DIELECTRIC PROPERTIES AND MICROWAVE ASSISTED SEPARATION OF EGGSHELL AND MEMBRANE

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ABSTRACT

Eggshell and membranes which are largely disposed of as waste are a reserve of many bioactive compounds with high economic and monetary value which can be extracted by the efficient separation of eggshell and membrane. Hence, this study concentrates on finding a suitable method for separating the eggshell from membrane.

First, the effect of microwave treatment on separation of eggshell and membrane was investigated. The response of a material to electromagnetic radiation depends upon its dielectric properties; therefore, the study of the dielectric properties of eggshell and membrane was carried out in the range of 200 MHz to 20 GHz and in the temperature range of 25 ^{0}C to 100 ^{0}C. Also, the possibility of using this technique for detection of protein denaturation in egg membrane and shell was investigated.

In the second part of the study, the effectiveness of microwave treatment on separation of eggshell and membrane was analyzed in terms of reduction in total energy required to separate the eggshell and membrane and was termed as bond energy. For all microwave treatments, three factors with three levels each were considered. Microwave treatment of eggs significantly reduced the bond energy between eggshell and membrane. A Model for calculating the bond energy between the eggshell and membrane for all microwave treatments was established.

Résumé

Généralement rejetées, les coquilles et membranes d'œuf représentent une importante réserve de composés bioactifs ayant une grande valeur économique et pécuniare, cette étude se concentre donc sur le problème de trower une méthode appropriée pour séparer la coquille de la membrane.

Premièrement, notre étude évalua l'effet d'un traitement aux micro-ondes sur l'aise de séparation de la membrane de la coquille. Comme la réaction d'un matériel aux rayonnements électromagnétiques dépend de ses propriétés diélectriques, les propriétés diélectriques de coquilles et membranes furent donc indépendamment évaluées dans une gamme de frequencies de 200 MHz à 20GHz, en combinaison avec des températures variant de 25°C à 100°C. De plus, la possibilité d'utiliser cette technique pour détecter la dénaturation des protéines membranaires fut évaluée.

En second lieu, l'efficacité du traitement aux micro-ondes à faciliter la séparation de la membrane de la coquille fut éprouvée en fonction de la réduction en énergie necessaire à cette séparation, soit l'énergie de liaison. Pour l'ensemble des traitements aux micro-ondes, trois facteurs à trois niveaux chacun furent évalués. Le traitement aux micro-ondes réduisit de façon significative l'énergie de liaison entre la membrane et la coquille. Un modèle fut développé permettant le calcul de l'énergie de liaison entre membrane et coquille, sous les divers traitements aux micro-ondes et selon les différents facteurs.

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CONTRIBUTIONS OF THE AUTHORS

The work reported here was performed by the candidate and supervised by Dr. G.S.V Raghavan of the Department of Bioresource Engineering, Macdonald Campus of McGill University, Montreal. The entire research work was carried out at the Postharvest Technology laboratory, Macdonald Campus of McGill University, Montreal.

The authorship for the papers are 1. A. Hussain, S.R.S Dev, Y. Gariépy and G.S.V Raghavan (Chapter 4); 2. A. Hussain, Y. Gariépy, S.R.S Dev and G.S.V Raghavan (chapter 5).

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CHAPTER 1

GENERAL INTRODUCTION

Eggshell which forms the outer crust of an egg is a non edible product with very limited use & value and is largely disposed of as a waste. Keeping in mind the high disposal costs which continue to rise due to increase in landfill taxes and increasing environmental concerns, it is necessary to find an alternative method which would transform the waste eggshells into a valuable item; giving financial benefits to the competitive egg processing industry. Apart from giving manufacturers a new profit stream it would help overcome the high disposal costs and environmental concerns (MacNeil 2006, 2001).

There are many uses of separated eggshell and membrane but not many when they are attached. It is established that the eggshell and membrane are a reserve of many bioactive components which can be utilized by efficient separation of the eggshell and membrane. However, the complex microstructure of the eggshell due to the strong interaction of the calcium carbonate crystals with organic matrix has made the separation of eggshell and membrane difficult (MacNeil 2005) limiting the value of the waste egg shells.

This manuscript gives a brief review of the structure of eggshell, various polypeptides and polysaccharides of monetary value present in the eggshell, the problems associated with efficient separation of eggshell and membrane and an alternative solution (hypothesis & objectives) to solve the problem.

CHAPTER 2

GENERAL HYPOTHESIS AND OBJECTIVES

2.1 PROBLEM STATEMENT

Eggshell and membranes which are largely disposed of as wastes are a reserve of many bioactive compounds with high economic and monetary value, which can be extracted by the efficient separation of eggshell and membrane. Many methods have been tried to separate the eggshell and membrane with minimal results. The extraction of the many bioactive compounds present in the egg membrane would not only benefit the egg processing industry by giving them a new source of revenue but also the cosmetic and pharmaceutical industry by reducing the processing cost significantly; making the product cheaper and hence affordable for a wider section of society.

2.2 HYPOTHESIS AND OBJECTIVES

"Alternative solutions which transform the waste product into salable item would be welcomed" (Abdullah 2000). There has been very little work done in the use of microwaves for the separation of eggshell and membrane. The study would be done in order to develop an alternative method for the separation of eggshell and membrane by using microwaves.

The study would be performed in two phases/parts:-

PART I. Study of the dielectric properties of the eggshell and membrane:

The study involves the investigation/analysis of the dielectric properties of the eggshell and membrane. The analysis of the dielectric properties would help in understanding the response of eggshell and membrane to microwaves.

Objectives:

1. To analyze the dielectric properties of the eggshell and the membrane in the frequency range of 200 MHz to 20 GHz and in the temperature range of 25 ^{0}C to 100 ^{0}C (the temperature at which the proteins in the eggshell and membrane would denature).

2. The changes in the dielectric properties would be compared to the denaturation temperature measured by differential scanning calorimetary (DSC).

PART II. Separation of eggshell and membrane:

In the second part of the study, microwave treatment would be used for the separation of eggshell and membrane. The study would be performed based on the following hypothesis:

The separation of the eggshell and membrane by microwaves would depend upon the fact that the membrane has higher moisture content than the eggshell which would lead to more absorption of the electro-magnetic waves by the membrane than the shell. The difference in the moisture content of the eggshell and membrane would result in a differential heating of the shell and the membrane leading to the expansion of the membrane, which would weaken the physical interaction between the shell and the membrane; thereby, assisting the separation of the membrane and the shell. Also the membrane is a protein matrix with relatively high concentration of polar amino acids which would also respond further to the electro-magnetic waves.

Objectives:

1. To investigate the possibility/efficiency of microwave treatment on separation of eggshell and membrane.

2. To investigate the effect of moisture content, varying temperature and power density on separation of eggshell and membrane.

CHAPTER 3

REVIEW OF LITERATURE

3.1 STRUCTURE AND COMPOSITION OF EGGSHELL AND MEMBRANE

The eggshell which forms the outer crust of an avian egg is a natural porous bioceramic, which has largely been studied since 1964. The structure of the eggshell and membrane is now very well understood due to scanning electron microscopy and microfocus X-ray scattering techniques (Lammie et al. 2005). However, ambiguities regarding its composition still exist.

The eggshell which consists of various different layers can be described as a well organized structure, the formation of which begins at different segments of the hen's oviduct. A number of different proteins (soluble and insoluble) and minerals are deposited during the process of eggshell formation which is later used up by the developing embryo. The insoluble proteins have been suggested to act as structural framework and the soluble proteins become embedded in the calcified layers. The deposited mobilized calcium is used for the development and formation of embryo's skeleton (Lammie et al. 2005; Stadelman and Cotterill 1996).

The eggshell which is largely made up of calcium carbonate (95%) and minor amount of organic matrix (3.5%) (Nys and Gautron 2007) can be divided into six different layers (inside to outside). The inner shell membrane forms the innermost layer (20 μm thick) and is in direct contact with the albumen. The outer membrane which lies just above the inner membrane is approximately 50 μm thick. Both, the inner membrane and the outer membrane are made up of interwoven protein fibers and lie parallel to the egg surface providing structural support to the eggshell as a whole (Lammie et al. 2005; Nys and Gautron 2007). The shell membranes greatly influence the shell strength and also prevent micro-organism penetration. The proteins of the shell membranes have been found to

have a high content of arginine, cystine, glutamic acid, histidine, methionine and proline (Stadelman and Cotterill 1996).

The calcified portion (consisting of calcium carbonate crystals) of the shell which precedes the outer membrane can be divided into three layers; the mammillary layer, palisade layer and the vertical crystal layer (Lammie et al. 2005).

The mammillary layer (70 μm thick) which forms the inner most layer of the calcified portion of the eggshell penetrates the outer membrane by means of numerous carbonate cones. The initiation of the formation of calcium carbonate crystals takes place at the mammillary knobs, which are organic cores deposited during the egg formation (Lammie et al. 2005).

The palisade layer (200 μm thick) lies above the mammillary layer and forms the major portion of the calcified layer of the eggshell. In this layer the calcite crystals grow perpendicular to the eggshell membranes. It also has a small portion (2-5%) of organic matrix incorporated in the calcite crystals. Pores formed in the palisade layer help in the exchange of gases. The formation of pores takes place when the adjacent crystals fail to fully join each other along their side surfaces, leaving a gap between the crystals. The palisade layer gives way to the vertical crystal layer (Lammie et al. 2005; Stadelman and Cotterill 1996; Nys and Gautron 2007).

The vertical crystal layer which is about 8 μm thick is a very narrow/thin layer and consists of the upper most part of calcite crystals which provides a surface for the formation of the cuticle (Lammie et al. 2005; Nys and Gautron 2007).

The cuticle is the outer most water insoluble layer of the eggshell (10 – 30 μm thick) (Lammie et al. 2005; Nys and Gautron 2007). The layer is largely an organic layer with protein contents as high as 90% and with a high content of cystine, glycine, glutamic acid, lysine and tyrosine. Fucose, galactose, glucose, hexosamines, mannose, and sialic

acid have been reported to be present as constituents of the polysaccharides (Stadelman and Cotterill 1996).

