Chapter

Risk Management of Egg and Egg Products: Advanced Methods Applied

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Abstract

Generally, foods are thermally processed to destroy the vegetative microorganisms for food preservation. However, only thermal treatment triggers many unwanted biochemical reactions, which leads to undesirable sensorial and nutritional effects. Therefore, a number of nontraditional preservation techniques are being developed to satisfy consumer demand. Ensuring food safety and at the same time meeting such demands for retention of nutrition and quality attributes has resulted in increased interest in emerging preservation techniques. The techniques are mainly focused on liquid foods and/or vegetable matrix, a lack of data is observed concerning animal food products. On this way, this chapter discusses about the alternative technologies developed and implemented considering sanitation and preservation of eggs.

Keywords: shell eggs, sanitization, pasteurization food safety, microbiological contamination, alternative technologies

1. Introduction

The chicken egg is as considered one of the nature's most complete foods because of its high nutritional value. It is composed of a variety of nutrients, vitamins, minerals, fatty acids, and protein, which makes it one of the most important foods in human nutrition. These nutrients are efficiently absorbed and essential for the proper functioning of the human body. In addition, it has low cost and high availability in most countries, which makes it possible to increase the consumption of a food of high nutritional value by the low-income population [1].

The world consumption of eggs increases each year with a consequent increase in production. In 2014, the world production of eggs was around 1.275 trillion of units. China is the main producer (36%), followed by the United States (7.9%), India (6.0%), Mexico (4.0%), Brazil (3.5%), and Japan (3%). It is noteworthy that these countries are among the world's top 10 chicken egg-producing nations [2].

Eggs are composed of approximately 65% water, 12% protein, 11% lipid, and 12% ash; it also has low carbohydrate content and provides only 72 calories [3]. In addition, it is a source of water-soluble and fat-soluble vitamins such as retinol, tocopherol, ascorbic acid, riboflavin, pantothenic acid, and vitamin D and minerals such as calcium, iron, phosphorus, copper, and zinc [4]. Egg is considered as a food of high biological value, because it has all the amino acids required in human nutrition [5].

The egg has three main components: shell (11%), egg white (58%), and yolk (31%). In **Figure 1**, more details about the structures of the egg can be observed.

The egg white, corresponding to 58% of the whole egg, has a main component the water (about 88%), being low in fat and rich in protein. The albumen (egg white) is constituted of around 40 different types of proteins, which are responsible for the functional and antimicrobial characteristics of the egg white. The main proteins present in the egg are egg albumin (corresponding to 50% of the proteins), conalbumin, ovomucoid, lysozyme, ovomucin, avidin, and ovoglobulin [7]. In the egg white, there is also the presence of carbon dioxide, which makes it cloudy, but this substance tends to disappear in aged eggs, making it look more transparent than fresh eggs. The albumen has the ability to form foams; it is fundamental in the formulation of soufflés, meringues, and omelets, which is denatured at temperatures above 58°C [8].

The egg yolk is a central part that lies within the egg white and it is yellow in color, representing 31% of the egg, and contains three-quarters of the total value of calories [7]. Pigmentation of egg yolks may vary depending on the feed of birds; however, this variation has no influence on the quality or nutritional value of the egg. The majority of the egg nutrients are present in the yolk, which is composed mainly of lipids (34%), and proteins, such as lecithin and globular proteins [9]. The lecithin protein is responsible for the emulsification of products such as mayonnaise and Hollandaise sauce [10]. The egg yolk consists of about 50% water, and its denaturation occurs at temperatures above 62.5°C [7].

The term "egg products" refers to eggs that have been removed from their shells to undergo processing operations, whether they are breaking, filtering, blending, stabilizing, pasteurizing, cooling, freezing, drying, and/or packaging. This definition includes whole eggs, yolk, or egg white that have been processed, pasteurized, and can be found in liquid, frozen, or dehydrated form [6].

Eggs are consumed worldwide because they are highly versatile, allowing them to be used in various culinary preparations. It can be served alone or as an ingredient to provide improvement on texture, flavor, structure, moisture, and increase nutritional value. Eggs also have great importance in the food industry, due to their technological characteristics, such as incorporation of air, gelatinization, and emulsification, which are desirable in meringues, biscuits, bakery products, and meat products [9].

The production route starts on farms, where the eggs are taken to the warehouses for washing, classification, and packaging into packages made with expanded polystyrene or cellulose pulp with capacity for 12 or 30 eggs.

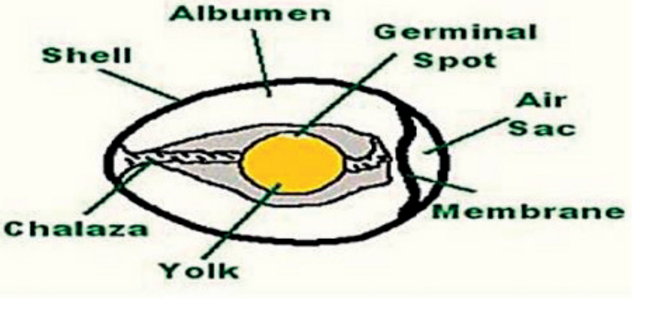

Figure 1.
Structure of the egg. Source: Souza [6].

Subsequently, they are packed in cardboard boxes and sent to the wholesale trade, in trucks, for retail resales [11].

Despite the nutritional value and functional properties of the egg, there are some problems resulting from its storage, which may interfere in quality. The fact that egg is a product rich in nutrients makes it conducive in the development of spoilage and pathogenic microorganisms [4]. Another important fact is that only 5% of the total chicken egg production in Brazil is destined for industrial processing. It is understood that the other 95% are intended for in natural consumption, where the eggs do not undergo quality control before being used in some preparation, as required in food industry [11]. Thus, the storage conditions of eggs, such as time and temperature are essential to ensure safety and quality, since they are packaged in their natural form and where there is a quality problem, it will be visible only to the consumer at the time of use.

