RADIOFREQUENCY HEATING OF SHELL EGGS - A PRELUDE

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ABSTRACT

Owing to their rich nutritive value, eggs serve as potential hosts to pathogenic microbes like *Salmonella enteritidis.* Administering heat treatments is the best solution for controlling these pathogens. However, heating affects the quality of the egg due to denaturation of proteins. Therefore a technique which causes minimal changes to the functional properties of the egg protein needs consideration.

In this study, the suitability of Radio frequency (RF) treatments of eggs is evaluated for effectiveness in terms of reducing/eliminating the microbial load. Finite difference time domains (FDTD) and Finite element models (FEM) were developed based on preliminary laboratory trials to simulate the electric field and temperature distribution in the egg components keeping in mind the dielectric properties, shape and composition of in-shell eggs.

Laboratory scale experiments were conducted to determine the dielectric properties of individual egg components followed by an investigation of the heating rates of individual egg components as well as intact in-shell eggs at different electric field strengths, where the electrode voltages are 2.5 kV, 3.5 kV and 4.25 kV with 60 mm spacing between the electrodes. The heating trends were then analyzed to obtain the time required to attain the targeted temperature. Models for calculating the dielectric parameters were presented.

As heat treatments invariably affect the functional properties of eggs the heat induced changes with respect to properties like foam stability, foam density, viscosity and turbidity were studied and compared with the physical properties of raw eggs. RF treated eggs showed minimal changes at lower power levels. However the change in properties was more pronounced at higher power levels. The coagulation taking place within the eggs at higher power levels with a view to

assessing the quality of heat treated eggs was studied by means of hyperspectral imaging (400-1700 nm).

The heat treatment process was validated by inoculating egg with a microbial contaminant and treating them in an RF applicator. The results indicated that RF heating may possibly serve as a way to pasteurize the eggs provided the process is improved and optimized to reach the required pasteurization temperature with minimum damage to the functional properties of the egg.

RESUME

En raison de leur riche valeur nutritive, les œufs frais peuvent potentiellement être contaminés par des microorganismes pathogènes comme la Salmonella enteritidis. Il est possible de contrôler les pathogènes alimentaires par l'utilisation judicieuses de traitements thermiques. Cependant, les traitements thermiques conventionnels causent la dénaturation totale ou partielle des protéines présentent dans le blanc et le jaune d'oeuf. Par conséquent, une technique qui permettrait de contrôler la contamination dans les œufs frais tout en maintenant les propriétés fonctionnelles des protéines de l'œuf doit être examinée.

Dans cette étude, l'adéquation de l'énergie des fréquences radios (RF) à 27 MHz pour le traitement thermique des œufs frais a été est évaluée. Des modèles en différences finies à dimension temporelle (FDTD) et des modèles d'éléments finis (FEM) ont été développés et utilisés pour simuler le champ électrique et pour prédire la distribution de la température dans les œufs frais en coquille et cela, en tenant compte des propriétés diélectriques, de la géométrie et la composition.

Les propriétés diélectriques des composants de l'œuf ont été mesurées en tenant compte de leur dépendance à la température. Par la suite, des essais en laboratoire ont été effectués pour déterminer les taux de chauffage des composants exposés à des niveaux de puissance de 2,5 3,5 et 4,25 kV. Les tendances de chauffage diélectrique ont été analysées et les temps requis pour atteindre la température cible ont été déterminés. Les modèles de prédiction des paramètres diélectriques ont aussi été développés.

Les effets des traitements thermiques sur les propriétés fonctionnelles des composants des oeufs ont été étudiés. Les paramètres retenus étaient : la stabilité et la densité de la mousse, la

viscosité et la turbidité des blancs d'œufs. Les résultats on indiqués que propriétés fonctionnelles des œufs traités aux niveaux de puissance de 2,5 et 3,5 kV étaient presque identiques à celles des œufs frais non-traités. Cependant, les changements observés étaient plus prononcés 4,25 kV. L'imagerie hyperspectrale (400 à 1 700 nm) a été utilisée pour déceler la coagulation du blanc d'œuf après le traitement thermique des œufs en coquille.

Le processus de traitement thermique a été validé par l'inoculation d'œufs frais avec une souche non-pathogénique de E. Coli. Par la suite, les œufs inoculés ont été traités thermiquement à l'aide du chauffage RF. Les résultats ont indiqué que le chauffage RF à 27 MHz était une méthode qui offrait un bon potentiel pour pasteuriser les œufs frais en coquille. D'autres études seront nécessaires pour optimiser la distribution de l'énergie RF et minimiser ses effets sur les propriétés fonctionnelles du blanc d'œuf.

CHAPTER I

Introduction

1.1 Food borne Diseases

Food borne diseases (FD) are illnesses that are acquired from eating contaminated food. In most of the cases, FD occurs as outbreaks rather than as individual cases, because a group of people who consumed the same food are infected within a short time period. Individual cases of FD are almost impossible to identify except for a few diseases like botulism, poisoning from shellfish and trichinosis. Mostly the food consumed immediately before the occurrence of disease symptoms is held responsible for causing the sickness. However; in most of the cases this assumption is erroneous because the incubation period of all the common food borne pathogens range from 18 to 72 hours. Food borne pathogens also invade other media and transmission from person-person or from an infected animal directly is also possible.

Food safety is considered to be an indispensable public health concern. Food Safety and Quality has become an area of precedence and necessity for consumers, retailers, manufacturers and regulators. Altering global patterns of food production, global trade, technology, public expectations for health security and several other issues have created a huge demand for food safety and quality auditing professionals.

1.2 Eggs

Eggs are considered to be one of the nature's nutritious diets. Eggs are one of the good sources of proteins, vitamins, minerals, folic acid and choline among many other micronutrients (Li-Chan *et al.*, 1995). Eggs are regarded as a complete meal as it contains all the essential amino acids. This rich nutritional profile of eggs makes it an important diet. In addition, eggs are an essential ingredient in many food processing and baking industries. This is attributed to the unique functional properties of eggs such as emulsification, foaming, coagulation and browning, rendered by the proteins present in the egg (McDonnell *et al.,* 1955). Unfortunately, many of the famous egg recipes involve the use of uncooked raw eggs. For instance hollandaise sauce, mayonnaise, egg nog are a few examples of such recipes. Eggs are perfectly safe when they are well cooked as most of the harmful virulent pathogens become unviable. When an individual consumes an improperly cooked egg or raw egg, there is a very good chance for the individual to succumb to illness. This usually means that the consumed egg is contaminated. One notable sickness is called Salmonellosis and is characterized by severe abdominal cramps, chills and fever. Even though healthy adults recover without any medication within a week's time, high risk populations like children, elderly people and people with compromised immune system almost always require hospitalization. As eggs are a popular dietary food in most of the countries and have constant demand in the market, it necessitates the presence of a safety surveillance system to establish the microbial safety of eggs and egg products. Although the Canadian eggs are regarded as one of the safest eggs in the market, it is better to undertake additional precautions as it can help in ensuring that the eggs are safe for human consumption.

1.3 Facts and Statistics

The probability of *Salmonella* penetrating fresh eggs is of the range 0.005 to 1 %. However, 20,000 cases of salmonellosis have been reported in Canada from the year 1993 to 1995 (Woodward *et al.,* 1997). Moreover 15,000 cases of hospitalizations and 500 deaths have been accounted every year in the United States due to the consumption of contaminated eggs

(Schroeder *et al.,* 2005). The economic losses caused due to these illness outbreaks have amounted to $200 million to $1 billion per year (Morales *et al.,* 1999). The average per capita egg consumption per year has gone down from 25 dozen in the year 1957 to 15 dozen in 2000. In addition to this the percentage of total eggs broken has seen a rapid increase from 5% in the year 1952 to more than 20% in the year 1998 (AAFC 2005). In the US in April 2010, there were 29 outbreaks of *Salmonella* in restaurants and at other events that has led to massive egg recall. *Salmonella* infections have not decreased during the past 15 years and have instead increased by 10 percent in recent years.

1.4 Problem Statement

Eggs are rich in nutrients that microbes need for their survival and reproduction. This attracts several microbes notably *Salmonella enteritidis* (SE). SE has been associated with several outbreaks of the disease salmonellosis in the recent past. Despite several safety measures taken up by the food safety regulating authorities, the incidence of SE in eggs has gone up and so has the SE outbreaks related to eggs. Egg shell acts as a natural barrier to the entry of microbes. It is interesting and unfortunate to note that most of the outbreaks of SE are related to shell eggs that are properly washed, candled to harden the shell, disinfected and met all other requirements laid down by the regulating authority. This has led the regulating authority to turn to recent advancements in technologies. As every pathogen needs an apt temperature to be viable, cooling the eggs immediately after they are laid is found effective to prevent SE contamination. Rapid cooling systems have been developed to cool the eggs faster. Also, ultrasound waves have been used to disinfect the eggs. Though these techniques were found effective for egg products, SE inside the egg shell is unharmed which when consumed led to SE outbreaks.

Heating the eggs is a better option as most of the FD causing pathogens is susceptible to heat. The main problem with the conventional water bath / steam heating is that the mode of heat flow in this process is via conduction. In other words, first the heat is transferred from the water bath /steam to the egg white and the egg yolk via the egg shell. Unfortunately, the pH and fat content of the egg yolk favors the bacteria to be more heat resistant in the yolk than in the white. This means that the egg yolk should be heated more than the egg white to make SE non-viable which is almost impossible by conduction mediated heat transfer operation. Because the egg white proteins coagulate at a lower temperature than the egg yolk, the desirable temperature to make SE non-viable cannot be reached without cooking the egg white partially or completely. This is very undesirable as the unique functional properties of the egg are attributed to the proteins in the egg white. Eggs are used as an essential ingredient in several food processing applications because of its functional properties notably foaming ability and whipping ability. Also, egg shell and shell membrane have poor thermal properties which increase the treatment time. This makes the process less energy efficient and the long treatment time increases the odds of the egg being cooked. So this operation is not cost effective and does not serve the purpose well. So other techniques that can heat the egg yolk more than egg white without harming the proteins in the egg white are considered necessary.

1.5 Hypothesis

It has been proven by Dev *et al.,* 2008 that microwaves can be used for in-shell heating of eggs to raise the temperature. Microwaves make use of the dielectric heating principle to heat the eggs from within. Like microwaves, radiowaves also have the capability to rotationally polarize the molecules present in the eggs and are thus suited for dielectric heating. Like microwaves, radiowaves also are non-ionising radiations and the heat generated while exposing the eggs to these waves are sufficient to bomb the microbes that are potential pathogens. Microwaves are shown to be suitable to heat the in-shell eggs by analyzing the temperature distribution of the in-shell eggs in a microwave environment. It is assumed that under radio wave environment, a similar temperature distribution can be achieved. There is an added advantage because the process will be much faster than the conventional water bath/ steam heating. The problem is that there is limited literature available on the behavior of eggs under radio waves and the suitability of the process in large scale industries. One other problem will be the non-uniformity in heating which can be corrected by proper positioning of eggs in the reactor and by choosing the proper length and correct functioning of the electrodes. In other words, the hypothesis is that it is purely an engineering problem that can be solved straight away.

1.6 Objectives

There is a need for an enormous amount of work to be put in prior to making it possible for the industries to employ this process on a large scale. Due to deficit of knowledge from basic literature, the present study has been undertaken. It is aimed at gaining fundamental knowledge that is essential to employ radiofrequency heating in industrial scale. Taking this into consideration, the objectives of this study are to:

(1) Perform mathematical modeling and simulation of the temperature distribution inside the in-shell egg and to evaluate the simulated results in lab scale.

(2) Study the dielectric properties (ε'- dielectric constant and ε''- dielectric loss factor) of the egg white and egg yolk in the radio wave frequency range from 10 MHz to 3 GHz and between temperatures 5˚C and 56˚C. Subsequent modeling of the dielectric

properties as a function of temperature and frequency to better aid in the construction of an RF applicator.

(3) Study the heating rates and time taken by the egg white and yolk to reach the 56°C temperature from ambient temperature (come-up time) for three electric field strength levels. The electrodes are in the parallel plate configuration (60 mm apart) and are operated at voltages 2.5, 3.5 and 4.25 kV and at frequency 27.12 MHz for albumen and yolk outside the egg shell as well as for the in-shell eggs. Also, a study to evaluate the efficacy of optimized heat treatment parameters in killing the microbes was conducted.

(4) Study the effect of heat treatment on the physical properties namely viscosity, turbidity, foam stability and foam density of the egg components as they are very essential to maintain the unique functional properties of the egg.

Chapter II

General Review of Literature

2.1 Egg – Importance

Eggs are considered to be one of nature's nutritious diets and have been consumed by mankind for over a million of years. Eggs are one of the good sources of proteins, vitamins, minerals, folic acid and choline among many other micronutrients (Li-Chan *et al*., 1995). This nutritional profile of eggs makes it an important diet and is shown in the Table 1. Chicken eggs are most frequently consumed by humans. Duck and Goose eggs, Quail eggs and Gull eggs are some other bird eggs consumed by humans (Roux *et al.,* 2006). Eggs are available all the year round and are an essential ingredient in many food processing and baking industries. The unique functional properties of eggs such as emulsification, foaming, coagulation and browning are vital in food chemistry applications (McDonnell *et al.,* 1955). Eggs are composed of translucent white and the yolk is covered by a protective shell and cuticle. Eggs consist of all essential nutrients and diverse defense mechanisms that enable the proper development of chick (Roux *et al.,* 2006).

2.2 Health Benefits of Eggs

For many decades, eggs have been perceived as somewhat unhealthy due to their cholesterol levels. Consumers assumed that the potential for ill-health coming from eggs was science based and it was in part the cause. In the last decade, several studies have been re-testing old recommendations and beliefs about the limits for egg intake as well as testing the cholesterol message in relation not only to coronary heart disease, but also to general health and survival. Recent studies emphasize the large number of nutritional benefits that may accrue from regular egg consumption.

Table 2.1 Nutritional Profile of Large Eggs

Nutrient (unit)	Whole Egg	Egg White	Egg Yolk
Calories (kcal)	70	16	54
Protein (g)	6.3	3.60	2.70
Carbohydrate (g)	0.36	0.24	0.61
Total fat (g)	4.8	0.06	4.5
Polyunsaturated fat (g)	1	0	0.71
Monounsaturated fat (g)	1.8	0	2
Saturated fat (g)	1.6	0	1.6
Cholesterol (mg)	185	0	185
Choline (mg)	126	0.04	116
Vitamin A (IU)	270	0	245
Vitamin D (IU)	41	0	37
Vitamin E (mg)	0.5	0	0.44
Vitamin B6 (mg)	0.09	0	0.06
Vitamin B12 (mcg)	0.45	0.03	0.33
Folate (mcg)	24	1	25
Thiamin (mg)	0.02	0	0.03
Riboflavin (mg)	0.2	0.14	0.09
Calcium (mg)	28	2	22
Sodium (mg)	71	55	8
Potassium (mg)	69	54	19
Phosphorus (mg)	96	5	66
Magnesium (mg)	6	4	1
Iron (mg)	0.88	0.03	0.46
Zinc (mg)	0.65	0.01	0.39

Source: Li-Chan *et al.,* 1995

Benefits from Protein

Including complete protein eggs in meals and snacks of human beings helps sustain the energy level. A complete protein contains the nine essential amino acids that the human body cannot produce naturally. Protein is the most filling nutrient. It helps to control the rate at which food energy (calories) is absorbed by the human body. Eggs are one of the few foods considered to be a complete protein. The learning ability of children depends upon their nutritional food intake. Eating healthy balanced diet enables to improve the concentration level, attention span, mathematical and reading skills. Egg recipes are a good formulation for the breakfast as eggs have all essential nutrients a person needs. Research indicates that high-quality protein may help active adults build muscle strength and middle-aged and aging adults prevent muscle loss. Consuming eggs following exercise is a great way to get the most benefits from exercise by encouraging muscle tissue repair and growth.

Weight Management

The protein in eggs can help human beings lose weight by controlling the rate at which the body absorbs calories. In a randomized controlled trial, 160 overweight or obese men and women were divided into 2 groups, one of which ate a breakfast including 2 eggs, while the other consumed a bagel breakfast supplying the same amount of calories and weight mass (an important control factor in satiety and weight loss studies). Participants ate their assigned breakfast at least 5 days a week for 8 weeks as part of a low-fat diet with a 1,000 calorie deficit. (Dhurandhar *et al*., 2007)

Compared to those on the bagel breakfast, egg consumers:

- Lost almost twice as much weight
- Had an 83% greater decrease in waist circumference
- Reported greater improvements in energy

Brain Development

Choline is an essential nutrient needed in good supply for good health. The flexibility and integrity of many fat containing structures in cell membranes is dependent upon choline (Zeisel *et al*., 2006). Choline is very important for the functioning of brain (Zeisel *et al*., 2006). Phosphotidylcholine and sphingomyelin account for a high percentage of the total mass of the brain. Methylation process in cells is highly dependent on the availability of choline (Zeisel *et al*., 2006). As choline contains three methyl groups, it is highly active in the process. Acetylcholine made up of key component choline is the primary neurotransmitter that communicates messages between nerves and muscles. But choline is not produced by the body in adequate amounts; a continuous new supply must be provided by our diet (Zeisel *et al*., 2006). Two large eggs provide an adult with the recommended daily intake of choline (Jensen *et al*., 2004).

Protection of Eyesight

Lutein and zeaxanthin are antioxidants found in egg yolks. Food containing antioxidants and food supplements with antioxidants help to protect eyes against damage due to ultraviolet radiation from the sun (Grando *et al*., 2003*)*. Research has shown that these antioxidants may be very important in reducing the risk of age-related macular degeneration (AMD) as well as the risk of cataracts (Moeller *et al*., 2000). AMD is the leading cause of blindness in people over age 65. Although eggs contain lesser antioxidants than green leafy vegetables, the antioxidants found in eggs are absorbed easily into the blood stream (Chung *et al., 2004*). Yet the enigma behind the increased bioavailability of antioxidants is not known.

Cholesterol Profile Improvisation

The link between egg consumption and raised cholesterol levels, which ultimately could lead to cardiovascular disease, was based on out-of-date information. One egg contains just 5 grams of fat and only 1.5 grams of that is saturated fat and 185 mg of cholesterol (Li-Chan *et al*., 1995). Cholesterol from an egg can affect blood cholesterol levels in two ways. First, there are individual differences in the way people respond to certain foods. People who have high cholesterol levels are more likely to show a greater increase for the same amount of cholesterol in food than those whose blood cholesterol levels are initially lower. Secondly, different food habits or patterns can also influence the effect of egg consumption on blood cholesterol levels. Individuals who eat eggs and have a diet that is high in saturated fat (mainly from animal foods) are more likely to elevate their cholesterol levels than people who eat eggs and have a diet that's low in saturated fat. In other words, the saturated fat content of our diet has a greater impact on our cholesterol levels than the cholesterol content of our diet (Ballesteros *et al*., 2004). This is because the absolute quantity of fat consumed in an average diet is much greater than the amount of cholesterol consumed and because saturated fat can be converted to cholesterol in the body. Not only have studies shown that eggs do not significantly affect cholesterol levels in most individuals, but the latest research suggests that eating whole eggs may actually result in significant improvement in one's blood lipids (cholesterol) profile - even in persons whose cholesterol levels rise when eating cholesterol-rich foods (Howell *et al*., 1997).

Helping to Prevent Blood Clots

Eating eggs may help lower the risk of a heart attack or stroke by helping to prevent blood clots. Proteins in egg yolk are not only potent inhibitors of human platelet aggregation, but also prolong the time it takes for fibrinogen to be converted into fibrin (Cho *et al*., 2003). Fibrin serves as the scaffolding upon which clumps of platelets along with red and white blood cells are deposited to form a blood clot. These anti-clotting egg yolk proteins inhibit clot formation in a dose-dependent manner-the more egg yolks that are eaten, the more clot preventing action.

Healthy pregnancy

Choline is an essential nutrient that contributes to fetal brain development and helps prevent birth defects (Zeisel *et al*., 2006). Two eggs provide about 250 milligrams of choline, or roughly half of the recommended daily intake for pregnant and breastfeeding women (Howe *et al*., 2004). In addition to choline, eggs have varying amounts of three other nutrients that pregnant women need most. Eggs are a good source of the highest quality protein, which helps to support fetal growth. Eggs also have a B vitamin that is important for normal development of nerve tissue and can help reduce the risk of serious birth defects that affect the baby's brain and spinal cord development (Shaw *et al*., 2004). The type of iron in eggs (a healthy mixture of heme and non-heme iron) is particularly well-absorbed, making eggs a good choice for pregnant and breastfeeding women who are at higher risk for anemia.

2.3 Structure of Eggs

The egg is a biological structure that protects and provides a complete diet for the developing embryo. Egg serves as the principal source of food for the first few days of the chick's life. Eggs are also one of the most nutritious and versatile human foods. The structure of the egg is shown if Figure 2.1. When the egg is freshly laid, the shell is completely filled with albumin. As the freshly laid eggs are hotter than the environment, the resulting cooling process leads to the formation of air cell due to the contraction of the contents inside the egg. Air cell is smaller in most of the high quality eggs. The colourless vitelline membrane covers the yolk and the yolk is well placed in the centre of the egg white *i.e* albumin. Fertilization occurs in the germinal disc which is attached to the yolk. The two twisted, whitish cord like objects present on the opposite sides of the yolk are known as chalazae. It enables the yolk to be suspended in the centre of the albumin. The size and density of the chalazae may vary between eggs. This does not affect the nutrition or the functional properties of the egg. A large portion of the albumen is thick. The albumin is surrounded by two membranes the inner shell membrane and the outer shell membrane. Further a thick shell covers the egg white. The shell contains several thousand pores that allow the entry of air into and out of the egg. The pores in the egg are covered by a thin layer of calcium called as cuticle. An average-sized egg weighs approximately 57 grams (about 2 ounces). Of this weight, the shell constitutes 11 percent; the white, 58 percent; and the yolk, 31 percent. Normally, these proportions do not vary appreciably for small or large eggs.

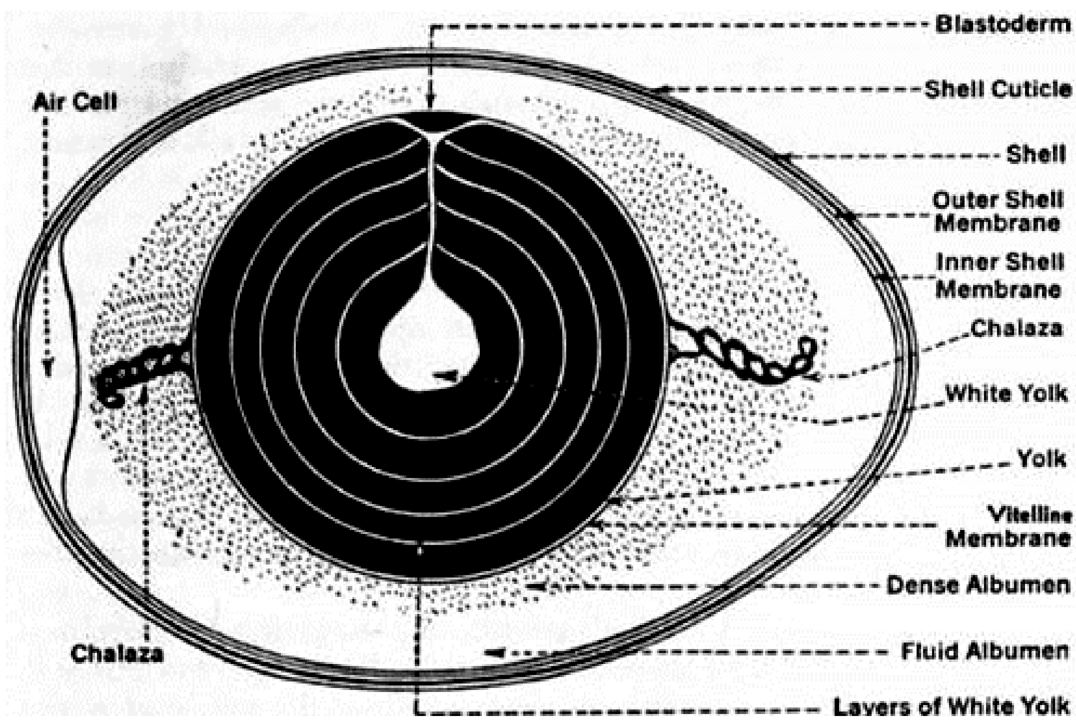

Figure 2.1 Structure of Egg (Adapted from Brown 1914)

2.4 Food Borne Disease and its Prevalence

In the last decade the incidence of food borne diseases has increased considerably. Food borne diseases are responsible for high levels of morbidity and mortality in the general population, particularly among the high risk groups, such as infants and young children, the elderly and the immune compromised (Bryan 1982). While most food borne illness cases go unreported to health departments, and are thus of unknown origin, the CDC estimates that 9.4 million of the illnesses are caused by 31 known food borne pathogens, and that 90% of all illnesses due to known pathogens are caused by seven pathogens: SE, norovirus, Campylobacter, Toxoplasma, E. coli O157:H7, Listeria and Clostridium perfringens (Archer *et al.,* 1985). According to the 2010 estimates, norovirus is the most common of the known pathogens, responsible for 5.4 million illnesses and 149 deaths each year (Scallan *et al.,* 2011). SE is now estimated to cause more than a million illnesses and 378 deaths annually. E. coli toxins are estimated to cause 176,000 illnesses and 20 fatalities a year. Campylobacter is estimated to cause 845,024 illnesses and 76 deaths. Listeria is one of the most lethal pathogens, estimated to cause 1,591 illnesses and 255 deaths. Some food borne diseases are well recognized, but are considered emerging because they have recently become more common (Mead *et al.,* 1999). For example, outbreaks of salmonellosis have been reported for decades, but within the past 25 years the disease has increased in incidence on many continents. In the Western hemisphere and in Europe, SE has become the predominant strain. Investigations of SE outbreaks indicate that its emergence is largely related to consumption of poultry or eggs (Mead *et al.,* 1999).

