agents are less effective and have a slower rate of action (Arya et al., 2018). It has been demonstrated that using neutral electrolyzed water (NEW), often referred to as slightly acidic electrolyzed water (SAEW), reduces *Salmonella Enteritidis* on egg surfaces at a rate that is comparable to or greater than that of using AEW (Cao et al., 2009; Yuan et al., 2022) Even if SAEW has potential, chlorine and other more conventional sanitizers are still more effective (Abadias et al., 2008; Huang et al., 2020).

2.5.3 Natural disinfectants

Research conducted on efficiency of disinfection by natural products compared to formaldehyde showed that the natural alternatives are valuable in the efficacy and safety of various compounds derived from plant extracts, essential oils, and bioactive substances.

Since natural antibacterial elements are biodegradable and non-toxic as opposed to chemicals, which are poisonous, non-degradable, and corrosive, their use as egg disinfectants opens the door to their usage as a safer alternative. Oils can be extracted using a variety of techniques, including low pressure distillation with boiling water or intense heat, liquid CO_2, and microwaves (Bakkali et al., 2008). During egg processing the use of egg washes and sanitizers can help to reduce the bacterial load during egg processing but improvements and more sanitizer options are needed.

Plant extracts are considered safe and efficient remedies for ailments and diseases in traditional medicine. The antimicrobial properties of these plant extracts are capable enough to act as egg disinfectants, due their biodegradable and non-toxic nature (Upadhyaya et al., 2013). Oils can be extracted using a variety of techniques, including low pressure distillation with boiling water or intense heat, liquid CO_2, and microwaves (Bakkali et al., 2008). The most important components are phenolic compounds, such as eugenol (the active component of cloves; Eugenia caryophillis), carvacrol from oregano oil (Origanumglandulosum), and trans-cinnamaldehyde (an aldehyde found in cinnamon bark extract (Cinnamomumzeylandicum). When compared to eggs that had been water-washed or exposed to chlorine, these substances have shown efficacy in decreasing *Salmonella enteritidis*.

2.5.4 Antimicrobial agents

A study conducted to understand the effectiveness of antimicrobial agents such as (Citric acid 3%, Sodium bicarbonate 3%, hydrogen peroxide (H_2O_2) 3%) against eggshells bacterial load caused by *E.coli* and *Staphylococcus.aureus*. The obtained results found that *Staph.aureus* has recorded the highest prevalence (90%) in table eggs. Both *E. coli* and *Staph.aureus* isolates showed high resistance to the tested antibiotics. The effect of tested antimicrobial agents on the experimentally contaminated eggs showed that H_2O_2 (3%) was more effective in reducing the load of *E.coli* on eggshells than the other antimicrobial agents tested, while bicarbonate 3% showed significant reduction of *Staph.aureus*. In *E.coli*, after treatments with citric acid, H_2O_2, and bicarbonate at concentration of 3% the reduction in bacterial count was 0.36, 1.16, and 0.80 Log CFU/ml, respectively. Similarly, the reduction in *Staph.aureus* count after the treatments was 0.90, 0.83 and 1.14 Log CFU/ml, respectively. However, *Staph.aureus* appeared more sensitive to bicarbonate 3% soution than other antimicrobial agents tested. Immersion in H_2O_2 solution showed major reductions in *E. coli* on shells compared to other tested antimicrobial agents. These results agreed with Maktabi et al. (2018) who reported that citric acid and H_2O_2 were same in reducing *E.coli* contamination in eggs as well as other foods. Fong et al. (2015) reported that Lemon juice (citric acid) and baking soda (bicarbonate) are easily available, ecofriendly and possess less toxicity. However, Citric acid has higher antimicrobial efficacy than sodium bicarbonate in the treatment of hard surfaces (Bjornsdottir et al., 2006).

2.6 Chemical disinfectants

To eliminate potential pathogens in eggs, the poultry industry has opted for the use of whole egg disinfectants, which includes Chlorine based disinfectants such as chlorine dioxide and sodium hypochlorite, sodium hydroxide, formaldehyde, and citric acid. However, among others such disinfectants can be corrosive and toxic.

2.6.1 Chlorine

Chlorine can be used in various forms such as chlorine dioxide or hypochlorite solutions, concentration range from 50 to 200 ppm. A suitable concentration and humidity of chlorine dioxide gas (ClO_2) can greatly lower the amount of Salmonella spp. on an eggshell. ClO_2 (40 ppm) gas disinfection is suggested as a safe disinfectant for the efficient control of bacteria on the egg surface, despite reports that it has no influence on the intrinsic quality of table eggs.

(Chung-Myeon et al., 2005). Considering relative merits and limitations, chlorine is one of the best sanitisers (Favier et al., 2001). By entering the bacterial cell wall and interfering with the sulfhydryl-containing enzymes involved in the metabolism of glucose, chlorine uses its germicidal ability to kill the bacteria (Banwart, 2012). Sodium hypochlorite (HCh) - (active chlorine; 4 percent), along with auxiliary and functional ingredients, make up the HCh agent, which is a transparent liquid ranging in color from colorless to light yellow with a mild chlorine odor. The *Salmonella enteritidis* (SE) bacterial suspension contaminated the shell of table eggs. It was discovered that by using a 0.04% solution for disinfection at a 2-minute exposure (the automated method) and a 0.02% solution at a 5-minute exposure (the manual method), HCh provides inactivation of SE on the shell of table eggs. Following the 15 days of

disinfection and storage, the eggs' shells stayed clean, undamaged, and discoloration-free; the air chamber remained stationary or barely moved, and the height did not rise above 7 mm; the whites stayed dense, light, and transparent, while the yolks were strong, barely perceptible, and only slightly deviated from the center in a few of the eggs. The experimental and control batches of eggs did not differ significantly in terms of pH content variations or weight loss after storage (Kozak).

2.6.2 Sodium hydroxide

Sodium hydroxide, commonly referred to as caustic soda or lye, is a potent alkali that is employed in many industrial processes, such as sanitation and cleaning. Because of its many benefits, most notably the alkaline hydrolysis of fats and oils (saponification), which also promotes surfactant activity by stabilizing oil-in-water emulsions, sodium hydroxide (NaOH) has a long history of use in cleaning (i.e. detergent) mixes. Moreover, NaOH has the ability to denature and agglomerate proteins by increasing the pH of solutions (Helbig et al., 2019) and enhance the action of chelators of hard water ions (Ca^{2+} and Mg^{2+}) such as Ethylenediaminetetraacetic Acid EDTA (Boziaris & Adams, 1999). These water softening properties improve surfactant activity and aid in stabilizing unattached surface dirt in suspension. NaOH, a highly reactive molecule, not only has cleansing properties but also possesses microbicidal activity. When sodium hydroxide encounters skin or other organic materials, it can burn severely due to its extreme corrosiveness. Because eggshells are mostly composed of calcium carbonate, using them for egg washing carries some danger. The egg's natural defense against microbial invasion may be compromised by the corrosive properties of sodium hydroxide, which can erode or destroy the shell. Although sodium hydroxide is an effective cleaner and disinfectant, it is typically not recommended to use it to wash eggs

because of the possibility of chemical exposure, the potential for egg destruction, the possibility of health risks from residues, and the availability of safer alternatives. It's dangerous and caustic character at realistic biocidal doses limits its promise as a general-purpose disinfectant (Wales et al., 2022).

2.6.3 Formaldehyde

Globally, formaldehyde fumigation has long been the standard procedure for sanitizing eggs (Steinlage et al., 2002). It was used in many hatcheries around the world as part of their disinfection procedures when it comes to fertile eggs; According to EU standards, formaldehyde has recently been subject to European Union standards due to its recognized carcinogenic properties. Furthermore, studies have demonstrated that even a 20-minute formaldehyde pre-incubation fumigation has a detrimental impact on the tracheal epithelial cells of 18-day-old embryos and one-day-old chicks (Hayretdağ & Kolankaya, 2008).

However, it also has genotoxic and cytotoxic properties that can affect humans, resulting in irreversible effects from its inhalation. The dose, exposure duration, application technique, and egg exposure period are the primary determinants of these effects (Oliveira et al., 2019). The issue with formaldehyde use is that it must be used at a concentration of at least 600 mg/m^3 (489 ppm) as a disinfectant, which exposes personnel to a high level of exposure (Cadirci, 2009). Significant attempts have been made to develop alternatives to using formaldehyde in light of the disadvantages connected with its use (Samberg & Meroz, 1995; Steinlage et al., 2002).