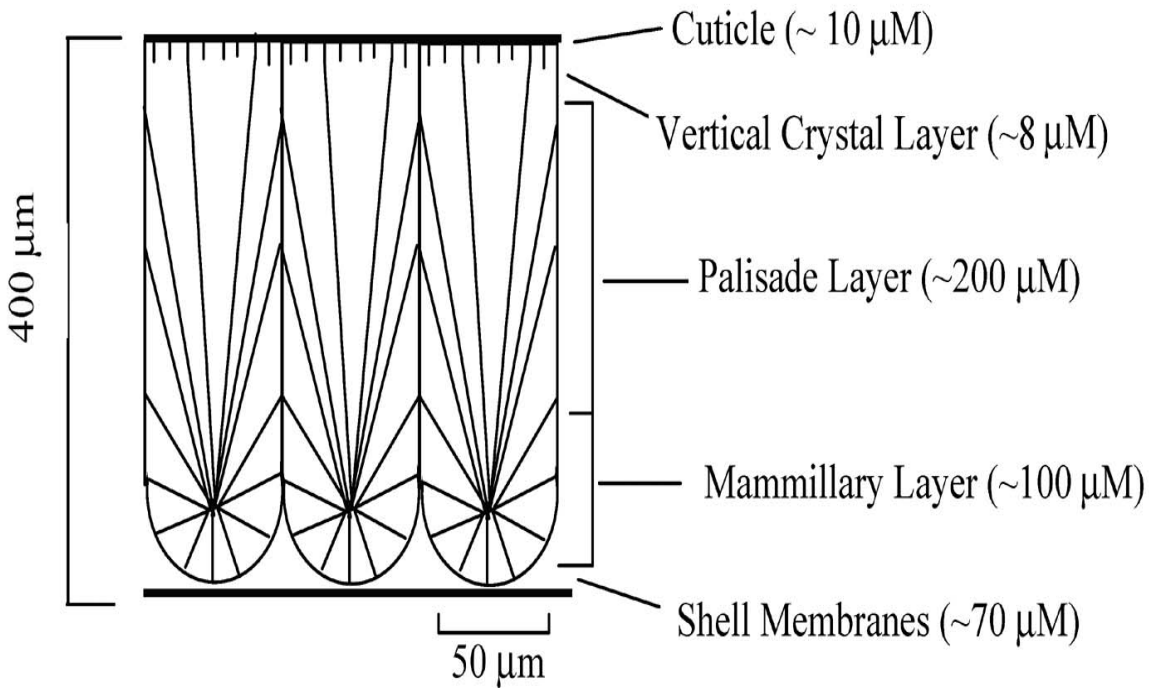

Figure 3.1 Schematic diagram of the structure and different layers within the eggshell (Source : Lammie et al. 2005)

3.2 POLYPEPTIDES AND POLYSACCHARIDES OF THE EGGSHELL AND MEMBRANE

Since 1990, numerous efforts have been carried out to identify and characterize the protein components of the calcified shell and the organic membrane. Matrix proteins have been identified using various biochemical and molecular biological techniques. The previous studies in combination with the recent development of the functional genome tools and the sequence of the chicken genome have led to the identification and characterization of a variety of eggshell matrix components (Gautron and Nys 2007).

The chicken eggshell matrix is a complex mixture of interwoven protein fibers and polysaccharides with at least 70% of the matrix being proteins (Gautron and Nys 2007). It was estimated that 11% of the matrix is polysaccharide that contains chondroitin sulphate A and B, dermatan sulphate, hyaluronic acids, keratan sulphate and uronic acids (Gautron and Nys 2007).

Ovalbumin, which is an egg white protein, has been observed to be localized in the mammillary knobs of the eggshell. Two other major egg white proteins, the lysozyme and ovatransferrin were identified at the basal parts of the eggshell (eggshell membranes, mammillae) (Gautron and Nys 2007). Various glycoproteins such as osteopontin (a phophorylated glycoprotein) and clusterin (a secretory disulphide-bonded heterodimeric glycoprotein), have been reported to be localized in the different layers of the mineralized and non-mineralized parts of the eggshell (Gautron and Nys 2007).

A number of proteins have been found to be novel and specific to the eggshell. Ovocleidin- 17, Ovocleidin-16 a 80 ka protein (742 amino acids) containing two N-glycosylation and two disulphide bonds, Ovocalyxin – 32 and 25, Ovocalyxin 36 , are localized in various layers of the calcified shell (Gautron and Nys 2007).

High contents of arginine, glutamic acid, methionine , histidine, cystine , hydroxyproline, hydroxylysine, and desmosine were found in proteins of the shell membranes (Gautron and Nys 2007).

Proteins such as collagen which hold high economic and monetary value have been reported to be present in eggshell membrane (Arias et al. 1990; Wong et al. 1984a).

3.3 POTENTIAL USES OF SEPARATED EGGSHELL AND MEMBRANE

Eggshell which forms the outer crust of an egg is a non edible product with very limited use & value and is largely disposed of as a waste. There has been an exponential growth in the processed egg industry with 30% of the egg produced in United States today, is

consumed by the processed egg industry. According to an estimate by the United States Department of Agriculture, the egg processing industry consumed 25.6 million cases of egg in 1984, to manufacture liquid and dry egg products. In 1997 the same industry consumed about 50 million cases of egg, producing more than 120,000 tons of unprocessed egg shell waste with disposal costs between $ 25,000 and $ 100,000 per year (MacNeil 2001).

Keeping in mind the high disposal costs which continue to increase due to increase in landfill taxes and increasing environmental concerns, it is necessary to find an alternative method which would transform the waste eggshells into a valuable item; giving financial benefits to the competitive egg processing industry. Apart from giving manufacturers a new profit stream it would help overcome the high disposal costs and environmental concerns (MacNeil 2006, 2001).

There are many uses of separated eggshell and membrane (MacNeil 2001) but not many when they are attached. The following section gives a brief review of various potential uses of separated eggshell and membrane.

Collagen

Collagen constitutes 10% of the total protein content of the egg membrane (MacNeil 2006, 2001). Collagens of type I, V and X have been identified in the eggshell membrane (Arias et al. 1990; Wong et al. 1984a). A lot of emphasis has been given to the presence of collagen in eggshell membrane due to its important economic and monetary value. Collagen finds wide scale usage in the field of biomedical applications, such as skin grafts, tissue replacement products, plastic surgery, cornea repair, prosthetic implants etc (MacNeil 2006, 2001; De Vore et al. 2007; Long et al. 2004). Apart from its biomedical uses it is widely used in food industry for production of gelatin. Keeping in mind the 1997 estimates, 120,000 tons of eggshell waste would yield 110,000 tons of eggshell and 10,000 tons of membrane. Considering that 10% of membrane is collagen, it would yield 1,000 pounds of collagen which is presently priced at $ 1000 per gram or about $ 454,000 per pound (MacNeil 2001). Collagen is generally derived from bovine tissues and to

lesser extent human collagen is also used. There are a number of issues associated with use of bovine collagen, such as the possible transmission of bovine spongiform encephalopathy (commonly known as the mad cow disease). Though the possibility/ risk of transmission are very low but it calls for the maintenance of a well isolated and expensive herds. Also it is estimated that 2% to 3 % of the population is allergic to bovine collagen. Therefore, extraction of collagen from the eggshell membrane would help to overcome the issues associated with the use of bovine collagen (MacNeil 2006, 2001).

Lysozyme and Avidin

Lysozyme finds wide scale usage in food and pharmaceutical industry due to its anti-bacterial properties. Lysozyme has been reported to be present in shell membranes and in the matrix of the calcified shell (Hincke et al. 2000). The main application of lysozyme in the food industry involves the inhibition of *clostridium tyrobutyricum* during cheese maturation. Its application in pharmaceutical industry, involves the preparation of "aerosols for the treatment of bronchopulmonary diseases and for its prophylactic functions relating to dental caries. It is also used in droplets for nasal tissue protection and various therapeutic creams designed for the protection and topical reparation of certain dystrophic and inflammatory lesions of the skin and soft tissues, e.g., burns, viral diseases such as Herpes and shingles, as well as for the treatment of recurrent aphthous stomatitis" (Lesnierkowsi and Kijowski 2007). Oral administration of lysozyme has been reported to induce immune stimulation effects in guinea pigs (Namba et al. 1981).

Avidin finds wide scale usage in biomedical industry. Due to its high affinity constant for biotin, it is widely used in molecular biology techniques such as Enzyme Linked ImmunoSorbent Assay (ELISA), molecular recognition and labelling, affinity chromatography, cytochemistry and histochemistry (Wilchek and Bayer 1990).

Ovotransferrin (Conalbumin)

An iron binding protein present in birds is widely used for its capability of delivering iron to cells and inhibiting/controlling bacterial multiplication. Also, the antiviral activity of ovotransferrin towards chicken embryo fibroblast infection by avian herpes virus has been reported. Ovotransferrin was found to be more effective than human and bovine lactoferrins in inhibiting the same (Giansanti et al. 2001). Ovotransferrin is also used as a nutritional ingredient in many iron-fortified products available in the market today such as iron supplements, iron fortified mixes for instant drinks, sport bars and protein supplements and iron-fortified beverages. Ovotransferrin was shown to be effective against acute enteritis in infants (Corda et al. 1983) .

Ovalbumin

Reported to be present in the mammillae of the eggshell (Gautron and Nys 2007). A purified ovalbumin finds widescale usage in molecular biology techniques, such as Enzyme Linked ImmunoSorbent Assay (ELISA), Western Blotting (used as blocking agent), in SDS- PAGE (Neova Technologies)

Chondroiton sulphate

It forms important structural components of the cartilage largely responsible for giving it the resistance against compression. Along with glucosamine it is now widely regulated as a dietary supplement in many countries including the US (National Center for Complementary and Alternative Medicine, USA). It also forms an integral component of the alternative medicines used to treat osteoarthritis and along with glucosamine, chondroiton sulphate finds application in veterinary medicine (Forsyth et al. 2006).