2.5 Reasons for Increased Prevalence

New FD threats occur for a number of reasons. These include increase in international travel and trade, microbial adaptation and changes in the food production system, as well as human demographics and behavior.

- **The globalization of the food supply**: A large outbreak of cyclosporiasis occurred in North America in 1996-7 linked to contaminated raspberries imported from South America.
- **The inadvertent introduction of pathogens into new geographic areas**: *Vibrio cholerae* was introduced into waters off the coast of southern United States when a cargo ship discharged contaminated ballast water in 1991. It is likely that a similar mechanism led to the introduction of cholera for the first time this century (1991) into South America.
- **Travelers, refugees, and immigrants exposed to unfamiliar food borne hazards while abroad**: International travelers may be infected by food borne pathogens that are uncommon in their countries. It is estimated that about 90% of all cases of salmonellosis in Sweden are imported.
- **Changes in microorganisms**: Changes in microbial populations can lead to the evolution of new pathogens, development of new virulent strains in old pathogens, development of antibiotic resistance that might make a disease more difficult to treat, or lead to changes in the ability to survive in adverse environmental conditions.
- **Change in the human population**: The population of highly susceptible persons is expanding world-wide because of ageing, malnutrition, HIV infections and other underlying medical conditions. Age is an important factor in susceptibility to food borne infections because those at the extremes of age have either not developed or have partially lost protection from infection. Particularly for the elderly, food borne infections are likely to

invade their blood stream and lead to severe illness with high mortality rates. People with a weakened immune system also become infected with food borne pathogens at lower doses which may not produce an adverse reaction in healthier persons. Seriously ill persons, suffering from, for example cancer or AIDS, are more likely to succumb to infections with SE, *Campylobacter, Listeria, Toxoplasma, Cryptosporidium*, and other food borne pathogens. In developing countries reduced immunity due to poor nutritional status render people, particularly infants and children, more susceptible to food borne infections.

- **Changes in lifestyle**: Greater numbers of people go out and eat meals prepared in restaurants, canteens, fast food outlets, and by street food vendors. In many countries, the boom in food service establishments is not matched by effective food safety education and control. Unhygienic preparation of food provides ample opportunities for contamination, growth, or survival of food borne pathogens.

2.7 Challenges In controlling Food Borne Disease

The following factors make controlling food borne pathogens particularly challenging (CAST Report, 2009):

- Emerging pathogens demand even greater food safety vigilance than what was required in previous generations because as pathogens are evolving and becoming more widespread, humans are becoming more resistant to treatments.
- The food supply has become global, with many different countries supplying food products to the United States.
- More food is prepared and consumed away from home. The U.S. Department of Agriculture (USDA) estimates that consumers spend 48 cents of every food dollar eating

out. Also, an increasing amount of food prepared away from the home is then taken home for consumption, thus creating more opportunities for contamination.

- Consumers may not always be consistent with hand washing and safe thawing habits.

In addition to this, microorganisms continue to adapt and evolve, thereby increasing their degree of virulence. For example, *E. coli* O157:H7 was first identified in 1982, but the bacterium has already been indicated as a cause for severe vomiting, bloody diarrhea, and even hemolytic uremic syndrome, which leads to kidney failure (Diez *et al.,* 1997).

2.7 Microbial Profile of Eggs

Common contaminants found are bacteria, moulds and yeast. Most common bacterial contaminants of the egg are the members of the genera: Micrococcus, Staphylococcus, Bacillus, Pseudomonas, Alcalgenes, Flavobacterium, Escherichia, Aerobacter, Acinetobacter, SE, Serratia and Enterobacter (Board *et al*., 1995). The common molds that can contaminate the eggs are the members of the genera: Mucor, Penecillium, Hormodendron and Cladosporium (James *et al*., 2005). The only yeast that is found to contaminate the eggs consistently is Torula. Rotting is the most common spoilage of eggs caused by bacteria. Bacteria can also cause mustiness in eggs. The molds cause pinspots; rightly called from appearance of mycelial growth in the inside (James *et al.,* 2005). Among the bacteria SE is potent and efficient in successful invasion of the eggs. It has led to several outbreaks of Salmonellosis in the recent past (James *et al.,* 2005).

Outbreaks of SE from 1985 through 1991 were determined by reports to the national SE surveillance system and through the foodborne disease outbreak surveillance system (Mishu *et al*., 1994). From 1985 through 1991, 380 outbreaks were reported involving 13,056 ill persons and 50 deaths (Mishu *et al*., 1994). The proportion of Northeast outbreaks fell from 81% in 1985 to 55% in 1991 as the number of outbreaks in other areas increased (Mishu *et al*., 1994). Grade

A shell eggs were implicated in 82% of outbreaks. Case-fatality rates in nursing homes and hospitals were 70 times higher than in other settings (Mishu *et al*., 1994). During 1998--2002, a total of 6,647 outbreaks of foodborne disease were reported (1,314 in 1998, 1,343 in 1999, 1,417 in 2000, 1,243 in 2001, and 1,330 in 2002) (Mishu *et al*., 1994). These outbreaks caused a reported 128,370 persons to become ill. Among 2,167 (33%) outbreaks for which the etiology was determined, bacterial pathogens caused the largest percentage of outbreaks (55%) and the largest percentage of cases (55%). Among bacterial pathogens, SE accounted for the largest number of outbreaks and outbreak-related cases. Cultures of environmental or animal specimens from all farms tested yielded SE. Egg borne SE infections are a major public health problem (Patrick *et al*., 2004). The SE incidence rate increased from 2.38 per 100,000 population in 1985 to 3.9 per 100,000 in 1995. Since then, there has been a decline of 49%, to 1.98 per 100,000 in 1999 (Patrick *et al*., 2004).

Salmonellae are gram-negative motile bacilli. As with the closely related bacterium *Escherichia coli, Salmonellae* are potential enteric pathogens and a leading cause of bacterial foodborne illness. The transmission of S*almonellae* to a susceptible host usually occurs via consumption of contaminated foods. In addition, human-to-human and animal-to-human transmissions can occur. Although the infectious dose varies among *Salmonella* strains, a large inoculum is thought to be necessary to overcome stomach acidity and to compete with normal intestinal flora (Chalker *et al.,* 1988). Large inocula are also associated with higher rates of illness and shorter incubation periods (Chalker *et al.,* 1988). In general, about 10^6 bacterial cells are needed to cause infection. Low gastric acidity, which is common in elderly persons and among individuals who use antacids, can decrease the infective dose to 10^3 cells, while prior vaccination can increase the number to 10^9 cells (Chalker *et al.,* 1988). After ingestion, infection with *Salmonellae* is characterized by attachment of the bacteria by fimbriae or pili to cells lining the intestinal lumen. *Salmonellae* selectively attach to specialized epithelial cells (M cells) of the Peyer patches (Jones *et al*., 1994). The bacteria are then internalized by receptor-mediated endocytosis and transported within phagosomes to the lamina propria, where they are released (Jones *et al*., 1994). The bacteria then produce an enterotoxin that causes inflammation and diarrhea, which can, at times, be fatal (Jones *et al*., 1994). While these Gram-negative bacteria grow best at temperatures between 8-46°C, in a pH range of 3.8 to 9.5, and at water activities above 0.94 (Bell *et al*., 2002), they are capable of surviving in conditions of low water activity, and extreme pH and temperature conditions. They are destroyed at temperatures of 70°C and above, so they are susceptible to ordinary cooking temperatures if applied sufficiently long (Guthrie 1992).

The foods that are cooked properly do not cause an infection as most of the bacteria are killed while cooking. But the consumption of raw eggs and recipes that use raw eggs like mayonaisse, hollandaise sauce, egg-nog, Caesar salad and Ice creams etc., are a few examples wherein the use of contaminated eggs can be hazardous. (Morrone *et al*., 2008; Zeilder *et al.*, 2002).

2.8 *Salmonella* Penetration in Eggs

There are two possible routes for the bacteria to contaminate eggs (Schoeni *et al*., 1995). One is trans-shell or horizontal transfer wherein the bacteria in the outside environment like feces or vaginal deposits on the shell later penetrate the egg (Schoeni *et al*., 1995). The other route is trans-ovarian or vertical transfer wherein the bacteria especially SE can infect the reproductive tissues like ovary and oviduct tissues which may result in the contamination of the egg even before the egg shell is formed. The Figure 2.2 shows the pathogenesis of SE contamination in eggs. SE enters the intestinal tract of the hen upon ingestion of the bacteria orally. Bacteria can colonize the gut by invading the intestinal epithelial cells upon prior successful colonization of the intestinal lumen. This infection stimulates the immune cells especially macrophages. The immune cells are attracted to the site of infection and enclose the SE bacteria. The bacteria survive and multiply in the intracellular environment of the macrophages. These infected macrophages migrate to the internal organs such as the reproductive organs (systemic spread). In addition to systemic spread, bacteria can also access the oviduct through ascending infection from the cloaca. The process is depicted in the Figure 2.2 a. This process may result in the direct contamination of the yolk, yolk membranes, albumen, shell membranes and egg shell originating from infection of the ovary, infundibulum, magnum, isthmus and shell gland, respectively as described in Figure 2.2 c. The Figure 2.2 b shows the other possible route of egg contamination by SE penetration through the eggshell and shell membranes after outer shell contamination (Gantois *et al.,* 2009). Surface contamination may be the result of either infection of the vagina or fecal contamination (Gantois *et al.,* 2009). SE bacteria deposited in the albumen and on the vitelline membrane are able to survive and grow in the antibacterial environment (Gantois *et al.,* 2009). They are also capable of migrating to and penetrating the vitelline membrane in order to reach the yolk (Gantois *et al.,* 2009). After reaching this rich environment, they can grow extensively as shown in Figure 2.2 d.

2.9 Barriers to Bacterial Penetration in Eggs

As described above, eggs apart from providing the nutrition to the growing embryo also protect the developing embryo from external agents. The barriers to the external agents can be classified broadly under two categories: Extrinsic and Intrinsic Factors.

Extrinsic factors

Bacterial strain

In a study (Jones *et al.*, 2002), it was demonstrated that SE and *Pseudomonas fluorescens* exhibited an ability to survive at different rates in the various portions of the egg. SE was more able to survive on the exterior surface, whereas *Pseudomonas fluorescens* was better able to traverse the shell membranes and infect the contents of the egg. SE and *Salmonella typhimurium*

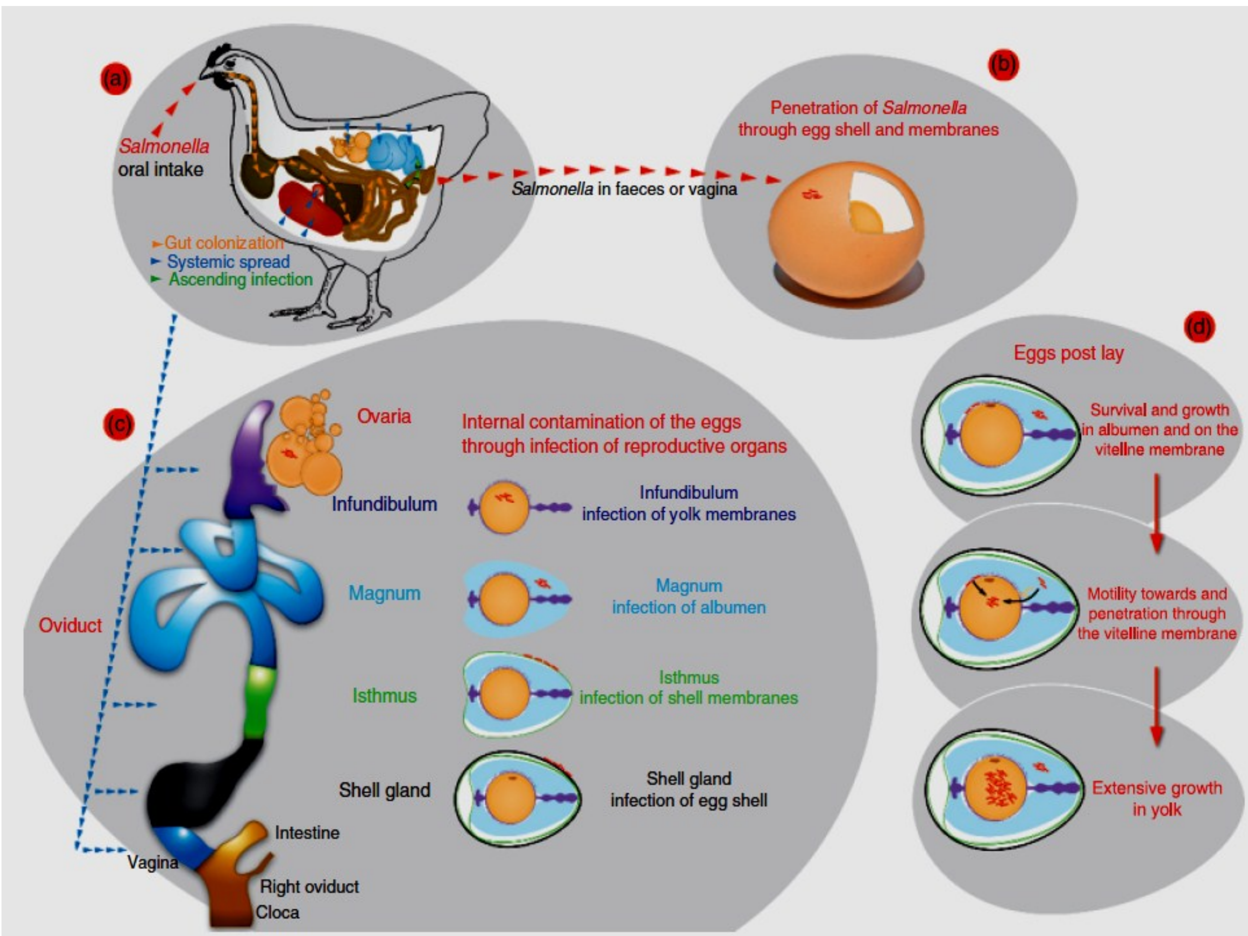

Figure 2.2 Pathogenesis of egg contamination by Salmonella

(Adapted from Gantois Inne *et al., 2009*)

were able to penetrate the eggshell similarly (Miyamoto *et al.*, 1998a). The growth of SE in albumen seems to rely upon the expression of flagella and curli fimbriae (Cogan *et al.*, 2004).

yafD gene codes for a 266 amino acid protein and has a molecular weight of 29.9 KDa. The function of the protein is yet to be deciphered. The protein is homologous to endonuclease-exonuclease-phophatase family. The bacterial strains like *Salmonella typhimurium* and *typhi* are susceptible to albumin. Albumin has shown to cause DNA damage by nicking the super coiled DNA, to linear DNA and then chopping it. The presence of *yafD* gene has shown to confer resistance to this activity of albumin. This confers advantage to SE over other strains.

Temperature differential between egg and bacterial Suspension

The highest percentage of contaminated eggs and the fastest penetration rates were observed when a positive temperature differential (egg warmer than the environment) was created between the eggs and the SE culture used for dipping the eggs. When the egg is warmer, the subsequent cooling caused its contents to contract. The resulting negative pressure aids drawing the bacteria through the pores (Bruce *et al.,* 1994).

Moisture

Moisture is needed to allow penetration according to some authors (Bruce *et al.,* 1994; Berrang *et al.*, 1999). Hence any stage of production where both moisture and a positive temperature differential may be present provides an opportunity for bacterial invasion (Berrang *et al.*, 1999). Although the presence of water on the shell enhanced ST penetration, its presence is not essential for penetration to occur.

The number of organisms in the suspension or in the feces

The higher the numbers of SE in the culture used for dipping the eggs, the higher the rate of contamination of the eggs as demonstrated by Chen *et al.,* 1996, Miyamoto *et al.,* 1998 and Braun *et al.,*1999. For example, when stored at 15°C and 60% RH, 10.1% of the eggs were contaminated after 20 days of storage using the higher inoculation dose compared to 1% using the lower inoculation dose (Braun *et al.*, 1999).

pH of immersion solution

The effects of the pH of the immersion solution on the penetration of eggs by three *Salmonella* serovars, *i.e. t*yphimurium, *St.-Paul* and *Derby* were studied by Sauter *et al.*, 1979. The pH ranged from 5.0 to 9.5 with 0.5 increments. For all strains, the eggshell penetration at pH 5.0 was significantly lower than at pH 5.5 and both were less than all other pH values studied. At these low pH values, strains remained viable during 4 h and decalcification did not occur (Sauter *et al.*, 1969).

Temperature and relative humidity during storage after Inoculation

The SE penetration upon storage at various temperatures and RH's has been studied by Braun *et al.*, 1999. The level of SE penetration to the egg contents increased with increasing temperature and RH. Recovery of SE from the contents was already observed by day 3 when eggs were stored above 15°C. Schoeni *et al.*, 1995 examined the potential for SE in contaminated feces to establish to the interior of eggs after 3 days of storage: 50% of contents were contaminated at 25°C, while no contamination was found at 4°C. In a study by Ruiz *et al.,* 1966 using a positive temperature differential, recoveries of SE from egg contents were low in eggs stored at 2°C.

Intrinsic factors affecting *Salmonella* penetration

Cuticle

The cuticle is the egg's first defence against bacterial infection. For the first minutes after oviposition, the cuticle is usually moist and immature, and less effective in the prevention of penetration than a mature cuticle, as demonstrated by Sparks *et al.,*1985, Padron 1990 and

Miyamoto *et al.*,1998. Moreover since the egg is warmer (42°C) than the environment, *i.e.* a positive temperature differential is present, bacteria migrate more easily through the eggshell and membranes (Bruce *et al.,* 1994). When mature, the cuticle is a very effective barrier to water and interferes with bacterial invasion by closing the pores, hence avoiding flooding of the egg (Berrang *et al.*, 1999). Removal of the cuticle by abrasion of the eggshell or by chemical treatment with EDTA increased the penetration of the eggshell by bacteria (Board 1980). The egg washing chemical, alkaline sodium carbonate, altered the egg surface, enhancing bacterial penetration. The cuticle can also be digested by Pseudomonas during storage in a humid atmosphere (Board 1980). The cuticle is not an inherent aspect of the true shell. A small fraction (3.5%) of eggs was found to lack a demonstrable cuticle, while 8.0% had no cuticle on the apex or blunt end.

Eggshell

Shell quality

The quality of eggshells is most commonly defined in terms of the amount of shell present and is assessed by measuring shell specific gravity, shell weight or shell thickness. The relative amount of shell explains only a part of the strength of an eggshell (Solomen 1997). Another vital consideration is how well the eggshell is constructed and this is where ultrastructural studies play an important role (Roberts *et al.,* 1994). Nascimento *et al.,* 1991 reported that eggs judged visually to have poorer quality eggshells were more likely to allow SE penetration. The greatest variation in eggshell ultrastructure occurred in the mammillary layer and various abnormalities have been described. Some of these abnormalities decreased, while others increased the resistance to bacterial penetration (Nascimento *et al.*, 1992).

Many factors have been found to affect eggshell quality: the strain of bird, the age of the hen, environmental temperature (> 21°C usually leads to deterioration in shell quality), dietary factors, dietary electrolytes, stress, disease and other chemical compounds (Roberts *et al*., 1994). Bacterial contamination of air cells, shells, and egg contents was more common in eggs from older hens than from younger hens (Jones *et al.*, 2002).

Porosity

The hen's eggshell has numerous pores estimated to range from 7,000-17,000 per egg (Mayes *et l.,* 1983) that are unbranched and capped with organic material (the cuticle) (Board 1980). Even in an egg having an undamaged cuticle, there are at least 10 to 20 pores that lack either an adequate cover or plug of cuticle. These uncovered, also termed "patent" pores, may provide the portals for bacteria to infect the contents of the egg (Board *et al.*, 1995). Current evidence suggests that, while pores represent portals of entry, their function as primary routes of transfer is of secondary importance to the structural defects that occur in many eggs, and that by virtue of their magnitude, offer a much easier route (Solomon *et al.*, 1997). On the other hand cracked eggs were subjected to extensive *Campylobacter jejuni* colonisation in defective areas (Allen *et al.*, 2001). The integrity of the eggshell can be affected by environmental conditions, such as the rate of cooling. The microscopical cracks due to rapid cooling of eggs might give rise to increased penetration by SE as observed by Fajardo *et al.,* 1995.

Membranes

At the initial stage of contamination by horizontal transmission, bacterial cells are located between the eggshell and the membranes, as demonstrated using luminescent SE (Chen *et al.*, 1996). The membranes act like filters and pose only a temporary barrier to the inward movement of bacteria (Berrang *et al.*, 1999), as demonstrated for *Pseudomonas* by comparing the penetration through the intact eggshell and the eggshell with membranes removed. It should be noted, however, that data by Jones *et al.,* 2002 suggest that *Pseudomonas fluorescens* is a primary invader of eggs that is more capable of contaminating egg contents through the membranes than SE. Of the two membranes, the inner membrane offers the greatest resistance to bacterial penetration, possibly because its inner surface is clothed with a limiting membrane (Board *et al*., 1995). The rate and extent of the infection was influenced by the size of the inoculum and the site of contamination relative to yolk movement. Storage with air cell uppermost gave a higher incidence of contamination compared to storage with air cell downwards. This has been attributed to the yolk moving upwards towards the infected air cell membrane as a consequence of the decay of internal structure with storage (Clay *et al.*, 1991).

2.10 Salmonellosis

Upon successful penetration, SE contaminates the egg white. As the pH and nutrients in the egg white is not favorable, the bacterial growth ceases. As the egg ages, the egg white becomes thin and the bacteria can now derive the nutrition from the yolk. Once the bacteria are in the egg yolk, SE can double every 20 minutes. Most persons infected with SE develop diarrhea, fever, and abdominal cramps 12 to 72 hours after infection (Tauxe *et al.,* 1991). The illness usually lasts 4 to 7 days, and most persons recover without treatment. However, in some persons, the diarrhea may be so severe that the patient needs to be hospitalized. In these patients, the SE infection may spread from the intestines to the blood stream, and then to other body sites and can cause death unless the person is treated promptly with antibiotics. The elderly, infants, and those with impaired immune systems are more likely to have a severe illness.

Salmonellosis is diagnosed based on a medical history and a physical exam. The health professional will ask questions about the symptoms, foods that we had recently eaten, and the work and home environments. A stool culture and blood tests may be done to confirm the diagnosis. People with severe diarrhea may require re-hydration, often with intravenous fluids. Antibiotics are not usually necessary unless the infection spreads through the bloodstream into other organs. In these cases, the infection can be treated with ampicillin, gentamicin, amethoxazole, or ciprofloxacin.

2.11 Prevention Strategies for Salmonella contamination

Canada's egg producers are dedicated to providing consumers with eggs that meet the highest standards of safety and quality. The Canadian industry's adherence to these standards has paid off; our egg producers are global leaders in the development of on-farm health and safety practices and Canadian eggs are recognized as among the safest in the world (AAFC 2005).

The Canadian Egg Marketing Agency and its provincial-territorial partners have worked to improve on the egg's natural safety with several measures. The *Hazard Analysis Critical Control Point (*HACCP)-based Start Clean-Stay Clean program, which is in place at the farm level in Canada, is considered a global model and complies with internationally recognized safety standards. HACCP principles ensure that potential hazards in production are analyzed and critical control points for reducing or eliminating hazards are identified. Federal and provincial officers' conduct inspections at the farm to advice on the Start Clean-Stay Clean safety guidelines. Start Clean-Stay Clean completed technical review by the Canadian Food Inspection Agency in 2004. Additionally, testing to detect the presence of SE in layer barns has been instituted in all 10 provinces and the Northwest Territories. If a positive environmental sample is confirmed in a layer barn, eggs are diverted directly to an egg breaker where they are broken and then pasteurized, even though none of the eggs is likely to have the pathogen. Pasteurization kills SE. The eggs are then sold as liquid, frozen or dried eggs and used in a variety of foods. However, in USA FDA emphasize the use of pasteurized In-Shell eggs for raw egg recipes. FDA along with FSIS-USDA has devised a temperature guideline for pasteurization of egg white and egg yolk. They recommend the heating of egg white and yolk to a temperature of 57.5 and 61.1˚C respectively for at least 2.5 minutes to ensure the safety of eggs against harmful pathogens USIS (FSIS-USDA., 2006).

2.12 Safety Measures - Treatments

Cooling of eggs

Several research studies have shown that cooling the eggs immediately after they are laid is effective in controlling the SE outbreaks. Since every organism needs a favorable temperature to be viable, cooling or heating the eggs makes it unfavorable environment for the organisms to grow. While there are no federal guidelines for how quickly eggs should be cooled, current industry procedures can take as long as six days to cool eggs to 45°F, the temperature at which SE can no longer grow. Keener's rapid-cooling technology would take 2–5 min. However, FDA studies show that if eggs were cooled and stored at 45°F or less within 12 hr of laying, there would be an estimated 78% fewer SE illnesses from eggs in the United States each year. Several attempts were made to rapidly cool eggs. In one such cooling technology, carbon dioxide "snow" is used to rapidly lower the eggs' temperature. Eggs are placed in a cooling chamber and carbon dioxide gas at about -110°F is generated. The cold gas is circulated around the eggs and forms a thin layer of ice inside the eggshell. After treatment, the ice layer melts and quickly lowers an egg's internal temperature to below 45°F. The eggshell does not crack during this process because the shell can resist expansion from a thin ice layer. Previous studies have shown the cooling treatment would increase shelf life by four weeks.