2.6.4 Citric acid

Naturally occurring in all living things, citric acid (Acidum citricum) is essential to the metabolism of carbohydrates. Furthermore, citrate is a significant indirect product of the Krebs cycle when it is in its ionic state. Citric acid, one of the fruit acids, is mostly present in citrus fruits like oranges and lemons. However, growing Aspergillus niger is the main way to obtain this preservative (Dhillon et al., 2013). Citric acid and other disinfectants work synergistically to reduce the number of germs (Park et al., 2009). A study conducted to evaluate the potential use of citric acid as a modifier of quality changes in table eggs during their storage showed that the degree of qualitative alterations was lessened by the application of citric acid. Eggs treated with citric acid showed less weight loss, a shallower air cell, higher structural albumen, and less intensive water transfer from the albumen to the yolk. All of it suggested that the vittelline membrane was more resistant. It appears that citric acid can be used to prevent quality changes in table eggs during storage because it is readily available, reasonably priced material that is accepted and recognized as a safe food preservative (Drabik et al., 2021). The degree of hardness in the water used for washing can affect how effective citric acid is. Because of interactions with minerals, hard water can lessen the efficiency of citric acid. This might complicate the disinfection process and call for concentration adjustments or additional treatment procedures. Citrus acid provides an environmentally friendly and generally safe substitute for disinfecting eggs, but it is not as effective as more concentrated and potent disinfectants made especially for egg processing due to its limitations, potential harm to equipment, and inability to preserve eggshell integrity. Because these constraints may be properly regulated, their use may be more acceptable in smaller or more specialized applications.

2.7 Plasma activated water.

A partially or completely ionized gas, such as a mixture of electrons, positive or negative ions, free radicals, excited atoms and photons, and so on, is referred to as plasma (Mandal et al., 2018)

Between solid, liquid, and gaseous states of matter, plasma is regarded as the fourth state of matter. One frequent natural occurrence of plasma is lightning. Plasma with ambient temperature or slightly above it is called cold plasma (CP). The effectiveness of atmospheric CP technology against bacteria found on food and medicinal products has made it very important (Ali et al., 2020; Chen et al., 2020; Pan et al., 2019). Water treated with plasma and having a plasma plume above or below the water's surface produces plasma-activated water, or PAW. The particles produced by plasma interact with water molecules to initiate a series of chemical reactions that result in the production of PAW, a distinct mixture of biochemically reactive chemicals. PAW has also been shown to have exceptional biological activity in the biomedical and agricultural domains in recent years (Kaushik et al., 2019; Sajib et al., 2020). During PAW generation, a number of primary and secondary reactive species dissolve in the liquid as a result of a sequence of reactions that are started at the gas–liquid interface while the energetic particles in the plasma phase are trapped in the aqueous liquids (Guo et al., 2017; Khlyustova et al., 2019). The chemical and biological impacts of PAW are caused by these reactive species, which include reactive oxygen species (ROS) and reactive nitrogen species (RNS). For PAW generation, a variety of setups have been used, including as plasma jet, dielectric barrier discharge, corona or spark discharge, pin/pins in water discharge, or plasma discharge in water with gas bubbles (Hamdan et al., 2020; Jin et al., 2020; Lu et al., 2017; Piskarev, 2019; Yoon et al., 2018).

2.7.1 System configuration

There are several system configurations used to produce NTP at atmospheric pressure (Conrads & Schmidt, 2000). They are categorized as Corona discharge, dielectric barrier discharge (DBD) and hollow electrode (HEDDBD) are the important systems used for the NTP-liquid processes. Most of the food-related applications were conducted using a batch type system in which the plasma is exposed to a fixed amount of liquid substrate.

When sinusoidal high frequency or nanosecond pulsed waveforms are used DBD discharges produce negligible thermal effects in water. However sinusoidal waves produce filamentary discharges while nanosecond pulsed waveform produces stable glow discharges which are more preferred over the other because of their energy efficiency (Neretti et al., 2016). Reducing the initial discharge voltage and improving system efficiency can be achieved by keeping the high voltage electrode covered by a dielectric substance above the water's surface (Liu & Li, 2014).

DBD is favored over the other variants because of its straightforward electrode setup and capacity to generate a steady plasma discharge (Zhou et al., 2018). The DBD discharge produces a higher concentration of reactive species than plasma torch glow which discharges because of its bigger surface area (Leduc et al., 2010).

Corona discharge: Among the extensively studied NTP discharge techniques are corona discharges. A viable substitute for the current sophisticated oxidation procedures in water treatment is pulsed corona discharge and its effectiveness in microbial inactivation in water has been well documented (Abou-Ghazala et al., 2002; Gupta & Bluhm, 2007). An extremely high voltage could be applied to a thin wire or electrode to cause a corona discharge. Shock waves, UV rays, and bubbling are produced when this streamer discharge is created inside the water with the reactive species (Locke & Thagard, 2012). Pulsed-corona streamer discharge

produced in-water provides superior reactive species diffusion and energy efficiency (Banaschik et al., 2015). The deliberate use of corona discharge produces an ion wind that aids in liquid mixing, improving the bactericidal properties of plasma activated water (PAW) and the diffusion of reactive species (Jirešová et al., 2022). According to Baroch et al. (2008) electrode degradation and unstable discharges are the main drawbacks of corona discharge systems. Electrodes corrode when a sharp needle is exposed to a strong electric field for an extended period of time (Sunka et al., 1999).

Electrode corrosion during in-liquid spark or streamer discharges generated by nano-pulsed corona discharges was observed by Hamdan et al. (2018). The existence of Cu+ and Cu2+ ions in the activated water when utilizing a copper electrode in a plasma micro jet that is powered by a power supply with a negative polarity and high voltage of either DC or 0.35 A and 100 kV was reported by Sun et al. (2012); (2017). The preferred oxidation and mineralization mechanism for contaminants in water is the Fenton reaction, which is triggered by these metal ions acting as metal-catalysts (Marković et al., 2015). Yet in culinary and medical applications, it is not advisable for metal electrodes to erode or electrolyze owing to plasma since this could pollute the liquid phase and shorten the system's lifespan and efficiency (Satoh et al., 2007).

NTP is also produced using radio frequencies (RF) and microwaves (MW) in addition to AC/DC electrical sources (Tendero et al., 2006). The Submerged Microwave Plasma Jet (MWPJ) has the ability to enhance the conversion of plasma species into water and inhibit electrode corrosion. PAW produced by DBD plasma was compared to MW plasma by (Niquet et al., 2018). Nitrous acid was the primary species in the MW NTP treated water, and it further broke down into nitrates. Because of the greater plasma temperature, no ROS were found. Hydrogen peroxide was the most significant and stable reactive species in DBD PAW. While the reactive species concentration in MW PAW rapidly decreased, DBD PAW's

bactericidal property remained steady during a 24-hour storage period, despite being significantly lower than that of MW PAW. High voltage creates the plasma in the space between two or more diverging electrodes, which cools as it moves toward the electrode's broader edge (Burlica et al., 2006; Tian et al., 2016). Water is activated by gliding arc discharges in two ways: either directly by exposing the plasma to the water, or indirectly (Benstaali et al., 2002; Burlica et al., 2006) or directly by misting a combination of water and air. The gliding arc has effectively activated an aerosol of water and gas (oxygen or argon) that is used to inactivate bacteria (Burlica et al., 2010). Indirect liquid exposure is preferred in any system configuration over direct exposure of water electrodes since the latter may result in the production of unwanted and unexplained reactive species in the water (Oehmigen et al., 2010; Satoh et al., 2007). The concentrations of reactive species in the activated liquid are strongly influenced by the contact mode of the gas-liquid interface (Aoki et al., 2008; Tian et al., 2015). Based on the mode of generation it can be classified into 1) In water 2) Above water 3) Aerosol and 4) Bubble (Bruggeman et al., 2021). The reaction efficiency is quite high when the plasma is formed immediately inside a liquid substrate, such as water. During in-water discharge, electric current and short-lived reactive species are also transported to the liquid, which is not possible in above-liquid application (Bruggeman & Locke, 2013). Owing to this abundant transfer of reactive species (content), the in-water approach yields highly reactive PAW and successfully sterilizes the contaminated liquid (Stoffels et al., 2008). But the kinetics of in-water reactions are extremely complicated and still poorly understood (von Woedtke et al., 2013). Furthermore, this approach is not recommended for treating living cells in food and medical applications since it causes severe cell damage (Stoffels et al., 2008). On the other hand, gas-phase plasma is produced in the plasma-liquid interface in above-water or indirect applications, when reactive species are only diffused into the liquid. By forcing convection through liquid mixing or raising the gas flow rate, the species transfer

rate can be increased (Bruggeman et al., 2021). Because long-lived reactive species in plasma above-water systems move mass less rapidly from the gas phase to the liquid phase, only neutral species and radicals can reach the liquid (Tian et al., 2015). When an oxygen and nitrogen gas mixture is utilized, this results in higher concentrations of nitrogen species in the liquid substrate than oxygen species (Thiyagarajan et al., 2013). In biological applications, in-direct exposure is preferable over direct in-water exposure despite low mass transfer and energy efficiency because it better controls reactive species and produces milder reactions (Stoffels et al., 2008). Either the gas or the water phase will diffuse into the other to speed up the reaction rate in multiphase systems such as aerosols, bubbles, and sprays. In these systems, the heterogeneous mass transfer rate of reactive species is enhanced because of the larger surface area and smaller droplet size. Compared to in-liquid systems, they have superior reaction kinetics and energy efficiency (Jiang et al., 2011). In comparison to the indirect treatment method, Burlica et al. (2006) suggested that a higher amount of H_2O_2 was created in PAW when water was sprayed over the plasma along with oxygen gas. Because liquid droplets evaporate and have a shorter residence period in the plasma area, the interactions between plasma and liquid droplets in aerosol systems are not fully understood (Maguire et al., 2015).