Hyaluronic acid

Hyaluronic acid is another substance of high monetary value which is naturally present in and is a constituent of eggshell membrane. The total hyaluronic content of eggshell membrane is estimated to be between 0.5 – 10% (Long et al. 2005). Hyaluronic acid is actually a glycosaminoglycan (GAG) which is found in many body tissues such as

cartilage and skin and is responsible for increased resistance to compression in some tissues. Because of high hydration capacity/ability of hyaluronic acid (Long et al. 2005), it finds wide scale usage in cosmetic creams claiming to make the skin appear smoother by hydrating the skin (wikipedia.org). Various studies have reported/ demonstrated hyaluronic acid to be an effective treatment for rheumatoid and osteoarthritis (FDA). Fastening of the wound healing process and reduction in the appearance of old and new scars by the administration of hyaluronic acid has also been reported. US. Pat. No. 6946551 held by Long et al. (2005) describes a method of preparation/extraction of hyaluronic acid from eggshell membrane.

Amino Acids

The eggshell membrane and the eggshell is known to be rich in arginine, glutamic acid, methionine, histidine, cystine, hydroxyproline, hydroxylysine, desmosine, lysine, leucine, isoleucine, tyrosine, phenylalanine and trytophan (vlad 2007) which when extracted would find wide scale application in the biomedical, food, cosmetic and pharmaceutical industry.

The eggshell has proteins like Ovocleidin- 17, Ovocleidin-16, Ovocalyxin – 32 and 25, Ovocalyxin 36 which are novel and unique to eggshell membrane and have new potential applications (Gautron and Nys 2007).

Therapeutic and cosmetic applications

Much importance has been given to the presence of various therapeutic and cosmetically active components such as collagen, hyaluronic acid, glucosamine, chondroitin sulphate present in eggshell membrane having potential applications in cosmetic and pharmaceutical industries. The following components when extracted from other natural resources demands for significant processing cost due to the presence of these compounds in low quantity or due to the additional costs levied to obtain these compounds in the desired purity. Therefore, the extraction of these compounds from egg membrane, which is typically a waste product, is expected to reduce the cost

considerably. Also, depending upon the targeted application the composition / percentage of the compounds can be altered to serve the purpose (Long et al. 2004).

US patent no. 2007/0178170 held by Devore et al. (2007), discusses the anti-inflammatory properties of eggshell membrane and processed eggshell membrane preparations. Eggshell membrane was reported to be an ideal split- thickness skin graft (STSG) donor site dressing. It exhibited properties of pain relief, wound protection, promotion of healing (Yang et al. 2000). Also, dried non-fibrous egg membrane products assisted and stimulated healing process in damaged mammalian tissues such as the tissues lost or damaged due to cuts, injuries, burns and ulcerations (Neuhauser 1965).

Source of Calcium/ Calcium Carbonate

Calcium carbonate forms the major constituent of the eggshell accounting to 91% of the total mass. The processed/separated eggshells could be turned into an excellent source of calcium. It can be used as a dietary supplement in animal feeds, making toothpastes and orange juice. Deriving calcium carbonate from eggshells would not only decrease the burden on landfills but would also serve as a significant partial substitute for mined calcium carbonate. Calcium carbonate finds widespread use in the manufacturing of paper, bio-plastics and as component in ink jet paper coatings. Membrane free eggshell powder can be used as a lime substitute or calcium supplement in agriculture (Abdullah 2000; Anton et al. 2006).

Eggshell Powder:

Chicken eggshell powder due to its high calcium content and the presence of other microelements such Fe, Se and controlled amounts of Pd, Cd and Al has the potential of serving as a good human dietary calcium supplement. It would serve as a dietary supplement not only for the general population, but also for the elderly population and postmenopausal women (Schaafsma et al. 2000).

Other Uses:

Shoji et al. (2004) reported the removal of heavy metals and gold from industrial wastewater using a greatly swollen eggshell membrane- conjugated chitosan beads. Lifshitz et al. 1965 reported the use of exterior layers of the egg such as cuticle, shell and shell membranes as a support for growth of bacterial cultures.

Also, it has been suggested that eggshells could assist in the process of producing pure hydrogen for hydrogen powered cars. The calcium carbonate which forms 91% of the eggshell could be used for soaking acidic carbon monoxide gas released during hydrogen producing reactions (New Scientist, 23 January 1999).

3.4 SEPARATION OF EGGSHELL AND MEMBRANE

In recent years there has been a growing interest in separation of the eggshell and membrane which is clearly visible by the growing number of patents describing/developing methods for efficient separation of the same, which is due to the presence of various bioactive compounds in the eggshell and the membrane. Extraction of these bioactive components is dependent upon the efficient separation of the eggshell and the membrane which would serve as a source of revenue for the egg processing industries.

The following section gives a brief overview of the various methods/ procedures developed / reported for the separation of eggshell and the membrane:

MacNeil (US. Pat. No. 6176376) developed a system for separation of eggshell and membrane by the abrasion of linking structure between the membrane and the shell particles and passively dissociating them in a tank filled with liquid (preferably water). The abrasion is achieved by employing a reducing device, cutting action of which reduces the size of the shell particles variably to between 0.5 mm to 4.0 mm. The industrial set up of the invention would require gallons of the liquid with continuous recycling of the same, adding to the production cost. As the process requires the

reduction in the size of eggshell particles, it puts a limit on the application of membrane generated, as there are many applications in which membranes of larger size are desired; for example the membranes used as biological dressings. The reduction in size may also reduce the efficiency of the process. The separated membrane and the eggshell will have to be first dewatered /dried before it can be put to any use, which further adds to the production cost, time and may increase the losses due to the difficulties in the handling of moist membrane pieces (MacNeil 2001). A system for separation of eggshell and membrane by the application of cavitations in a fluid mixture was developed by Vladimir Vlad (US. Pat No. 0159816).

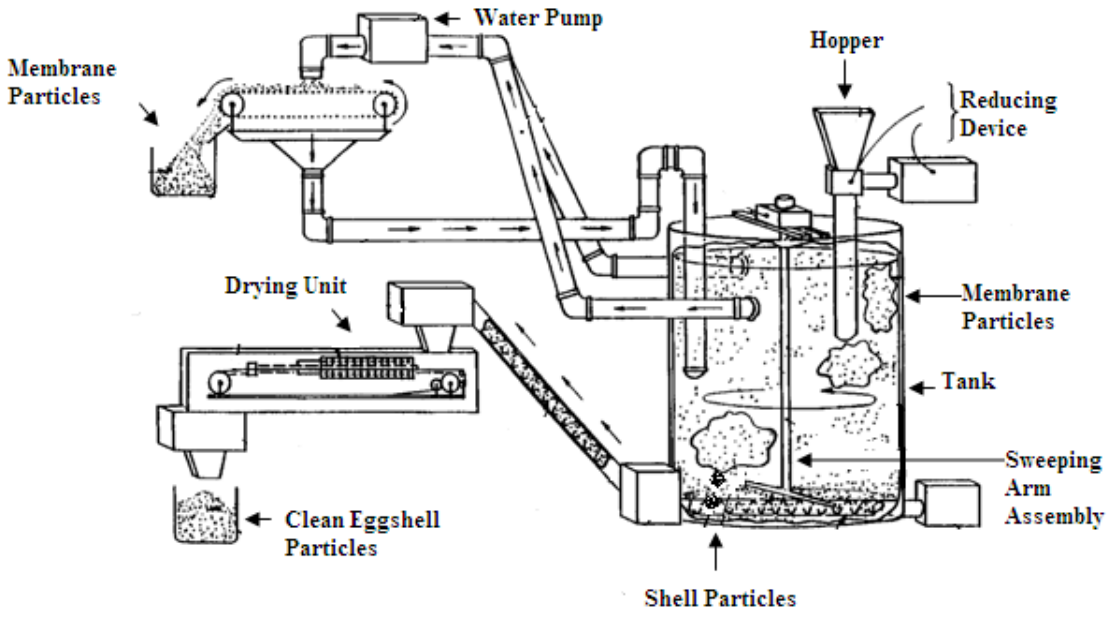

Figure 3.2 Apparatus for separation of eggshell and membrane (Source: MacNeil 2001)

Successful separation of eggshell from hard boiled eggs has been demonstrated by use of chemical means with separation achieved by acid treatment, alkali treatment or by the combination of both. However, the process has its own drawbacks. Boiling acid regardless of dilution effect produces noxious fumes and is thus difficult to handle. It is also corrosive in nature causing damage to the equipment. Boiling in acid also produces foam which can be controlled only by the addition of defoamers, thereby increasing the

cost of the process. Also the acid or the alkali has to undergo continuous treatment, further adding to the production cost and the time. The acid and the alkali have to be effluent treated before it can be discarded. Such treatments add to the cost of labor, energy and equipment (Zeidler et al. 1991).

3.5 MICROWAVE AND ITS GENERATION

Microwaves are short non-ionizing electromagnetic waves lying within the frequency band of 300 MHz to 300 GHz. Electromagnetic waves such as microwaves and radio waves are finding wide scale applications in food processing such as Rf/Microwave drying, baking, sterilization and pasteurization. They are also been applied for extraction of organic compounds (microwave assisted extraction), processing of ceramics and many more (Orsat et al. 2005).

Particular frequency bands have been assigned/reserved for industrial, scientific and medical purposes, collectively called as the ISM bands. The bands are located at 433 MHz, 915 MHz and 2450 MHz. The microwaves create an alternating electric and magnetic field which are at right angles to each other. This particular nature/ property make the usage of microwaves in the field of food processing possible. The application of microwaves greatly reduces the process time, making the process faster. It also makes the process cheaper (though initial set-up cost might be high), increases the efficiency and makes the process greener (Orsat et al. 2005; Datta et al. 2005).