Pasteurization

A process named after scientist Louis Pasteur which uses the application of heat to destroy human pathogens in foods. This process renders the harmful organisms such as bacteria, viruses, protozoa, molds, and yeasts non-viable. Unlike sterilization, pasteurization is not intended to kill all microorganisms in the food. Instead, pasteurization aims to achieve a "log reduction" in the number of viable organisms, reducing their number so they are unlikely to cause disease (assuming the pasteurized product is refrigerated and consumed before its expiration date). Eggs are pasteurized in two ways. Initially, eggs are broken; egg white and yolk are separated and then pasteurized individually. Then the products are sold as liquid or frozen pasteurized eggs. Alternatively, In-shell eggs can be pasteurized (Hou *et al.,* 1996). The major problem associated with the conventional pasteurization technique is the partial cooking of eggs. This is very undesirable as eggs are famous because of the incredible functional properties. This has led researchers to improvise on the available pasteurization techniques and also to develop alternative techniques like microwave heating and radio frequency heating that works based on the principle of dielectric heating.

Recently, researchers and manufacturers have developed a technique for the pasteurization of In-Shell eggs. So far, National Pasteurized Eggs, Inc. (NPE) is the only commercial egg company that has obtained a patent for its In-Shell egg pasteurization process. NPE is the world’s largest producer of in-shell pasteurized eggs, has perfected an all-natural, multi-patented process to ensure that eggs are pasteurized to meet U. S. Food and Drug

Administration (FDA) and World Health Organization (WHO) standards of SE-free assurance. Within North America, NPE has already produced and sold more than one billion in-shell pasteurized eggs. The process was invented by Dr. James Cox and it took ten years to patent the process.

Dielectric Heating

When a dielectric material is brought into a rapidly altering electrical field, heat is generated inside the material. This is known as heating by dielectric hysteresis or, in short, dielectric heating. Radio frequency and microwave heating are both applications of this principle (U.I.E 1992). In technological terms, however, there is a clear distinction between the two techniques. The essential advantage of dielectric heating resides in the generation of heat within the material to be heated. In comparison with more conventional heating techniques (hot air, infrared) in which the material is heated via the outer surface, dielectric heating is much more rapid (U.I.E 1992).

Dielectric heating works on the principle of rotational polarization. The dielectric molecules between the electrodes tend to rotate in an alternating electric field. While the molecule rotates, they collide with each other, as a result of which heat energy is liberated. Thus released heat energy is used to heat the material between the electrodes (Petrescu 1994). The material gets heated within unlike conduction in conventional heating. The pictorial representation of this phenomenon is shown in the Figure 2.3.

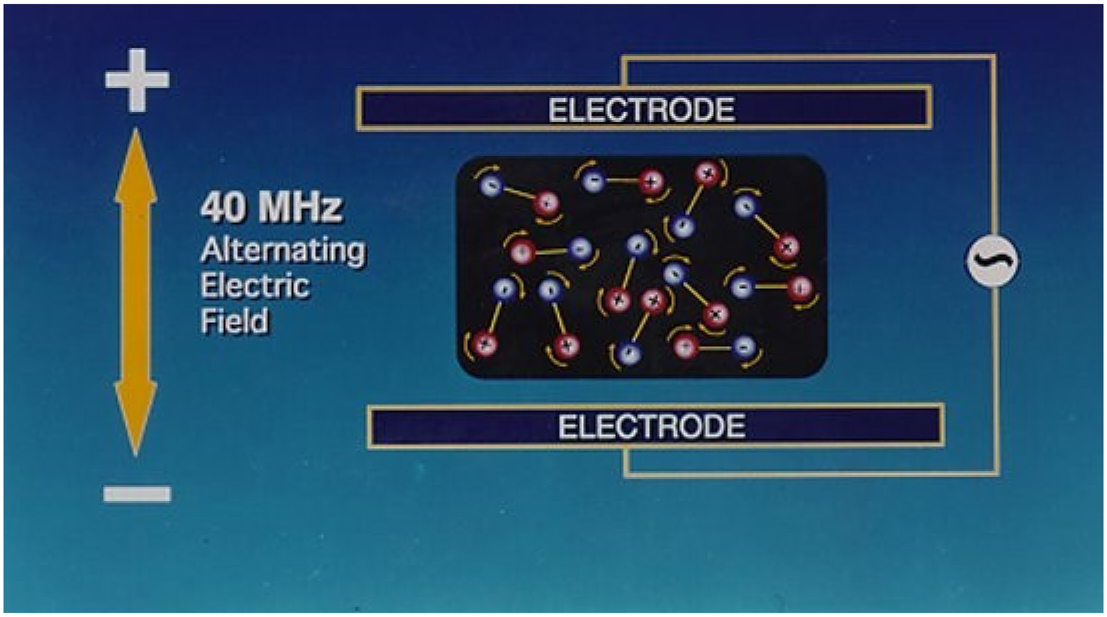

Figure 2.3 Rotational Polarization – Principle of Dielectric Heating (Source: Clark 1997)

2.13 Safety of Treated Eggs

The most heat sensitive protein in the albumin, namely globulin G3A denatures at 56°C, which typically causes whitening of the inner thick albumin due to protein aggregation. The denaturation stage can be delayed somewhat, making it possible to expose the eggs at the target temperatures for limited time with a minimum amount of damage to the albumin. Eggs, as being biological material, also differ from each other and each egg will have a unique shape and weight. Eggs are usually classified by weight, and eggs within a weight class can differ by as much as 10 grams. It is this variability, linked to the need to transfer heat efficiently into the egg that has posed the challenge for the design of a combination microwave/air pasteurization unit to allow for controlled heating of the eggs. The primary objective is to heat the eggs as quickly as possible to the target temperature where the microorganisms are being destroyed, and to hold the eggs in a controlled temperature environment for a fixed time period to allow for equilibration of the egg temperatures to take into account the variability in egg shape and weight. The total exposure time must be sufficient to allow significant reduction in pathogen counts, but cause the minimum amount of damage to the egg albumin and yolk. In order to evaluate the temperature distribution within the eggs to determine the effect of treatment on egg components, mathematical simulation were conducted.

2. 14 Simulations

Thermal processing of an egg is a straight forward approach though measuring temperature at different points inside the shell is almost impossible due to the poor integrity and brittleness of the egg shell. One possibility is to insert several probes within the egg shell and measure the temperature at different points. However, egg shell can host only a threshold level of holes after which egg shell breaks. Also, the repeatability of the experiment is very difficult as it is impossible to place the probes in the exact same previous positions. This task becomes even more challenging in a radiowave environment. It is mandatory to measure and analyze the temperature distribution to evaluate the efficacy of the egg heating process. Theoretical, mathematical, numerical modeling and simulation helps understanding and predicting the temperature distribution inside biological medium. Therefore modeling and simulation of radiowave heating of eggs was carried out.

Simulations of electromagnetic energy distribution and heat generation involve solving sets of complex Partial Differential Equations (PDEs). Numerical approximation techniques like the Finite Difference Time Domain (FDTD) and Finite Element Method (FEM) are commonly used to solve for different variables in PDEs. The FEM technique competes very favorably with other numerical methods, as it is based on reducing the Maxwell's equations to a system of simultaneous algebraic linear equations (Delisle *et al*., 1991). FEMs can readily model heterogeneous and anisotropic materials as well as arbitrarily shaped geometries. It can also provide both time and frequency domain analyses, which are important in dealing with radiowave heating problems like field distribution, scattering parameters and dissipated electric field strength distribution for various materials and geometries (Dai 2006).

A three-dimensional finite element model needs to be developed using proprietary software namely, MATLAB (Version 7.7) from Mathworks Inc, USA and COMSOL (Version 3.5a) from COMSOL Inc, Boston, USA. COMSOL is a finite element modeling software package, in which most of the modeling and simulation is done with the help of its graphical user interface, but this package lacks certain features like simulation of a rotating/moving object in an electromagnetic field. This gap is bridged by MATLAB coding specifically developed for a given object geometry. This model will be useful for determining radiowave energy distribution and for the prediction of temperature profiles inside the in-shell eggs during radiowave processing. This model will be developed by taking into consideration the complex shape, dielectric properties and heterogeneous composition of the in-shell egg.

There is poor understanding of the mechanisms involved in the actual energy distribution inside the eggs when subjecting them to electromagnetic field. The electromagnetic field distribution inside the radiowave oven can be traced out by solving the Maxwell's equations (Dev *et al.,* 2008). The FEM is commonly used for solving Maxwell's equations to get the energy distribution in a complex object or within a multimode cavity and it is capable of simulating electric field strength density distribution in a 3-D space. (Fu *et al.*, 1994; Zhou *et al.*, 1995).

2.15 Finite Element Method

The finite element method (FEM) (sometimes referred to as finite element analysis) is a numerical technique for finding approximate solutions of PDEs as well as of integral equations. The solution approach is based either on eliminating the differential equation completely (steady state problems), or rendering the PDE into an approximating system of ordinary differential equations, which are then numerically integrated using standard techniques such as Euler's method, Runge-Kutta, etc. In solving PDEs, the primary challenge is to create an equation that approximates the equation to be studied, but is numerically stable, meaning that errors in the input data and intermediate calculations do not accumulate and cause the resulting output to be meaningless. There are many ways of doing this, all with advantages and disadvantages. The Finite Element Method is a good choice for solving PDEs over complex domains (like cars and oil pipelines), especially when the domain changes (for instance situations such as during a solid state reaction with a moving boundary), when the desired precision varies over the entire domain, or when the solution lacks smoothness.

2.16 Radio Waves - Properties

Radio waves are electromagnetic waves that have frequency in the range of 3 kHz to 300 GHz. They are called as Radio waves as they are used in the radio communication and as other electromagnetic radiations radio waves obey laws of reflection, refraction, diffraction, polarization and interference. Radio waves have no mass and are neither affected by electric nor magnetic fields. These waves do not require any medium for transmission and travel with the velocity of light in vacuum and in presence of medium. Also, radio waves are two dimensional transverse waves as they transfer the energy perpendicular to the direction of the oscillations. Radio waves have the lowest frequency compared to other types of waves. Radio frequencies spectrum is further divided into eight frequency bands namely, 1. Very low frequency, 2. Low frequency, 3. Medium frequency, 4. High frequency, 5. Very high frequency, 6. Ultra high frequency, 7. Super high frequency and 8. Extreme high frequency. Each of these frequencies is ten times higher in frequency as the one immediately below it.

2.17 Radio Waves - Applications

Radio is one of our most important ways of communicating. Since the late 1800s, when radio was invented, it has played a huge role in our lives. Communication between two far distant places became quick and much more inexpensive than stringing telegraph wire. Suddenly, ship-to-ship and to-shore radios were saving thousands from disaster at sea, radio entertainment broadcasts were going into peoples' homes, and soldiers in the field were able to keep in touch with friendly units. Broadcasting is the most well-known use of radio. Two-way radios are also very important. Emergency personnel such as police, fire fighters, and ambulance crews use radio to stay in contact with their bases and with each other. They send and receive reports with radios in their vehicles and carry smaller portable devices with them. An EMT can send descriptions of wounded individuals ahead to the doctors at a hospital so they can prepare to treat them. Besides being used for transmitting sound and television signals, radio is used for the transmission of data in coded form. In the form of radar it is used also for sending out signals and picking up their reflections from objects in their path. Long-range radio signals enable astronauts to communicate with the earth from the moon and carry information from space probes as they travel to distant planets. For navigation of ships and aircraft the radio range, radio compass, and radio time signals are widely used. Radio signals sent from global positioning satellites can also be used by special receivers for a precise indication of position. Digital radio, both satellite and terrestrial provides improved audio clarity and volume.

Various remote-control devices, including rocket and artificial satellite operations systems and automatic valves in pipelines, are activated by radio signals. The development of the transistor and other microelectronic devices led to the development of portable transmitters and receivers. Cellular and cordless telephones are actually radio transceivers. Many telephone calls routinely are relayed by radio rather than by wires; some are sent via radio to relay satellites. Some celestial bodies and interstellar gases emit relatively strong radio waves that are observed with radio telescopes composed of very sensitive receivers and large directional antennas.

2.18 Radiofrequency Heating – Basics and Considerations

The microwave assisted pasteurization of eggs is described briefly in the Rehkopf *et al.,* 2005. The functional and physical properties of the shell eggs pasteurized by two different processes namely conduction heating and microwaves were compared for the retention of the unique quality attributes. It was found that the protein denaturation was more in the conduction mediated heat pasteurization process. Also, the treatment time of the eggs was greatly reduced to 8-12 seconds. Despite the advantages, the formation of clumps in the treated eggs is undesirable. Further advancements in the instrumentation are needed to minimize this problem so that the process can be scaled up. The basics and the considerations for design of the RF applicator is discussed below. A basic schematic of a Radio Frequency heating is shown below in Figure 2.4. The device receives standard power (i.e. 480V, 60 Hz) through the Switchgear. In the Power Supply section, line voltage is stepped up to high voltage AC through a transformer and then changed to high voltage DC through rectifiers. In the Oscillator section, high voltage DC is changed to high frequency, high voltage RF energy and transmitted to the applicator or electrodes where it is applied to the work. All of this is controlled by a modern control system.

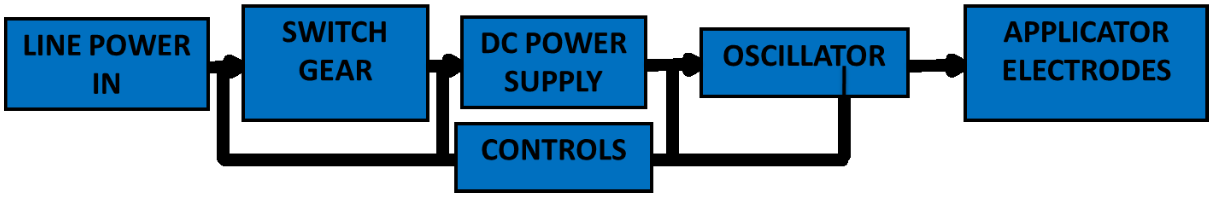

Figure 2.4 RF Heating Setup

The key to effective application of RF energy for heating is the right applicator, or electrode design (Kinn 1947). Traditionally, heating was accomplished by creating a uniform electric field between two parallel plates. This approach is capable of heating thicker materials uniformly because a high voltage gradient can be established in the material. For thin materials, the strayfield electrode design was developed. This design creates an electric field between alternating parallel rods that gives a higher voltage gradient in the material for faster heating. A variation on this electrode design for thicker material is the staggered strayfield design. This allows for more uniform heating of thicker materials (Kinn 1947). This has also been used for thin beds of ceramic powders. As a general rule, materials that are under 1/4” thick use the strayfield design, materials 1/4 ” - 1/2” use the staggered strayfield design, and materials over 1/2” use the parallel plate design. In all of these electrode designs, the material can be either self-supporting or can be transported on a conveyor.

Materials have a major effect on the success of RF heating. Some materials heat very well and some do not heat well at all. The key measure of “heatability” is the loss factor of the material. The loss factor is a material property that determines how well the material absorbs the RF energy (Kinn 1947). If the material has a high loss factor, it absorbs energy quickly and thus heats quickly. If a material has a low loss factor, it absorbs energy slowly and thus heats slowly.

In general, polymers tend to have low loss factors and thus do not heat well. Water, on the other hand, has a high loss factor so it heats rapidly. This is why RF lends itself to heating so well, it heats the water quickly but does not heat most base materials.

It is important to remember every material reacts differently and loss factors (the ability to absorb RF energy) can change with frequency and temperature. A material that does not absorb RF energy at room temperature might absorb the energy at higher temperatures. This is especially important in a composite product with a high loss factor material (RF heats rapidly) and low loss factor material (RF heats slowly). As the high loss factor material is heated by the RF energy, it will heat up the low loss factor material through normal conduction. If this heat raises the temperature of the low loss factor material to where it now absorbs RF energy, both products are heated and could be overheated. In rare cases, this can lead to a runaway situation where as the temperature increases, it absorbs more energy, which increases the temperature, which increases the energy absorbed, and it continues until the material overheats (Kinn 1947).

In most cases, the product can be heated faster than the solvent can be removed so the heating rate must be scaled back to get the right balance of heat transfer and mass transfer. If the heat transfer rate is too high, steam will be generated which can damage the product. The complexity of the interaction between materials and the RF field is why it is critical to consult with an expert in RF heating and conduct trials on the product (Kinn 1947).

Among the egg white and the yolk, which are the two primary components of the egg, the albumen is the primary infection site as SE needs only indirect contact with the yolk for its growth and multiplication and hence the albumen is the primary target of radio wave heating (Fleischman *et al*., 2003). The first hindrance for the application of radio wave frequency pasteurization is the high risk related to the pressure build-up within the eggs. However, with proper control of the process parameters, radio frequency heating can provide efficient and rapid heating for thermal pasteurization. The issue of heating uniformity can be overcome with the proper orientation of the egg and a specially designed waveguide, which is an engineering issue (Fleischman 2004) and also by the precise design of the container.

A complete understanding of the dielectric properties and egg curvature on power distribution will help design a system highly specific and efficient for this application. There are several ways of measuring the dielectric properties of different materials like perturbation technique, transmission line technique, open ended probe technique, time domain reflectometry, free-space transmission technique, microstrip transmission line etc. (Venkatesh *et al.,* 2005). Among these the open ended coaxial probe technique was found to be more appropriate and precise for measuring the dielectric properties of the egg components. Theoretical mathematical studies have shown that even though albumen exhibits better dielectric properties than yolk, the egg's curvature has a focusing effect which leads to a suitable power distribution.

2.19 Need for RF Heating as an Alternative

Salmonella enteritidis had caused several outbreaks of salmonellosis in the near past (Mead *et al.,* 1999). It is essential to prevent the bacteria from causing outbreaks in the future. One straight forward approach to ensure safety is to eat safer eggs. The microbial contamination in the eggs can be kept in check if the in-shell eggs are pasteurized and stored under refrigerated conditions. The conventional conduction mediated heating renders the eggs partially or completely cooked which is undesirable. This destroys the unique functional properties of the egg which makes egg impeccable in the food industry for various applications (Van der Plancken *et al.,* 2006). Several modern techniques had been proposed and are being evaluated as alternatives for conduction mediated heat pasteurization of eggs. One interesting alternative is the dielectric heating of eggs accomplished by using either the micro waves or the radio waves (Rehkopf *et al.,* 2005). The preliminary experiments in microwave pasteurization of eggs indicate that the technology can be scaled up with further advancements in the design, engineering and instrumentation in the near future. One major problem is the unequal heating of eggs that may lead to clumps in the treated eggs which can be easily surpassed with proper instrumentation (Rehkopf *et al.,* 2005). Another possible alternative for pasteurization of in-shell eggs is the RF heating. This power of RF heating as a potent alternative for pasteurization process has been depicted successfully in the pasteurization studies of ham (Orsat *et al*., 2004). The ham samples were heated by RF applicator so as to reach an internal temperature of 75˚C and 85˚C for 5 minutes (Orsat *et al*., 2004). Interestingly, such a high internal temperature was achieved in 5 minutes. In addition to the microbial safety, the quality attributes of the ham were little or unaltered after storage at 4˚C for 28 days in repacked plastic films (Orsat *et al*., 2004). However, literature for experimental validation of the efficacy of the pasteurization of eggs is scarce. RF heating has been used for the pasteurization of egg products. A patent for a pasteurization setup where the flowable egg product is exposed to high frequency radio waves for a predetermined time so as to pasteurize the egg product was filed (Hamid-Samimi *et al*., 2002). In addition to this, the application of radio waves in food processing is wide. The same technology can be used for drying. There is a chance for the industries to minimize the cost incurred with proper design and instrumentation wherein the same setup (or a slightly modified version) can be employed for both the drying and pasteurization process.

CHAPTER III

Dielectric properties of Egg Components and Optimization of Radio Frequency Heating Parameters

3.1 Abstract

Eggs are a potential host for microbes to invade, feed and multiply because of their rich nutritive value. *Salmonella enteritidis* is one such microbe that is highly pathogenic and is the causative agent for the disease Salmonellosis. Heat treatments are the best solution to control these pathogens. However the functional properties of the eggs should not be affected by these treatments. Thus a technique which does not cause pronounced changes to egg proteins needs consideration.

In this study, radiofrequency (RF) heating has been considered for in-shell egg pasteurization. In the first part, effects of temperature (5˚C-56˚C) and frequency (10 MHz-3GHz) on the dielectric properties of egg components were investigated. In the second part, individual egg components as well as intact in-shell eggs were bought to a temperature of 56˚C in a laboratory scale RF heating set up working at 27.12 MHz using three different electric field strength levels. The electrodes are 60 mm apart and operated at voltages 2.5, 3.5 and 4.25 kV. The heating curve was analyzed to determine the heating time required for different electrode voltage levels. Under the conditions studied, it was demonstrated that the albumen had higher dielectric constants and loss factors than the yolk. This suggested that the albumen was more efficient in converting Radio energy into heat than the yolk.

Key Words: Radiofrequency Heating, Dielectric Constant, Dielectric loss factor, Heating Rate

3.2 Introduction

Eggs are considered to be one of the nature's nutritious diets. Eggs are one of the good sources of proteins, vitamins, minerals, folic acid and choline among many other micronutrients (Li-Chan *et al*., 1995). Eggs are regarded as complete meal as it contains all the essential amino acids. This rich nutritional profile of eggs makes it an important diet. Eggs are an essential ingredient in many food processing and baking industries. This is attributed to the unique functional properties of eggs such as emulsification, foaming, coagulation and browning; rendered by the proteins present in the egg (McDonnell *et al.,* 1955). Unfortunately, many of the egg recipes involve the use of uncooked raw eggs for instance hollandaise sauce, mayonnaise, egg nog etc. Eggs are consumed in most of the countries and have constant demand in the market. This calls for a safety surveillance system that establishes the microbial safety of eggs and egg products.

Due to the potential nutritional value, eggs are potential hosts of moulds, yeasts and several bacteria notably *Salmonella enteritidis* (SE). SE is potent and efficient in successful invasion of the eggs that has led to several outbreaks of Salmonellosis (more than 90%) in the recent past that has been traced back to the Grade A shell eggs (St. Louis *et al.*, 1988). SE after ingestion finds its way into specialized intestinal epithelial cells where they release an enterotoxin that causes inflammation and subsequent diarrhea. This might be severe which requires hospitalization in high risk population that includes children, elderly people and persons with compromised immune system. As the bacteria is not able to withstand high temperature, proper cooking of the egg rules out the infection. As many recipes use raw egg, special treatment of eggs is mandatory to establish the microbial safety. The initial safety protocol insisted on the proper surveillance of SE in eggs by constantly checking the egg quality like shell integrity, washing and disinfection of the shell and environment of the egg farm. Further outbreaks due to SE in eggs have led to the consideration of alternative techniques like pasteurization for eggs. For effective pasteurization the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) recommends heating the egg white and egg yolk to a temperature of 57.5˚C and 61.1˚C for 2.5 minutes respectively to aid the SE and other food borne pathogen in eggs inactive (FSIS-USDA 2006). Egg yolk needs to be treated at high temperature than egg white as it has high solid content and at the pH of the yolk (6) SE is most heat resistant (Garibaldi *et al.,*1969). When the egg white and egg yolk are separated by breaking the eggs, the pasteurization becomes easier. However, reassembling the egg white and yolk aseptically adds to additional processing costs. This has led to the evaluation of several methods for the pasteurization of the in-shell eggs (Hou *et al*., 1996).

The prescribed pasteurization temperature as per FSIS-USDA can be achieved by conventional conduction heating methods i.e. water-bath heating (Schuman *et al*., 1997). However, the egg white gets heated up more than the egg yolk that eventually leads to the denaturation and coagulation of egg white proteins. Also the egg white gets partially cooked in the process that affects the functional properties of the egg, thus making it undesirable for use in food processing industries. On evaluating the alternative techniques of heating for the pasteurization of eggs, the principle of dielectric heating serves as the best candidate as it heats the material from within. Dielectric heating can be achieved by the application of microwaves or radiowaves.

Dielectric properties of the material are dependent on the temperature, moisture content and composition of the food; and the frequency of the applied electric field (Datta *et al.*, 2005). Evaluation of the dielectric properties of the egg white and egg yolk with respect to frequency and temperature and subsequent modeling is mandatory to facilitate better designing of the pasteurization equipment. Knowledge of these properties also helps in overcoming issues like non uniformity and complexity in scaling up such equipments (Debard 2004). It has been established that the egg white has better dielectric properties than egg yolk in microwave frequencies (200 MHz to 10 GHz) that led to better heating of the egg white under laboratory conditions (Dev *et al.,* 2008). Interestingly, within the egg shell, it was found that the electric field distribution is not so different that egg white and yolk were heated at similar rates. This may be attributed to the focusing effect rendered by the non-ellipsoidal and non-spherical architecture of the eggs. Also a thorough understanding of the dielectric properties of the egg in radio wave frequency range will enable us to design better pasteurization procedure that will eventually affect the end product quality to a minimum extent.

This study aims at establishing the following objectives: (i) Studying the effect of dielectric properties (dielectric constant (ε') and dielectric loss factor (ε'') in the frequency range of 10 MHz to 3 GHz and at temperature range of 5˚C to 56˚C. (ii) To determine the heating rates and heating time from ambient temperature to a temperature near pasteurization of egg components both in-shell and out of the shell at 27.12 MHz (approved radio frequency to be used in food industries).