2.7.2 PAW Chemistry

2.7.2.1 Gas composition

The feed gas or carrier gas composition is another important factor which affects the composition of NTP activated liquid (Mao et al., 2013; Thirumdas et al., 2018). Because noble gases have low breakdown voltages at atmospheric pressure, they are utilized in conjunction with nitrogen and oxygen to create stable glow discharges. Examples of these gases are Argon and helium. Reactive oxygen species (ROS) and reactive nitrogen species

(RNS) are the two primary NTP reactive species, depending on the composition of the feed gas. The antibacterial actions of PAW are thought to be primarily mediated by reactive oxygen and nitrogen species (RONS). Numerous primary and secondary species are produced as a result of the chemical reactions that occur between reactive species and water. Singlet oxygen, superoxide's, excited ions, electrons, atoms, ozone, UV, and local electric fields make up the majority of plasma (Liao et al., 2017; Misra et al., 2011). Upon encountering water, plasma initiates dynamic chemical reactions that result in the formation of several aqueous reactive species. Electron paramagnetic resonance (EPR), UV-vis spectrophotometer, chemical dosimeter, liquid chromatography, in addition to colorimetric and fluorescence methods (Griess reagent, nitrate assay kit, titanium (IV) oxysulfate, amplex red hydrogen peroxide [H_2O_2] assay kit) have all been used to identify and quantify the reactive species generated in plasma-treated liquids (Hoeben et al., 2019; Jo et al., 2020; Kučerová et al., 2020; Pandiyaraj et al., 2020; Xu et al., 2020). While gas sensors or analyzers, mass spectrometry, laser-induced fluorescence (LIF), and Fourier-transform infrared (FTIR) spectroscopy are employed for product identification in the gas phase (Gorbanev & Bogaerts, 2019; Khlyustova et al., 2019). Several activities, including the movement of gaseous species into the liquid and chemical interactions between gaseous species and liquid molecules, take place at the interface layer when PAW is formed (Wende et al., 2018)

2.7.2.2 Reactive oxygen species

According to Bruggeman et al. (2016), the discharges that occur within the liquid are very dynamic and fleeting, involving quick breakdown processes that are mostly triggered by

intense electric fields, bubble implosions, or laser pulses. When water is treated with plasma, the water molecules dissociate out of equilibrium, forming short-lived species including hydroxyl ions (OH^-) and hydrated (solvated) electrons (e_{solv}) (Khlyustova et al., 2019). Hydroxyl ions (OH^-) and hydrated (solvated) electrons (e_{solv}) react quickly afterward to produce more stable species such as superoxides (O_2^-), ozone (O_3), and H_2O_2. The reactions are as follows:

$$H_2O + e^- \rightarrow OH^\circ + H^\circ + e^-$$

$$H_2O + e^- \rightarrow OH^\circ + H + 2e- - -$$

$$OH^\circ + OH^\circ \rightarrow H_2o_2$$

$$H + O_2 \rightarrow Ho_2$$

$$H^\circ + H_2O_2 \rightarrow OOH + H_2O$$

$$O_2^- + H^+ \leftrightarrow OOH$$

$$O_2 + e^- \rightarrow O^+ + O^\circ + 2e^-$$

$$O_2 + O^\circ \rightarrow O_3$$

The extremely reactive, short-lived species known as hydroxyl radicals (OH˚) have a very high redox potential. When there is organic material in the liquid, OH• combines with the organic material to create new radicals, which set off other processes. Depending heavily on the organic materials in the liquid, this sequence may result in the transition of reactive species to radicals. Lipid per oxidation, for instance, results in the production of lipid peroxide and lipid peroxyl radicals from the reaction of OH• with the lipids. In general, singlet oxygen and ozone are short-lived species. When dissolved in a liquid, ozone is not very stable, and the pH of the liquid has a significant impact on this stability. Ozone production is inhibited in the presence of water vapor because of its interaction with OH•. When ozone content in liquids was measured, it was found to be quite present and below the detection limit (Ayala et al., 2014).

Although H_2O_2 is a reasonably stable species, depending on the liquid environment, it can transform into superoxides in the form of hydroxyl radicals or hydroperoxy radicals. Niket and his coworkers demonstrated, using the dielectric plasma system, that H_2O_2 concentrations in PAW rose with the duration of the plasma treatment and stayed constant throughout a prolonged post treatment storage period (Laurita et al., 2015; Lukes et al., 2014). In plasma-activated liquids, H_2O_2 is a biologically active substance with notable antibacterial and cytotoxic qualities (Julák et al., 2018; Niquet et al., 2018).

2.7.2.3 Reactive nitrogen species

In addition to ROS, PAW also includes low-level transient RNS, such as nitrogen dioxide (NO_2•) and peroxynitrous acid (ONOOH)/peroxynitrite ($ONOO^-$) radicals, as well as nitric (HNO_3) and nitrous acid (HNO_2) radicals. Nitrogen oxide (NO) is created when nitrogen and oxygen from the gaseous phase separate in the presence of air. NO then combines with water to produce acids.

$$NO_2^- + H^+ \rightarrow HN0_2$$

$$NO_2° + OH° \rightarrow HNO_3$$

Either NO_2's electron capture or NO's oxidation results in the formation of nitrite ions. The pH and acidic surroundings are reduced as a result of its creation. Rapid pH decrease speeds up nitrite disproportionate or nitrite breakdown to nitrates, as well as H_2O_2 interacting with nitrites to create $ONOO^-$s.

$$3NO_2^- + 3H^+ \rightarrow 2NO + NO_3^- + H_3O^+$$

$$2HNO_2 \rightarrow NO° + NO_2° + H_2O$$

$$2NO_2 + H_2O \rightarrow NO_3^- + NO_2^- + 2H^+$$

$$NO_2^- + H_2O_2 + H^+ \rightarrow ONOOH + H_2O$$

$$ONOOH \rightarrow NO_2^{\circ} + OH^{\circ}$$

$$ONOOH \rightarrow HNO_3 \rightarrow NO_3^{-} + H^{+}$$

Since ONOOH has a pKa of 6.5 to 6.8, physiological pH contains both the acid and the anion (Gupta et al., 2009). In acidic solutions, $ONOO^-$ has a lifetime of <1 s before decaying to OH• and NO_2• or altering into the more stable nitrate ion (Tarabová et al., 2019).

Due to their potent oxidizing characteristics, both $ONOO^-$ forms are thought to contribute significantly to the germicidal actions in PAW. They have the ability to oxidize cellular constituents, diffuse past the cell membrane, function as a nitrating agent, and mediate cytotoxic effects (Ikawa et al., 2016; Naïtali et al., 2012). Because of their high reactivity, short lifespan, rapid decay, and low concentrations, $ONOO^-$s are challenging to detect. However, using direct or indirect measuring methods, some studies have shown the presence of ONOOH/$ONOO^-$ in plasma-activated liquids (Girard et al., 2016; Tarabová et al., 2019). Other short-lived but highly reactive carbonate radical anions exist as well, such as CO_3•, which is created when carbon dioxide reacts with $ONOO^-$ anion (Bonini et al., 1999). Strong oxidizers and carbonate radicals can start a variety of harmful processes that are typically linked to hydroxyl radicals in biological literature. Strong oxidizers, carbonate radicals can start a variety of harmful processes that are typically linked to hydroxyl radicals in biological literature (Pacher et al., 2007).

2.7.3 PAW disinfection properties.

The use of PAW as a disinfectant has numerous benefits. When compared to some conventional chemical sanitizers, whose by-product has caused growing worries for the environment and public health, it is more environmentally friendly (Ölmez & Kretzschmar,

2009). After generation, PAW is typically employed as a source of the antibiotic or disinfection solution; this is useful for heat-sensitive samples since it excludes heat, electric fields, and UV rays from direct plasma treatment. Furthermore, PAW's responsiveness and antibacterial qualities can hold steady for a while under the right storage circumstances, allowing for scale-up applications (Shen et al., 2016). Thus, it is crucial to develop an understanding of PAW's intricate structure and the mechanism of action that triggers the inactivation process.

Because fresh foods have irregular shapes and textures, it may be difficult to use PAW to remove bacteria (Herianto et al., 2021). According to Tian et al. (2017) the fruit's surface and its rough skin surface may be able to stop reactive species from PAW from penetrating.