In general, the eggs present little contamination at the moment of the posture, which usually occurs after the oviposition [12]. Eggs can be contaminated in contact with feces: by transovarian contamination (when the chicken's ovaries are infected) or by microorganism penetration through the pores and microscopic cracks in the shell, whether in the washing process, packaging transport, or storage [13]. The genera of bacteria that contribute most to the deterioration of eggs are *Pseudomonas*, *Acinetobacter*, *Proteus*, *Aeromonas*, *Alcaligenes*, *Escherichia*, *Micrococcus*, *Serratia*, *Enterobacter*, and *Flavobacterium*. Meanwhile, the pathogenic bacteria associated with eggs are *Salmonella*, *Staphylococcus*, *Campylobacter jejuni*, *Listeria monocytogenes,* and *Yersinia enterocolitica* [14].

In this way, eggs need to go through some treatment that prolongs their shelf life and also reduces the risk of contamination by foodborne microorganisms. These treatments might be thermal or nonthermal, and the latter are better known as alternative techniques to thermal treatment in eggs.

2. Heat treatment in eggs: pasteurization

In order to extend the shelf life of eggs and their products, and to reduce consumer risks related to foodborne pathogens such as *Salmonella*, it is necessary that these products go through pasteurization. Thereby, to avoid the deterioration of this food, the method with wide application is the thermal pasteurization [4].

The pasteurized egg is preferably used in the food industry, when compared to the product in nature. Besides maintaining flavor, color, nutritional value, and functional properties, this method presents operational advantages, such as reduction of losses and wastes, ease of measuring portions and less space for storage, and it saves time and labor [15].

Hot water, steam, microwave, radiofrequency (RF), and freeze-drying are some of the thermal methods used for the decontamination of eggs and egg products. Each of the methods use different range of temperature conditions [16].

Although decontamination methods using heat are efficient for microbial reduction, they can negatively affect the physical-chemical characteristics, nutritional content, and also sensorial properties, such as color and texture, making this type of food and its products less attractive to the consumer [4].

2.1 Hot water method

Currently, the use of hot water is the main method of pasteurization in whole eggs, but less than 1% of all shelled eggs are pasteurized [17]. Normally, this processing is carried out in an equipment known as a water bath (**Figure 2**). In this

Figure 2.
Water bath equipment. Source: Pombo [11].

equipment, the water is heated to a certain temperature, the eggs are placed in the equipment and submitted to heating with defined time intervals [11].

Studies have shown that the load of inoculated *Salmonella typhimurium* cells was significantly reduced after the pasteurization process of shelled eggs in a circulating water bath at 57°C for 15 minutes [17].

Whole eggs submitted to pasteurization in water bath at 57°C for 20 minutes maintained their quality and showed a reduction of the microbial load [18].

In liquid eggs, the efficacy of the circulating water bath for *Salmonella enteritidis* inactivation at 65°C was verified in an interval of 0–7 minutes, already showing a reduction of the contamination at 3 minutes. This same process exerted less impact on the egg viscosity when compared to the high-pressure treatment, which has a positive effect on functionality and allows the use of liquid eggs in various products [19].

Decontamination by this method can also be accomplished by emerging the eggs (without cracking) in water at 95°C for 10 seconds. Studies have claimed that when liquid eggs are subjected to temperatures above 70°C for 1.5 seconds, there is a significant reduction of *Salmonella enteritidis* [20, 21]. However, despite the efficiency in egg decontamination, this method affected the egg quality, altering the texture, yolk membrane strength, albumin contents, and yolk characteristics [22].

However, egg washing may decrease or remove the cuticle layer that surrounds the eggshell (responsible for antimicrobial defense), increasing the probability of microbial invasion, reducing the quality and life of the washed eggs [23].

2.2 Steam method

Steam pasteurization may be a valuable alternative to egg surface decontamination, also in relation to the ban of the use of water by the European Union in eggs. However, further studies are made on the efficacy of decontamination of this technique on eggs. Among the available studies, there are studies that investigated the applicability of a steam gun treatment to pasteurization of the egg surface. They investigated the temperatures inside and outside the egg and identified that 180°C for 8 seconds as the best treatment corresponding to the surface temperature, the highest that can be achieved without detrimental changes to egg quality. Unfortunately, no microbiological investigation was performed [24].

Whole egg pasteurization can be completed by using steam generators with 60°C for 8 seconds, while eggs spin and swirl through the aid of mechanical engineering. Then, the eggs are treated with cold air through hot air generators (20–25°C) for 32 seconds. This treatment was effective in reducing *Salmonella enteritidis* and *Salmonella typhimurium* in egg shells and did not affect the egg quality [18, 24].

Whole egg pasteurization can be done using steam generators at a temperature lower than previously reported (heating at 60°C for 8 seconds) while the eggs roll through the aid of mechanical engineering. Then, the eggs are treated with cold air through hot air generators (20–25°C) for 32 seconds. This treatment was effective in reducing *Salmonella enteritidis* and *Salmonella typhimurium* in egg shells and did not affect egg quality. This method is further recommended for pasteurization of egg yolk, egg white, and whole egg liquid [17, 23].

After evaluation and comparison of the quality characteristics of eggs treated with steam and eggs in nature, after 28 days of storage at 20°C, it could be observed that the quality parameters (pH and color) were not different, indicating that the treatment of steam does not exert negative effects on the main quality characteristics of the egg. These parameters, along with the microbial results in experimentally inoculated eggs, suggest that the industrial application of steam treatment in eggs prior to the packaging is useful to achieve a reduction of approximately 90% of the population of *Salmonella enteritidis*, which naturally infects the surface of the eggs [24].