3.3 Materials and Methods

In this study, the suitability of RF heating for heating fresh in-shell eggs for the purpose of pasteurization was investigated. Initially, the dielectric properties of egg albumen and yolk were measured at temperatures ranging from 5ºC to 56˚C, and at frequencies ranging from 10 MHz to 3 GHz. With the help of the empirical relationships established, the dielectric properties were expressed as a function of temperature and frequency. Secondly, in-shell eggs were heated from 24ºC to the temperature to a temperature of 56˚C in Agilent 34970A radio wave applicator. The heating rate and time taken to reach the desired temperature were calculated.

Study on the Dielectric properties of Egg components

Egg Samples

The whole eggs used in this study are of Grade A, small size with an average weight of 40 g. These egg samples were purchased from the local market, fresh and within three days of grading [determined from the best before date] (CEMA 2004). The eggs were stored in a refrigerator at 5˚C until they were used in the study.

Equipment

Measurements of the dielectric properties were made with an Agilent E4991A RF Impedance/Material Analyzer (1 MHz – 3 GHz) using a High temperature probe sensitive in the frequency range of 10 MHz – 3 GHz.

Experimental Design and Procedure

Prior to measuring the dielectric properties, the impedance analyzer was turned on and allowed to warm up for about 30 minutes. The instrument was calibrated using Agilent 16195B 7 mm Calibration kit that consisted of four calibration standards (an open circuit, a short circuit, a 50 Ω load and a low loss capacitor).

Eggs were cracked carefully prior to the measurements and the egg white (25 g) and egg yolk (15 g) were collected separately in small glass tubes. All the measurements were made in triplicates (for the egg white and yolk at each temperature).

The probe was mounted on the stand facing downwards. Calibration was done using a shorting block, air and water before each experiment and then checked by measuring distilled water at room temperature (selected standard load) to ensure that the calibration was stable.

The samples were heated using a heating block set at the required temperatures and placed right beneath the probe on the platform of the stand used to mount the probe. Measurements were taken every 5°C interval from 5°C to 56°C for egg white and egg yolk. The temperatures of the sample were measured using a digital thermometer.

Data Analysis

CurveExpert Basic 1.4 Software was used to analyze the collected data and to establish the mathematical relationships for the dielectric constant and loss factor as a function of frequency and temperature.

Radio Frequency Heating of In-shell eggs

Egg Samples

Commercially available small size fresh eggs of an average weight of 40 g were acquired from the local market in a refrigerated condition. These egg samples were purchased from the local market, fresh and within three days of grading [determined from the best before date]. (CEMA 2004). Prior to RF heating, the eggs were brought to room temperature of about 24°C by placing the opened carton on the laboratory counter for a period of three to four hours (tested by breaking and measuring inner temperatures of three representative samples) before giving the heat treatments.

Equipment

A computer controlled RF applicator was used for this study. The generator operates at a frequency of 27.12 MHz and has a maximum power output of 600 W for a maximum applied voltage of around 5 kV. The surface of application of a container which is 9 cm in diameter and 8 cm in depth is ~ 60 cm^2.

The generator is a circuit made of free running oscillator whose oscillations are sustained by a triode valve. The two galvanometers indicate the incident and reflected power. If the reflected power is more than 10% of the incident power, the generator will automatically shut down to prevent any damage to the generator.

To maximize the energy transfer between the power source and the applicator, there should be equal impedance offered by the applicator and the generator. This is maintained by the automatic impedance adaptor between the generator and the applicator. This further aids in maximizing the power transfer at the same time minimizing the reflected power.

Temperature measurements were made by optical fiber sensor probes. These probes can be inserted directly into the material and are transparent to electromagnetic interferences. A schematic figure of the applicator in standard parallel plate configuration is shown in Figure 3.1 and the RF applicator set up is shown in Figure 3.2. Egg samples are heated between square shaped electrodes. The distance between the two plates is 60 mm. A metal cabinet maintained at ground potential is used to contain the applicator so as to cease radiations from escaping to the immediate environment. A silver plated connector connects the high voltage electrode of the applicator to a voltage regulator and it aids in the accurate measurement of the voltage. The inputs were sent to a data acquisition unit (Agilent 34970A, Santa Clara, USA) which in turn is connected to a computer.

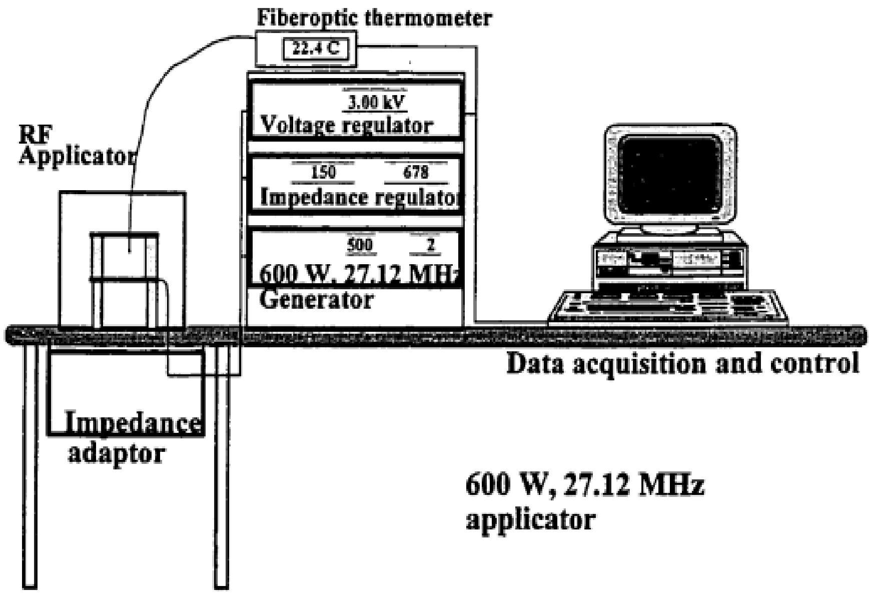

Figure 3.1 Schematic view of RF system

Figure 3.2 RF Applicator Setup

Experimental Design and Procedure

The temperature was recorded by the optic probe that was introduced through the shell of the eggs. The experiment was repeated at three different electric field strength levels by operating the electrodes at voltages 2.5, 3.5 and 4.25 kV (electrodes are 60 mm apart). Any non-uniformity in heating was not investigated for. Individual egg components were heated in a cylindrical borosilicate glass reactor. From preliminary experimental trials with Glass eggs it was determined that there was increased amount of coagulation upon heating the eggs to a temperature more than 56˚C. This might be attributed to the non-uniform temperature distribution in the eggs that leads to the development of hot spots and cold spots within the shell eggs. Even though the instrument was switched off at 56˚C to maintain the eggs at the holding temperature (56˚C) for 2.5 minutes, there was a slight increase in temperature by 1-2˚C. This might further increase the non-uniformity in the in-shell eggs. In order to control the coagulation level, the maximum heating temperature was fixed at 56˚C. The shell eggs were held 56˚C temperatures for 2.5 minutes. All the measurements were made in triplicates.

Data Analysis

Temperature measurements obtained for the in shell eggs for a given electrode voltage level were plotted as a function of temperature versus time. This was to observe the heating curves within the target temperature to study the actual heating time required for in-shell eggs to attain such temperatures. The heating rates for different electrode voltage levels were also compared. The data obtained were used to calculate the come-up time (the time taken to reach the target temperatures) for shell eggs at different electrode voltage levels as mentioned above.

3.4 Results and Discussion

Dielectric Properties of the Egg components

As shown in the Figures 3.3 and 3.4, the dielectric constant and loss factor of the egg white and egg yolk decreases in the radio frequency range (10 MHz – 300 MHz) and a linear relationship is observed in the microwave frequency range (300 MHz – 3 GHz). The decrease in the dielectric constant and the loss factor can be explained upon consideration of the polarization mechanisms in detail. This trend can be explained based on the phenomenon of dielectric relaxation which is the lag in the dielectric constant of the material in response to an external field. Here the dipoles are unable to keep up with the alternating electric field. The time delay between the electric field and dipole polarization suggests a permanent degradation of free energy. The relaxation frequency is the frequency at which dielectric parameters attain the maximum value above which these parameters gradually decrease with increase in frequency. In accordance with the Debye model this frequency is dependent on the viscosity of the liquids, such that more viscous liquids relax at lower frequencies as compared to the less viscous ones (Bircan *et al.,* 2002). In eggs the suspended matter are biomolecules and electrolytes and have much lesser relaxation frequencies than water. Dielectric loss factor is the measure of the leakage in the stored charge in any form of energy. Since the net dielectric constant decreases with increasing frequency, the total charge stored decreases and as a result the dielectric loss factor also decreases with increasing frequency.

It is evident that the egg white possess higher dielectric constant than the yolk which can be attributed to the high water content of the egg white (>90%) than egg yolk (<50%). Since the water content is high, there is high rotational polarization in the egg white than the egg yolk; as a result the net dielectric constant is higher.

As shown in the Figure 3.5, the dielectric constant of the egg white and the egg yolk slightly decreased with increasing temperature. This is due to the loss in the rational polarization as the molecules deviate from aligning to the alternating electric field due to high thermal energy readily available. This thermal energy facilitates random motion of the molecules and therefore the molecule deviates from properly aligning to the alternating electric field. Rightly so, the dielectric loss factor of the egg white increases with increasing temperature which may be due to the increase in the ionic polarization due to increase in the number of free ions available. This can be duly confirmed by measuring the ionic conductance of the egg white at different temperatures. However, the dielectric loss factor of the egg yolk follows a random pattern. The loss factor initially decreases and then increases. The initial decrease may be due to the massive decrease in the orientational polarization. At high temperatures, due to high solid content in the yolk, the thermal energy may render several free ions which may contribute to the increased loss of energy. Ionic conductance has been proven to play a major role at RF frequencies while water molecules affect dielectric properties at higher frequencies (Wang *et al.,* 2009). Determining the ionic conductance may help in proving the above hypothesis.

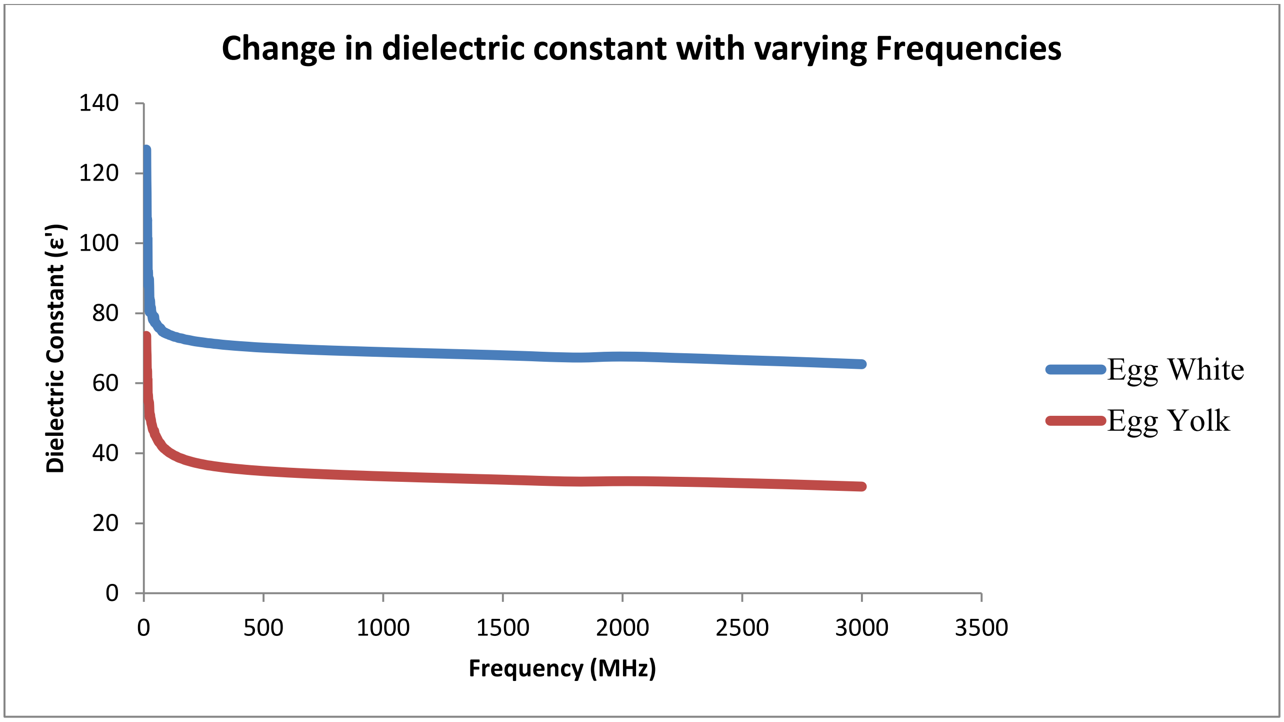

Figure 3.3 Change in the Dielectric constant of Egg Components with varying Frequencies

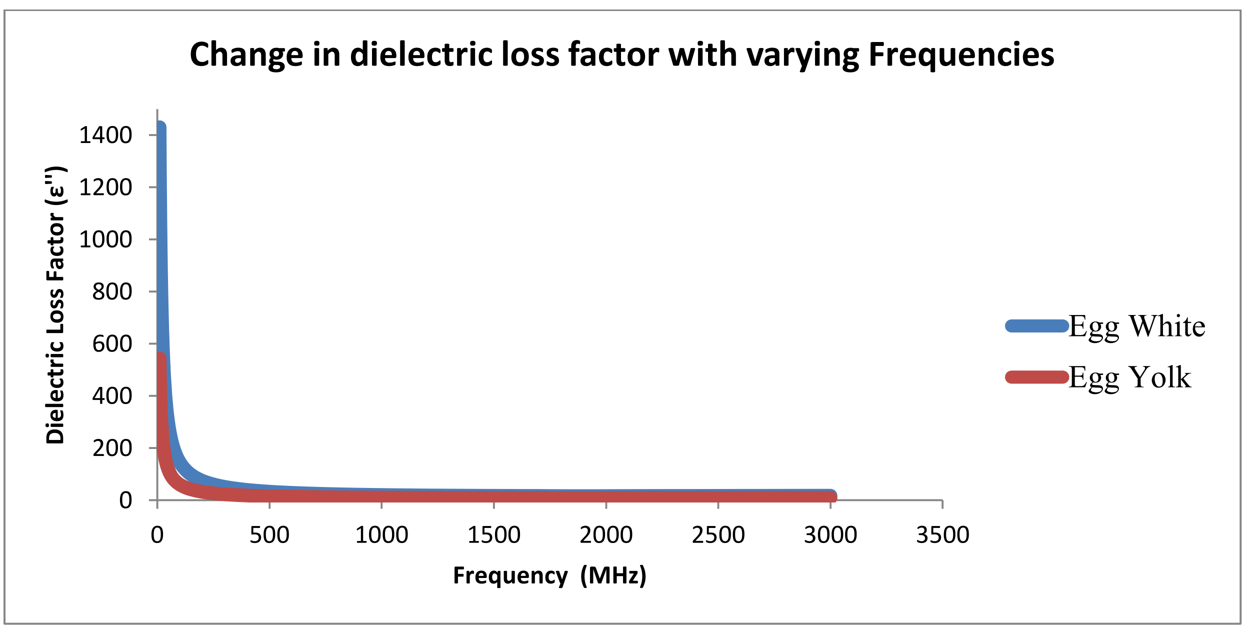

Figure 3.4 Change in the Dielectric Loss Factor of Egg Components with varying Frequencies

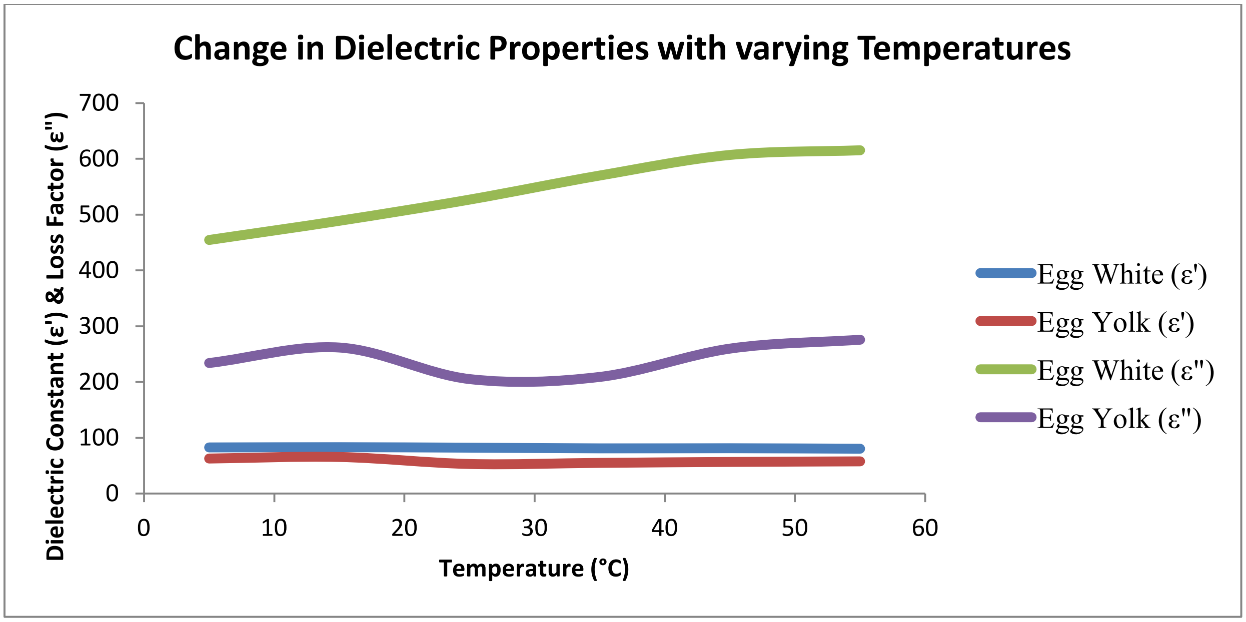

Figure 3.5 Change in Dielectric Properties of Egg Components with varying Temperatures

Upon fitting the data in the Curve Expert software and subsequent modeling, the following equations **(1)** – **(4)** are derived for the dielectric parameters as a function of the temperature and frequency. These equations can help determine the dielectric constant and dielectric loss factor at a point between the frequency 10 MHz to 3 GHz and at temperature from 5˚C to 56 ˚C.

$$\varepsilon'(EW) = (71.2 - 0.12\,T + 0.0004T^2)e^{\frac{(4.84+0.03\,T)}{f}} \qquad R^2 = 0.98 \tag{1}$$

$$\varepsilon'(EY) = (21.53 + 6.36T - 0.0435T^2 + 0.010T^3 - 0.00008T^4)e^{\frac{(13.73-1.40T+0.086T^2-0.0019T^3+0.00001T^4)}{f}} \qquad R^2 = 0.97 \tag{2}$$

$$\varepsilon''(EW) = 6.18 - 0.00026T - 0.00024T^2 + \frac{(12349.8+60.57T+0.54T^2)}{f} \qquad R^2 = 0.98 \tag{3}$$

$$\varepsilon''(EY) = 10.57 + 8.30T - 0.535T^2 + 0.012T^3 - 0.00095T^4 + \frac{(12.83-1.28T+0.07T^2-0.0018T^3+0.000013T^4)}{f} \qquad R^2 = 0.95 \tag{4}$$

Where,

EW- Egg White,

EY- Egg Yolk,

f - Frequency and

T- Temperature.

Radiofrequency heating of Individual Egg Components

In order to optimize the in-shell heating of eggs, the behavior of the individual components in the radiofrequency range is studied. As expected the egg white heated at a faster rate than the egg yolk because of better dielectric properties. It can be observed from Figures 3.6 and 3.7, that in RF heating the egg white is heated at a faster rate than the egg yolk. For the three different operating electrode voltages tested, egg yolk heated to 56˚C in 44.5, 20 and 17 minutes respectively at 2.5, 3.5 and 4.25 kV while the egg white heated at 12.6, 6.24 and 3.5 minutes respectively at 2.5, 3.5 and 4.25 kV. The heating rates for the egg yolk were 0.674, 1.48 and 1.76˚C/min at 2.5, 3.5 and 4.25 kV respectively. The heating rates for the egg white were 2.3, 4.8 and 8.7˚C/min at 2.5, 3.5 and 4.25 kV respectively. The non uniform heating and hence the coagulation was more at higher electrode voltage levels.

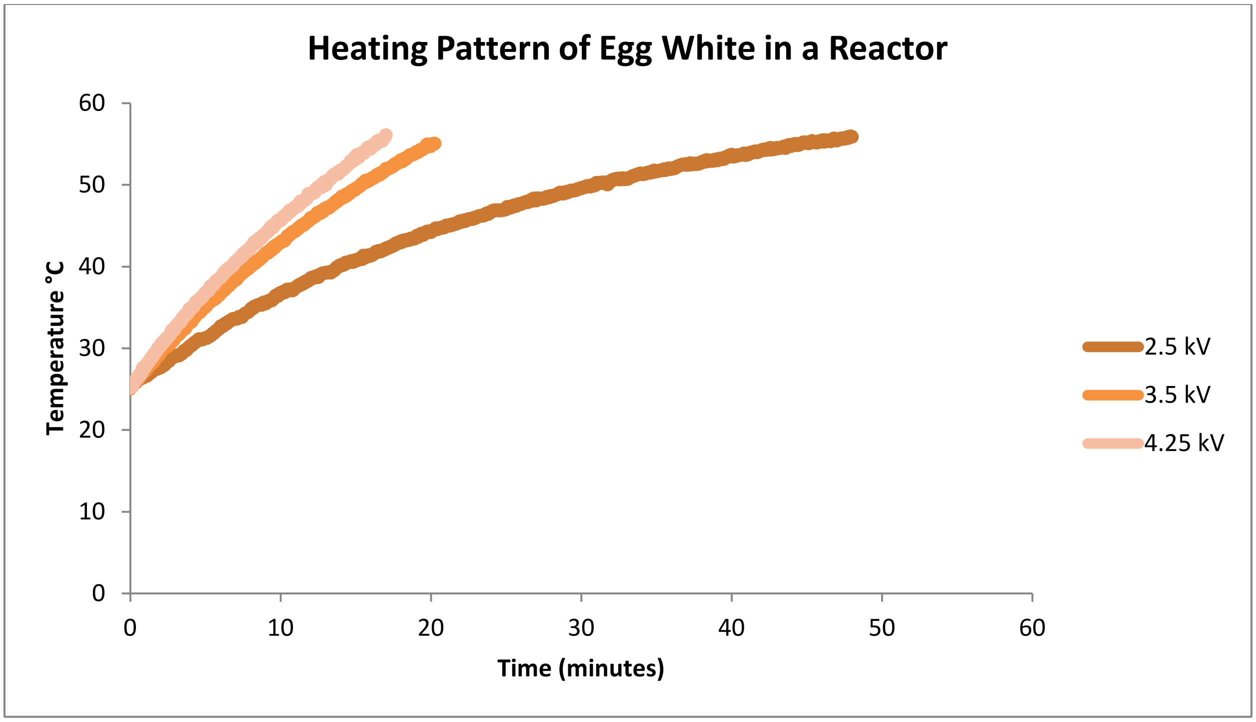

Figure 3.6 Heating Pattern of Egg White in Reactor at 27.12 MHz

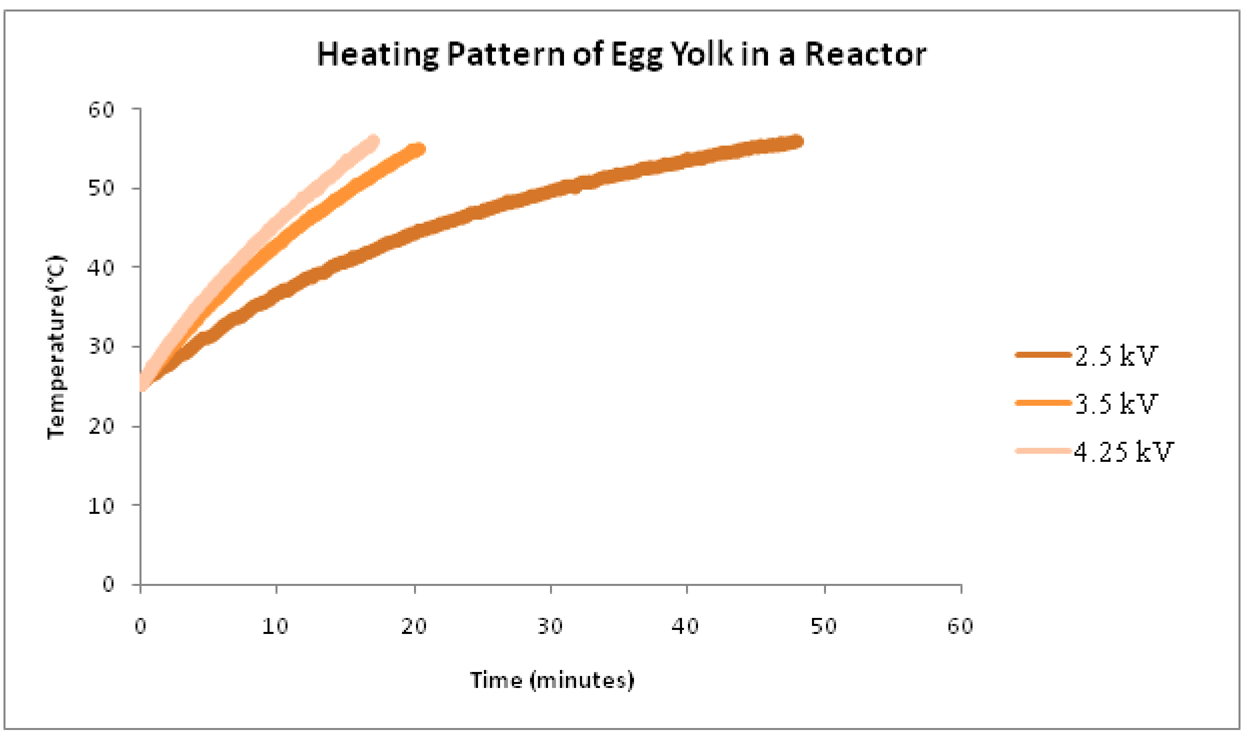

Figure 3.7 Heating Pattern of Egg Yolk in Reactor at 27.12 MHz

Radiofrequency heating of In-shell Eggs

The heating curves of in-shell egg were studied using the laboratory scale parallel plate radiofrequency applicator. An optic fiber sensor, carefully inserted through the egg shell, was used to monitor the temperature of the egg during processing. The heating pattern of the in-shell egg is shown in the Figure 3.8. It is evident from the graph that even after 35 minutes of heating the egg at electrode voltage 2.5 kV, it only reached a temperature of 45˚C. Further heating of the egg resulted in pronounced coagulation of the egg white. The heating time for the eggs at electrode voltages of 3.5 and 4.25 kV were 14 and 24 minutes respectively. However, higher the electrode voltage level, greater is the electric field strength and consequently the coagulation of the egg protein is found to be greater. In other words, the coagulation lumps seen at a setting of 3.5 kV is much lesser than that observed at the 4.25 kV setting.