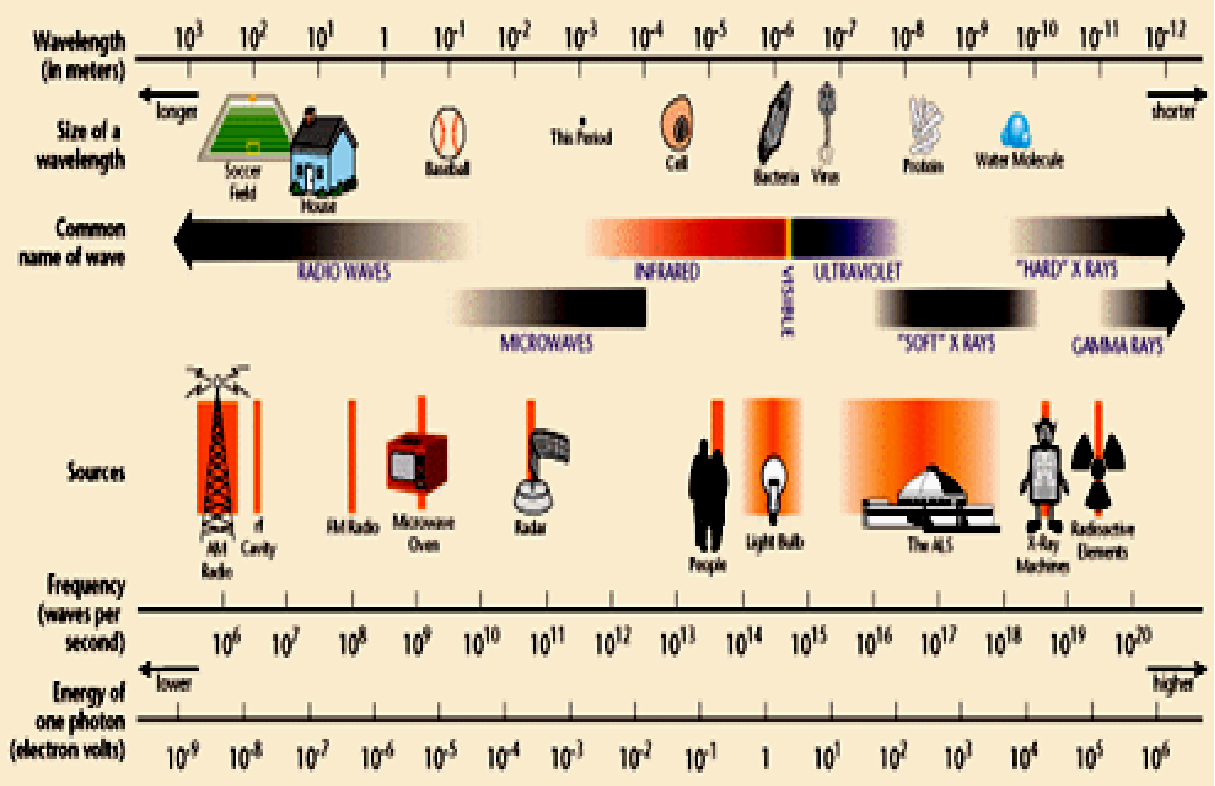

Figure 3.3 The Electromagnetic Spectrum (Source: Wikipedia.org)

3.5.1 Microwave Generation

A typical microwave generation system consists of three basic parts: magnetron tube (microwave source), waveguide and the applicator.

Magnetron tubes are the most commonly used source for microwave generation (about 98%). A magnetron consists of an electron cathode/filament located at the center of the magnetron. The cathode is surrounded by an anode, which consists of a hollow cylinder made up of iron having even number of vanes tending inwards (Orsat et al. 2005).

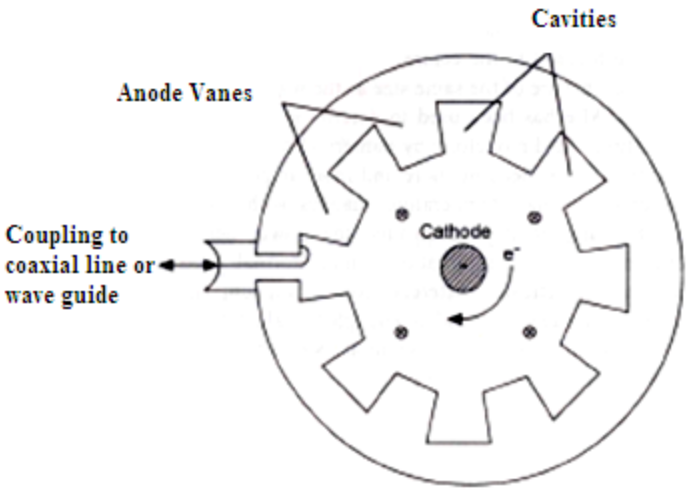

Figure 3.4 Schematic diagram of a magnetron tube (Source: Anonymous, 2007)

The areas between the vanes form the resonant cavities and control the output frequency of the magnetron tube. The tube is also mounted by strong permanent magnets which are responsible for the magnetic field (Orsat et al. 2005).

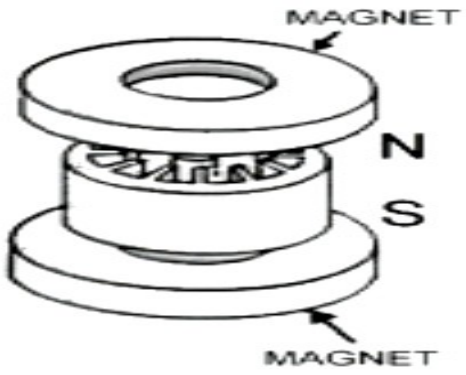

Figure 3.5 Permanent magnets mounted on the magnetron (Source: Anonymous, 2007)

The electrons emitted by the cathode move towards the anode (from negative to positive potential) and are influenced by the combined electric and magnetic field. The magnetic field is parallel to the axis of the cathode i.e., the magnetic field is at right angles to the path of the electron, due to which the electrons move towards the anode in a curve rather than a direct path (Orsat et al. 2005).

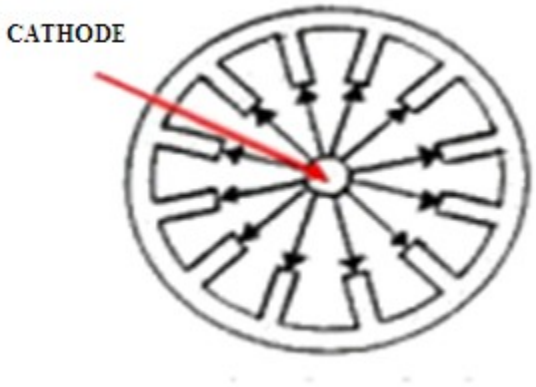

Figure 3.6 Motion of electrons in an electric field (Source: Anonymous, 2007)

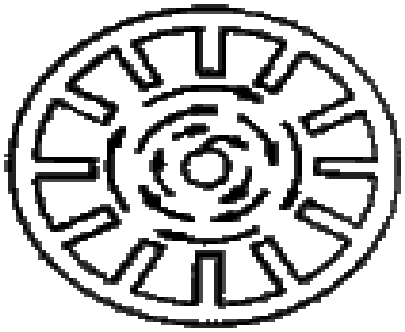

Figure 3.7 Spiral motion of electrons under the influence of combined electric and magnetic field. (Source: Anonymous, 2007)

The spiral rotating electrons interact with the resonant cavities which transfer the energy from the electrons into a waveguide or coaxial line via circular loop antenna (Orsat et al. 2005).

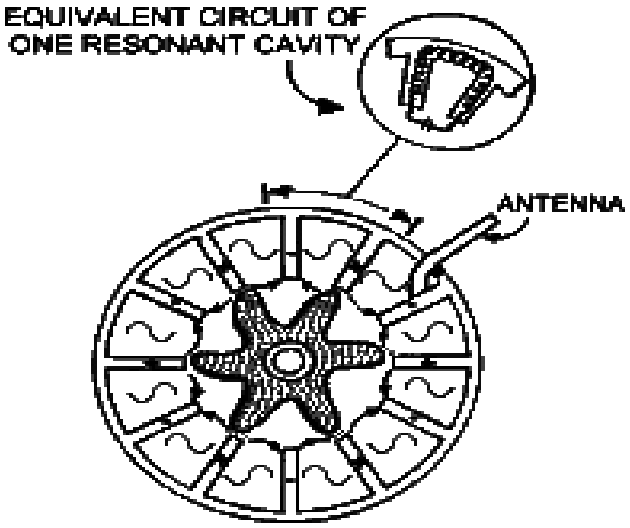

Figure 3.8 Rotating patterns of electron under electric and magnetic field effect. (Source: Anonymous, 2007)

Waveguides or coaxial lines can be used for guiding electromagnetic waves such microwaves. The waveguides typically consist of hollow cylinders of rectangular or circular cross section, with width double in dimension to that of the height in case of rectangular wave guides. Depending upon the direction of the electric and magnetic field inside the waveguide, the microwaves may split into transversal electric (TE) or transversal magnetic (TM) modes (Orsat et al. 2005).

Tuners are components of the waveguide which match the load impedance to the waveguide impedance and try to minimize the reflected power for the most efficient transfer of power to the load (Orsat et al. 2005).

3.6 MECHANISM OF MICROWAVE HEATING

The absorption of microwave energy in the food is greatly dependent upon two mechanisms: dipolar rotation and ionic conduction.

Dipolar Rotation:

The imbalance caused in the re-arrangement of electrons during the formation of a molecule leads to the creation of a permanent dipole moment and molecules with such arrangements are called as polar molecules. Molecules as water exhibit strong permanent dipole moment and are hence primarily responsible for dipolar rotation. In the absence of an electric field, the dipole moment are oriented in a random manner but they experience a rotational force (due to the torque exerted by the electric field on the electric dipole) when an alternation electric field is applied. The water molecules try to align themselves to the direction of the alternating field, resulting in random collision between the neighbors. The same process is repeated when the field gets reversed leading to thermal agitation and heating takes place (Datta et al. 2005).

Ionic Conduction:

The application of an electric field leads to the migration of ions present in a salty food. The net electric field in the oven accelerates the particle in one direction while the opposite charged particle gets accelerated in other direction leading to a random collision between neighboring particles. Such collisions impart kinetic energy to the particles, resulting in an increased agitated motion leading to a temperature rise of the particle. As more agitated particles collide or interact with each other, the agitation gets transferred to the adjacent particles causing an increase in the temperature of the material (Datta et al. 2005).