2.3 Microwave method

Microwave-assisted thermal method is a new thermal processing technology that provides rapid volumetric heating [25]. Electromagnetic waves are able to reduce *Salmonella enteritidis*, which is often found in shell eggs. The microwave frequency ranges from 300 MHz to 300 GHz, while the wavelength ranges from 1 mm to 1 m. In order to generate heat, the microwaves interact with dielectric materials and stir the molecules in an alternating electromagnetic field. Generally, foods have excellent microwave absorption capacity due to high water or carbon content, which can result in a faster temperature increase, thus requiring less time to inactivate the present microorganisms [26].

Microwave is an easy and affordable method to heat up food. However, the way absorbed energy is distributed depends on the shape, surface area, and food matrix, besides the type of equipment used. The eggs tend to burst when using this method to warm and sanitize, if the equipment exhibits high levels of energy. Therefore, while using this type of procedure, it is ideal to use low energy levels and slowly heating up the product [27, 28].

In **Figure 3**, it is possible to visualize a representative microwave scheme adapted at the laboratory level, which enables the measurement and control of the dielectric properties of the equipment from computer software.

Studies have shown that eggshell and eggshell membrane presented transparency to the microwave. The pasteurization of whole eggs, placed with the largest extremity face up, was achieved, when the shell was heated and the yolk reached the temperature of 61.1°C. A microwave oven with power 9 for 15 seconds showed efficiency in the reduction of previously inoculated S*almonella* strains [18]. However, further investigations should be conducted regarding changes in egg rheology, viscosity, emulsifying property, and protein denaturation [16].

2.4 Radio-frequency heating

The radio-frequency band (RF) of the electromagnetic spectrum covers a wide range of high frequencies, typically in the kHz band (3 kHz $< f \leq$ 1 MHz) or MHz band (1 MHz $< f \leq$ 300 MHz) [30].

Unlike conventional systems, where thermal energy is transferred from a hot medium to a colder product resulting in large temperature gradients, radio-frequency heating involves the transfer of electromagnetic energy directly to the product, initiating heating due to friction, and interaction between molecules (heat is generated within the product). The RF heating is also known as high-frequency dielectric heating. During RF heating, the product to be heated forms a "dielectric" between two metal capacitor plates (electrodes) (**Figure 4**), which are alternately charged positively and negatively by a high electric current field [30].

RF heating is a promising application in food processing, due to the rapid and uniform spread of heat, better penetration, and low energy consumption. Researchers conducted on eggs using RF heating (10 MHz–3 GHz) using temperatures of 5–56°C indicated the eggshell and eggshell membrane are extremely transparent to this technology. The more transparent is the product investigated and pasteurized, and the more efficient is the decontamination [30, 32].

The immersion of the eggs in deionized water combined with the RF focused on the egg yolk and surface cooling showed a high security potential from the microbiological point of view [33]. The combination of RF (60 MHz) in water at 35°C for 3.5 minutes resulted in a temperature of 61°C inside the egg yolk. After that, the egg was again heated for another 20 min with water at 56.7°C. Performing this two-step process, with a total duration of 23.5 minutes, the *Escherichia coli* population significantly reduced (6.5 log); however, comparing with pasteurization only with

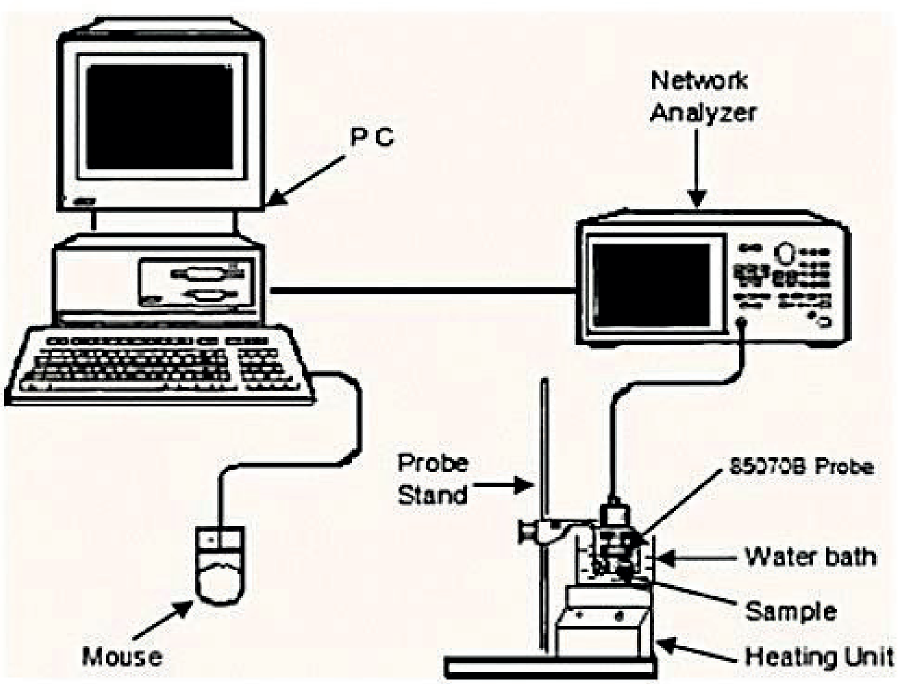

Figure 3.
Representative microwave scheme adapted at the laboratory level. Source: Dev et al. [29].

Figure 4.
Schematic representation of the RF heating process. Source: Marra et al. [31].

hot water, it took 60 minutes to reduce this microbial population by 6.6 log. The combination of the RF and hot water method was faster than the existing commercial process, using only hot water [34].

However, there is a disadvantage of RF, if it is not uniform, it can be observing the formation of coagulation rings around the egg air cell, thus damaging the decontamination process due to the impact on product quality [35].