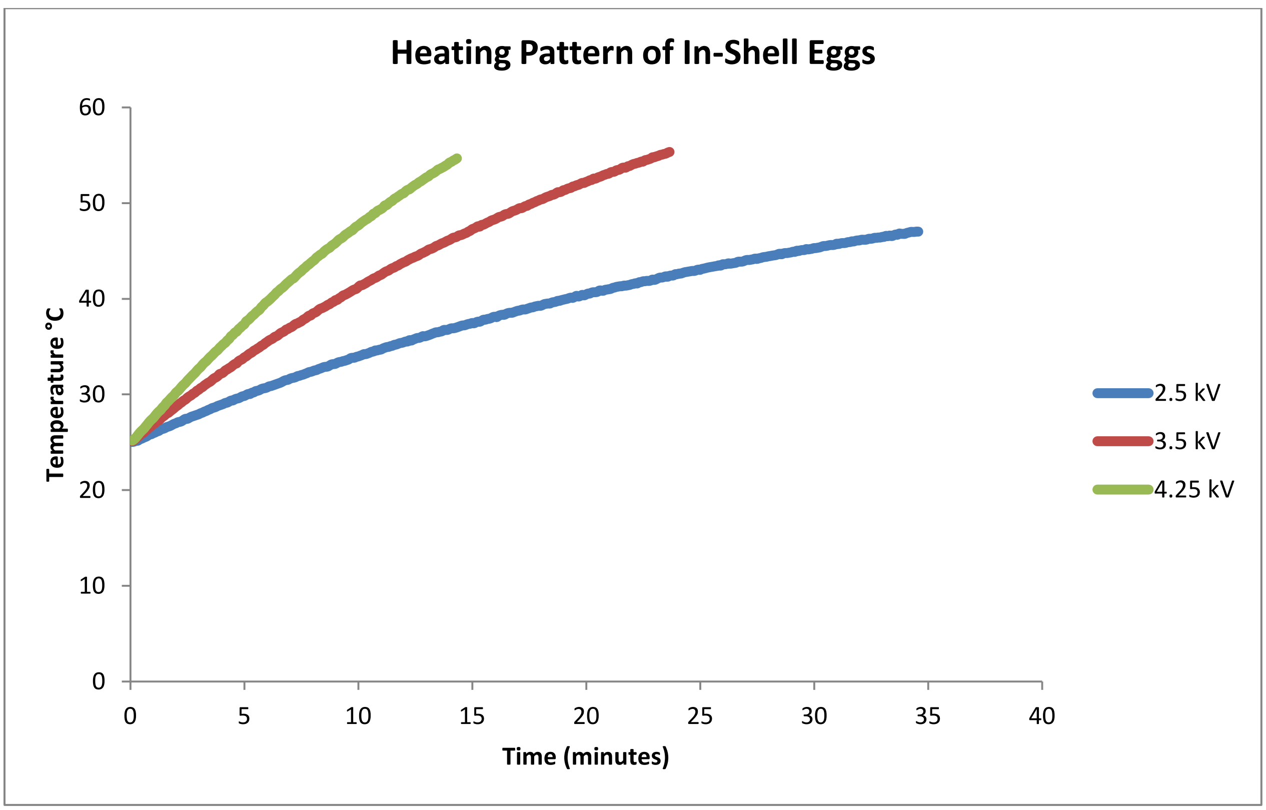

Figure 3.8 Heating pattern of In-shell Eggs at 27.12 MHz

3.5 Conclusion

The study of dielectric properties yielded results that offered an excellent qualitative understanding of the egg constituents under RF heating. The egg white displayed better dielectric properties as compared to the yolk. Also, the heating curves of the egg components as well as the shell eggs provided the actual heating time required for different electrode voltage levels. The non-uniformity in heating was found to be more pronounced at higher electrode voltage levels. This information could be utilized in designing or improving the design of existing equipments to achieve RF pasteurization of in-shell eggs with minimal damage to the product quality.

3.6 Acknowledgements

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Connecting text

It is clear from Chapter III that numerical modeling and simulation is inevitable to design and develop a radiofrequency applicator for shell eggs. One can take different numerical approaches for predicting the temperature profile inside a food material subjected to dielectric heating for instance FDTD and FEM. FDTD modelling and simulation, restricted to handle rectangular shapes and simple alterations thereof, is relatively simple and less computationally intensive approach. The most attractive feature of FEM is its ability to handle complex geometries (and boundaries) with relative ease. The handling of geometries in FEM is theoretically straightforward. There are reasons to consider the mathematical foundation of the finite element approximation sounder, because, for instance, the quality of the approximation between grid points is poor in FDTD, and so, the quality of an FEM approximation is often higher than the corresponding FDTD approach. In the following chapter, FEM is used to simulate the RF heating of shell eggs. Also process optimization and lab scale experimental validation needs to be carried out using validated simulation approaches before fabrication.

CHAPTER IV

Optimization of Radiofrequency Heating of In-Shell Eggs through Finite Element Modeling and Experimental Trials

4.1 Abstract

Considering Radio Frequency (RF) heating as a viable alternative for the in-shell heating of eggs, Finite Element Modelling and simulation of RF heating of in-shell eggs at 27.12 MHz were carried out to assess the feasibility and heating uniformity of the process. According to the recommendations of USDA-FSIS for the pasteurization of eggs, egg white must be heated upto 57.5ºC and the egg yolk has to be heated upto 61.1ºC. The objective of the simulation was to determine the location of hot spots and cold spots generated due to non-uniform heating. A parallel plate setup for Radio Frequency heating was simulated for different electric field strength levels and orientations of the egg (long axis parallel and long axis perpendicular to the plates). The simulation results were experimentally verified and the simulation procedure was validated using a laboratory parallel plate RF setup. A coaxial cavity design was simulated with a similar approach. Results indicated that both the parallel and coaxial cavity designs were suitable for in-shell pasteurization of eggs provided that the eggs were rotated to maintain the uniformity in heating. After the simulation of RF heating process, the process optimization was carried out to determine the most effective procedure for the process. The varying parameters obtained by using different modeling techniques for radiofrequency heating of in-shell eggs, were optimized using MATLAB. Laboratory scale experimental trials were conducted to test the validity and effectiveness of the optimized parameters. The optimal parameters set forth were found to be more efficient in terms of heating time and uniformity.

Keywords Radio frequency heating, pasteurization, eggs, food safety

4.2 Introduction

Eggs are considered to be one of the natural complete meals available as it has all the essential amino acids. In addition, eggs are one of the good sources of vitamins, minerals, folic acid and choline among many other micronutrients (Li-Chan *et al.,* 1995). The unparalleled functional properties of the egg make it an essential ingredient in the food processing and baking industry. Unfortunately, there are several of the egg recipes which involve the use of uncooked raw eggs. Due to their high nutritional value, eggs are potential hosts of several bacteria notably Salmonella enteritidis (SE) (FSIS-USDA, 2006). SE is potent and efficient in successful invasion of the eggs that has led to several outbreaks of Salmonellosis (more than 90%) in the recent past most of which has been traced back to the Grade A shell eggs (Schroeder *et al*, 2005; St. Louis, *et al.,* 1998). This calls for advanced safety protocols that regulate the safety of eggs. The present techniques for pasteurization of eggs primarily using hot water bath or steam are not effective, as they adversely affect the functional properties of the egg. Dielectric heating with the aid of Electro Magnetic Waves (EMW) like micro waves and radio waves are being evaluated to pasteurize the whole in-shell eggs (Dev *et al.*, 2008).

This study examines the use of radiofrequency (RF) heating to pasteurize the eggs. In pasteurization uniform heating is essential to maintain the quality of the egg. There are several factors that are to be considered while designing the wave applicator to uniformly heat the egg. For instance, placement of electrodes, distance between the electrodes, shape of the electrodes, length of the electrodes and spatial placement of the egg between the electrodes are a few such factors that affect the uniformity in heating. To aid in the designing of the applicator, Finite Element Method (FEM) has been employed to determine the optimized parameters (Zhou *et al.*, 1995).

FEM has been employed successfully to solve complex partial differential equations which represent a non-homogenous, irregularly shaped system (Jia *et al.*, 1992). In this study, FEM is used to model the temperature distribution in the in-shell egg using a parallel plate RF heating system. This modeling helps to determine the hot and cold spots for a particular process parameter. Further, fine tuning can be employed by varying the process parameters to arrive at an optimized condition that renders more uniform heating. Experimental verification of the optimized parameters is done subsequently. Also, coaxial cavity method of RF heating was also simulated in a similar manner and the efficacies of the methods were compared.

4.3 Materials and Methods

Mathematical Model

Electromagnetics

The Maxwell's equations that govern the electromagnetic phenomena involving a given configuration resolved in 3D space are given by equations **(5)-(10)** (Dai 2006).

$$\frac{\partial E_x}{\partial t} = \frac{1}{\varepsilon_0 \varepsilon'}\left(\frac{\partial H_z}{\partial y} - \frac{\partial H_y}{\partial z}\right) - \frac{2\pi f \varepsilon''}{\varepsilon'} E_x \quad \textbf{(5)}$$

$$\frac{\partial E_y}{\partial t} = \frac{1}{\varepsilon_0 \varepsilon'}\left(\frac{\partial H_x}{\partial z} - \frac{\partial H_z}{\partial x}\right) - \frac{2\pi f \varepsilon''}{\varepsilon'} E_y \quad \textbf{(6)}$$

$$\frac{\partial E_z}{\partial t} = \frac{1}{\varepsilon_0 \varepsilon'}\left(\frac{\partial H_y}{\partial x} - \frac{\partial H_x}{\partial y}\right) - \frac{2\pi f \varepsilon''}{\varepsilon'} E_z \quad \textbf{(7)}$$

$$\frac{\partial H_x}{\partial t} = \frac{1}{\mu_0}\left(\frac{\partial E_y}{\partial z} - \frac{\partial E_z}{\partial y}\right) \qquad \textbf{(8)}$$

$$\frac{\partial H_y}{\partial t} = \frac{1}{\mu_0}\left(\frac{\partial E_z}{\partial x} - \frac{\partial E_x}{\partial z}\right) \qquad \textbf{(9)}$$

$$\frac{\partial H_z}{\partial t} = \frac{1}{\mu_0}\left(\frac{\partial E_x}{\partial y} - \frac{\partial E_y}{\partial x}\right) \qquad \textbf{(10)}$$

As shown in Chapter III, the dynamically changing dielectric constant and loss factor were calculated using equations **(1) to (4)**, modified to SI units.

$$\varepsilon'(EW) = (71.2 - 0.12\,T + 0.0004T^2)e^{\frac{(4.84+0.03\,T)}{f}} \qquad \textbf{(1)}$$

$R^2 = 0.98$

$$\varepsilon'(EY) = (21.53 + 6.36T - 0.0435T^2 + 0.010T^3 - 0.00008T^4)e^{\frac{(13.73-1.40T+0.08\text{[illegible]})}{f}} \qquad \textbf{(2)}$$

$R^2 = 0.97$

$$\varepsilon''(EW) = 6.18 - 0.00026T - 0.00024T^2 + \frac{(12349.8+60.57T+0.54T^2)}{f} \qquad \textbf{(3)}$$

$R^2 = 0.98$

$$\varepsilon''(EY) = 10.57 + 8.30T - 0.535T^2 + 0.012T^3 - 0.00095T^4 + \frac{(12.83-1.28T+0.07T^2-0.0018T^3+0.000013T^4}{f} \qquad \textbf{(4)}$$

$R^2 = 0.95$

Where,

EW- Egg White,

EY- Egg Yolk,

f - Frequency and

T- Temperature.

Constant values of 5.0 and 0.5 were taken for the ε' and ε" values of both the shell and shell membrane (Dev *et al.,* 2008).

The time average electric field strength dissipated in each element in a dielectric material was obtained by integrating the Pointing vector over the closed surface S for each tetrahedral element (Jia *et al.,* 1992):

$$P_{av} = -\frac{1}{2}\int_S P_c.dS \qquad \textbf{(11)}$$

Where $P_c = E \times H$

Volumetric heat generation can be expressed in terms of electric field strength intensity in three orthogonal directions (Lin *et al.,* 1989):

$$Q = \frac{\partial P_{av(x)}}{\partial V} + \frac{\partial P_{av(y)}}{\partial V} + \frac{\partial P_{av(z)}}{\partial V} \qquad \textbf{(12)}$$

Boundary conditions

Perfect Electrical Conductor boundary conditions (n x E = 0) were used for the walls of the cavity and Perfect Magnetic Conductor boundary condition (n x H = 0) was used for the symmetry boundaries (Fu *et al.,* 1994).

Boundary conditions at the port were as follows:

$$H_y = ACos(\frac{\pi x}{a})\ Cos(\omega t + \beta y) \qquad \textbf{(13)}$$

$$E_z = (\omega\mu_0 \frac{a}{\pi}) ASin(\frac{\pi x}{a})\ Sin(\omega t + \beta y) \quad \textbf{(14)}$$

$$H_x = (\frac{\beta a}{\pi}) ASin(\frac{\pi x}{a}) Sin(\omega t + \beta y) \quad \textbf{(15)}$$

Heat transfer

For an incompressible food material heated under constant pressure, the thermal energy equation is given by equation (16) (Dai 2006)

$$\rho C_p \frac{\partial T}{\partial t} = \nabla \cdot (K \nabla T) + Q \quad \textbf{(16)}$$

Simulation

A Finite Element Model was developed using COMSOL Multiphysics version 3.4 (COMSOL Inc., USA) software package to simulate a parallel plate setup for Radio Frequency heating simulation at different electric filed strengths. This was achieved by operating the electrodes (60 mm apart) at 4.2, 5.0 and 5.7 kV. The parameters tested are the orientations of the egg (long axis parallel and long axis perpendicular to the plates). Also a simulation for a coaxial cavity design was carried out. Figure 4.1 shows the flow diagram of the simulation technique. A time domain based iterative solver was used which iteratively solves for the electromagnetic equations and the heat transfer equations for each time step. The time steps were chosen to be linear or logarithmic in real-time, adaptive to the convergence of the solution. This approach coupled with the bilateral symmetry of the cavity and waveguide was taken advantage in the simulations, thereby greatly reducing the resources required for running these simulations.

Considering a statistical accuracy of 95% in reality, for the simulations, any change less than 5% in the dielectric properties was considered statistically insignificant and therefore ignored. This helped performing the simulation like a step function wherein the electric field strength distribution was calculated only for every 5% change in dielectric properties, reducing the number of iterations by nearly 5 orders of magnitude, while still being able to account for transient dielectric properties.

Different element sizes were used for different sub-domains based on the dielectric properties of the sub-domain and the precision required in the sub-domain of interest. Also egg rotation was simulated by moving meshes with an angular velocity of $\frac{\pi}{18}$ rad.s^{-1}, programmed using COMSOL Script version 1.2. Different configurations of RF applicators like a parallel plate applicator and a coaxial cavity applicator were simulated. Figures 4.2 (a) and (b) give the FEM structure of the cavities with parallel plate applicator and a coaxial waveguide applicator respectively.

A custom-built computer with Intel Core 2 Quad 2.4 GHz processor and 8 GB primary memory was used to run the simulations.

Experimental Validation

Equipment

Simulations were also computed for an all-white egg in order to be able to verify the simulation approach with an artificial egg (a transparent glass egg made with 40 ml of real egg white as shown in Figure 4.9). The simulation results were experimentally verified by heating the artificial egg in a custom built, computer controlled parallel plate RF applicator (Figure 4.3). The generator operates at a frequency of 27.12 MHz and has a maximum power output of 600 W for a maximum applied voltage of around 5 kV. The aluminum applicator plates are separated from each other by 44 mm. The RF generator was a circuit made of free running oscillator whose oscillations are sustained by a triode valve. The two galvanometers indicate the incident and reflected electric field strength. To maximize the energy transfer between the electric field strength source and the applicator, there should be equal impedance offered by the applicator and the generator. This is maintained by the automatic impedance adaptor between the generator and the applicator. This further aids in maximizing the power transfer at the same time minimizing the reflected power.

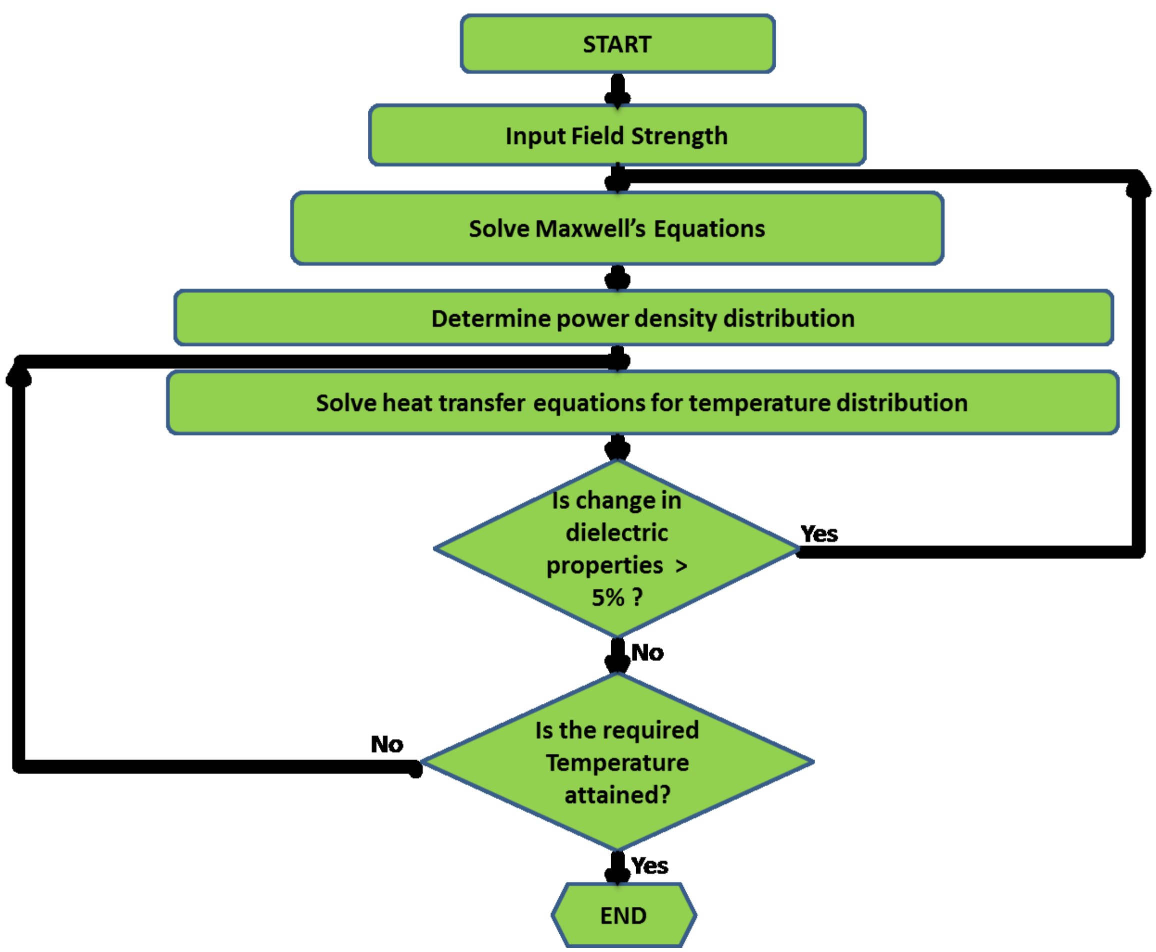
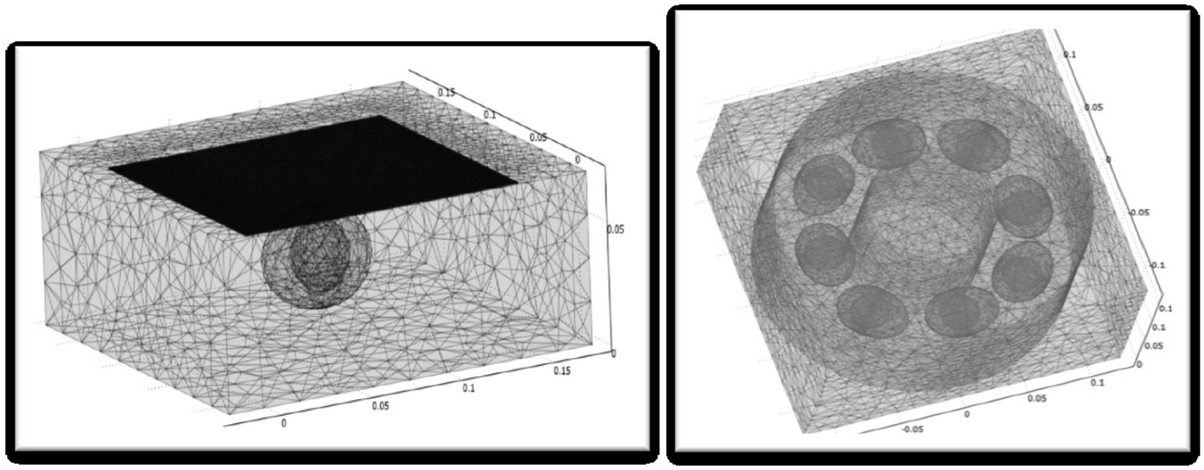


Figure 4.1 Flow Diagram of FEM Simulation Technique



(a) (b)

Figures 4.2 FEM structure of the (a) Parallel plate applicator (b) Coaxial cavity applicator

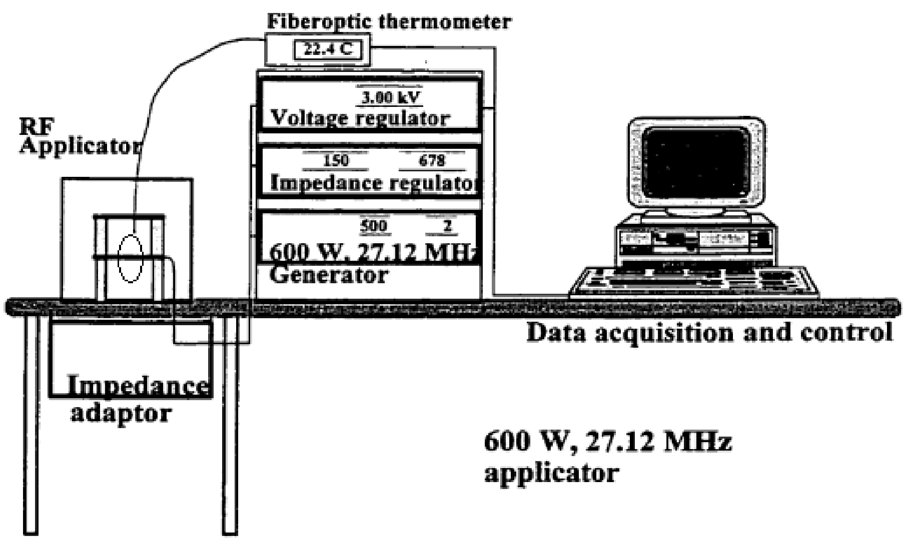


Figure 4.3 Schematic view of RF system

Experimental Design and Procedure

The temperature was recorded by the fiber optic probe that was introduced through the cap of the glass eggs. The experiment was repeated at different electric field strength densities.

The electrodes are operated at voltages 4.2, 5.0 and 5.7 kV and the electrodes are 60 mm apart. Any non-uniformity in heating was not investigated. The artificial eggs were held at the pasteurization temperatures (±0.5°C) for 2.5 min. All the measurements were made in triplicates.

Data analysis

Temperature measurements obtained for the glass eggs for a given electric field strength level were plotted as a function of temperature versus time. This was to observe the heating curves within the pasteurization temperatures to study the actual heating time required for glass eggs to attain such temperatures. The heating rates for three different operating electrode voltages 4.2, 5.0 and 5.7 kV were also compared. The data obtained were used to calculate the come-up time (the time taken to reach the pasteurization temperatures) for the eggs at different electrode voltages as mentioned above.