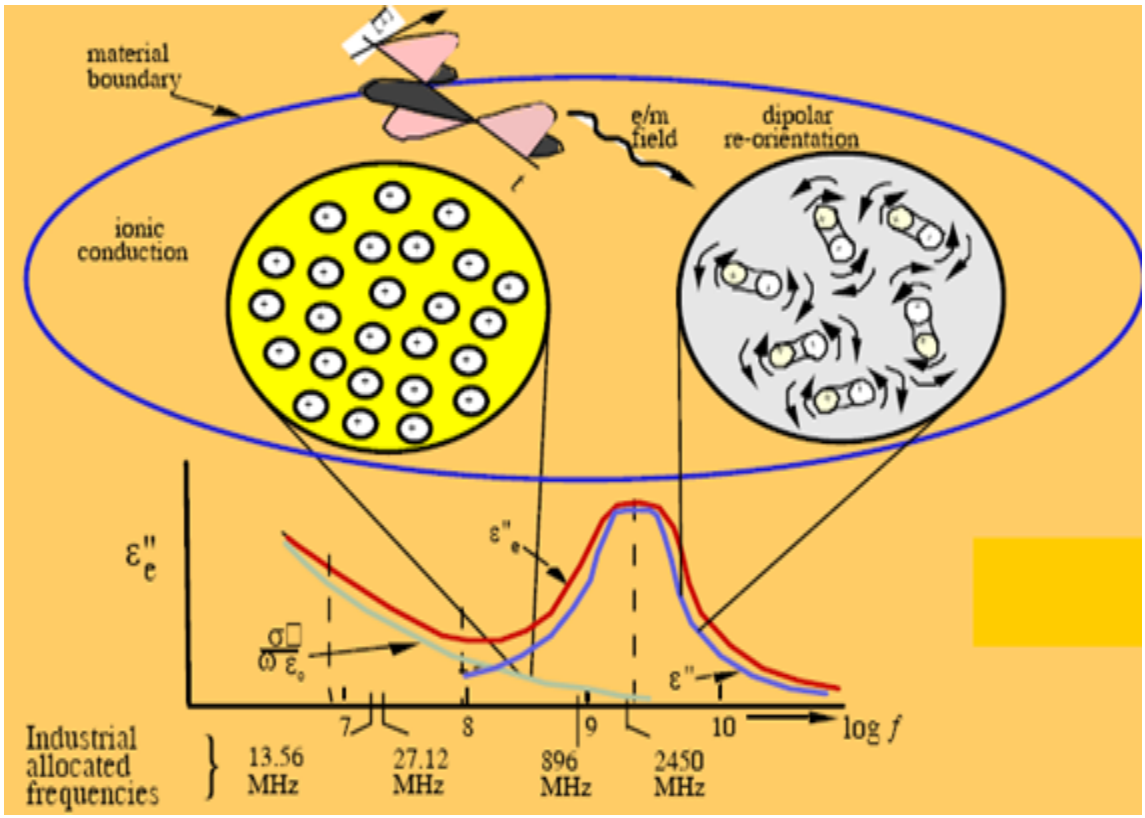

Figure 3.9 Schematic diagram representing the mechanism involved in dipolar heating and ionic conduction in dielectric heating of food (Source: Clark et al. 1996)

3.7 DIELECTRIC PROPERTIES FOR DETECTION OF PROTEIN DENATURATION IN FOOD SYSTEMS

When microwave is incident on a food material, part of the energy is absorbed by the food, leading to its temperature rise. Electromagnetic waves are composed of an electric and a magnetic field. The dielectric properties determine the response of a material to an electromagnetic field. The dielectric properties are analyzed with respect to a complex number consisting of a real portion (dielectric constant) and an imaginary number (dielectric loss) (Orsat et al. 2005; Datta et al. 2005).

Both the dielectric constant and the dielectric loss are the measure of the ability of the material to interact with the electric field of the microwaves. The dielectric constant gives the measure of the food material's ability to store electromagnetic energy, which depends

upon the polarizability of the molecules present in the food. The dielectric loss is related to the energy absorption and dissipation of the electromagnetic energy from the field. The dielectric constant decreases by the presence of ions, which bind water, decreasing the mobility. However, the dielectric loss factor is increased by the presence of ions (Orsat et al. 2005; Datta et al. 2005).

Lately, dielectric properties have been used to detect protein denaturation. Bircan et al. (2002; 2001) determined whey protein denaturation and egg protein denaturation by analyzing the dielectric properties. Brunton et al. (2006) used dielectric properties for assessing protein denaturation in beef biceps femoris muscle. Protein denaturation can be triggered or caused by the application of heat, ultraviolet or agitation, during which the protein undergoes physical changes resulting in loss of crystallizability, reduction in protein solubility and increase in solution viscosity. The changes in the physical state of the protein leads to the disturbance of protein structure and an increase in asymmetry of charge distribution resulting in large dipole moment and polarization, which ultimately affects the dielectric properties (Datta et al. 2005).

3.8 SEPARATION OF EGGSHELL AND MEMBRANE USING MICROWAVES

The major problem in profitable utilization of eggshell and membrane is the complete separation of the two with minimal damage.

3.8.1 Difficulties in separation of Eggshell and Membrane

Many methods such as acid treatment, drying, abrasion, crushing, etc, have been tried with minimal results (Abdullah 2000), which puts a limit on the utilization and the salable value of the eggshell and organic membrane. The recent inventions in this regard leads to the generation of moist shell and membrane which have to be dried before they can be put to any use (MacNeil 2006, 2001), which increases the production cost and the losses. The difficulties in handling of moist membrane pieces have to be kept in mind.

A major problem with profitable utilization of the waste eggshell is ensuring the complete separation of the shell and the membrane. Many methods have been tried to completely separate the membrane from the shell, as when separated both the items can have significant value (MacNeil 2006, 2001).

The presence of high density continuous distribution of mammillary knobs at the outer shell membrane provides an optimal interface for the establishment of a firm attachment/bond between the shell and the membrane. The membranes which are a matrix of interwoven protein fibers, act as a structural reinforcement contributing significantly to shell strength. This strong physical attachment between the shell and the membrane makes their separation extremely difficult (MacNeil 2001; Orberg 1990) . "Alternative solutions which transform the waste product into salable item would be welcomed" (Abdullah 2000).

CHAPTER 4

DIELECTRIC PROPERTIES OF EGGSHELL AND MEMBRANE

Abstract

Dielectric properties of eggshell and membrane were investigated from 25 ^{0}C to 100 ^{0}C and in the frequency range of 200 MHz to 20 GHz. Differential Scanning Calorimetary (DSC) was used for the determination of protein denaturation. DSC indicated two major endotherms for egg membrane, at 72 ^{0}C and 92 ^{0}C. The dielectric constant for egg membrane increased at the initial protein denaturation and decreased during complete protein denaturation. Dielectric constant was observed to be more sensitive to protein denaturation than dielectric loss. The dielectric constant and loss for eggshell decreased gradually with temperature.

Keywords: dielectric properties, eggshell, egg membrane, denauration

4.1 INTRODUCTION

Dielectric properties can be described as the electrical properties of a material, which governs its behavior to electromagnetic radiations. The dielectric properties are analyzed with respect to a complex number consisting of a real portion (dielectric constant) and an imaginary number (dielectric loss). Both of these parameters give an insight about the ability of the investigated material to interact with the electric field of the applied electromagnetic wave such as microwaves (Orsat et al. 2005).

Electromagnetic heating such as radio-frequency and microwave heating finds wide scale usage in the field of food processing, drying, sterilization, pasteurization and cooking (Datta et al. 2005). The absorption of microwave energy in the food is greatly dependent upon two mechanisms: Dipolar rotation and Ionic conduction.

Dipolar Rotation:

The imbalance caused in the re-arrangement of electrons during the formation of a molecule leads to the creation of a permanent dipole moment and molecules with such arrangements are called as polar molecules. Molecules such as water exhibit strong permanent dipole moment and are hence primarily responsible for dipolar rotation. In the absence of an electric field, the dipole moment are oriented in a random manner but they experience a rotational force (due to the torque exerted by the electric field on the electric dipole) when an alternating electric field is applied. The water molecules try to align themselves in the direction of the alternating field, resulting in random collision between the neighbors. The same process is repeated when the field gets reversed leading to thermal agitation and heating takes place (Datta et al. 2005).

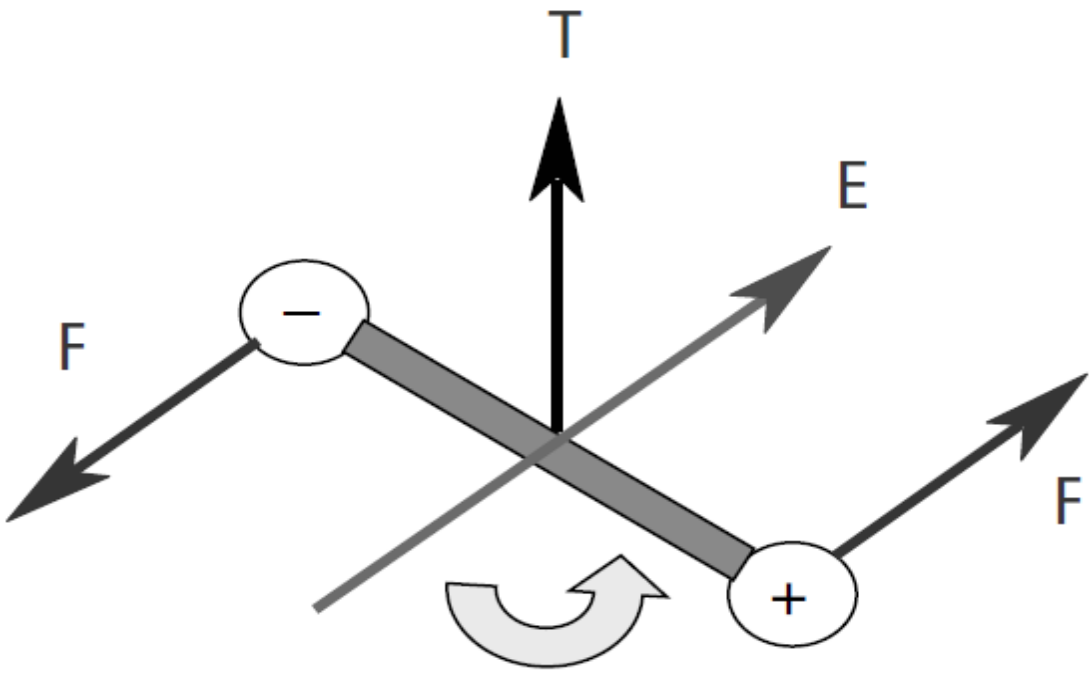

Figure 4.1 Dipolar rotation in an electric field. (Source: Agilent Technologies 2005)

Ionic Conduction:

The application of an electric field leads to the migration of ions present in a salty food. The net electric field in the oven accelerates the particle in one direction while the opposite charged particle gets accelerated in the other direction leading to a random collision between neighboring particles. Such collisions impart kinetic energy to the particles, resulting in an increased agitated motion leading to a temperature rise of the particle. As more agitated particles collide or interact with each other, the agitation gets transferred to the adjacent particles causing an increase in the temperature of the material (Datta et al. 2005).