2.5 Freeze-drying or cryodesiccation

Dehydration is a successful method of preserving eggs, it presents the advantages as follows: occupies less space in stock, provides ease of transport, good uniformity, easier use (ready-to-use product), and presents stable microbiological quality [36]. One of the main procedures used to dehydrate egg and turn it into powder is the freeze-drying or cryodesiccation method. This process consists of the rapid freezing (−50 to −60°C) of the liquid egg or part previously pasteurized and subsequent dehydration: the water contained in the product passes directly from the solid state to the vapor state by sublimation, under low temperature and vacuum conditions [36, 37].

A freeze-dryer or lyophilizer is used for the process of freeze-drying; it consists of a vacuum chamber, a heat source, a condenser, and a vacuum pump (**Figure 5**). The main function of the vacuum chamber (where the food is contained) is to resist the differences in pressure so the ice melting does not occur, and the pump helps to maintain this difference by removing noncondensable gases. The heat source is responsible for producing the energy that will evaporate the ice and is the type of source that determines the type of freeze-dryer used. The condenser retains moisture from the food and prevents its increase from inside the chamber and returning to the food [15].

The freeze-drying method applied on eggs occurs in three stages: (a) initial freezing of egg, (b) primary drying, in which the water is removed by sublimation that takes place under vacuum and the addition of heat and ends when the increase of the temperature of the egg is found in a value close to the environment or when it starts to defrost, and (c) secondary drying (also called desorption), which occurs after all ice has already been removed from the egg, but still retains an amount of liquid water (called tightly bound water), requiring a reduction of moisture to about 2–8%. For moisture reduction, the partially dried egg should be kept in the freeze-drying for about 2–6 hours and heated until its temperature equals that of the plate (20–60°C), maintaining the vacuum and evaporation of much of the wastewater [37, 38].

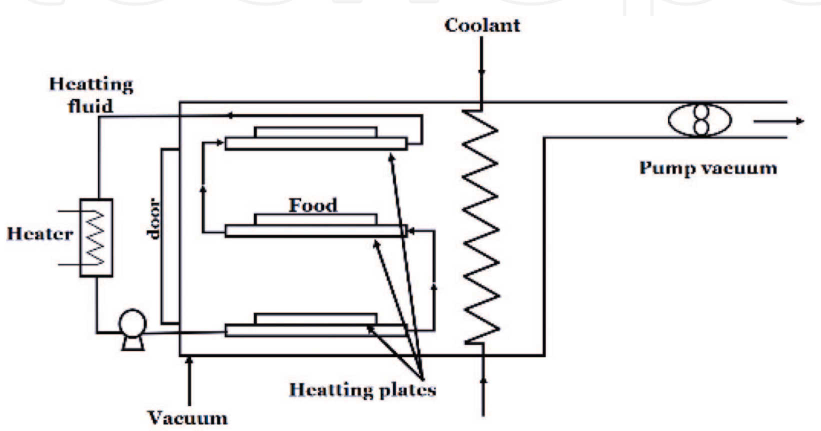

Figure 5.
Main components of the freeze-dryer. Source: Cunha et al. [15]—adaptado.

By going through the freeze-drying process, the egg and its products retain the sensory characteristics and the nutritional quality, because the temperature is not too high. In addition, they have an extended shelf life when packaged correctly. The volatile compounds are not absorbed by water vapor and are retained in the matrix of the products, allowing the retention of the egg aroma of about 80–100% [15, 39].

The main alteration that occurs in egg composition is the alteration in the quaternary and tertiary structures of the proteins. After water removal, changes occur in these structures due to exposure of the hydrophobic parts of the protein, previously protected inside the tertiary and quaternary structures, due to nonaffinity with water. The nutritional content and aroma of the eggs do not present significant changes. In addition, with the removal of water, preservation of egg powder is maintained, due to the low humidity, which reduces microbial proliferation [15, 37].

The main disadvantages of this method is that eggs may be susceptible to oxidation reactions (lipids, carotenoids, fat-soluble vitamins, and aromatic substances) if not packed into a vacuum, oxygen-impermeable, and opaque packaging [37, 39]. The freeze-drying process is time-consuming and may last up to 48 hours, depending on the batch size and the units to be processed, increasing the cost of the process. In addition, the freeze-dryer is a costly equipment [37, 40].

3. Alternative techniques for the thermal treatment in eggs

Considering that microorganisms are naturally present in any raw food, there are concerns regarding egg contamination. These products have the potential for contamination with bacteria from the animal's intestinal tract, feces, and the surrounding environment. In addition, eggs are an ideal growth medium for pathogenic bacteria that are dangerous to humans (*Salmonella, Escherichia, and Enterobacter*) [41].

Pasteurization techniques are used to prolong shelf life and maintain the quality of egg products. However, thermal techniques can have negative impact on the functional properties of this food, in the amount of nutrients, taste, and texture. Although heat processes used in egg pasteurization can ensure food safety by eliminating heat-sensitive pathogens, some heat-resistant microorganisms can survive the process, spoiling the product even under refrigerated conditions [42, 43]. In this way, new techniques are being developed and applied in the food industry.

New preservation technologies are an interesting option for producing high-quality food and extend its shelf life [44]. These technologies present a moderate impact on the sensory profile and quality attributes of the processed foods (such as flavor, color, aroma, and nutrients), giving food producers the opportunity to offer safe and high-quality food.

However, emerging techniques in the egg industry must be further studied, so they can be considered a successful processing and thus produce on a commercial scale. In this way, advantages and safety are provided not only to industry, but also to supermarkets and consumers [15, 45].