4.4 Results and Discussion

Simulation

The Figure 4.4 shows the simulation result of the temperature distribution in the in-shell egg in three different planes XY, YZ, ZX when heated in a parallel plate RF applicator with 5 mm air gap between the eggs and the electrodes. The eggs are kept static between the electrodes. The Figure 4.4 legend shows the temperature in K. It is clearly evident from the Figure 4.4 that the heating is highly non uniform and this may lead to the generation of cold spots and hot spots within the shell eggs. Egg white gets heated up faster than the egg yolk in the parallel plate RF applicator which is not desirable for the pasteurization process.

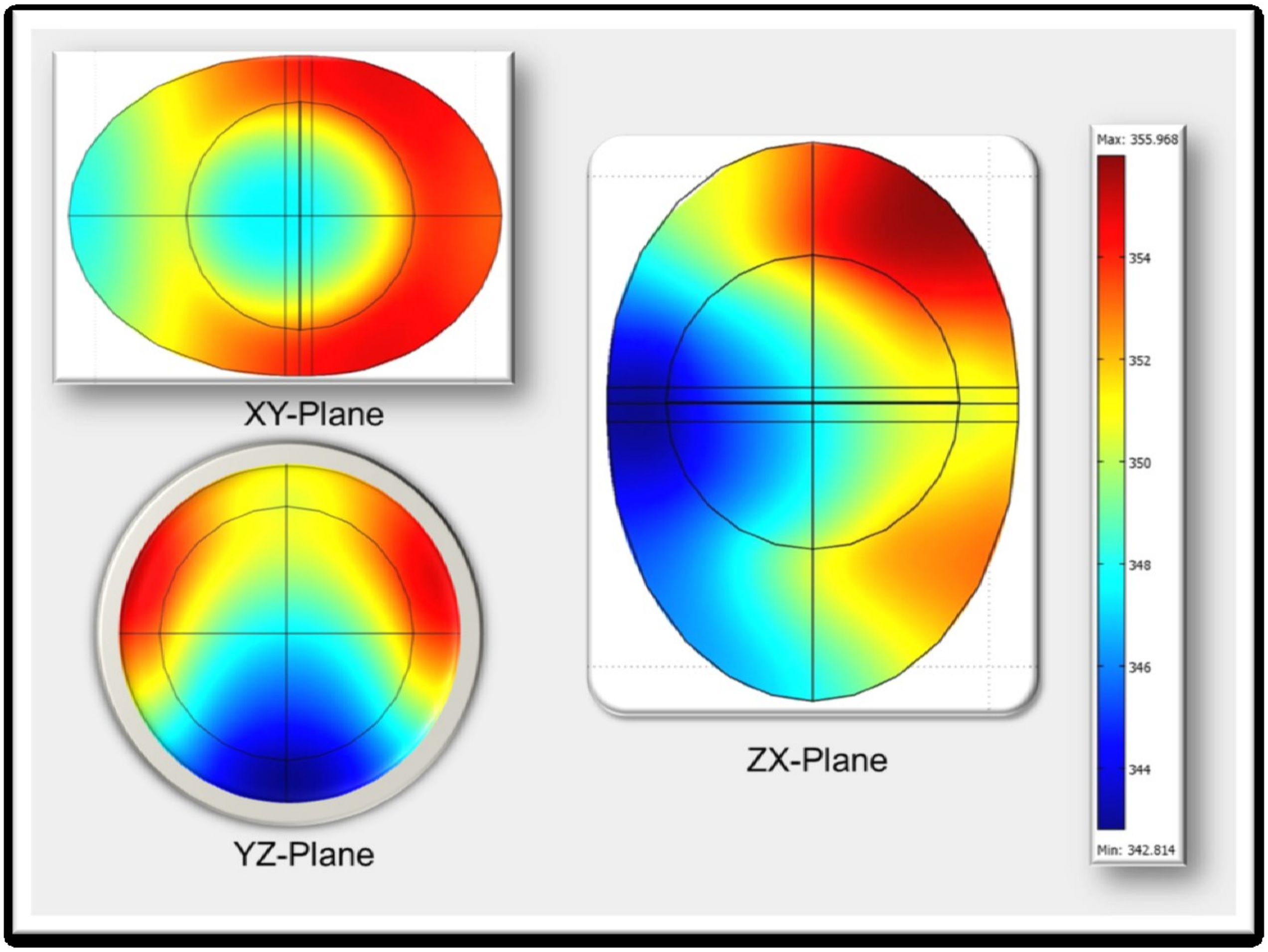


Figure 4.4 Temperature Distribution of the In-shell Egg heated in a Parallel Plate RF Applicator (5 mm air gap, Eggs Static)

The Figure 4.5 shows the simulation result of the temperature distribution of the in-shell egg heated in a parallel plate RF applicator with 0.5 mm air gap between the eggs and the electrodes. The eggs are kept static between the electrodes. On comparing the Figures 4.4 and 4.5, it is clearly evident that the non uniformity in heating is greater when the air gap between the egg and electrodes is 0.5 mm. Closer the eggs get to the electrodes, the faster the egg white gets heated up as compared to the egg yolk. It might lead to the increased coagulation of the egg white proteins which is a major drawback as the functional properties of the egg will be affected.

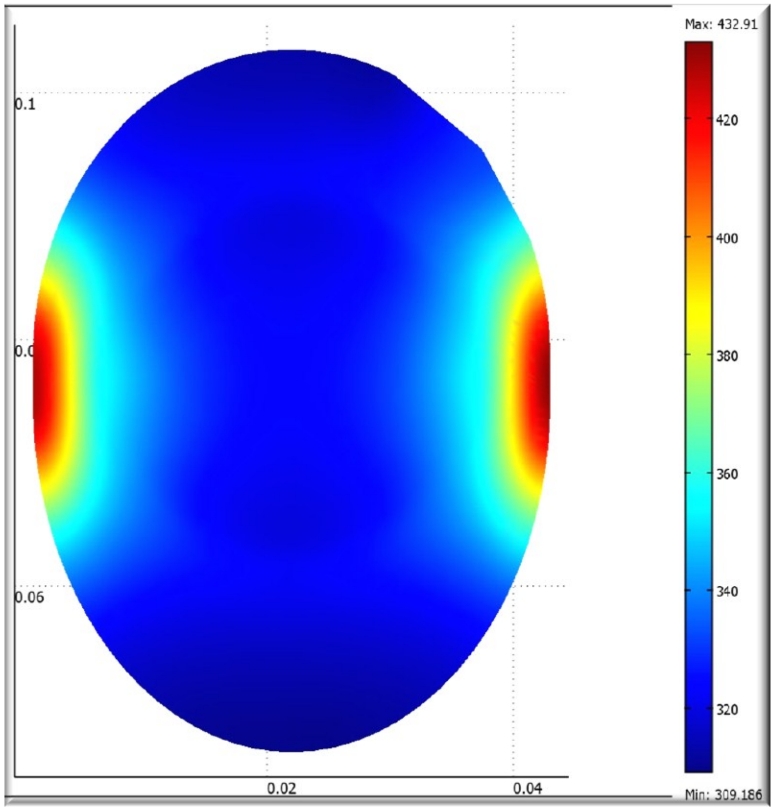


Figure 4.5 Temperature Distribution of the In-shell Egg heated in a Parallel Plate RF Applicator (0.5 mm air gap, Eggs static)

The Figure 4.6 shows the simulation for the temperature distribution of the eggs heated in a parallel plate RF applicator with 5 mm air gap between the eggs and the electrodes. However, the condition here is that the eggs are rotated between the electrodes. In comparison with the previous Figures 4.4 and 4.5 where the eggs were held static Figure 4.6, shows the temperature distribution of the eggs to be convincingly uniform. The egg yolk is heated more than the egg white as preferred. This is of great advantage in the in-shell egg pasteurization process where heating the egg yolk more than the egg white is essential. Also as the heating is uniform, there will be no coagulation of the proteins in the egg white thereby maintaining the unique functional properties of the egg even after the heat treatment.

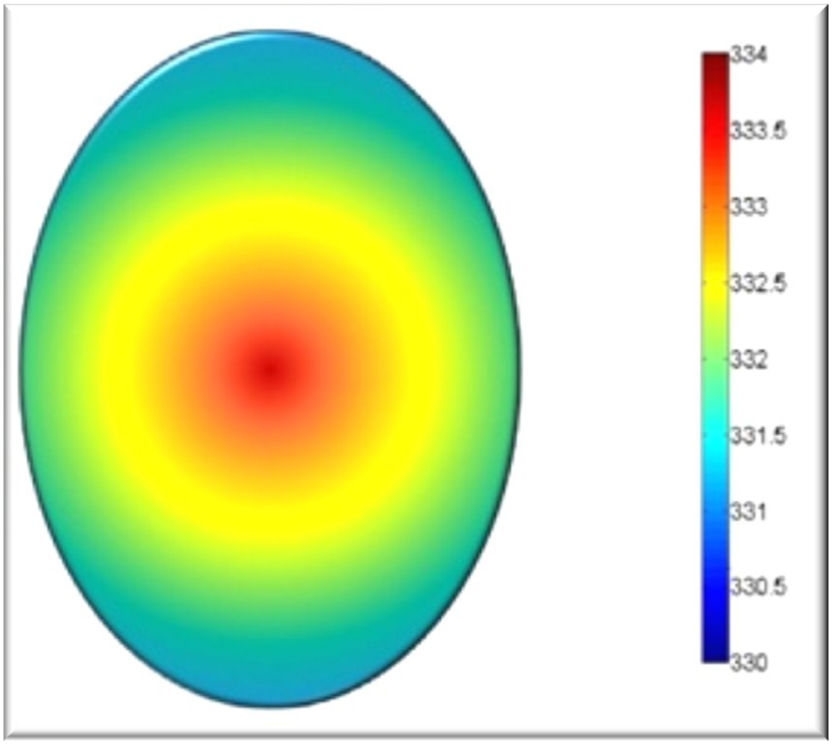


Figure 4.6 Temperature Distribution of the In-shell Egg heated in Parallel Plate RF Applicator (5 mm air gap, Eggs rotating)

The Figure 4.7 shows the simulation results of the electric field distribution in a coaxial cavity RF applicator when the eggs are heated. As it can be clearly seen from the Figure 4.7 that the inside of the coaxial cavity has low voltage and the outside of the cavity has higher voltage. The temperature distribution of the in-shell eggs that are kept static with 5 mm air gap heated in a coaxial cavity RF applicator is shown in Figure 4.8. When the eggs are kept static and heated in the coaxial cavity RF applicator, the temperature distribution in the in-shell egg is similar to that of the eggs when heated in a parallel plate RF applicator with 5mm air gap. This is obvious while comparing the Figures 4.4 and 4.8. The only difference between the temperature distributions of the two treatments observed is that there is a swap over between the temperature distribution in

XY plane and the ZX plane. This is because the eggs are kept in a position where the heating takes place from top to bottom in the parallel plate RF applicator whereas in the coaxial cavity the heating takes place from side to side. This is a 90˚ switch in the direction of the heat application between the two treatments and this has led to the switch over of the distribution patterns between the XY and ZX planes. Even when the eggs are kept static and heated in the coaxial cavity RF applicator, the heating is highly non uniform.

The simulation of the RF heating in a coaxial cavity RF applicator when the eggs are rotated and heated is very tedious and practically cumbersome. This is because the eggs are constantly fed into the RF applicator and at a time there are multiple eggs to simulate. So there is a need for a lot more resources and the process has numerous mathematical and numerical issues. We expect that by heating the eggs that rotate within a Teflon cylinder in a coaxial cavity RF applicator with 5 mm air gap between the electrodes and the eggs, it is possible to achieve more heating of the yolk than the white as the uniformity in the heating is maintained. This set up would be suitable for applications at an industrial scale.

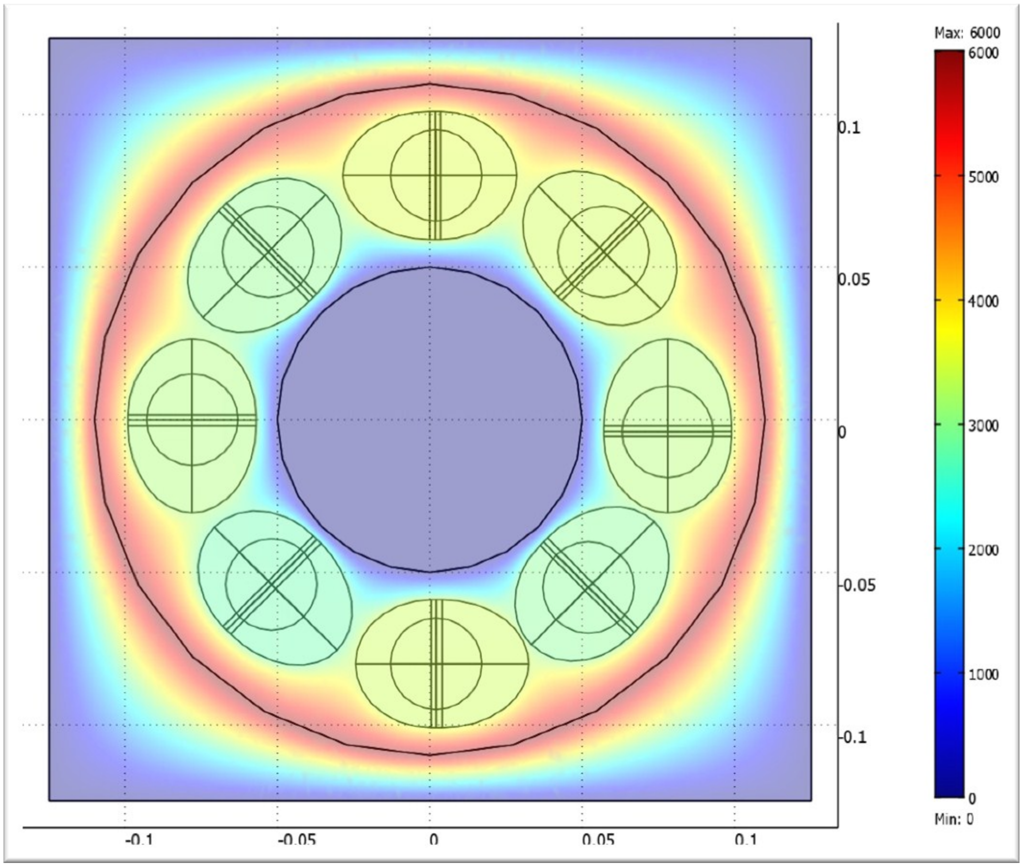


Figure 4.7 Electric Field Distribution of the In-shell Egg heated in a Coaxial Cavity RF Applicator (5 mm air gap, Eggs Static)

Experimental Validation

Numerically simulated results corroborated well with the experimental data. The extent of coagulation obtained in the simulation and in the experiment (eggs are kept static and heated in a parallel plate RF applicator with 5 mm air gap) was in agreement. The Figure 4.9 shows the levels of coagulation observed upon heating egg white in specially designed glass eggs. It is clear from the picture that the extent of coagulation depends on the electric field strength level used. As the electric field strength level increased, the extent of coagulation also increased. As the egg white is heated the protein is denatured and loses its 3D confirmation which leads to a decrease in the density, as a result of which the coagulation lumps float to the top and forms a cooked layer at the top.

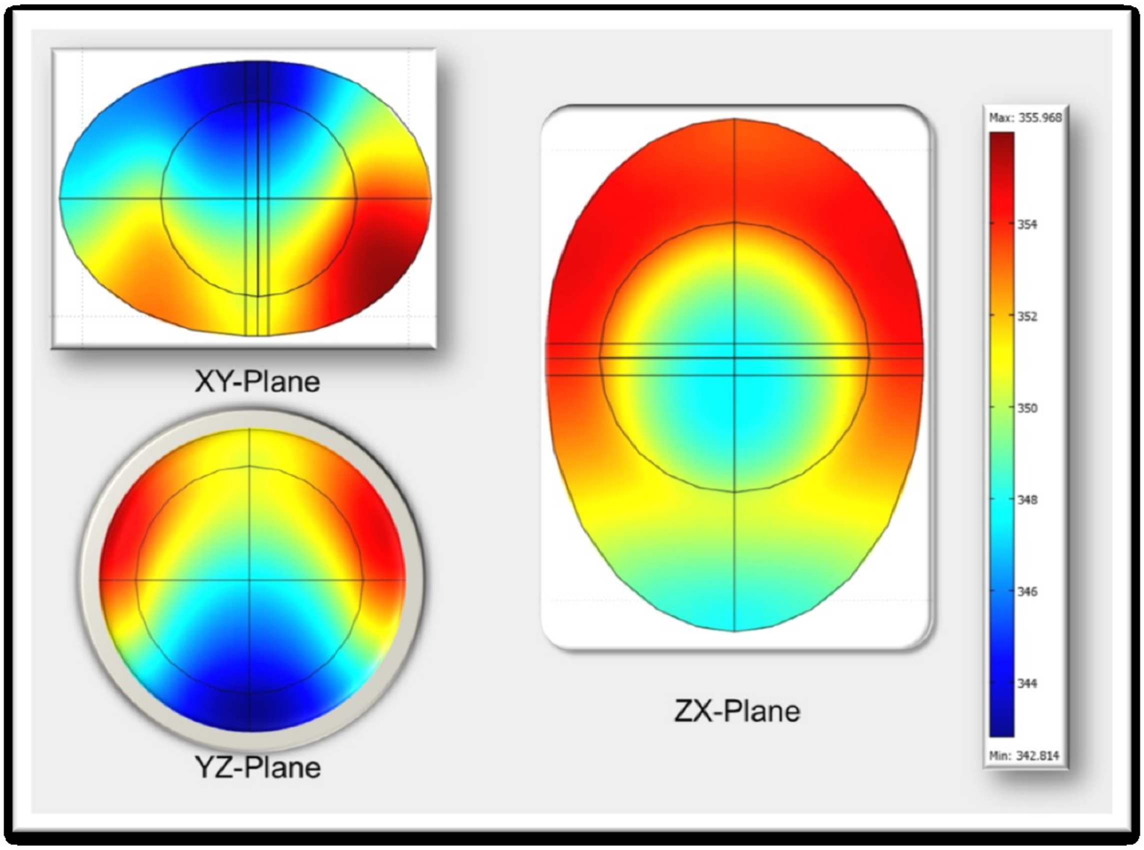


Figure 4.8 Temperature Distribution of the In-shell Egg heated in a Coaxial Cavity RF Applicator (5 mm air gap, Eggs Static)

4.5 Conclusion

Results of the actual RF heating and mathematical simulations corroborate each other very well, thereby helping to configure the accuracy of this approach. The non uniformity in heating was more evident at higher electric field strength levels leading to coagulation of egg white. This suggests that lower electric field strength levels are a better option in producing quality pasteurized products.

Experimental Verification of Simulation at Different voltage Levels

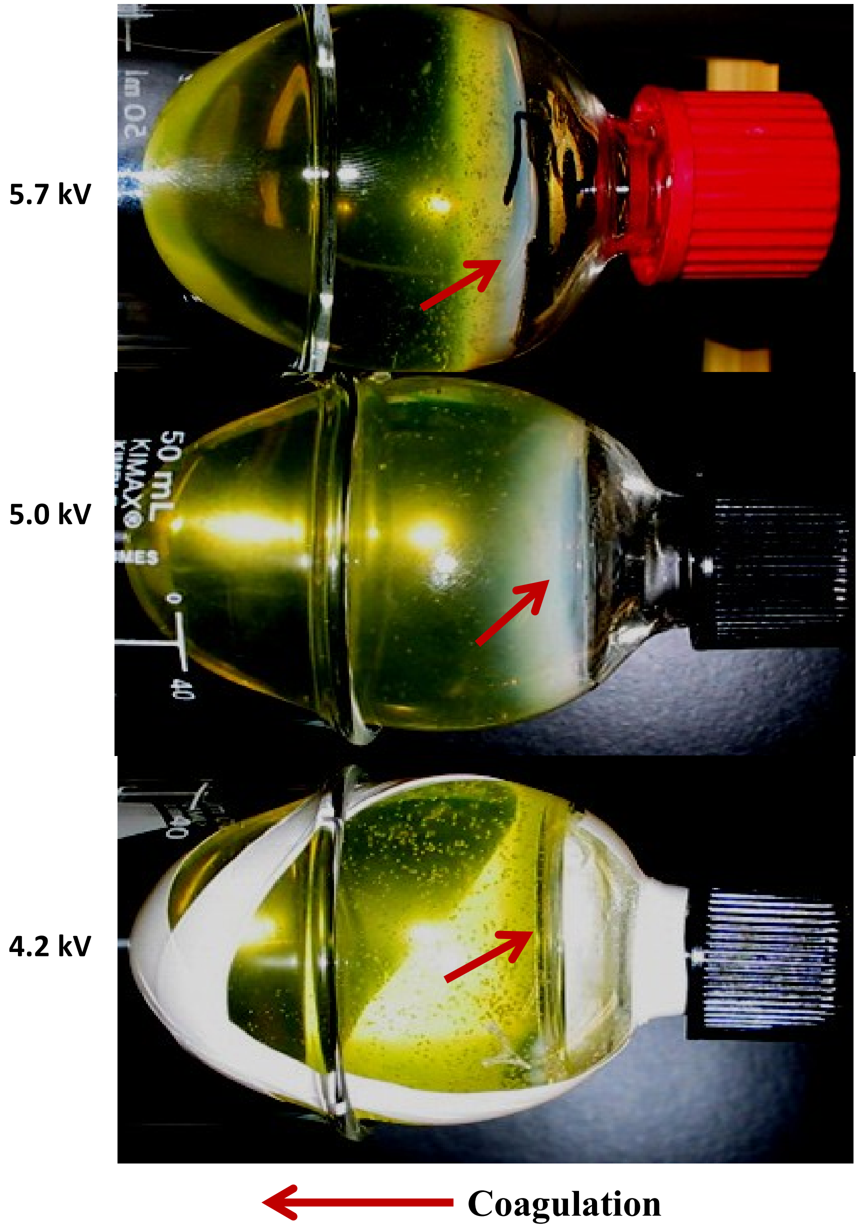


Figure 4.9 Experimental Verification of the Optimized Simulation results

4.6 Acknowledgements

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Nomenclature

E	Total Electric field intensity (V m^{-1})
E_x	Electric field intensity x component (V m^{-1})
E_y	Electric field intensity y component (V m^{-1})
E_z	Electric field intensity z component (V m^{-1})
H	Total Magnetic Field Intensity (A m^{-1})
H_x	Magnetic field intensity x component (A m^{-1})
H_y	Magnetic field intensity y component (A m^{-1})
H_z	Magnetic field intensity z component (A m^{-1})
F	Frequency of microwaves (Hz)
ε'	Dielectric constant
ε''	Dielectric loss factor
ε_0	Permittivity of free space (F m^{-1})
μ_0	Permeability of free space (H m^{-1})
P_{av}	Time average power dissipated (W)
P_c	Poynting Vector – power dissipated over unit area (W m^{-2})
P	Density of the material (kg m^{-3})
C_p	Specific heat capacity of the material (kJ kg^{-1} °K^{-1})
T	Temperature (°K)
T_c	Temperature (℃)
K	Thermal conductivity (W m^{-2} °K^{-1})
Q	Power Source Term (W m^{-3})
V	Volume (m^3)
N	Unit vector normal to the surface
A	Cross sectional area of the waveguide
A	Length of the long side of the rectangular waveguide
Β	Phase constant

Connecting text

After studying the dielectric properties of the egg components and simulating and then subjecting to radiofrequency heating, it was found that radiowaves are exceptionally suitable for heating the eggs. As it is well known that heating causes egg proteins to denature and thereby affects the physical properties like foaming ability, viscosity and turbidity, further study was undertaken to evaluate these changes with respect to the raw eggs and compare it to the untreated eggs. The following chapter will explain this assessment in a detailed manner.

CHAPTER V

Study on the Effect of Radio Frequency heating on the Physical Properties of the Egg White

5.1 Abstract

As already stated above, eggs is one of the most nutritious foods available in nature. Eggs are rich in all the essential amino acids, minerals and vitamins. This has made eggs an important part of human diet. The dominance of eggs is not restricted to households but also extends to food processing industries especially baking industries. Eggs are known to be used in food processing industries as a whipping agent, foaming agent and also to make the food more viscous. These unique attributes are due to the proteins that constitute the egg white. But the conventional conduction mediated heating partially/completely cooks the egg white which eventually destroys the above unique nature of the eggs. The proposed alternative, radiofrequency heating should ideally leave the egg white undisturbed while pasteurizing the eggs. This study evaluated the effect of the radio frequency heating on the physical properties of the egg white in terms of viscosity, turbidity, foaming ability and foam stability. The hyperspectral study for evaluating the quality of the heat treated egg is conducted by scanning the eggs in-shell using ten informative wavelengths in the visible and near infra red (NIR) range. Results have demonstrated that RF heated eggs showed limited changes from that of raw eggs at lower electrode voltage levels in the properties tested for, when compared to in-shell heating at higher electrode voltage levels.

Keywords: RF in-shell heating, Shell eggs, Egg quality

5.2 Introduction

Eggs are one of the most important ingredients in several food processing applications in industries. This is attributed to the unique properties of the proteins of the egg white and yolk, in particular egg white. Some of the noteworthy properties of the egg white are its whipping ability, foaming ability, emulsification and gelling/binding abilities. Unfortunately, the eggs due to their rich nutritive value, serve as a potential source for microbes to invade and multiply (Schroeder *et al.,* 2005; Woodward *et al.,* 1997). To ensure the safety of the eggs, several thermal methods are in practice as of today (Griffiths 2005). Unfortunately, the proteins that contribute to the functional properties of the egg are the most heat sensitive egg proteins. Higher temperatures render them denatured due to which the functional properties of the eggs are affected (Van der Plancken *et al.,* 2006).