Joshi et al., 2018 inactivated *Enterobacter aerogenes* on a variety of fruit surfaces using PAW and plasma activated acidified buffer (PAAB). They found that, after three minutes of treatment, the inactivation efficiency of PAW was independent of the fruit surface's texture, but not that of PAAB. The results of PAAB treatment indicated that the smoothest surface, glass, had the biggest reduction (6.32 ± 0.43 log CFU per surface), whereas the roughest surface, spiny gourd, exhibited a much lower reduction (2.52 ± 0.46 log CFU per surface). They postulated that surface characteristics such as hydrophobicity and the existence of cuticular waxes could impact bacterial adherence, hence changing the functionality of PAW and PAAB. However, Joshi et al. (2018) suggested using PAW to wash fruits and vegetables in dump tanks.

According to (Xu et al., 2016), button mushrooms treated with PAW washing for seven days at 20°C showed a significant delay in microbial development. When stored, PAW produced by plasma exposure for 5 and 10 minutes demonstrated superior microbiological disinfection compared to PAW produced by plasma exposure for 15 minutes. They conjectured that the oxidative stress brought on by the high quantities of ROS species was what caused the

mushroom deterioration. Ready-to-eat salted and shredded cabbage was sequentially subjected to PAW washing and mild heating, as described by Adhikari et al. (2019) in a recent study. They asserted that, without compromising quality, little heating of cabbage following PAW washing greatly decreased the microbial load.

2.7.4 Factors affecting Inactivation efficiency of PAW.

2.7.4.1 Microbial properties

Like other sterilizing procedures, the bactericidal effects of NTP are significantly influenced by the innate elements-microbial qualities (Azharonok et al., 2009). Using a high-frequency barrier discharge plasma, it was discovered that the survival rate of *E.coli* was less than 0.2% at an initial inoculum (N0) of roughly 3 logs, but it was more than 71% at a N0 of roughly 4.1 logs. *S. aureus* also yields the same results; the survival proportion was 36% with N0 of 3.9 logs and 83% with N0 of 4.4 logs. Higher bacterial concentrations were thought to have the effect of clustering more cells together, which reduced the likelihood that plasma active substances would reach the bacterium cells. Microbial species also have a major impact on the rate and extent of bactericidal effects by NTP, except for initial inoculums. Bacterial spores with hard, multilayered coatings are generally more durable than vegetative cells so they are not easily inactivated by NTP (Tseng et al., 2012). Furthermore, fungi typically have better NTP resistance than bacteria. Since fungi's cell walls are made of chitin, which is more stiff than bacteria's cell walls made of peptidoglycan (Hong et al., 2009). Both the physiological phase and species diversity are impacted by microbial resistance to NTP. Deng et al. (2007) found that when exposed to NTP for the same amount of time, *E.coli* cells in the 12-hour growth state reduced more than those in the 24- and 48-hour growth states. This

outcome was comparable to the research report of Yu et al., 2006 where inactivation of a cold atmospheric gas plasma on *S. Typhimurium* was done. However, it appears this to be independent of the microbial development phase, according to Fernandez et al. (2013). In the future, additional research is needed to clarify the connection between the physiological states of microorganisms and NTP, as the cause of these variations is currently unknown.

Table 2.2 Microbial properties affecting microbial inactivation by non-thermal plasma.

Micro-organisms	**Matrix studied**	**Plasma type**	**Microbial properties**	**Main result**	**Reference**
E.coli	Almonds	Non-thermal plasma	Growth stages	Cells of 12-h growth phase showing slightly higher inactivation rate than those of 24h & 48h growth.	(Deng et al., 2007)
E.coli NCTC 12900, E.coli ATCC 25922, L.monocytogenes NCTC 11994	Sterile phosphate buffered saline (PBS)	DBD plasma	Gram characteristics	$Gram^+$ microbe more sensitive than $Gram^-$	(Lu et al., 2014)
			Antioxidative activity	Higher antioxidative capacity, more sensitive	

Salmonella, E.coli and L.monocytogenes	Tomato and strawberry surface	Atmospheric cold plasma (ACP)	Gram characteristics	Gram$^-$ bacteria ore susceptibility than Gram$^+$ bacteria	(Ziuzina et al., 2014)
E.coli, B. atrophaeus and B. atrophaeus spores	Petri dishes	One atmosphere uniform glow discharge plasma	Gram characteristics	Gram$^-$ microbe inactivated easily than Gram$^+$	(Sharma et al., 2005)

2.7.4.2 Processing parameters

The food matrix, pH, relative humidity, and other environmental conditions all have a big impact on how well NTP sterilizes. For example, it has been discovered that the acidity of the feeding matrix affects the bacteria's ability to withstand various stresses, such as heat, pulsed electric fields, and so forth (Dasan et al., 2016). Notice that increased voltage and frequency of DBD plasma had resulted in a higher decrease of *Escherichia coli* on the surface of unshelled almonds. DBD plasma with 20 kV and 25 kV produced around 2.43 and 4.12 log reductions, respectively, at a constant frequency and treatment duration, whereas those with 16 kV produced just 1.0 log reduction. One explanation could be that increased input energy density is produced by higher voltage and frequency. Another important aspect affecting the effectiveness of NTP antibiotics is exposure mode, yet different research produced differing findings. Numerous investigations revealed that the antibacterial effect of direct exposure was greater than that of indirect exposure (Dobrynin et al., 2009). (Lu et al., 2014), however,

suggested the exact opposite outcome. They clarified that it was most likely the outcome of reactive radicals recombining before they reached the sample. Furthermore, the category and quantity of reactive species generated by NTP are determined by the working gas types selected (Basaran et al., 2008). Discovered that when DBD plasma with He/O_2 input gas was used to treat the cheese slices, the amount of *E. coli* and *Staphylococcus aureus* decreased more than when DBD plasma when He alone was used. They stated that increasing the amount of oxygen contributed to the production of additional O_2^-based radicals (such as OH, O_2^-, $^1O_{2-}$, etc.), which strengthened the antibacterial action (Eto et al., 2008). Using a flexible sheet-type DBD plasma to act on *Geobacillus stearothermophilus* spores positioned on a stainless steel disk in the shape of a bowl and using varying gas mixture ratios of N_2 and O_2, they discovered that a larger O_2 gas level produced more ozone and accelerated the inactivation of spores.

Table 2.3 Processing parameters affecting microbial inactivation by non-thermal plasma.

Microorganism	Matrix studied	Plasma type	Processing parameters	Results	Reference
Aspergillus parasiticus	Nut surface	Low Pressure cold plasma	Gas type	Higher inactivated effect of SF_6 than that of air	(Basaran et al., 2008)
E.coli, Salmonella stanley	Golden delicious apples	Gliding arc plasma	Flow rate	Higher Flow rate, higher bactericidal effect	(Niemira & Sites, 2008)

E.coli & *L.monocytogenes*	Pork loins	Dielectric barrier discharge plasma(DBD plasma)	Gastype & treatment time	He/O_2 showing higher efficiency of microbial reduction when compared to He alone Higher inactivation with longer treatment time.	(Kim et al., 2013)
E.coli	Almonds	Non-thermal plasma	Voltage & frequency	Higher voltage & increasing frequency, greater bacterial reduction.	(Deng et al., 2007)
E.coli, *L.monocytogenes & S.Typhimurium*	Bacon	Atmospheric pressure plasma (APP)	Input power & gastype (He & He/O_2)	Higher microbial reduction with Higher input power. Addition of	(Kim et al., 2011)

				O_2 improving microbial inactivation.	
S. aureus	Beef, jerky, agar plates, and polystyrene plates.	Radiofrequency atmospheric pressure plasma	Input power & exposure time	Higher microbial reduction with higher input power and longer treatment time.	(Yun et al., 2010)
L. monocytogenes, E.coli 0157 : H7, and S.typhimurium	Pork-butt and beef-loin	DBD plasma	Treatment time	Higher inactivation with longer treatment time.	(Jayasena et al., 2015)

2.7.4.3 Environmental elements

The food matrix, pH, relative humidity, and other environmental conditions all have a big impact on how well NTP sterilizes. For example, it has been discovered that the acidity of the feeding matrix affects the bacteria's ability to withstand various stresses, such as heat, pulsed electric fields, and so forth (Kayes et al., 2007; Manas & Pagán, 2005). According to Kayes et al. (2007), after 30 seconds of plasma treatment, the *Bacillus cereus* decrease was only 2.1 logs at pH 7, compared to roughly 4.9 logs at pH 5. Concerning distinct matrixes, according to Perni et al., 2008, microorganisms put on the filter membrane exhibited a higher

inactivated capacity in a cold ambient plasma than those on the fruit surface (mango and cantaloupe melon). They suggested that it has to do with bacteria migrating at a specific speed from the mangoes' outer to internal tissues. Additionally, Yong et al. (2015) found that a DBD plasma exhibited greater inactivated efficiency of *Salmonella typhimurium, Listeria monocytogenes,* and *E. coli* O157:H7 on agar plates compared to a slice of cheddar cheese.