The dielectric constant depends upon the polarizability of the molecules present in the investigated material and is a measure of the materials ability to store electromagnetic energy. Dielectric loss on the other hand, is related to the materials ability to absorb energy and dissipate electromagnetic energy from the field (Orsat et al. 2005).

Measurement/Analysis of dielectric properties of materials such as food systems not only helps in designing of efficient dielectric heating equipments, but also helps in monitoring of physiological processes. Dielectric properties are increasingly being used as a non-destructive technique for the assessment of food quality (Orsat et al. 2005).

Lately, dielectric properties have been used for the detection of protein denaturation in food systems as an alternative to differential scanning calorimetry (DSC). Protein denaturation can be triggered or caused by the application of heat, ultraviolet or agitation. During denaturation the protein undergoes physical changes resulting in loss of crystallizability, reduction in protein solubility and increase in solution viscosity. The changes in the physical state of the protein leads to the disturbance of protein structure and an increase in asymmetry of charge distribution resulting in large dipole moment and polarization, which ultimately affects the dielectric properties (Datta et al. 2005).

Bircan et al. (2002; 2001) determined whey protein denaturation and egg protein denaturation by analyzing the dielectric properties. Brunton et al. (2006) used dielectric properties for assessing protein denaturation in beef biceps femoris muscle.

The chicken eggshell matrix is a complex mixture of interwoven protein fibers and polysaccharides, with at least 70% of the matrix being proteins (Gautron and Nys 2007).

The study involved the investigation/analysis of the dielectric properties of the egg shell and membrane to determine if dielectric properties could be used for the detection of protein denaturation in egg membrane and shell. The research was carried out with the following **objectives:**

1. To study the dielectric properties of the eggshell and the membrane in the frequency range of 200 MHz to 20 GHz and in the temperature range of 25 ^{o}C to 100 ^{o}C .

2. Compare the changes observed in the dielectric properties to the denaturation temperature measured by differential scanning calorimetary (DSC).

4.2 MATERIAL AND METHODS

Commercially available eggs were used in the study. All eggs were of large size with an average weight of 58 g each. The eggs were stored at 4 ^{0}C until they were used.

4.2.1 Egg Membrane Sample

The eggs were carefully broken and egg white and yolk were discarded. Membrane and shell were carefully separated by manual peeling. The samples consisted of both the inner and outer membrane. The membranes were washed thoroughly with distilled water to remove any egg white sticking onto the surface of the membrane. The membranes were then placed between two absorbent papers for 15 minutes in order to remove surface water and were allowed to be air dried at room temperature for 15 minutes. The membranes were carefully folded to form a cup shaped sample (Fig 4.4) of 20 mm (height) x 20 mm (diameter). It was made sure that there were no air gaps/sockets present

between the folded membranes. It took approximately 10 large sized eggs to form one sample i.e. one replicate. The samples were placed in air tight vials and stored at 4 ^{0}C until used. All the measurements were performed on the same day of peeling.

Figure 4.2 Peeled Egg Membranes

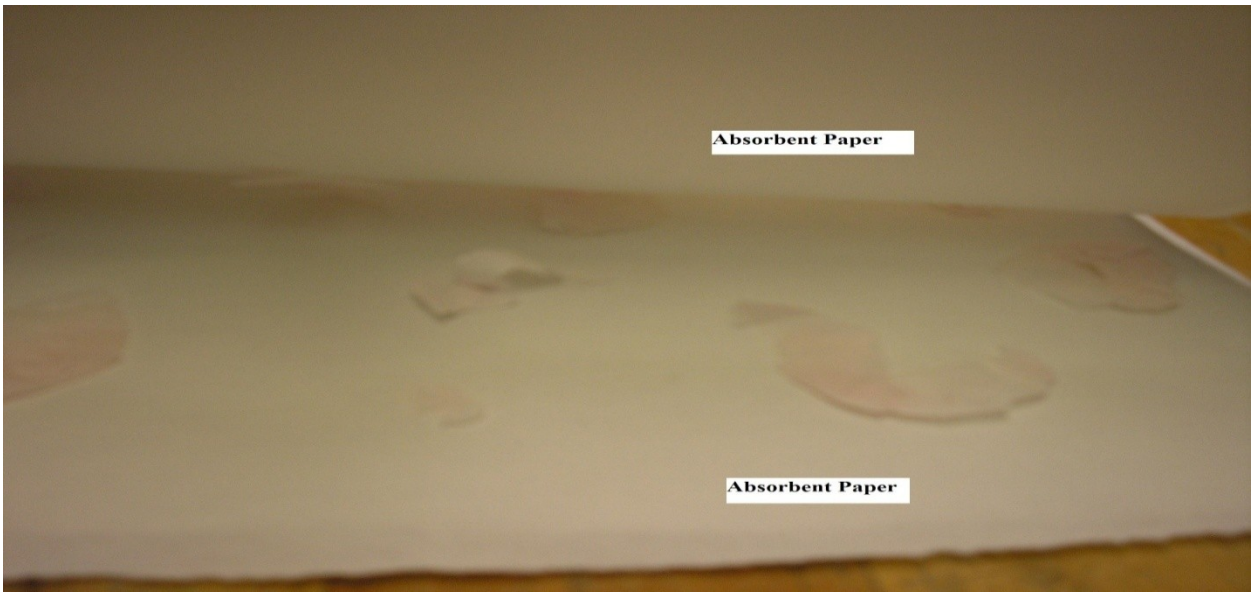

Figure 4.3 Membranes placed between absorbent paper

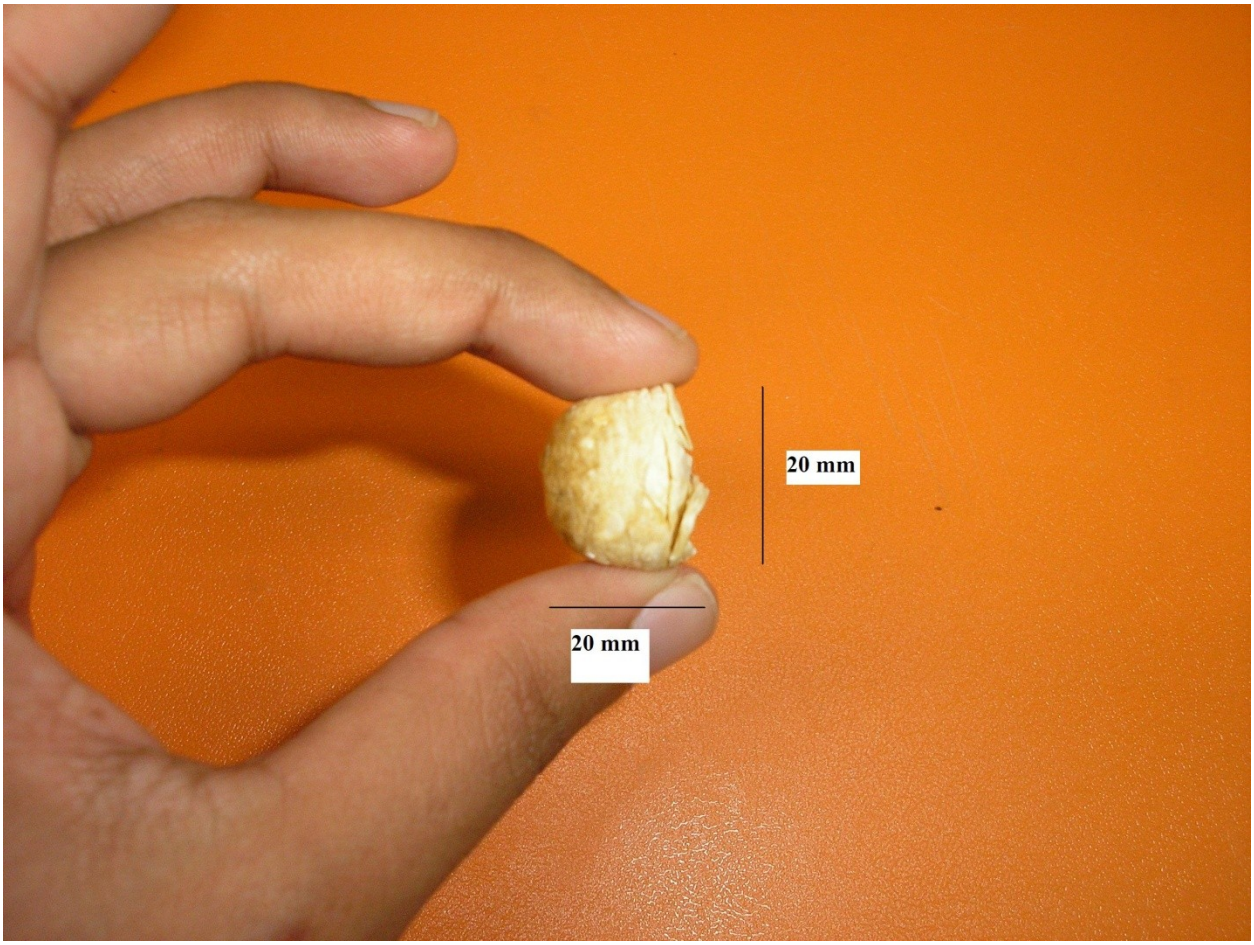

Figure 4.4 Cup shaped Membrane Sample

4.2.2 Shell Samples

Shell samples from which the membrane was removed were used. The inner surface of the shell was first cleaned with a slight damp paper towel and then immediately wiped with a dry paper towel. The shells were then left to dry at room temperature for 15 minutes. The shells were first manually broken into small pieces and then later finely powdered using a mortar and a pestle. The shells were powdered to have an average particle size of less than 250 µm (USA standard sieve E-11 specification No. 60). Again, all the measurements were performed in triplicates. It took two large size eggs to form one sample i.e. one replicate. The samples were placed in an air tight vial and stored at 4 ^{0}C until used. All the measurements were performed within 36 hours of powdering.