Eggs attract different microbes as it is a nutrient rich medium. Among the microbes that the egg attracts, *Salmonella enteritidis* (SE) is the one microbe that has led to several outbreaks in the recent past. The symptoms appear between 18-72 hours after the ingestion of the SE contaminated eggs. The common symptoms are abdominal cramps, chill and fever. Children, aged people and persons with compromised immune system form the high risk population and for almost every infection in this case of the population, hospitalization becomes vital. However, the healthy adults recover from the disease within a week. Most of the outbreaks have been traced back to the Grade A large size eggs which are disinfected, washed and candled (St. Louis *et al*., 1988). This has led the safety governing authority to adopt several preventive measures to contain the SE outbreaks from eggs.

One such preventive measure is the thermal processing of eggs to render SE non viable. This also increases the shelf life of eggs. Among thermal processes, pasteurization is the most important process. In contrast to sterilization, pasteurization aims at reducing the number of viable microbes to a concentration that is not harmful to humans. The process of pasteurization is well-known and has been successfully employed to many food products. Pasteurized foods guarantee microbial safety to the consumers for a particular storage period. Several ways of heating are available for pasteurizing foods. In the conventional water bath heating, the mode of heat transfer is conduction. In this case, the egg white is heated prior to the egg yolk. It has been determined from several experiments that egg yolk is to be heated more than egg white as SE is more resistant to heat when present in egg yolk. So heating the egg yolk to the required temperature without compromising the quality of the egg white is almost impossible.

Egg white is comprised of several proteins like ovalbumin, ovotransferrin, ovomucoid, ovomucin, globulins, avidin etc. that render their unique functional properties as a whole (McDonnell *et al*., 1955; Cunnningham 1995). Some of these proteins have their denaturation temperature as low as 45ºC. The USDA- FSIS recommends heating the egg white to a temperature of 57.5ºC and egg yolk to 61.1ºC for 2.5 minutes to make the potential pathogenic microbes non-viable (FSIS-USDA 2006). In the conventional water bath heating set up, the prescribed temperature for the egg yolk cannot be achieved without partially or completely cooking the egg white. Even a slight coagulation in the egg white drastically affects the functional properties. In addition research has shown that at lower temperatures (< 50ºC) the physio-chemical changes are dependent on the temperature but at higher temperatures (> 50ºC) the changes depend on the treatment and holding time (Van der Plancken *et al.,* 2006). Therefore, the total time of heating plays a crucial role in the pasteurization process as well as in determining the quality of the eggs.

It has been established by simulations in Chapter IV that with the proper fabrication of the RF applicator and optimized length of electrode, position and distance between the electrodes and positioning of the eggs between the electrodes RF heating can be used to pasteurize the in-shell eggs without cooking the egg white. A detailed study on the effect of the RF heating on the physical properties of the egg is mandatory to assess the efficacy of the process. With this in mind, the present study evaluates any such effects on the egg white with respect to viscosity, turbidity, foam density and foam stability.

5.3 Materials and Methods

Egg Samples

The fresh whole eggs were obtained from the market within three days of grading and packing (identified from the date stamped on the eggs, which is usually thirty five days from the date of packing) (CEMA 2004) and refrigerated at 5˚C until used. They were of Canadian Grade A and of small size, having an average mass of 40 g. Prior to heat treatment, the eggs were brought to room temperatures of 25˚C by placing it on the laboratory counter for a time period of about three hours (confirmed by measuring the temperature of three representative samples) before administering the heat treatment.

Evaluation of Physical Properties of Eggs after RF heating

In-shell eggs were pasteurized using a laboratory optimized RF process. Effects of heat treatments on the physical properties affecting the functional quality of the egg white (recovered from the treated eggs) were measured and compared to that of fresh untreated egg white.

Heat treatments for pasteurization

Heat treatments for the pasteurization of in-shell eggs were done in triplicates (i.e) three eggs were used for each treatment for the measurement of each parameter within the scope of this study. The treatment consisted of heating in-shell eggs in a laboratory RF heating set up working at a frequency of 27.12 MHz operating at three different electrode voltages 2.5, 3.5 and 4.25 kV. The In-shell eggs were heated to a temperature of 56˚C with a holding time of 2.5 minutes. The total time taken to reach this temperature was 45, 30 and 10 minutes respectively for the electrode voltages 2.5, 3.5 and 4.25 kV. The temperature was measured using a fiber optic probe inserted into the egg white through the shell and the RF heating operation was controlled by the computer running HPVEE (Agilent) object oriented programming language to maintain the desired process temperature.

Measurements of the physical properties

In order to evaluate the effect of RF heating on the physical properties of the egg, an experiment that assesses Viscosity, Foam Density, Foam Stability and Turbidity was conducted. Heat treated eggs and untreated eggs were evaluated for the above discussed physical parameters. The comparison between heat treated and untreated eggs will help determine if RF heating is desirable for pasteurization with minimal damage to the eggs. Eggs were subjected to RF heating as described in Chapter III. Eggs were cracked carefully after the heat treatment and the egg white and yolk were collected in small cylindrical beakers. The egg shell was discarded. The measurements were made in triplicates.

Viscosity

To evaluate the viscosity of the heat treated and untreated egg white and yolk sample, Brookfield viscometer and a temperature controlled water bath were used. Measurements were made for each sample treated at different electrode voltage levels in triplicates. The measured viscosity values were compared with the values of the untreated white and yolk samples.

Foam density and foam stability

Foam density is a unique property of the egg that is responsible for the aerating property of the eggs in food industries. Foam density is a measure of thickness of the foam. The foam thickness indicates the amount of the air that is trapped in the foam. The functional quality of the egg is determined by the foam stability. The commercial use of egg white in food industrial applications is highly dependent on its foam density and stability (McDonnell *et al.,* 1955).

The egg white samples were foamed using Braun 60 Egg Beater (USA) in a 500 ml cylindrical beaker. The foaming process consisted of beating 40ml of egg white for 2 minutes at a speed of 2000 rpm. The mass of the foam is calculated using a weighing balance. Then the density of the foam is calculated using the equation **(17).**

$$Foam\ density = \frac{mass\ of\ the\ foam}{volume\ of\ the\ egg\ white\ taken} \qquad \textbf{(17)}$$

To determine the foam stability, an experimental set up devised by Dev *et al.,* 2008 as shown in the Figure 5.1 was used. The foam stability was taken as the quantity of liquid drained as a function of time from the completion of foaming. Foam stability measurements were taken for 180 minutes after foaming.

Turbidity

Turbidity is a direct measure of the extent of protein coagulation. As proteins coagulate, the transmittance of light through the egg white reduces. The amount of light absorbed (absorbance) is a function of the turbidity of a liquid. The absorbance of the heat treated and untreated egg white samples was measured at 650 nm (Van der Plancken *et al.,* 2006) using a

Biochrom Ultra spec 2100 Pro spectrophotometer at 24ºC. Plain demineralised water was used for calibration. An absorbance value of 0 % corresponded to a totally clear solution.

Figure 5.1 Experimental setup for measurement of foam stability

Hyperspectral Study

The potential use of Vis/NIRS in real-time assessment of radiofrequency heated egg quality in terms of variation in transmittance was investigated. Transmittance characteristics in the spectral range of 400 to 1700 nm for radiofrequency heated eggs at three different electric field strength levels by operating the electrodes spaced 60 mm apart at voltages of 2.5, 3.5 and 4.25 kV was compared with that of the boiled and RF heated eggs. Studies related to prediction of egg albumen quality using NIRS values. The feasibility of using visible transmission spectroscopy as a non-destructive method to assess the freshness of an egg was investigated by Kemps *et al.,* 2006. The spectral data of 600 white-shelled eggs were compared with the pH and the HU (Haugh unit, a unit for describing egg freshness, based on the thickness of the albumen) and showed that the light transmission spectrum of an egg can provide quantitative information about egg freshness. Kemps *et al.,* 2007 combined visible and near-infrared transmission spectroscopy with low resolution nuclear magnetic resonance (LR-NMR) and concluded that combining the two spectroscopic techniques did not improve the assessment of egg quality when compared to the use of the transmission spectroscopy alone. Thus the accuracy of prediction can be improved by choosing appropriate wavelengths. On the contrary Abdel-Nour *et al.,* 2009 concluded that combining the Vis/NIRS is effective in determining the quality of the inshell microwave pasteurized egg by choosing appropriate informative wavelength for the analysis. Informative wavelengths were identified using subset selection by multiple linear regression analysis. An unsupervised k-means classification was performed to classify the spectral data within a 95% confidence interval. Thus it was established that the presence of any heat damage to proteins inside the shell egg can be quantified in terms of its reduction in transmittance.

Instrument

The hyperspectral imaging system used for the study consisted of 2 line-scan spectrographs, namely ImSpector (ImSpector, V10E, Spectral Imaging Ltd., Finland) with the spectral range of 400 to 1000 nm and HyperspecTM (Headwall Photonics Inc. USA) with a spectral range of 900 to 1700 nm.

The ImSpector and HyperspecTM were connected to a CMOS camera and InGaAs cameras respectively, both mounted above a moving conveyor driven by a stepping motor with a user-defined speed (MDIP22314, Intelligent motion system Inc., USA). A tungsten halogen lamp was used to back illuminate the eggs as they are moved across the cameras' field of view.

Data Analysis

MATLAB Version R2010a (Mathworks Inc, USA) was used in merging the hypercubes and multiple linear regression for subset selection. An unsupervised k-means classification of the spectral data was performed using the ENVI version 4.7 software (ITT Visual Information Solutions, CO, USA).

5.4 Results and Discussion

Viscosity

The viscosity of egg white and yolk measured after breaking open the heat treated eggs and the untreated eggs are shown in the Figure 5.2. As expected the viscosity of the egg white of the untreated egg is lesser than that of the egg yolk. This is because egg yolk has 50% solid matter which includes fatty acids that adds up to the viscous nature of the yolk. The viscosity of the egg white and egg yolk of the heat treated eggs decrease with increasing electrode voltage levels i.e. 2.5 > 3.5 > 4.25 kV. This can be correlated to the denaturation level of the protein. If the denaturation is high, then the viscosity decreases drastically. Viscosity is very essential in the emulsifying properties of the egg white. The viscosity of egg white treated at 2.5 kV is close to that on the untreated eggs which in turn shows that there is minimum protein coagulation at 2.5 kV, whereas at 4.25 kV, the decrease in viscosity is significantly higher (one-third of the untreated eggs) that can be attributed to the increased coagulation of the protein.

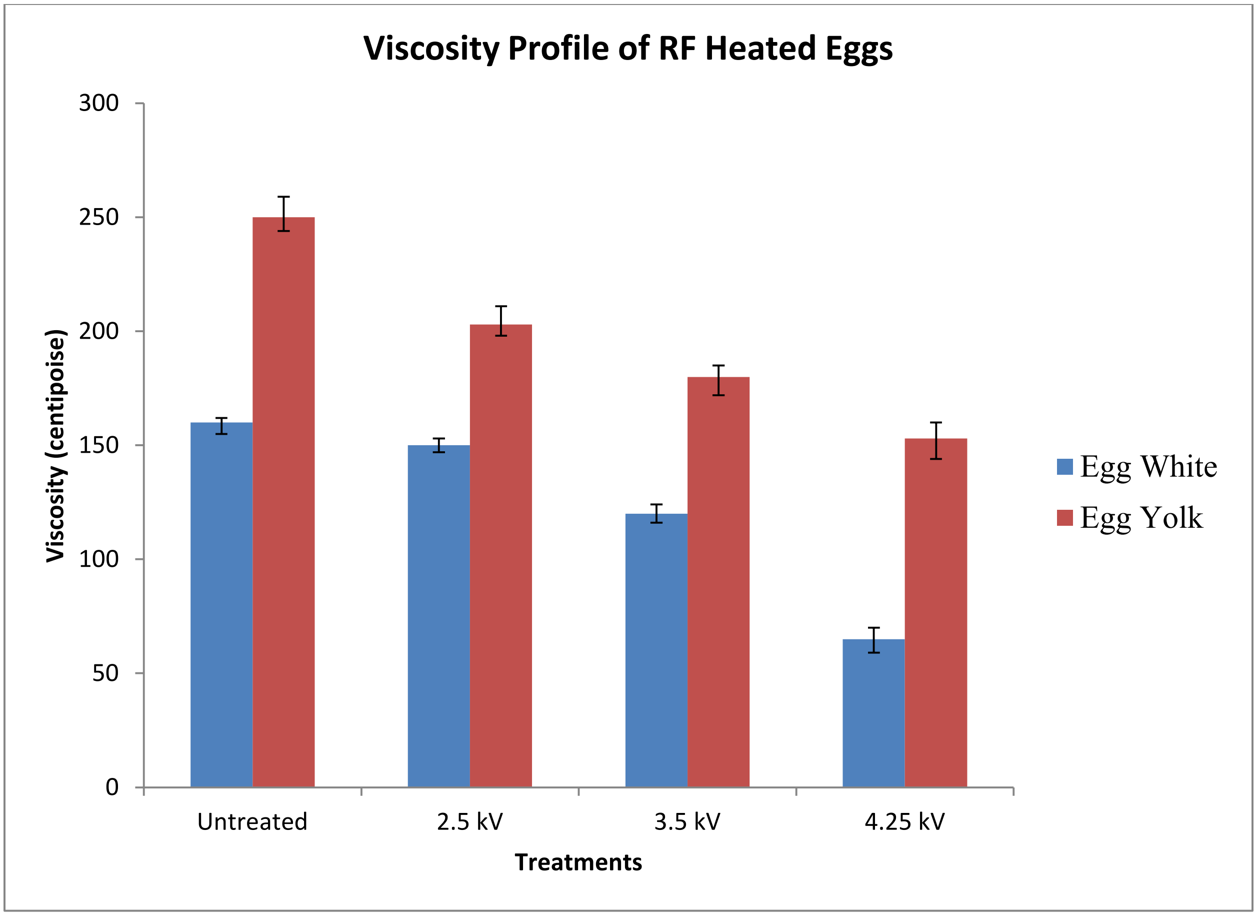


Figure 5.2 Viscosity Profile of the Eggs after RF heating at different Electrode Voltage Levels

Foam Density and Foam Stability

Foaming ability of the egg is very important in industrial application. Better foaming ability is associated with lower foam density. The obtained foam density data at different electrode voltage levels of heat treatment is shown in the Figure 5.3. The data clearly shows that the density has increased after the heat treatment. Interestingly, the varying electrode voltage levels seem to have no effect on the foaming ability.

Foaming stability is reported as the amount of liquid drained with time. The foam stability of the egg white treated at 2.5 kV behaved closer to that of the untreated egg white (Figure 5.4). Though there is huge difference between the foam stability of the egg white treated at different electrode voltage levels and untreated egg white, there is not much difference within the eggs heated at different voltage levels. In summary, the different electrode voltage levels of heat treatment are found to have minimal effect on the foaming ability and foam stability of the protein of the egg white.

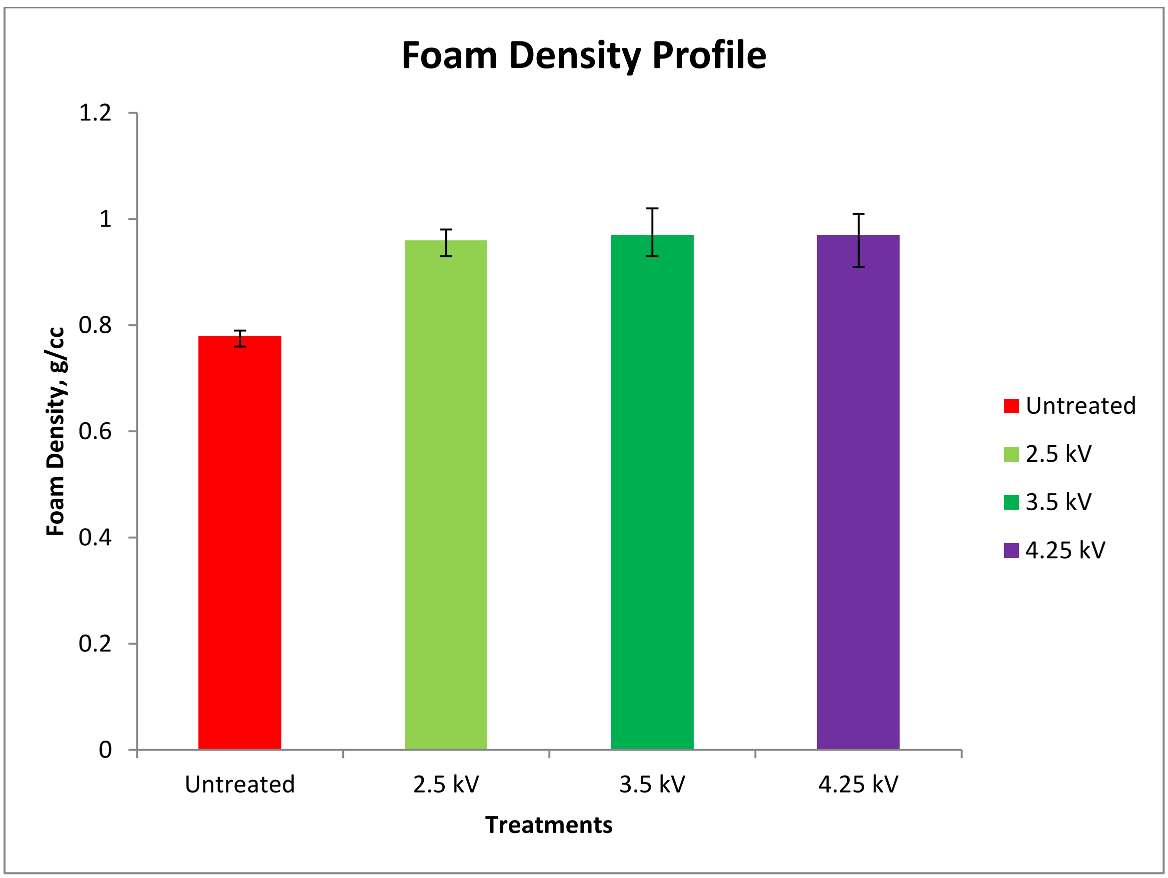
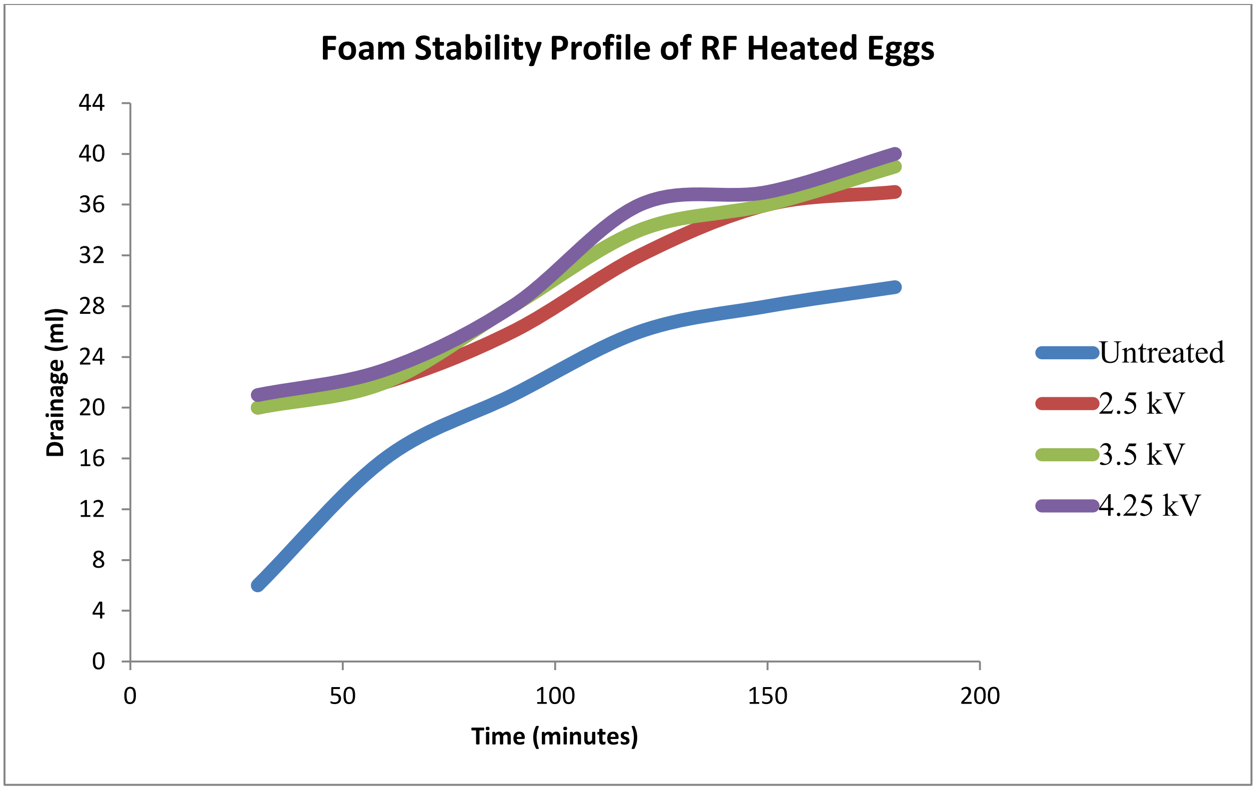
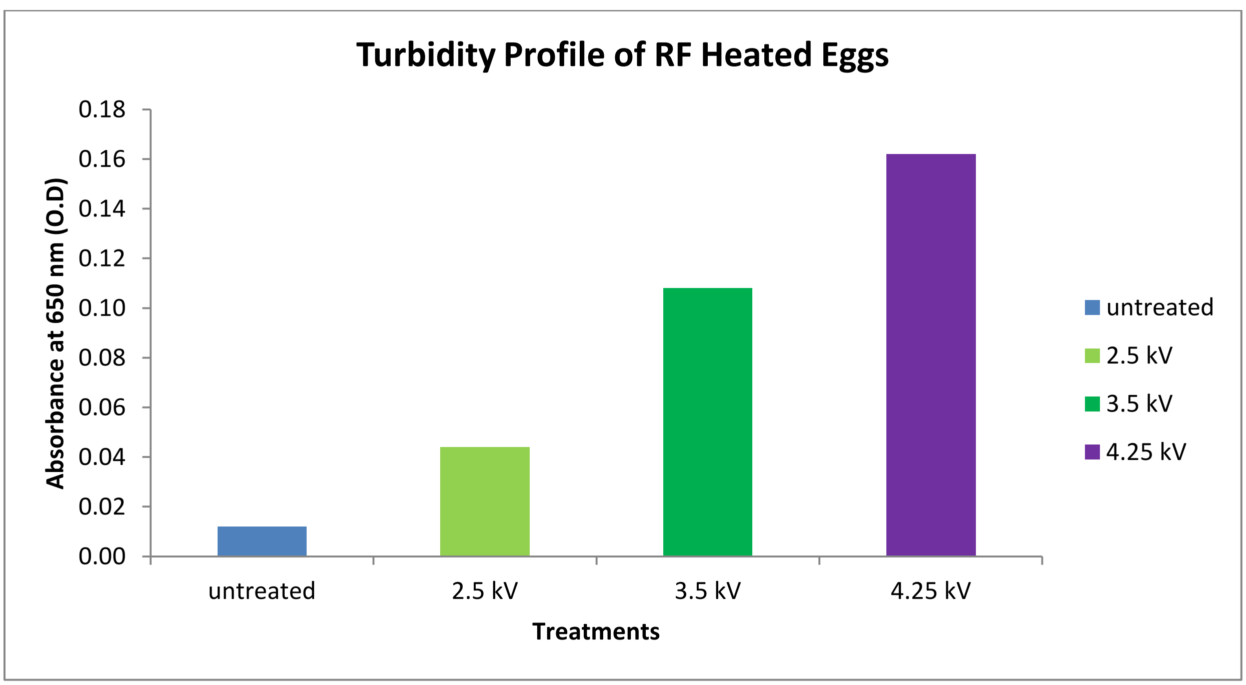


Figure 5.3 Foam Density Profile of the Eggs after RF heating at different Electrode Voltage Levels



5.4 Foam Stability Profile of the Eggs after RF heating at different Electrode Voltage Levels



5.5 Turbidity Profile of the Eggs after RF heating at different Electrode Voltage Levels

Turbidity

The turbidity analysis on the egg whites of the treated eggs revealed that there was significant protein coagulation occurring at all the electrode voltage levels of heat treatment (2.5, 3.5 and 4.25 kV). As expected, the turbidity values increased with increasing electrode voltage levels (2.5< 3.5<4.25 kV) (Figure 5.5). In other words the protein coagulation increased with increased electrode voltage levels. Though the heating time decreased with increasing field strength, protein coagulation level is still of major concern. The functional properties of the eggs directly depend on the proteins that are in proper structural orientation and stability. So, an optimized method of heating the eggs to the required temperature without compromising the protein quality is essential for industrialization of the process.

Hyperspectral Study

The two spectral data hypercubes obtained from the two cameras were merged using MATLAB R2010a and multiple linear regression analysis was done for subset selection (Ventura et al., 1998). From 2151 wavebands scanned, 10 wavelengths (5 from the visible spectral range and 5 from the NIR range) were chosen as informative wavelengths as they have an $R^2 > 0.90$ in the multiple linear regression analysis for maximum R^2.