Table 2.4 Environmental elements affecting microbial inactivation by non-thermal plasma.

Microorganism	Matrix studied	Plasma type	Microbial properties	Results	Reference
E.coli, Sacharomyces cerevisiae	Cantaloupe melon & Freshcut mango	Cold atmospheric gas plasma	matrix	Higher inactivated efficiency on membrane filter than that on fruit surface	(Perni et al., 2008)
S.Typhimurium	Lettuce & strawberry & potato tissue	Nitrogen cold atmospheric plasma	matrix	Diff inactivated performances on lettuce, strawberry & potato surface	(Fernandez et al., 2013)
L.monocytogenes & E.coli	Celery radicchio & deionized water	DBD Plasma	matrix	Reduction in inactivation efficacy with the presence	(Berardinelli et al., 2016)

				of vegetables in the deionised water	
G. stearthermophilus	Wheat grains & polypropylene surfaces	DBD plasma	matrix	Inactivation more efficient on polyethylene surface than on wheat grains	(Butscher et al., 2016)
S.aureus	Beef jerky, agar plates, and polystyrene plates	Radiofrequency atmospheric pressure plasma	Matrix	Microbial inactivation rate slower on the beef jerky than that on the agar plates or polystyrene plates.	(Kim et al., 2014)
E.coli	Lettuce, baby carrots & cocktail tomatoes	Atmospheric pressure cold plasma (APCP)	Matrix	Tomato easier to disinfect than carrots lettuce.	(Bermúdez-Aguirre et al., 2013)

2.8 Conclusion

In conclusion, the review of egg disinfection methods and the exploration of of plasma-activated water (PAW) disinfection; it shows how crucial it is to guarantee the quality and safety of eggs throughout the food supply chain. Although pathogen contamination risks have been reduced by using traditional disinfection techniques, new technologies such as PAW, with their strong antimicrobial capabilities and environmentally friendly nature, they can offer viable alternatives.

Every approach has its own benefits and drawbacks; therefore, it is important to carefully weigh aspects like safety, usability, and effectiveness in practical applications. The findings show PAW's exceptional antibacterial activity, which has been shown to successfully lower the load of dangerous pathogens on egg surfaces, including *Salmonella spp.* and *E. coli*, without leaving behind toxic residues. This feature responds to the increasing demands from consumers and authorities for food processing techniques that reduce the use of chemicals and improve food safety. The capacity of PAW to produce reactive oxygen and nitrogen species, which efficiently target a variety of diseases while minimizing environmental damage, sets it apart from the other techniques.

2.9 Connecting text

Chemical disinfectants can become less effective over time when germs get resistant to them. It may become more difficult to control infections as a result of this resistance, which can cause resistant strains to multiply. Several sanitizers have been used or discussed in egg-washing methods, including organic acids and chlorine-based sanitizers (Wang & Slavik, 1998). In the food industry, conventional chemical disinfectants, including those based on chlorine, raise the possibility of cancer, which is a public health problem (Bai et al., 2020). Thus, the development of novel disinfectants - like PAW - that can provide defense against spoiling microbes, preserve nutritional content, and be sustainable in terms of energy efficiency and environmental safety is desperately needed (Perinban et al., 2019). The prior analysis focused on conventional disinfection methods such the use of chlorine, UV light, and organic acids and PAW efficiency as a disinfectant. These methods, while successful in different ways, frequently have drawbacks in terms of their effectiveness, their impact on egg quality, and their environmental implications. Building on this basis, the investigation of PAW as a novel and eco-friendly substitute takes center stage. Plasma is applied to water to create PAW, which is a combination of reactive species with strong antibacterial characteristics. This technology provides a viable answer that satisfies growing consumer and regulatory demands for disinfection techniques that are more environmentally friendly and leave no trace.

Chapter 3

Plasma activated water for disinfection of *E.coli* inoculated eggs and changes in quality attributes upon storage.

3.1 Abstract

The study investigates the disinfection efficiency of Plasma activated water on *E.coli* inoculated shell eggs. The effect of PAW treatment times (5, 10, 15, 20 minutes) and dip times (30, 90, 150 seconds) on the microbial load of *E.coli* inoculated eggs was shown. Furthermore, this study assessed the impact of PAW treatments (5, 10 minutes) on egg quality during storage intervals of 0, 7 and 14days. Based on the results longer exposure times of PAW significantly improved the disinfection efficacy, demonstrating the powerful bactericidal effects of PAW. Quality analysis showed no significant effects in albumen turbidity, weight loss, color index, or eggshell strength during the 14-day storage period. The yolk index showed significant changes after the treatment. More research is necessary to determine how this alteration may affect the sensory characteristics of eggs. This work demonstrates a potential approach in dealing with microbial contamination without losing egg quality, highlighting PAW's feasibility as a secure and efficient substitute for traditional ways of disinfecting eggs.

3.2 Introduction

One of the most prevalent facultative anaerobic bacteria found in both animal and human digestive tracts is *Escherichia coli*. Although they typically coexist in commensals, certain serotypes can seriously infect humans, animals, and birds (Vinayananda et al., 2017). The

risk of outbreaks related to eggs and egg products has increased due to the ongoing rise in both egg production and consumption (Choi et al., 2015). Horizontal transmission, commonly referred to as external contamination of eggs, is more likely to happen than vertical transfer (Miyamoto et al., 1998). The most common horizontal transmission occurs right after oviposition via the eggshell (Miyamoto et al., 1998). After oviposition, the temperature of the eggs is about 42°C. When an eggshell cools, its contents contract and separate from the shell, creating a vacuum between the membrane of the shell and the shell (M. L. Hutchison et al., 2003; Miyamoto et al., 1998). Through tiny holes in its shell, the egg draws in water and air from the medium around it to balance this pressure differential (Haines & Moran, 1940). Bacteria from tainted water may potentially enter as a result of this water intake (Board, 1980).

In order to inactivate Salmonella, shell eggs must be cleaned in the US with sanitizers that have been approved by the FDA. Nonetheless, shell eggs are not cleaned in Europe to preserve the egg cuticle layer, which is the egg's natural barrier. Keeping the cuticle layer intact helps stop Salmonella from entering the body. To lower the incidence of *salmonellosis*, industrial washing of fresh eggs has been enforced in the United States (Hutchison et al., 2003). Because they are inexpensive and very effective, quaternary ammonia (QA) and chlorine-based sanitizers are frequently employed in industrial egg washing. These sanitizers have the potential to produce waste streams that could contaminate the environment and leave chemical residues on eggs' surface (Cao et al., 2009). The egg rule permits the use of QA and chlorine-based sanitizers, but their application has also been shown to degrade the proteinaceous egg cuticle, impairing the eggs' built-in defense against bacterial infiltration (Bialka et al., 2004). Additional research has shown that mechanically brushing eggs can result in abrasions, which can harm the cuticle of the egg as well (Ball et al., 1975; Laukkanen et al., 2006). Reusing wash water can raise the risk of cross-contamination

between batches of eggs even more (Board, 1980; Hutchison et al., 2003). It has been demonstrated that eggs exposed to cold plasma, or atmospheric gaseous plasma, reduce *Salmonella enteritidis* by more than 5 log CFU/egg-shell, without altering the eggs' shell strength or cuticle layer (Ragni et al., 2010). However, the industry would need to employ processing times of up to 90 minutes if this nonthermal technology was to be used, which would not be practical on a wide scale. In recent times Plasma Activated Water (PAW) has been explored for its suitability for fresh food disinfection. Plasma-activated water is produced by exposing water to nonthermal plasma and the subsequent transfer of reactive species from the gas phase plasma to the water. Though PAW has been reported to be an effective disinfectant against various food pathogenic microorganisms, information on its stability, process optimization, and reactivity with food components is still largely unexplored. The Public Health Agency of Canada (PHAC) collaborated with provincial public health partners, the Canadian Food Inspection Agency (CFIA), and Health Canada to investigate the outbreak of *Salmonella* infections that occurred in Newfoundland Labrador, and Nova Scotia. The outbreak appears to be over, and the investigation is winding down. Investigation findings identified exposure to eggs as a likely source of the outbreak. Many of the individuals who became sick reported consuming, preparing, cooking, and baking at home with eggs. Some individuals reported exposure to eggs at an institution (including nursing homes and hospitals) where they resided or worked before becoming ill. Although this outbreak appears to be over, it serves as a reminder that eggs can sometimes be contaminated with *Salmonella* bacteria on the shell and inside the egg. The bacteria are most often transmitted to people when they improperly handle, eat, or cook contaminated foods. Salmonellosis in humans is still the second most commonly reported bacterial disease. It has been observed that egg and egg products are still the most frequently reported vehicles (47.2%) for *S. Enteritidis* food-borne outbreaks. For the egg industry, ensuring the quality

and safety of shell eggs is essential. A rising number of people are interested in creating innovative nonthermal egg-washing techniques because they are safer than existing procedures, more environmentally friendly, and produce higher-quality eggs. Therefore, the objective of this research was to determine how efficient PAW is in inactivating the *E.coli* on inoculated eggs. Subsequently, the freshness of PAW treated eggs also changes in certain quality parameters during storage, and hence it was also investigated.