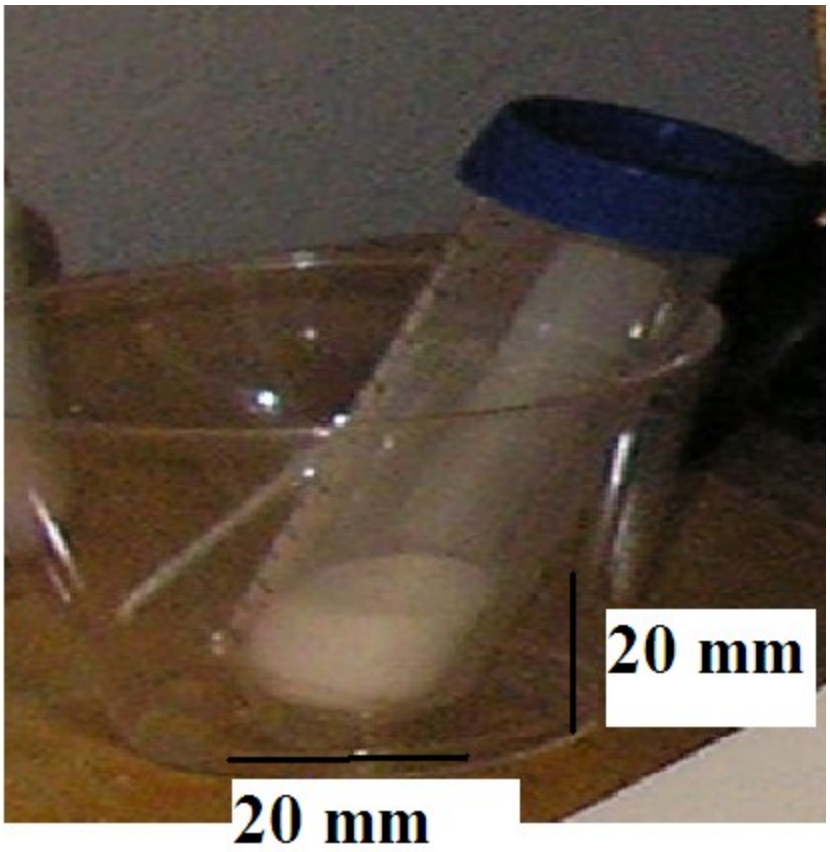

Figure 4.5 Eggshell Powder

4.2.3 Differential Scanning Calorimeter

Differential scanning calorimeter (DSC) measurements were performed by TA instruments Q_{100} DSC (TA instruments, New Castle, Delaware, USA), controlled by a computer installed with Q_{100} DSC 7.0 built 244 software. A hermetically sealed empty aluminum pan was used as a reference. Determination of the sample size was done by first weighing the sealed empty and then the sealed filled pan. The pans were then transferred to the instrument pan holder with the help of tweezers. The temperature scan range was from 25 ^{0}C to 100 ^{0}C with a heating rate of 10 ^{0}C/min.

4.2.4 Equipment and Procedure

Dielectric properties of the samples were measured by open ended coaxial probe technique. Agilent 8722 ES s-parameter Network Analyzer (Santa Clara, USA) equipped

with a high temperature probe (model 85070B) was used for this study. The equipment was controlled by a computer software (Agilent 85070D dielectric probe kit, software version E01.02, Santa Clara, USA) (Dev et al. 2008).

The sample to be measured was taken in a small cylindrical test tube (20 mm in diameter, 50 mm height and 2 mm thickness) made of borosilicate glass. The high temperature probe was mounted on the stand with the flange of the probe facing downwards. Before each experiment, the flange and the aperture of the probe were cleaned with ethanol and then wiped with paper towel. Three point calibration of the probe was performed using a shorting block, air and distilled water. The stability of the calibration was ensured by measuring distilled water as a test sample. The cable and the probe were so fixed that they could not be moved during the sample measurement (to avoid any error in the measurement).

The samples were heated using a heating unit incorporated with a metallic sample holder (Fisher scientific, USA). The dielectric properties were measured at 301 different frequencies from 200 MHz to 20 GHz, with a temperature range of 25 ^{0}C to 100 ^{0}C. The samples were heated at approximately 0.5 ^{0}C/min. The temperature of the sample was monitored using a K type thermocouple, placed parallel to the probe at a distance of 3 mm. Once the probe and the sample were in contact, the face of the test tube (holding the sample) was sealed in order to avoid any moisture loss during heating.

Since a certain amount of shrinkage of the sample is expected during heating, good contact between the probe and sample was insured by using a laboratory jack (Fisher Scientific, USA) which was adjusted to maintain a constant force of 14.7 N (monitored using a weigh balance, Denver Instruments, USA). The weigh balance, heating unit and sample were placed above the jack (Fig 4.6).

Measurement of dielectric properties of denatured sample was done by cooling the heated sample (heated up to 100 ^{0}C during the course of the experiment) to room temperature

without the removal of the sample from the test tube. The sample was then again heated from 25 ^{0}C to 100 ^{0}C and dielectric properties were measured.

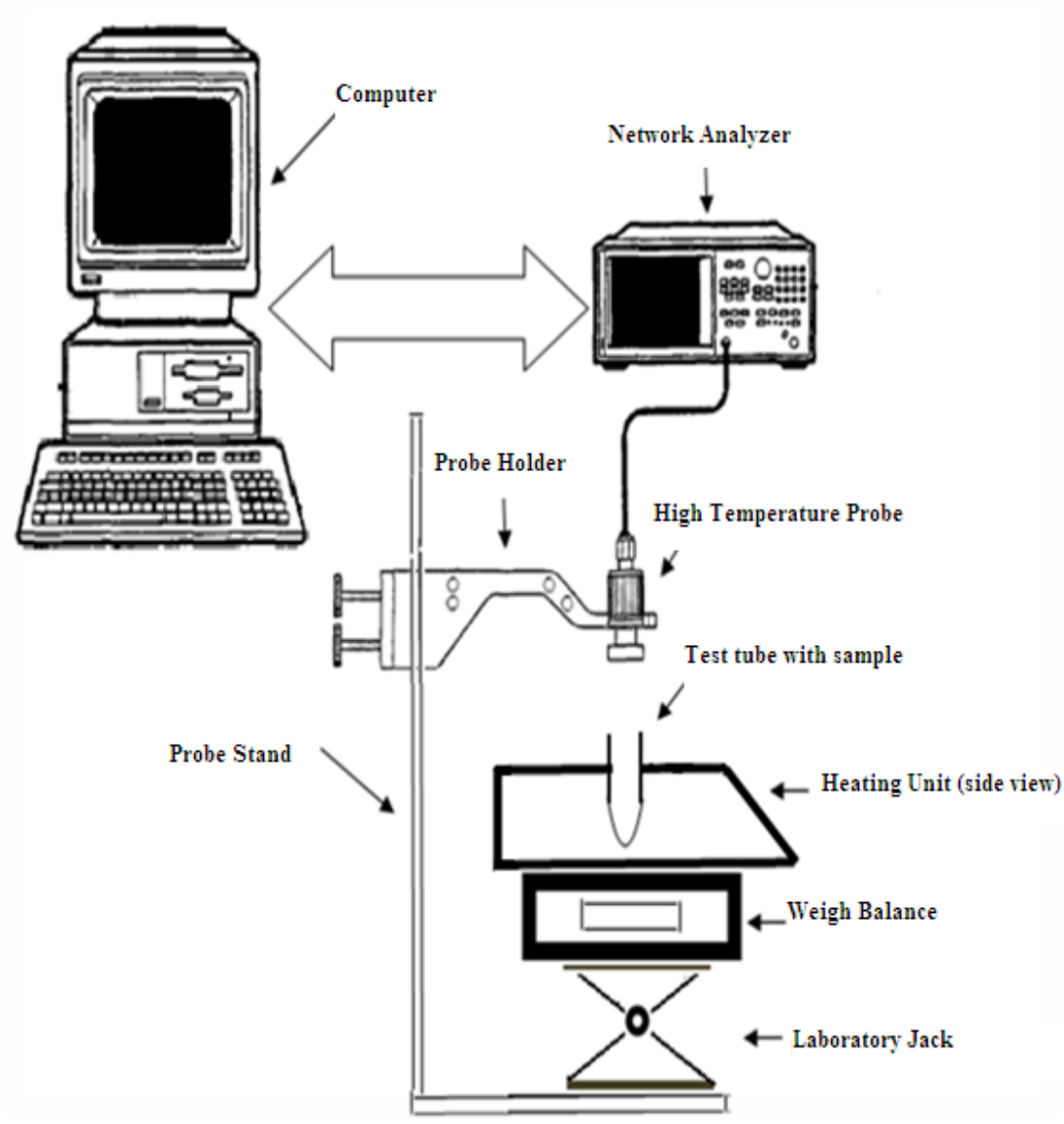

Figure 4.6 Experimental Setup for Measurement of Dielectric Properties of Eggshell and Membrane (Source: Adapted from HP Dielectric Probe Kit, user's manual)

4.3 RESULTS AND DISCUSSION

The dielectric properties of eggshell membrane and shell were measured at 301 different frequencies ranging from 200 MHz to 20 GHz. All the measurements were performed in triplicates and were reproducible ± 6%.