Figure 5.6 shows an unsupervised k-means classified mosaic made from two eggs from each treatment. It is clear that as the electric field strength level of heating increases, the quality of the egg decreases appropriately. From the Figure 5.6 it is clear that, the eggs heated at 4.25 kV have more pronounced coagulation. At 2.5 kV, the quality of eggs was close to that of the untreated eggs. Eggs treated at 3.5 kV had moderate coagulation in the egg. The Figure 5.7 shows the coagulated profile of eggs after breaking open the heat treated eggs. While comparing the Figures 5.6 and 5.7 it is clear that the results of the hyperspectral data are in accordance with observed results.

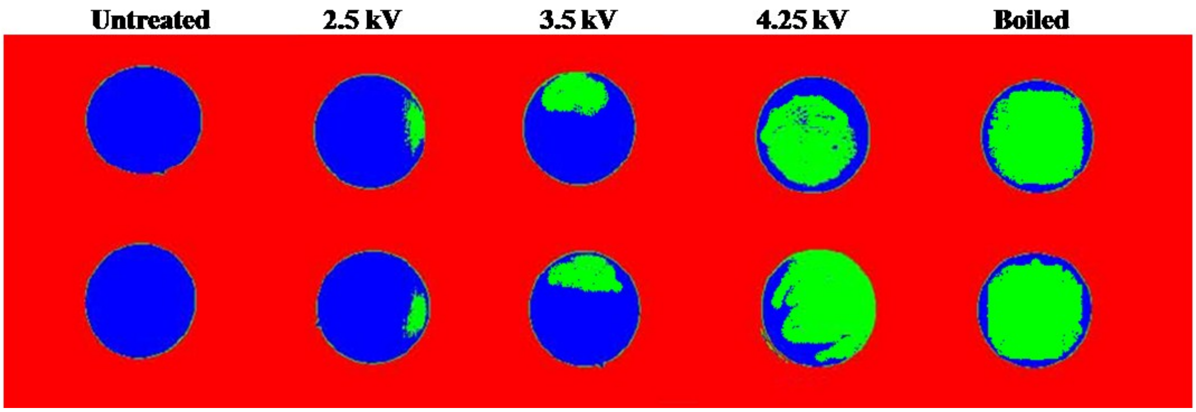


Figure 5.6 Hyperspectral Image showing the Coagulation Status of Eggs in duplicates

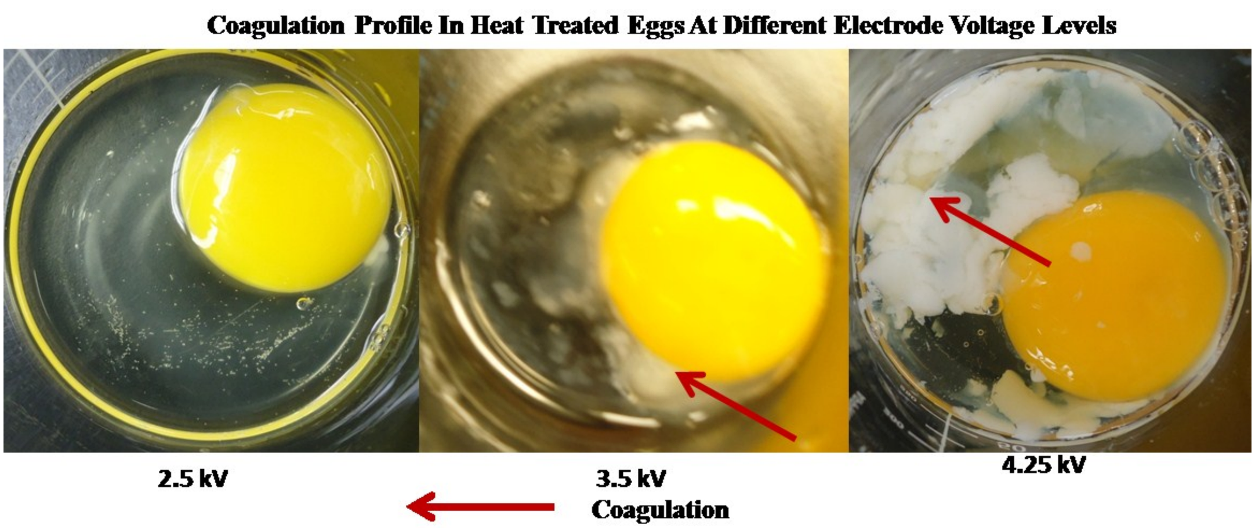


Figure 5.7 Coagulation Profile of the Heat treated eggs at Varying Electrode Voltage Levels

5.5 Conclusion

The effects of the RF treatments of in-shell eggs on their functional properties was determined and compared to that of untreated raw eggs. It was demonstrated that RF heating had minimal effects on the foam forming ability and stability of eggs. Heating at higher electric field strength levels by varying the electrode voltage appropriately, produced eggs with lesser foam stability as compared to eggs heated at lower electric field strength levels. From the data obtained from the turbidity and Hyper spectral studies it was confirmed that there was fair amount of coagulation at higher electric field strength levels which could be minimized with better equipment design as explained in Chapter IV.

5.6 Acknowledgements

The financial support by the Canadian International Development Agency (CIDA) and Natural Sciences and Engineering Research Council (NSERC) of Canada is greatly acknowledged.

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Connecting text

After designing and optimizing the RF heating process, it was necessary to verify the effect of heating on the microbial safety. RF heating being a thermo-biological process requires inoculation of microbial cultures, incubation, heat treatment and assessment of the microbial load after heat treatment. Handling pathogens like SE requires great care and additional safety equipment. Therefore, using surrogate non-pathogenic bacteria is the best practical approach. Hence a microbial validation with such non-pathogenic surrogate bacteria could validate the effectiveness of the optimized RF heating process.

CHAPTER VI

Microbial Validation of Radiofrequency Heating of Shell Eggs

6.1 Abstract

To authenticate the efficacy of the radiofrequency (RF) heating of eggs non-pathogenic *Escherichia coli* K12 was used as a substitute for *Salmonella enteritidis* the latter being highly pathogenic. Escherichia coli K12 (ATCC 23716) was cultured in *E. coli* (EC) broth for a time period of two days. Grade A shell eggs were inoculated with 0.1 ml of the 10^8 CFU/ml cultured *E. coli* K12 and incubated at room temperature for 48 hours. RF heating of the eggs was carried out using a laboratory optimized RF process. The eggs were then broken and plated in EC agar and incubated overnight at 37˚C. The inoculated but subsequently untreated eggs had a count of 10^8 CFU/ml, whereas all the RF heat treatments yielded eggs with a count of 10^4CFU/ml. This indicated that RF heating may possibly serve as a way to pasteurize the eggs provided the process is improved and optimized to reach the required pasteurization temperature with minimum damage to the functional properties of the egg.

Keywords Microbial Validation, RF heating, In-shell egg heating

6.2 Introduction

Eggs due to their rich nutritional content serve as potential hosts for pathogens to invade, feed and multiply. Eggs can contract microbes in two ways (Schoeni *et al*., 1995). First is the trans-ovarian route where the infected ovaries and oviduct tissues enable the microbes to enter the egg yolk even before ovulation. The other route is the trans-shell route. As the eggs are laid in the same passage as the fecal matter is excreted, any fecal matter deposit on the egg can render the eggs infected in due course of time. This process is hastened when the shell is damaged.

Contamination of the egg with one serotype of the bacteria *Salmonella,* namely *Salmonella enteritidis* (SE) has adverse implications for the poultry industry (Bruce *et al*., 2003). The likelihood of fresh eggs having SE has been reported to vary from 0.005 % (Mermelstein 2001) to 1 % (Griffiths 2005). But the incidence of SE outbreaks has gone up in the recent past (Shah *et al*., 1991; CDC 2001). More than 90 percent of food-borne Salmonellosis, caused by SE, occurs through shell eggs (Schroeder *et al.*, 2005; Woodward *et al*., 1997). Most SE outbreaks involved Grade A eggs that were washed and disinfected and also met the State requirements for shell quality (St. Louis *et al*., 1988). Thus contaminated eggs if consumed are hazardous to human health. There were several outbreaks in the past that were traced back to SE infection in eggs cooked for the public (Todd *et al.,* 2001). In order to provide safer eggs to the consumers, the egg farms are governed and frequently checked by appropriate regulating authority. For instance, FDA and FSIS- USDA regulate the egg safety in the US. It has been made mandatory that commercial egg products must be subjected to pasteurization processes to reduce pathogens to a reasonably acceptable level.

FSIS-USDA recommends heating of egg white and egg yolk to 57.5 C and 61.1 C for at least 2.5 minutes to inactivate any microbe that can cause disease in humans. This heating process assures a 5- log reduction from the initial bacterial population (FSIS-USDA 2006). The conventional methods of pasteurization result in heating of the periphery of the food before the material in the centre can get heated up. This is a vital problem in pasteurization of eggs.

Dev *et al.,* 2008 had proven that microwave heating is a viable alternative to overcome the disadvantages of conventional methods of pasteurization. With this system the yolk was heated to a higher temperature (61.1˚C) without cooking the egg white.

Non-pathogenic bacteria can be used as substitutes in place of pathogens to validate thermal processes (Eblen *et al*., 2005). However the substitute organisms should bear similar tolerance levels as that of the target pathogen. *E. coli* K12 was found to exhibit similar kinetic behavior, but higher thermal resistance than SE by Jin *et al*., 2008. Thus *E. coli* K12 can serve as a substitute in evaluating the thermal heating process to reduce/eliminate SE.

The objective of this study was to evaluate if RF heating would be suitable in reducing the load of the non pathogenic *E. coli* K12 strain in in-shell eggs in the place of the pathogenic SE and thus to determine if RF heating like microwave heating can be applied in pasteurization of eggs.

Safety Emphasis

The study was performed with a non-pathogenic strain of bacteria (*E. coli* K12). The inoculation and plating were conducted in a laminar flow chamber (Fisher Scientific, USA) equipped with a bunsen burner and sterilized with UV radiation. A biological safety cabinet (Fisher Scientific, USA) was used to store the plates.

6.3 Materials and Methods

In order to evaluate the RF heating efficiency the *E. coli* K12 was cultured, inoculated, incubated and subjected to three different electric field strength levels by varying the electrode voltage appropriately (2.5, 3.5 and 4.25 kV; electrodes are 60 mm apart) and then plated to assess the reduction in the microbial load (CFU- Colony Forming Unit).

Egg Samples

The fresh whole eggs were obtained from the market within three days of grading and packing(identified from the date stamped on the eggs, which is usually 35 days from the date of packing), (CEMA 2004) and refrigerated at 5˚C until used. They were of Canadian Grade A and of small size, having an average mass of 40 g.

The Culture

The *E. coli* K12 ATCC 23716 strain was obtained in vials from Cedarlane ® Laboratories Limited, ON, Canada in the lyophilized form. This was rehydrated in EC broth (Oxoid Canada) and cultured for 2 days to obtain an initial population of 5.74×10^8 CFU/ml.

Inoculation and Incubation

Inoculation was done in triplicates for the three optimized RF heating treatments and for the untreated control. The egg samples were inoculated by drilling a hole of less than 1 mm diameter on the blunt end of the egg using a drill bit sterilized with alcohol and injecting 0.1 ml of the above mentioned culture directly into the yolk, using a syringe and needle. This is to prevent any inoculums from entering the egg white, as the white contains an antibacterial enzyme called lysozyme. The hole made for the inoculation was sealed with sterile masking tape.

The four sets of inoculated eggs (12 eggs in total) were incubated at room temperature (23 ±2 ºC) for 2 days to allow the bacteria to grow and spread within the yolk.

Heat treatments for pasteurization

Heat treatments for the pasteurization of in-shell eggs were done in triplicates (i.e) three eggs were used for each treatment for the measurement of each parameter within the scope of this study. The treatment consisted of heating in-shell eggs in a laboratory RF heating set up working at a frequency of 27.12 MHz operating at three different electrode voltage levels 2.5, 3.5 and 4.25kV. The In-shell eggs were heated to a temperature of 56˚C with a holding time of 2.5 minutes.

Estimation of Microbial Population

After incubation period of two days the eggs were broken and plated. 0.1 ml of yolk from raw un-inoculated eggs (negative control) was diluted to 4, 5 and 6 logs and subsequently plated onto EC agar. There was no *E. coli* expected to be present initially. Dilutions of 4, 5 and 6 logs were used to plate the eggs treated with the electrode voltage levels of 3.5 kV and 4.25 kV, whereas the samples from eggs treated at a lower electrode voltage level of 2.5 kV was diluted to 5, 6 and 7 logs. The samples from the un-treated inoculated eggs (positive control) were diluted to 5, 6 and 7 logs. The dilutions were made with sterile water and subsequently plated on EC agar.

6.4 Results and Discussion

Growth curve

The initial revival of the bacteria from the culture was confirmed by measuring the optical density of the culture medium at 600 nm, plotting a growth curve and correlating it with the population in CFU/ml. The results of the growth curve modeling (Figures 6.1 and 6.2) illustrate the CFU/ml in the inoculums.

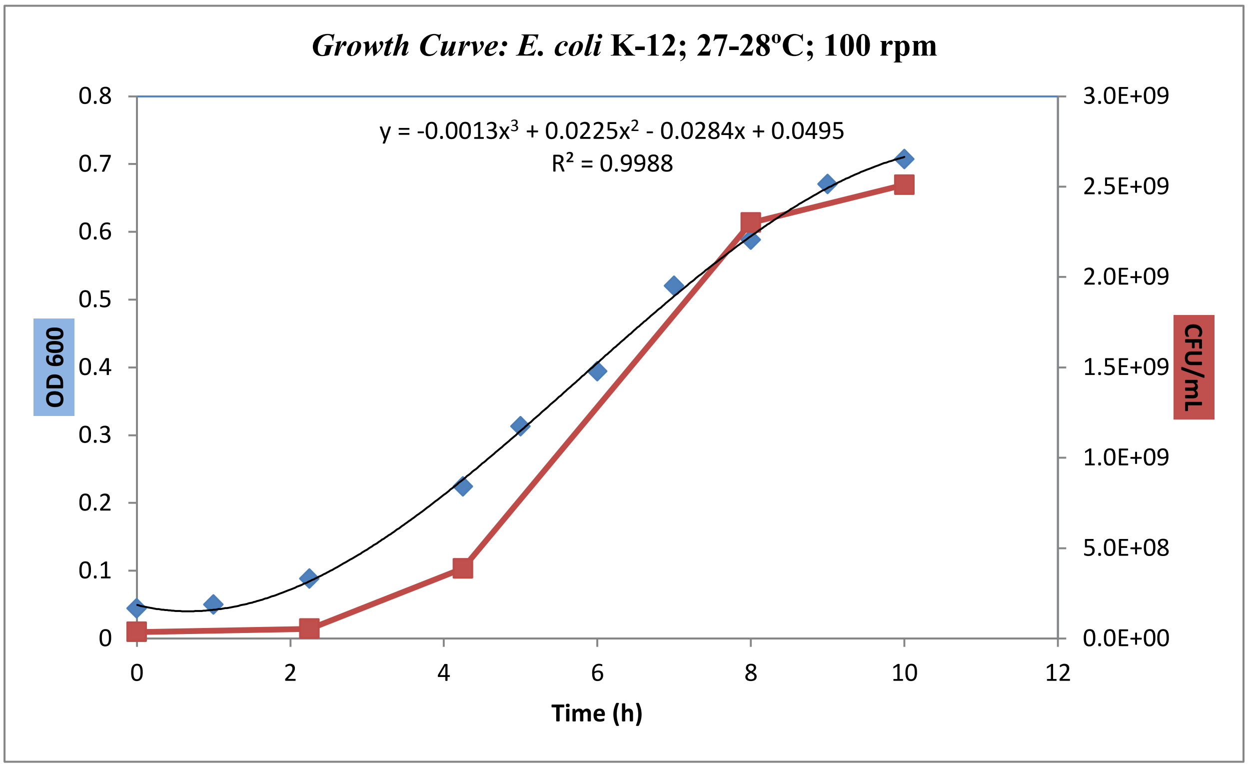

Figure 6.1 Change in Optical Density (OD at 600 nm) over time for E.coli K-12

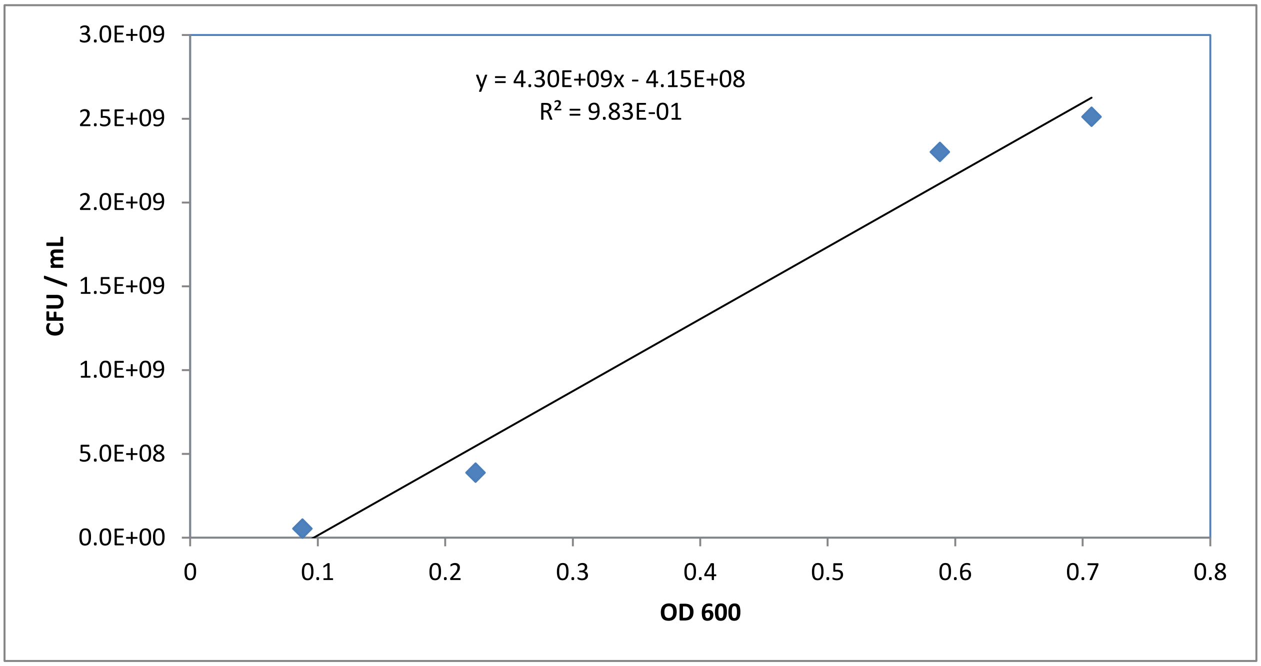

Figure 6.2 Correlation of OD to CFU/ml

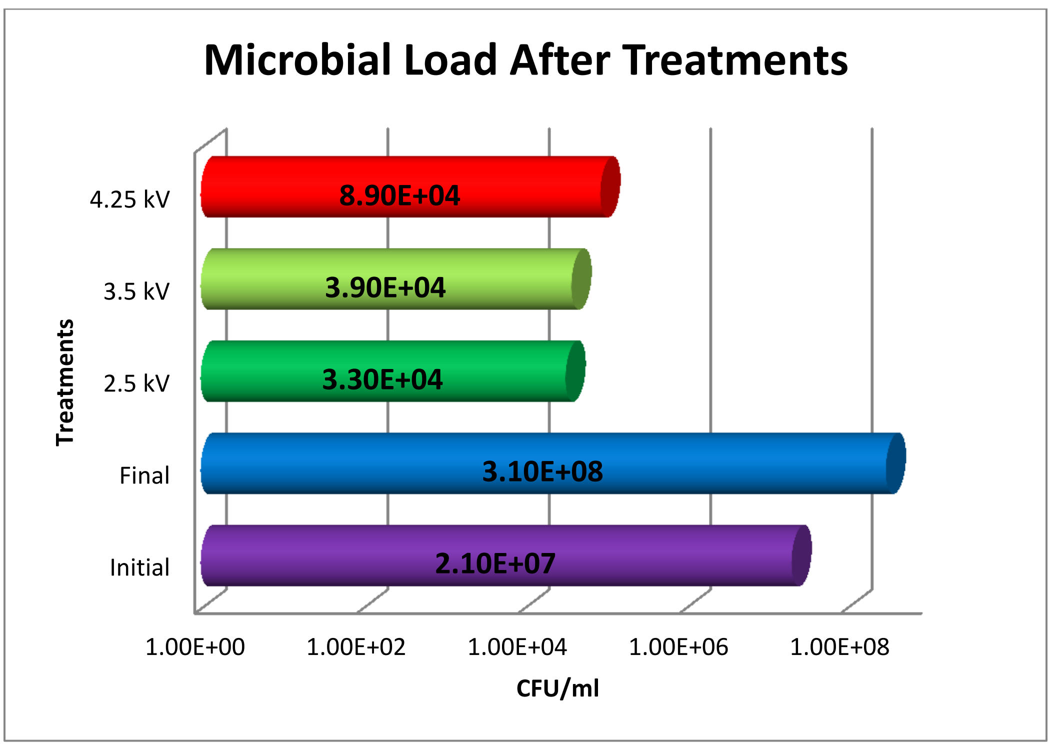

Figure 6.3 CFU/ml of egg yolk after heat treatment using different electrode voltage levels

Figure 6.3 gives the CFU/ml after heat treatment using the RF setup operating at different electrode voltage levels (2.5, 3.5, 4.25 kV) along with the initial and final count of the positive control. The control showed a count of a little more than 10^8 CFU/ml. There were a little over 10^4 CFU/ml present in the RF heated eggs which correspond to a 4 log reduction from the value of the untreated control which hardly meets the pasteurization standards set up by the FSIS-USDA. Also, some coagulation was observed indicating uneven heating as discussed in the hyperspectral study in Chapter IV.

Discussion

With respect to whole eggs, for a heating process to be considered as pasteurization, the temperature of the in-shell eggs should be at least 60˚C and there should be at least five log reduction from the initial microbial load. With this particular experimental setup the in-shell egg temperature achievable was only 56˚C. The RF heat treated eggs showed a 4 log reduction in the bacterial load for this particular configuration of parallel plate RF applicator having the distance between electrodes as 60 mm. Devising a better RF applicator setup which enables the eggs to be rotated may minimize non uniformity in heating. It may also enable the eggs to be heated to the prescribed temperature of 57.5˚C for egg white and 61.1˚C for egg yolk for 2.5 mins. Also, the temperatures of the in-shell eggs were measured by optical probes inserted at one particular point in egg white or yolk. The measurements made were taken as the representative temperature of the egg white and yolk. In this study, it is evident that there is non-uniformity in heating that might lead to cold spots and hotspots within the shell egg. A better configuration is ultimately needed to enhance the uniformity in heating so that the required temperature can be reached with minimal coagulation. Similar results were further confirmed by the simulation studies discussed in the Chapter IV. In summary, the non-uniformity of heating in the parallel plate set up and the difficulty in maintaining the temperature throughout the holding time could be accounted for the lower efficacy of the RF treatment configuration used in this study.

6.5 Conclusion

The RF heat treatments administered to the eggs did not achieve the targeted 5 log reduction in the bacterial load. In addition some amount of coagulation was observed at higher electric field strength levels due to protein denaturation. However, with improved equipment design that facilitates rotation of eggs within the cavity, pasteurization temperatures can be reached while simultaneously maintaining the uniformity in heating. Further research needs to done in developing other such configurations which can be used to perform industrial-scale heating of eggs.

6.6 Acknowledgements

The financial support provided by the Natural Sciences and Engineering Research Council (NSERC) is gratefully acknowledged.

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CHAPTER VII

Summary and Conclusion

It is well known that eggs are an important part of our everyday diet. Owing to their rich nutritive value, they serve as potential hosts to a number of microbes. The bacteria *Salmonella enteritidis* is one such microbe that is highly pathogenic and gives rise to the disease called salmonellosis. Hence it is important that the microbial safety of eggs be given prime consideration particularly for the reason that most of the egg recipes warrant the use of raw eggs. Ensuring the microbial safety of eggs can be done by administering heat treatments to the raw eggs. However the technology currently practiced adversely affects the functional properties of the egg. It requires the immersion of eggs in water bath and takes hours to complete the process and results in partial cooking of the eggs.

An alternative technique to heat the eggs is required to minimize the disadvantages of the conventional technique. Dielectric heating with electromagnetic energy like Radio frequency (RF) waves can be used to heat the eggs to the required temperature within a short time period. However, it is important to predict the heating pattern of the eggs by choosing an experimental and modeling approach so as to understand the behavior of the egg components under conditions of high temperature.

The present study consisted of analyzing the dielectric behavior of the egg components and establishing their relationship with respect to change in frequency and temperature. In addition, the heating rates of the egg white and yolk as well as the whole eggs were studied and the effects of RF heating on the functional properties of the egg were evaluated through certain physical properties. Finally, a microbiological study was undertaken to analyze the efficacy of the heat treatments in reducing the bacterial load and thus rendering the egg safe for consumption.

From the simulation studies it was evident that to maintain uniformity in heating in-shell eggs, the design of the equipment need to be improved such that rotation of the egg is feasible. Also, a coaxial cavity could serve as the solution to this problem as it facilitates rotation of the egg thereby facilitating uniform heating. This set up has an advantage that it could be scaled up to be used in industries. However, it is presently not practical to simulate the heating pattern of rotating eggs in a coaxial cavity.

The results of this study can be utilized in the development of a continuous RF egg pasteurization system. As pasteurized eggs and egg products are not widely available in Canadian markets, a successful establishment of a commercialized RF pasteurization unit can help supply safer eggs and egg products that will not only benefit the high risk population but also the general population.

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