3.3 Materials and Methodology

3.3.1 Plasma equipment and PAW preparation

An atmospheric pressure dielectric barrier discharge non thermal plasma system was used for the generation of PAW (Fig. 3.1). Briefly, the non thermal plasma (NTP) system consists of two copper electrodes placed over a glass tube and connected to a high voltage generator (PVM 500, Information Unlimited, and USA). The glass tube also acted as a dielectric barrier and the internal gap in the tube was 3 mm. Argon (Ar) and oxygen (O_2) gas mixture (98 %: 2 %) was used as a working gas and the plasma was generated at 10 kV (peak-peak) voltage and 20 kHz Frequency. 150 mL of deionized water was circulated inside the glass tube and exposed to plasma above water in the discharge gap at 300 mL/min flow rate to produce PAW. PAW was activated with NTP for 5 min (P5), 10 min (P10), 15 min (P15), and 20 min (P20).

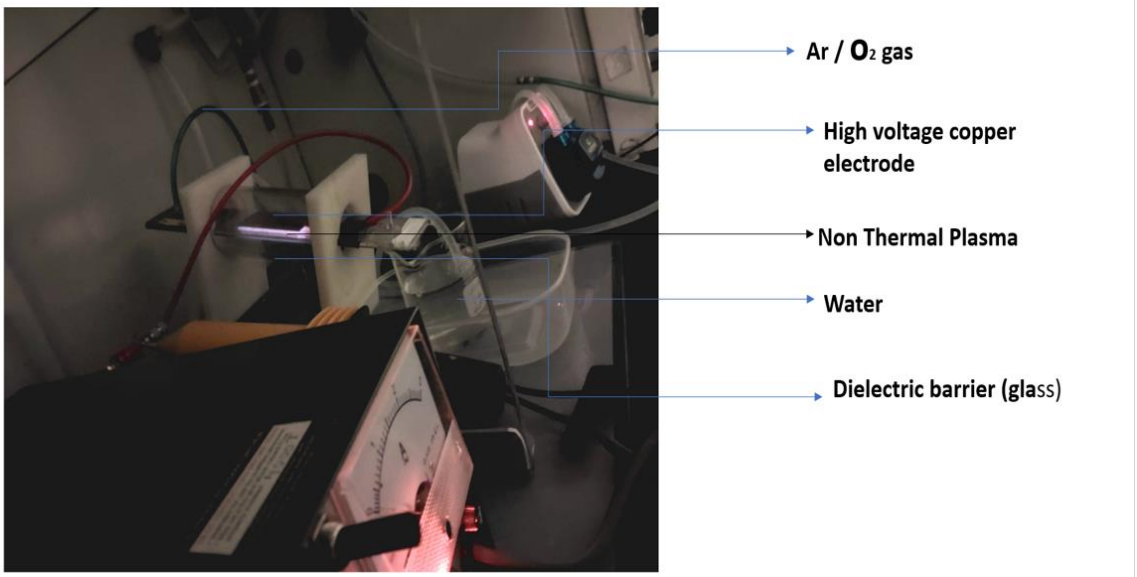

Figure 3.1 Schematic diagram of the PAW generation system.

3.3.2 Preparation of bacterial inoculums

Overnight culture of Escherichia coli K12 strain (ATCC 15597; C-3000) in LB broth (Oxoid, Canada) is used as a target bacterium in this study. It is the most common strain to cause illness in people. The bacterial culture was grown overnight (20-24 h) at 37^0C to reach a stationary phase in LB (Luria-Bertani) Broth. The inoculated culture was incubated for 37^0c and shaken at 200-250 rpm to obtain an initial bacterial concentration or cell density of approximately 9 log colony-forming units per mL (CFU/mL) before use.

Figure 3.2) Overnight *E. coli* culture b) Eggs used for inoculation.

3.3.3 Inactivation of *E.coli* on Eggs by PAW

Commercially available eggs (Burn brae brand) were purchased from Costco. Before the study commenced, the natural microorganisms on all the surfaces of the eggs were removed via the following procedure, whose effectiveness was verified by preliminary studies: All eggs were washed with RO water, disinfected with 75% ethanol for 20–30 s, rinsed by sterile water, wiped with a sterile paper towel, and finally dried in a laminar hood. The *E. coli* culture was incubated at 37^0c for 24hrs to achieve a population of 9 log cfu/ml; a 100-µL bacterial suspension was placed onto the top surface of each egg in 15–30 drops and then air dried for 20–30 min in a laminar hood. The inoculated eggs were placed into beakers containing pre-activated water for 5 minutes, 10 minutes, 15 minutes, and 20 minutes. The water amount was 150 mL. The inoculated eggs placed in sterile water were used as the control. After treatment, the egg sample was transferred into a sterile bag with 100 mL of phosphate buffer saline (PBS, pH 7.2) and then gently rubbed by hand for 2 min. The PBS was decimally diluted serially, and 0.1 mL was spread onto plate count agar (PCA). Duplicate plates were used for each dilution, and the plates were incubated at 37^0C for 18–24 h.

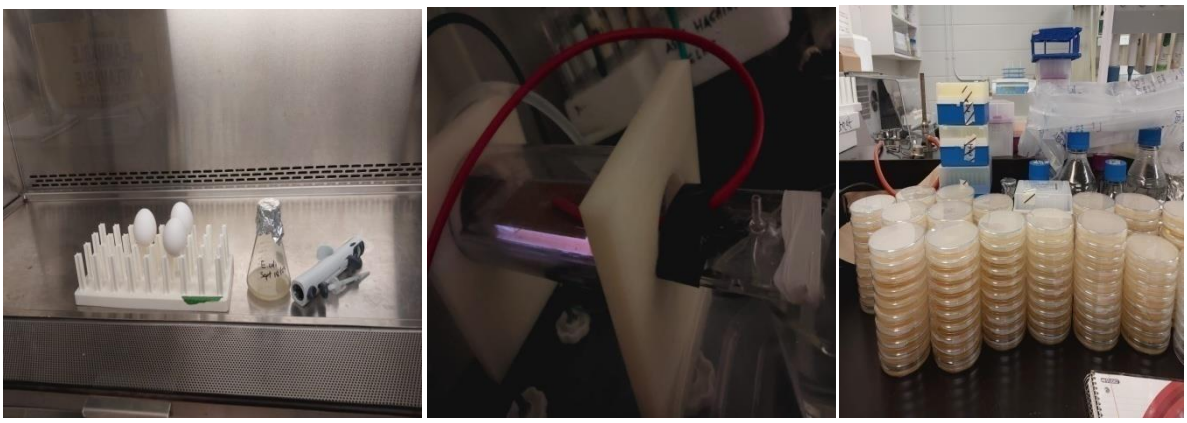

Figure 3.3 (a) eggs contaminated with *E. coli* (b) PAW treatment (c) Plating after serial dilution.

3.3.4 Determination of Egg Freshness

After obtaining the optimal procedure for inactive *E. coli* on the egg's surface, a fresh batch of eggs was treated using the optimal procedure without inoculation for storage studies. The eggs were treated with PAW 5 and PAW 10, and the unwashed eggs were used as controls. All the treated eggs are exposed to PAW water for 180 seconds and were air dried for some time and then stored in the egg cartons in an undisturbed place in the lab at room temperature, and the freshness of the eggs was determined on the testing day (Day 0), as well as 7 and 14 days after treatment.

3.4 Preparation of solutions with Different specific Gravities

Three amounts of NaCl (50, 35, or 15 g) were added to 500 mL of water to produce saline at three gravities (1.078, 1.050, and 1.020 g/mL). The eggs that did not float in the saline with higher specific gravities were determined to be very fresh.

Figure 3.4 Different concentrations of saline to determine freshness of eggs.

3.5 Measurements of yolk Index and Albumen Turbidity

The eggs were cracked and placed on a plate. The height and diameter of the yolk and albumen were measured. Yolk and albumen indices were defined as the ratio of the height (mm) and diameter (mm). Higher indices indicated higher degree of freshness. The eggs were deemed to be non-fresh if the yolk index was less than 0.300. In fresh eggs, the albumen turbidity is a measurement of thermocoagulation or protein denaturation, and it should have a steady viscosity and specific turbidity. A spectrophotometer is commonly used to measure it (Yang et al., 2019).

The minimum albumen turbidity in raw eggs is around 0.100 units measured at 600 nm (Perry et al., 2011; Yang et al., 2019).