New food processing technologies include the use of physical factors to process and preserve food [46]. Among the new technologies, high hydrostatic pressure (HHP), pulsed electric fields (PEF), treatment with ozone, ultraviolet light (UV), and gamma radiation are nonthermal technologies with application in eggs and egg products.

3.1 High hydrostatic pressure

The application of HHP technology has attracted the interest of the food industry due to its microbial destruction capacity at very low or moderate temperatures, the preservation of bioactive nutrients, the improvement of the extraction of bioactive compounds, and the reduction of the allergenic potential of foods, such as eggs [47].

The HHP technology applies high pressures (usually in the range of 100 and 1000 MPa) with or without heat treatments, in order to eliminate different microorganisms and to guarantee the microbiological safety of the final product. This process is operated on a batch system, usually using water as a pressure transmission medium. The food products are packaged, loaded into the pressure vessel, and then pressurized by water [47, 48].

The HHP equipment is generally made of high-strength steel alloys, making it resistant to oxidation and rupture. HHP mainly is applied on batch equipment; however, semicontinuous systems are available. Generally, an HHP batch equipment consists of a pressure vessel (thick wall cylinder), two covers that close the pressure vessel, a yoke which controls the closing cover under the pressure condition, a pump and intensifier to create high pressure, and a process control system for loading and unloading the products [43]. An HHP batch system can be used for liquid and solid foods, while a semicontinuous HHP process can be used only for pumpable foods.

During the process, the food is packed, sealed, and loaded into a sample basket. The packaging shall consist of flexible materials, which will resist to pressurization. The sample baskets then enter the pressure vessel, which contains the pressure transmitting fluid. Water is usually used as pressure transmitting fluid on industrial scale equipment. The pump and the intensifier provide a desired pressure by compression of the pressure transmitting fluid. Thereafter, the product is maintained under the right time and pressure to achieve the desired treatment. At the end of the treatment, the vessel is depressurized and the product is unloaded from the sample basket [42, 43].

The application of heat combined with HHP can cause physical, chemical, or biological changes on the food product. These changes depend on the applied pressure, treatment time, and temperature and can include protein denaturation, changes in enzyme activities [42].

Many attempts have been made to verify if the HHP technique can be used as a substitute for thermal pasteurization, and to identifying the structural changes in the components of the egg as a result of the high pressure [44]. This technique has been evaluated as an alternative to methods already used for liquid eggs, and it has been verified that the processing conditions must be well studied, as this can cause a protein coagulation [45]. It has also been reported that pressure-induced protein denaturation may occur in eggs due to the entry of water into wells of the protein molecule [46]. However, HHP at a pressure between 200 and 350 MPa did not cause detectable protein denaturation in liquid eggs [47]. Other research has shown that HHP treatment on liquid eggs is a successful preservation opportunity. The application of 600 Mpa for a 2 minutes cycle in boiled eggs was able to extend the shelf life of these products during refrigeration [49].

HHP present important advantages for food processing, the fact that this technology does not produce deterioration of thermolabile nutrients (such as vitamins) and does not alter low molecular weight compounds, fundamentally those responsible for flavor and aroma. The high pressure does not favor the Maillard reaction or enzymatic browning; thus, it does not alter the natural flavor or color of the food [50, 51].

The application of HHP causes a number of changes in the morphology, cell membrane and biochemical reactions of microorganisms, and all these processes are related to microbial inactivation. In particular, the cell membrane is considered the main target for inactivation of microorganisms induced by pressure, and it is generally accepted that leakage of intracellular constituents across the permeabilized cell membrane is the most direct reason for cell death by high-pressure treatment [50, 52].

Research shows, in addition to the applied pressure level and treatment time, the critical parameters for microbial inactivation are pH, water activity (aw), and treatment temperature: (a) microorganisms become more susceptible to pressure at lower pH [53]; (b) water activity reduction exerts a protective effect on microorganisms against high-pressure treatments [54, 55]; and (c) thermal processing with temperatures above or below room temperature tends to increase the rate of inactivation of microorganisms [56].

The effect of microbiological inactivation on egg products by HHP was reported in a study that showed the low-pressure ranges are used to reduce the microbial load of liquid eggs by 3 log. The study concludes that increased pressure may increase the effectiveness of the treatment and thus lead to the processing of microbiologically safe products [57].

This technology has great potential for use in food processing, since it is efficient in the elimination of microorganisms, thus providing microbiological safety and increased shelf life, maintaining the nutritional and sensorial characteristics of foods [50].

3.2 Pulsed electric field

Pulsed electric field (PEF), or high-intensity electric field (HELP), is one of the nonthermal processing technologies of interest to scientists and the food industry; it is new and alternative method for preserving liquid foods. In addition, it is a promising alternative to traditional heat treatments, which presents good results, not only by enabling the destruction of microorganisms and the inactivation of enzymes, but also by maintaining the flavor, color, texture, vitamins and not only by enabling the destruction of microorganisms and the inactivation of enzymes, but also by maintaining the flavor, color, texture, vitamins, and functional thermolabile components [58].

Food processing by applying PEF involves subjecting the product to repeated electric fields (constituting the number of pulses) for short time intervals (microseconds) in order to inactivate enzymes and destroy microorganisms [59].

This method uses high voltage pulses on a treatment chamber containing food between two electrodes. The high electric intensity is acquired by accumulating a large amount of energy in a condenser, which supplies and discharges the energy in the form of pulses, for short periods of time, uniformly and with a minimum increase of temperature [60, 61]. **Figure 6** shows two types of treatment chambers used in the PEF process.

The PEF technology can be one of the most suitable methods for liquid food processing. In the last years, the technology received considerable attention from scientists, governments and interested industries as a potential technique to be fully expanded in the future year [45].

Research shows that PEF technology has been used successfully to pasteurize foods such as dairy products, a variety of fruit juices, liquid eggs, and creamy soups

Figure 6.
Types of treatment chambers used in the PEF process. Source: Fani [62].