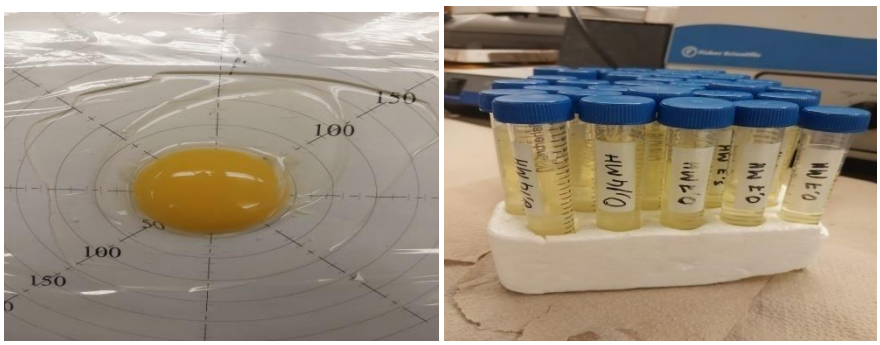

Figure 3.5 (a) Yolk index (b) Albumin turbidity.

3.6 Eggshell strength and measurement of weight-loss rate

To assess the structural integrity of the entire egg, a compression test was made using Instron 4502 Universal testing machine (Instron, Norwood, USA). which was assessed on days 1, 7 and 14. In a compression test, the egg is subjected to pressure until it breaks. It evaluates how well the egg withstands force applied from multiple directions. The egg was placed in a plastic egg tray vertically and the eggshell was punctured respectively at the top (small end) and bottom (large end) under the following conditions: a crosshead speed (5 mm/min), Sample rate 10 (pts/sec), Temperature 22^0c, Humidity (50%) and a force of 500N. Weight loss (%) of eggs during storage was measured on days 0, 7 and 14. The eggs were weighed using a digital scale, highly fresh eggs showed a lower ratio of weight loss.

Weight loss (%) = $\frac{(m_0 - m_1)}{m_0} \times 100$

Where m_0 and m_1 represent the eggs' initial weight and final mass, respectively.

3.7 Color Measurement and Observations via Scanning Electron Microscope (SEM)

Color values of the eggs in CIE (L*, a*, b*) color scale was measured using a chroma meter (CR-300 Chroma, Minolta, Japan). The L* value measures lightness, the a* value is the greenness-redness, and the b* value of the blueness-yellowness. The Whiteness Index was calculated using the formula WI = L* - 3b* Park (1994). The images of the surface cuticles of the eggs were captured through SEM (TM300, Hitachi High- Technologies Corporation., Tokyo, Japan.

3.8 Statistical analysis

A 3*3 full factorial design was used for microbiological disinfection studies and central composite design was used for storage studies. All analyses were conducted in triplicates and the results are presented as mean standard deviation. The data was analyzed by one-way ANOVA and the significant difference in the treatment means at the 5% level is compared by Student's test of the difference range test using JMP Pro (Version 15, SAS Institute Inc, NC, USA). The results of SEM imaging were analyzed descriptively.

3.9 Results and Discussion

3.9.1 Impact of PAW Treatment Time and Dip Time on *E.coli* Disinfection

Distilled water was used as the water source due to its softness and availability. Furthermore, the PAW that was generated at 5 mins, 10 mins, 15 mins, and 20 mins were used immediately after activation and the *E. coli* inoculated eggs were dipped for 150s, 90s, and 30s (Table 3.1). Thus, these combinations were used to generate the PAW used in the antibacterial test on the shell eggs. The log reduction of E. coli tends to rise with prolonged exposure to PAW, suggesting that PAW becomes more effective at reducing germs with longer contact times. Longer treatment times result in higher log reduction (5.35 logs) at PAW 20 minutes (150 s), which supports this (Table 3.1). Various dipping times also demonstrate various effects on the reduction of *E. Coli* logarithmic. Longer dip periods typically lead to higher levels of bacterial reduction, which is consistent with the theory that more antimicrobial agent exposure leads to better disinfection.

These results indicated that the PAW should contain antibacterial Substances that enables the PAW washing to achieve higher reduction than the water washing.

Table 3.1 Populations (log CFU/egg) of E. coli on the surfaces of eggs with different PAW treatment times.

PAW Treatment	Dip time	Log reductions
Control	150	1.6 ± 0. 54
Control	90	1.5 ± 0.58
Control	30	0.5 ± 0. 36
PAW 5	150	2.3 ± 1. 95
PAW 5	90	2.2 ± 1.65
PAW 5	30	1.54 ± 0.73
PAW 10	150	4.1 ± 2.85
PAW 10	90	3.21 ± 2.36
PAW 10	30	3.02 ± 1.97
PAW 15	150	5.2 ± 4.65
PAW 15	90	3.21 ± 2.93
PAW 15	30	1.9 ± 1.5
PAW 20	150	5.35 ± 4.39
PAW 20	90	4.38 ± 4.26
PAW 20	30	2.57 ± 1.99

In some cases, the standard deviations are rather high, particularly for the longer treatment and dip periods (5.2 ± 4.65 for the longest treatment time at the longest dip time, for example). This large diversity raises the possibility that there are uncontrolled variables influencing the effectiveness of PAW treatment. These could be variances in the starting bacterial load, changes in the surface characteristics of the eggshell, or irregularities in the PAW production process.

Results of a study conducted to inactivate *E.coli* using per acetic acid and chlorine showed the average log reductions for 200 mg/kg of CL and 100mg/kg of PAA were 2.38 (37.7%)

and 2.58 (41.2%) log cfu/ml, respectively (Vinayananda et al., 2017). A similar study conducted by using cold plasma (atmospheric gaseous plasma) on salmonella reduction showed 5 log CFU/egg-shell reduction, without affecting shell strength and cuticle layer on the eggs shell (Ragni et al., 2010).

3.9.2 Effects of storage on Different Quality Attributes

The PAW-treated eggs were stored in batches in the lab at room temperature to study the changes after storing for 7 days and 14 days. Also, 0-day storage was the control.

3.9.2.1 Freshness Index of the eggs

The eggs treated with PAW for 5 minutes maintained almost the same freshness as the eggs treated for 10 minutes. On the same test day, all the PAW-treated eggs had a specific gravity of >1.078. As the storage days increased to 7 and 14 days, only 2 of 3 eggs had a specific gravity of >1.078.

3.9.2.2 Yolk Index, albumen turbidity and weight loss

Statistical analyses showed that the independent variables (paw time and storage duration) and the interaction for the independent variables did not significantly ($p<0.05$) affect the dependent variables except Yolk index. Irrespective of the paw time, storage duration significantly affected the Yolk index. A pair wise comparison showed that increased storage duration from 0 to 7 did not significantly affect the Yolk index; however, an increased storage of 14 days had a significant effect on the Yolk index. For PAW time of 5 minutes, the

yolk index increased by 4.3% when the storage duration was increased from 7 days to 14 days. Pair wise comparison showed no significant differences for the other quality attributes i.e. Albumen Turbidity, weight loss, and Eggshell strength.

Table 3.2 Changes in yolk index, albumin turbidity, and weight loss after PAW treatment and storage.

PAW	**control**			**5 min**			**10 min**		
St	control	7 days	14 days	control	7 days	14 days	control	7 days	14 days
YI	2.29±0.02[a]	2.21±0.06[a]	2.25±0.02[b]	2.27±0.007[a]	2.28±0.08[a]	2.26 ± 1.8[b]	2.36±0.03[a]	2.38±0.019[a]	2.41±0.039[b]
AT	0.117±0.03	0.51± 0.61	0.11±0.01	0.09± 0.075	0.037± 0.015	0.26 ± 0.206	0.03±0.02	0.28±0.07	0.09±0.02
WL	64.28±0.75	64.87± 1.107	64.12± 1.18	63.61±1.55	64.58± 1.47	65.52± 0.66	63.01± 0.65	64.96±1.12	63.56± 2.12

Data are presented in the form of Mean ± standard deviation. Values for the storage conditions within each plasma-activated water (PAW) condition with the same superscript are not significantly ($p<0.05$) different from each other.

3.9.2.3 **Eggshell Strength**

The egg shell strength is directly related to the quality of the shell, which plays a crucial role in protecting the eggs from external mechanical shocks (Yüceer et al., 2016). A study conducted to analyze egg quality during storage using three alkaline sterilizing agent solutions, viz., Calcium hypochlorite, Hydrogen peroxide, Sodium percarbonate showed that the puncture strength was used to indicate the maximum stress at the breaking of the eggshells. It was observed that the samples CW, HW, SW had no significant differences in breaking strength (Pan et al., 2022), this was similar to results that 100 ppm NaClO (PH 7.5) washing had no adverse influences on eggshell breaking strength (Xie et al., 2002). In general, washing an eggshell or cuticle with chemicals or sanitizers can cause physical damage to the eggshell surface, weakening the eggshell and ultimately making it less strong (Hutchison et al., 2004). However, in this study using Both PAW time and storage duration did not have a significant effect (<0.05) on Eggshell strength. This shows that washing with PAW could effectively remove contaminants and microorganisms from the eggshell surface but did not affect eggshell strength. Therefore, it was beneficial to keep the freshness and quality of eggs.