[63]. The application of PEF in the control of spoilage or pathogenic microorganisms in different egg products has been highlighted. It has been reported that this method effectively reduces the activity of numerous microorganisms in egg products [64].

Liquid egg is widely used by the food industry and other commercial food manufacturers due to the convenience, ease of handling, and longer shelf life compared to shell eggs. Egg is a polyfunctional ingredient because of its thickening, gelling, emulsifying, foaming, coloring, and flavoring attributes, which can be used to modify the organoleptic and technological properties of many food products. In addition, liquid egg products are also valuable because of their high-quality protein content and low cost [65, 66].

Although thermal treatments represent the most available pasteurization methods for liquid eggs, they can affect their functional properties and degrade the quality of the products. Thus, the application of PEF, as a nonthermal food processing technology, might be an alternative to conventional thermal preservation methods. Combined methods with PEF, such as homogenization, show a great potential to preserve the liquid egg with small modifications of its native color, viscosity, and foaming capacity [45].

Microbial inactivation by electrical pulses depends on several factors that are critical to treatment efficacy. These factors can be classified by process parameters (pulse intensity, treatment time, and temperature), product attributes (pH, ionic compounds, and conductivity), and characteristics of microorganisms (type, concentration, and growth stage) [67].

Gram-positive bacteria are more resistant to electrical pulsed treatment than Gram-negative bacteria; this factor may be due to the rigidity of the peptidoglycan layers present in its cell wall. Due to their larger size, fungi are more sensitive to this treatment than bacteria [68].

The exposure of a biological cell to a high-intensity pulsed electric field leads to a phenomenon of membrane permeabilization. This leads to pore formation, which is reversible if the electric field is below a certain critical value and for a short period of time. This phenomenon is called electroporation and is used in genetic engineering. However, overcoming certain values of field strength and processing time, this process becomes irreversible, results in loss of cellular material, and inactivation of the cell [69].

This method has advantages such as the treatment time, which is relatively short, provides a low-temperature pasteurization, is efficient in liquid products, maintains the sensorial characteristics of the product, and shows no evidence of toxicity. Thus, this technology can complement a heat treatment, or completely replace it. However, the PEF method is not indicated for solids or liquids containing air pockets [70].

It is not clear whether the food industry will fully accept PEF as a processing technology. However, the PEF is already being used industrially in some fruit juice industries in Europe. However, the PEF method has been used industrially in some fruit juice companies in Europe, presenting a number of applications growing over the year. Nevertheless, its potential to replace or complement conventional methods comes from research related to the use of PEF in all fields of food processing [71].

3.3 Ozone

In 2001, the Food and Drug Administration (FDA) approved the use of ozone (O_3), either in gas or liquid form, as a disinfectant to be applied in food processing and product stock. Since then, special attention has been given to the use of O_3 as a potent disinfectant to be used in a variety of environments, such as hospitals, candy factories, cheese maturation rooms, and poultry hatcheries. Besides its disinfectant performance, O_3 in gaseous form has the same properties for disinfecting eggs, fresh fruits, and vegetables. In liquid form, O_3 can be used to wash poultry and fish carcasses in order to reduce or even eliminate the microbial load [72, 73].

O_3 is a triatomic form of oxygen that has been gaining space on food processing due to its high sanitizing power and rapid degradation, leaving no waste on treated foods, and known as a highly reactive antimicrobial agent. Therefore, a hypothesis to increase the shelf life of eggs would be the exposure of it to O_3. In many research, O_3 has been shown to be very efficient in the inactivation of microorganisms that could degrade food [74–76].

Research has shown that the concentration of gaseous O_3 between 4 and 6 $mg.L^{-1}$ could be used to maintain the internal quality of the eggs and extend their shelf life. Concluding, gaseous O_3 present great potential as an emerging technology to maintain fresh egg quality and also extend shelf life during storage at room temperature [77].

Due to its high instability, O_3 must be produced at the place of disinfection and its use must be immediate because it decomposes rapidly into oxygen. O_3 is generated by the exposure of air, or other gas containing normal oxygen, to a high energy source. The production forms are by the method of electric discharge (corona discharge method), electrochemical methods, and UV radiation, all of them inspired by its natural formation in atmosphere. The electric discharge method is the most commercially used, even though it has low efficiency (2–10%) and high electricity consumption. The other methods are less cost effective, but the O_3 production by the UV method is less than by the electric discharge method because it only produces O_3 in a concentration of 0.1% by weight [78].

Besides the microbicidal effect, characteristics of lower toxicity and easy handling give the O_3 advantages of use. Added to these factors, its decomposition into nontoxic oxygen and rapid degradation characterize O_3 as a nonwaste-producing disinfectant [75].

The existence of several methods of measurement of O_3 in the environment is also one of the advantages of its use as a disinfectant. Physical, physical-chemical, and chemical methods are available in the market. Physical methods measure the direct absorption in the region of the electromagnetic spectrum UV, visible light, and infrared, while physical-chemical methods are dependent on effects such as heat or chemiluminescence caused by reactions. Chemical methods refer to the quantification of products when O_3 reacts with chemical reagents, such as potassium iodide (KI), the Indigo method the most recommended in this case [72].

However, O_3 cannot be considered universally beneficial to food, because in high concentrations, it can promote oxidative rancidity, so it can cause modification on taste and color of the food product. Changes in sensory or physical-chemical attributes depend on the chemical composition of the food, the O_3 dosage, and the treatment conditions [79].

3.4 Ultraviolet

UV radiation stands out as one of the few technologies that does not generate residue to the environment and is effective in reducing the microbial load when applied correctly. The application of the germicidal effects of UV radiation covers three categories: (a) inhibition of microorganisms on the surface, (b) destruction of microorganisms in the air, and (c) sterilization of liquids. Based on these effects, the use of UV light is widely used for sanitizing water and food processes [44].

Characteristics of practicality and low cost, combined with the advantage of not producing chemical residues, coproducts or radiation at the end of the process give UV-C an excellent alternative for disinfecting environments and products [45].

UV radiation comprises the portion of the electromagnetic spectrum ranging from about 100 to 400 nm. UV radiation is composed of different wavelengths greater than the X-ray (200 nm) and smaller than visible light (400 nm). The true UV radiation is actually invisible to the human eye, but its larger portion (around 400 nm) has a violet color, hence the name ultraviolet [44].

The wavelength of UV light can be divided into three bands: long-wave (UV-A, 400–320 nm), which occur in sunlight and has little germicidal value; medium-wave (UV-B, 320–280 nm), also found in sunlight and germicidal effect; while the short-wave (UV-C, 280–100 nm), has the greatest germicidal effects and does not occur naturally, which is produced by the electric energy conversion [46].

As mentioned, sunlight can be a source of UV rays; however, it is known that the range of solar UV-C radiation with greater germicidal potential is blocked by stratospheric ozone. Artificial sources of this radiation are obtained by mercury medium pressure and low pressure lamps, which produce energy in the germicidal region and which are electrically identical to fluorescent lamps, except for the absence of phosphorus cover. These lamps consist of an airtight silica or quartz tube (both UV transmitters), with the ends endowed with tungsten electrodes with a mixture of alkaline earth metal, which facilitates the formation of the electric arc inside the lamp. Inside the tube is introduced a small amount of mercury and an inert gas—usually argon. The voltage between the electrodes produces an excitation of mercury atoms, then when they return to a level of less energy, the excited molecules emit UV light. Low-pressure UV lamps—or monochromatic—emit 85–90% of radiation at the wavelength of 254 nm, with a higher germicidal effect. Thus, in the kinetic studies of UV disinfection, the mean intensity of the germicidal radiation considered is 254 nm. On medium-pressure lamps—or polychromatic lamps—the contributions of each radiation of different wavelength shall be taken into account when determining the dose [44, 47, 48]. **Figure 7** shows a model of UV radiator.

There are two ways of applying UV light: pulsed and continuous light. The continuous mode is the conventional method; the light being emitted continuously without interruption. In pulsed UV-light mode, the UV-light is released as intermittent pulses using a capacitor, which allows to increase the energy intensity per pulse. Therefore, the pulsed mode is more effective for microbiological inactivation and the most used method [81].

The extent of UVC radiation to the microorganisms is conditional on the dose of radiation, which they can absorb. The dose required for destruction of the bacterial cell is relatively low and depends on the intensity and time of exposure. The impact of various obstacles can affect the optimum dose of UV light, because the light emitted by the germicidal lamp may not be absorbed by most of the microorganisms. Spores of microorganisms exhibit high UV resistance, and the sublethal dose may favor its growth rather than inhibiting it, so its use is important in environments and products with absence of organic matter and obstacles. In this way,

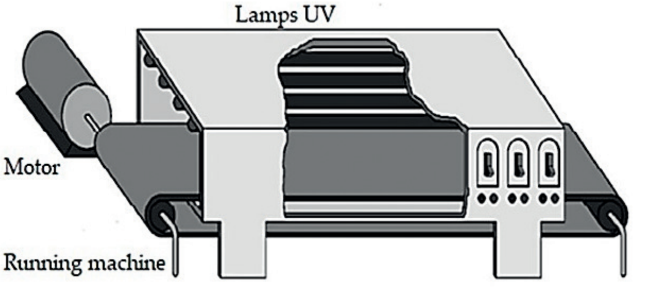

Figure 7.
UV radiation model. Source: Alexandre et al. [80].

microorganisms present on smooth and regular surfaces are more susceptible to the effects of UV light than those present on irregular surfaces [82–84].

Short-wave ultraviolet radiation (UV-C) has shown prospects for the pasteurization of liquid foods on appropriated reactors. Several studies have shown that the organoleptic properties of UV-treated liquid egg products are comparable to those untreated; therefore, those are excellent candidates for UV-C application. Regarding to the increase of the microbial load, research shows that the long-term microbial stability of liquid eggs was positively influenced by UV-C treatments and the shelf life was extended to 8 weeks in refrigerated storage. Thus, UV-C treatment is a promising technology to prolong the shelf life of liquid egg products [85, 86]. The time-temperature binomial is crucial to produce pasteurized eggs with high microbiological quality [87].

The biocidal effect occurs when UV-C radiation reaches the surface of the microorganism by overcoming the cell membrane and damaging its DNA genetic material. The DNA damage occurs through the formation of thymine dimers. The thymine dimer formation is the process of rupture of the nitrogen bases adenine and thymine (A-T), from the DNA. The rupture establishes a new chemical bond between two thymine, thus constituting the thymine dimers (T-T). The new binding prevents DNA replication and transcription, leading to the death of the microorganism [88–90].

Among the advantages of using UV-C radiation for food sterilization and disinfection are non-by-product production, does not alter sensory characteristics (taste, color, or odor), does not transmit radioactivity, it is a dry application process, and does not generate heat beyond the equipment and it is of low cost [91–93].

The main limitation of this technology involves the low degree of penetration that hinders the reach of the radiation by all the microbial load in the food. Thereby, it is more widely used in surface sterilization, for example in food packaging and in edible films. However, in liquid foods, the turbulent flow is recommended during processing [83, 94, 95].