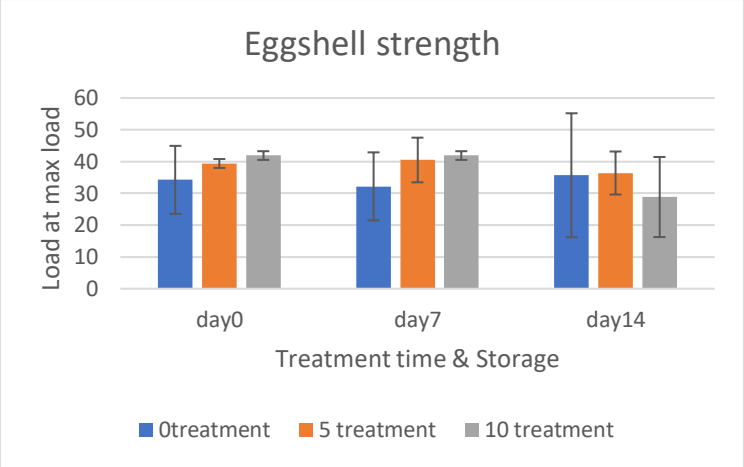

Figure 3.6 Graph depicting Egg shell strength upon PAW treatment and storage days.

3.9.2.4 Color measurement and SEM observations.

Statistical analyses showed that the independent variables (paw time and storage duration) and the interaction for the independent variables had a significant difference (<0.05) on L* a* b* values of the Color index. At the same time, the whiteness Index remained unchanged with their interaction as well as their effect on the independent variables.

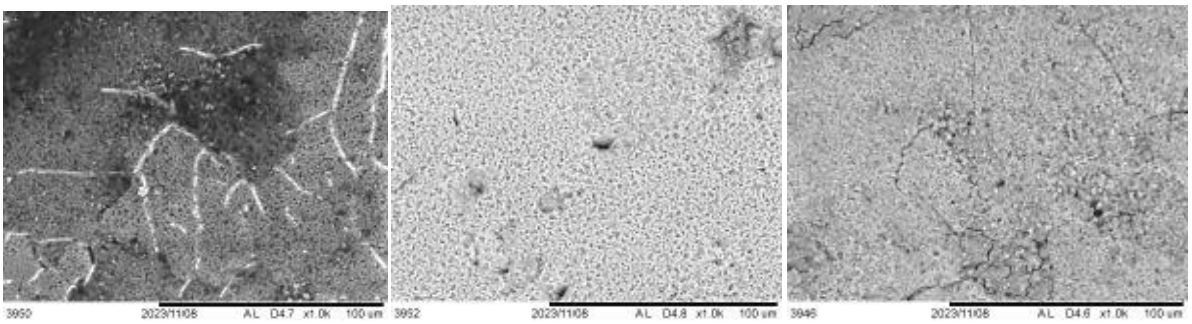

Figure 3.7 Appearance of the egg surface under SEM after different treatments. (a) Unwashed; (b) PAW treated for 5 minutes (c) PAW treated for 10 minutes.

Fig 3.7 shows SEM observation of *E. coli* inoculated eggs which are washed for PAW 5 minutes and PAW 10 minutes. The surface cuticle of eggs was used for observation under different magnifications. It is observed that the bacterial cells on the surface of the PAW-

treated eggs were deformed or broken, but clearly, there were not many differences seen between PAW washed and unwashed eggs. According to a study by Chen et al. (2019); Dominguez-Gasca et al. (2017), Egg freshness quality and surface cuticle quality are closely associated. Because the cuticle of these eggs suffered less damage and had a higher freshness index, bacteria were less likely to infiltrate through it. As a result, compared to the control, PAW treatment did not result in cuticle damage, making it an appropriate method for pathogen inactivation. However, a similar study conducted to inactivate *Pseudomonas deceprionenesis* by PAW 10 minutes treatment showed severe morphological changes and significantly higher permeability was observed (Xiang et al., 2019).

Table 3.3 Color index components L*, a*, b* and Whiteness Index (WI).

PAW	**control**			**5 min**			**10 min**		
St	Control (0) day	7 days	14 days	Control (0) day	7 days	14 days	Control (0) day	7 days	14 days
L	94.8±0.11 [b]	95.6±0.27 [a]	94.7±1.45 [b]	94.4±0.47 [b]	96.5±0.35 [a]	94.63±0.12 [b]	94.72±0.54 [b]	96.93±0.47 [a]	94.65±0.19 [b]
a *	-0.11±0.09	2.58 ±0.09	-0.24 ±0.4	-0.05 ±0.05	0.07± 0.13	-0.33 ±0.14	-0.48± 0.711	0.07 ±0.09	-0.26± 0.16
b *	2.13± 0.13 [b]	2.58± 016 [a]	2.66 ±1.21 [ab]	1.91± 0.6 [b]	2.41± 1.12 [a]	1.77 ±0.433 [ab]	0.9± 0.117 [b]	2.58±0.42 [a]	1.87±0.31 [ab]
w	88.4± 0.5	87.9 ±0.7	86.7± 5.04	88.7 ±2.27	89.3 ±3.7	89.32 ±1.23	92.006 ±0.67	89.19±1.73	89.03± 0.73

Data are presented in the form of Mean ± Standard deviation. Values for the storage conditions within each PAW treatment time with the same superscript are not significantly ($p<0.05$) different from each other.

3.9.3 Effects of PAW Treatment time and storage duration on Quality parameters

PAW treatment time, storage duration and their interaction effects on various quality parameters are determined by ANOVA. Some factors show marginal effects on certain parameters, but most of the effects are not statistically significant. The interaction between PAW and Storage duration significantly (<0.05) affects the yolk index. Some components of the color index (L*, a*, b*) also had significant effects due to the PAW treatment and Storage duration as well as their interaction. However, there was no change in the whiteness index of the egg. Other parameters of Eggshell strength and Albumen turbidity as well as SEM information did not show any significant effects from the independent variables i.e. PAW treatment time and Storage duration as well as their interactions.

Overall, the data point to the effectiveness of plasma-activated water in lowering the amount of E. coli on egg surfaces. This finding is encouraging for food safety since it suggests that PAW might be a workable way to sanitize eggs and possibly other food items. There is a need for optimization because of the variability and the fact that significant levels of E. coli are still present even after the longest treatment times and dip durations (as evidenced by log reductions less than 6, which is frequently regarded an objective for pasteurization operations). This could mean modifying the parameters of PAW production, treatment intervals, and dip times in order to attain more uniform and elevated disinfection levels. Considering the Quality and Safety Factors: Any application of PAW for food treatment must take into account potential effects on food safety and quality, even though these are not

specifically covered by the data. For instance, it would be crucial to make sure that the PAW treatment leaves no adverse residues and does not negatively impact the flavor, texture, or nutritional value of eggs.

Chapter 4

SUMMARY AND CONCLUSION

The goal of the study was to determine how well plasma-activated water (PAW) disinfected shell egg surfaces in order to lower the level of *Escherichia coli* contamination. To evaluate any effects on egg quality parameters, the experimental setup examined different PAW treatment durations (5, 10, 15, 20 minutes) and dip periods (30, 90, 150 seconds). This was followed by a storage analysis conducted over 0, 7, and 14 days. The significant decline in *E.coli* concentrations noted for all PAW treatment durations and dip times offers conclusive evidence of PAW's bactericidal characteristics. Extended exposure periods to PAW showed higher bacterial count reduction, indicating that PAW is helpful in improving the microbiological safety of shell eggs. The result holds significant importance for food safety, particularly in reducing the likelihood of food-borne illnesses linked to egg intake. Furthermore, PAW treatments do not negatively impact the chemical and physical characteristics of eggs, according to a quality examination of eggs treated with PAW for five and ten minutes throughout a maximum 14-day storage period. Important quality indices showed no obvious changes and remained constant, including albumen turbidity, weight loss, color index, and eggshell strength. These findings imply that PAW treatment can be used without affecting the eggs' structural integrity or appearance, two crucial aspects of quality from the perspectives of consumers and businesses. A considerable variation was noticed in the yolk index, which showed post-treatment modifications. While the change in yolk quality may give rise to questions about the sensory qualities of eggs, more research is needed to determine the extent of these changes and their practical effects to assess whether or not they have a major impact on the overall quality of eggs or customer acceptance. Overall, the results of this thesis show the potential of PAW as a reliable and secure substitute for

conventional techniques of disinfecting eggs, which sometimes require chemicals that may leave residues or cause unfavorable changes in the properties of the eggs. PAW is a viable method for improving the microbiological safety of eggs without sacrificing their quality, as evidenced by the efficacy and safety profile of the technique shown in this study. To maximize the benefits of PAW in the egg industry, future studies should investigate treatment condition optimization and evaluate the sensory effects of changes in the yolk index. These results pave the way for additional investigation into determining the best PAW application conditions and thoroughly evaluating its effects on all sensory aspects of eggs.

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