3.5 Gamma radiation

Ionizing radiation, in the form of gamma rays, is obtained from isotopes or, commercially from X-rays and electrons, and it is applied in food preservation through microbial elimination or inhibition of biochemical changes. It has several advantages, such as low or no heat generation, low energy requirements, food preservation in a single operation, irradiation of packaged or frozen products, besides those it can cause changes in the nutritional value of food similar to other conservation methods [96].

Gamma rays are a type of electromagnetic radiation produced in nuclear decay processes. These are highly energetic due to their high frequency and consequently low wavelength. Generally, the frequency of the gamma rays is above 1019 Hz, which implies wavelengths below 10–12 m and energies above 0.1 MeV (the energy of the visible radiation ranges from 1 to 4 eV, about 50,000 times smaller) [97].

The irradiation is done in a special processing room or chamber for a certain time. The food is treated in an installation known as an irradiator. This equipment (**Figure 8**) consists of a cobalt-60 source installed in a bunker, which is an irradiation chamber whose walls are concrete shields, in the form of labyrinths. The radiation source, when the plant is not in operation, is stored in a pool (water well) with treated and demineralized water. The well is lined with a stainless steel coating, inside the shield. The food product to be irradiated is placed in containers and through a monorail are conducted into the irradiation chamber, where they receive

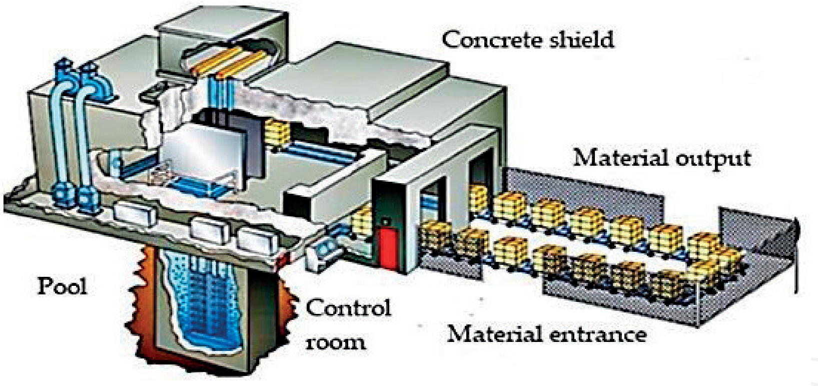

Figure 8.
Irradiation plant with cobalt-60 source. Source: Caldeira et al. [99]—adaptado.

the programmed dose of gamma radiation. Qualified operators electronically monitor the source of radiation and the treatment of the products from a console located in a room outside the irradiation chamber [98].

The gamma radiation sources commonly used on commercial plants are cobalt 60 and cesium 137. These isotope sources cannot be switched off, which is why they are kept in a water tank located below the processing area to allow an approximation of the machine operator. When the irradiator is in operation, the source is elevated and the packed food is transported by an automated conveyor through the irradiation field in a circular route that allows uniformity and efficiency of the process [96].

Regarding the inactivation of the microbial load, the efficiency of the treatment on the microorganisms depends on several factors: (a) the number of microorganisms: the higher the amount of microorganisms presents in the food, the higher the radiation dose required; (b) the food composition: microorganisms on rich media are more resistant than in buffer solution; (c) oxygen: the presence of oxygen makes the microorganisms less resistant to radiation; (d) state of matter: dehydrated or frozen cells are more resistant to radiation than in the normal state; (e) the condition of the microorganism: microorganisms in the lag phase are more resistant; and (f) the microorganism radioresistance: overall the more complex the DNA, the greater is the sensitivity of microorganisms to irradiation [96, 100].

The use of ionizing radiation is an alternative method in the reduction of pathogenic microorganism on eggs (such as *Salmonella* spp.) when the use of heat is impractical or undesirable for food preservation. Irradiation in an appropriate dose eliminates foodborne pathogens in frozen and unfrozen liquid eggs, powder egg white and egg yolk, fresh whole egg with intact peel, and cooked egg [101].

The advantages of the use of irradiation as a method of conservation over the other methods are: (a) time, since the irradiation can be applied in a few minutes; (b) the method does not leave residues in the food, because only the gamma rays come into contact with the food, without any risk of radioactive contamination; (c) it can be applied on a wide range of fruits; (d) it prevents food recontamination, since the product is already packed during the process; and (e) cold process, which avoids damages caused by the temperature increases and enables the irradiation of cooled and frozen products.

The disadvantages of the use of irradiation are high initial cost and difficulty in establishing the right doses [102].

Although these are all the benefits, there are several barriers that still persist and prevent irradiated foods from reaching a wide commercialization, mainly related to the cost and consumer resistance due to lack of information [103].

4. Conclusions

The main concern regarding to food safety of eggs is related to the presence of pathogenic microorganism. As an attempt to reduce problems resulting from egg contamination, in addition to prolonging shelf life and ensuring greater safety for consumers, they are subjected to thermal and nonthermal processes. Pasteurization is a thermal method widely used, and it has efficiency in the decontamination of this food, but the use of heat can alter the nutritional quality, flavor, and texture of the products. The alternatives to the traditional pasteurization are the new technologies. In this way, alternative methods of food preservation that minimize the likelihood of outbreaks of food poisoning leading to improvements in food safety are of great importance. The new technologies are efficient in reducing the microbial load, if well used, cause minor alterations in the nutritional and organoleptic properties, contributing to the offer of a fresher product, besides being safe from the microbiological point of view. In addition, the combination of pasteurization methods with other alternative methods needs to be studied in order to provide quality to eggs and their products without affecting their properties and functionalities.

Acronyms and abbreviations

FDA	Food and Drug Administration
HHP	high hydrostatic pressure
HELP	high-intensity electric field
O_3	ozone
KI	potassium iodide
PEF	pulsed electric field
RF	radiofrequency
UV-C	short-wave ultraviolet radiation
UV	ultraviolet

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