4.3.1 Eggshell Membrane

The eggshell membrane can be considered to be a complex mixture of interwoven protein fibers and polysaccharides with at least 70% of the matrix being proteins and 11 % being polysaccharides (Gautron and Nys 2007).

Collagen constitutes 10% of the total protein content of the egg membrane. Collagen of the type I, V and X have been found and identified in the eggshell membrane (Arias et al. 1990; Wong et al. 1984b).

Also, proteins such as avidin and ovalbumin have been identified in the mamillary knobs of the eggshell matrix. Ovalbumin was one of the first egg white proteins to be identified in the eggshell matrix and is secreted in large amounts in the uterine fluid during the early stages of eggshell formation (Gautron and Nys 2007).

A number of different proteins which are novel and specific to eggshell matrix such ovocleidin-17, ovocleidin -16 have been reported. Also, a number of glycoprotein's have been isolated and characterized in the eggshell membrane (Gautron and Nys 2007)

Though the structure of the eggshell and membrane is now well understood but ambiguities regarding its composition still exist (Lammie et al. 2005) making the measurement and understanding of its dielectric properties very challenging.

Dielectric Properties

The dielectric constant and loss of the eggshell membrane decreased from 25 ^{0}C to 70 ^{0}C, increased sharply from 70 ^{0}C to 75 ^{0}C and again decreased sharply from 75 ^{0}C to 80 ^{0}C (Figs 4.7, 4.8). The dielectric constant continued to decrease from 80 ^{0}C to 95 ^{0}C, but a slight increase was observed from 95 ^{0}C to 100 ^{0}C (Fig 4.7). In contrast, the dielectric loss remained mostly constant from 80 ^{0}C to 100 ^{0}C (Fig 4.8).

The dielectric constant remained positive (positive value) for all temperatures and frequencies but the dielectric loss value was negative for temperatures above 55 ^{0}C at higher frequencies. In case of frequencies above 15 GHz, the negative dielectric loss value was observed at 45 ^{0}C (Fig 4.8), possibly due to the effect of temperature and the failure of the dipoles to align themselves to the fast alternating electric field at higher frequencies.

The initial decrease in dielectric constant and loss from 25 ^{0}C to 70 ^{0}C (Fig 4.7) could be due to the low moisture content and high ash content (Biova-ovacore, LLC, Ames, IA, USA) of the eggshell membrane. Ash content which is mostly composed of salts have been found to be negatively related to dielectric constant, which is due to the binding of water by salts, thereby decreasing their ability to orient themselves to changing electric field direction (Sipahioglu and Barringer 2003).

The DSC thermogram for eggshell membrane showed two main peaks, one at 72 ^{0}C and the other at 92 ^{0}C (Fig 4.9). The peak observed at 72 ^{0}C was much larger than that observed at 92 ^{0}C. The size of the peak varies depending upon the heat flow required to denature the proteins at that temperature. The size of the peak is the product of the enthalpy of denaturation of the protein, the concentration of the protein in the sample and the total weight of the sample. Therefore, a detailed examination/analysis of the thermogram can give quantitative information about the amounts of various components present in the sample (Donovan et al. 1975). Larger the amount of a protein in the sample, the bigger would be its peak. Therefore, it can be assumed that the protein undergoing denaturation at 72 ^{0}C is present in larger amount in the eggshell membrane

than the protein undergoing denaturation at 92 ^{0}C. As 10% of the total protein content of the eggshell membrane is collagen, it can be assumed that the peak observed at 72 ^{0}C is collagen peak. Co-relating to the DSC thermogram, it can be said, that the changes observed in dielectric constant and loss between 70 ^{0}C to 80 ^{0}C is due to the denaturation of collagen.

The denaturation of collagen due to the application of heat leads to the unfolding and shrinkage of collagen and the breakage of hydrogen bonds which stabilized the protein. This unfolding and breakage of the bonds leads to extensive water- ion interactions, which increases the water binding capacity of the protein (Mangino 1984), resulting in a decrease in dielectric constant and loss between 75 ^{0}C to 80 ^{0}C.

As the collagen in eggshell membrane is buried in a proteoglycan matrix (Arias et al. 1991), the initial increase might be due to the interactions of the glycans (containing oligosaccharide chains) with the electric field during the initial bond breakage and unfolding of the protein. The interaction of the hydrogen bonds and hydroxyl groups with water are known to play a significant role in sugar based foods (Roebuck and Goldblith 1972). Also, primary hydration water is set free during the denaturation of collagen (Wright and Humphrey 2002).

Co-relating to the DSC thermogram, another peak was observed at 92 ^{0}C. The dielectric constant decreased from 80 ^{0}C to 95 ^{0}C, with a sharp decrease between 90 ^{0}C to 95 ^{0}C. The decrease in dielectric constant could be due to the denaturation of a protein such as ovalbumin or avidin. As mentioned earlier, ovalbumin is secreted in large amounts in the uterine fluid during the initial stages of eggshell formation (Huopalahti et al. 2007). A more stable conformation of ovalbumin called as S-ovalbumin is known to denature at around 90.2 ^{0}C (Bircan and Barringer 2002). However, it should be noted that in the study conducted by Bircan et al, the dielectric constant for egg white ovalbumin peaked at 90 ^{0}C. But as observed from various sources, the dielectric properties for a particular protein can vary depending upon its source.

Avidin, which is also present in the eggshell membrane have shown to match ovalbumin transition (Bircan and Barringer 2002) and have been reported to denature at 95 ^{0}C in one of the studies (Donovon and Ross 1973).

The decrease from 80 ^{0}C to 95 ^{0}C was observed only in dielectric constant and not in dielectric loss which remained mostly constant after 80 ^{0}C. From which, it can be said that dielectric constant in this study was more sensitive to protein denaturation than dielectric loss.

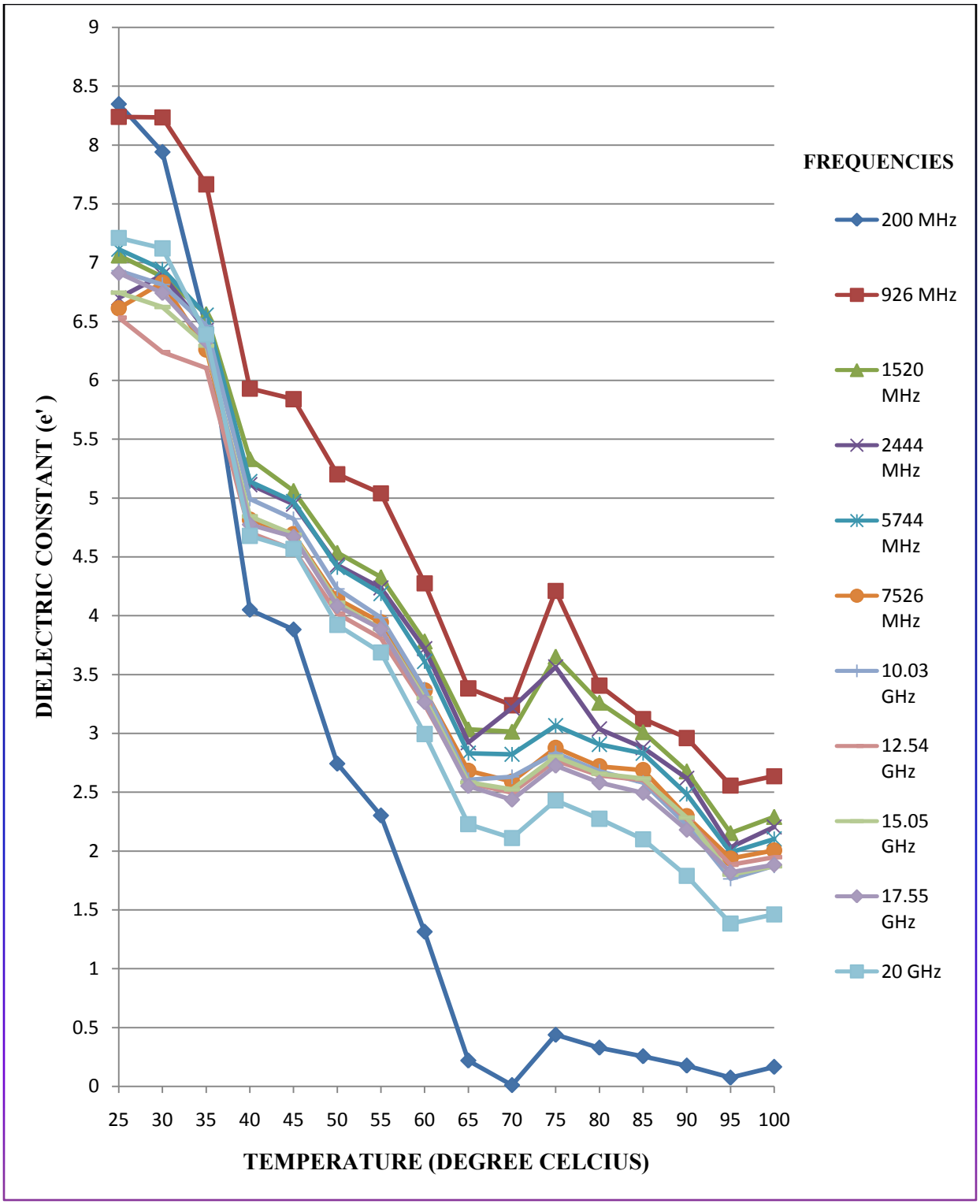

Figure 4.7 Dielectric Constant Vs Temperature for Eggshell Membrane at 11 frequencies

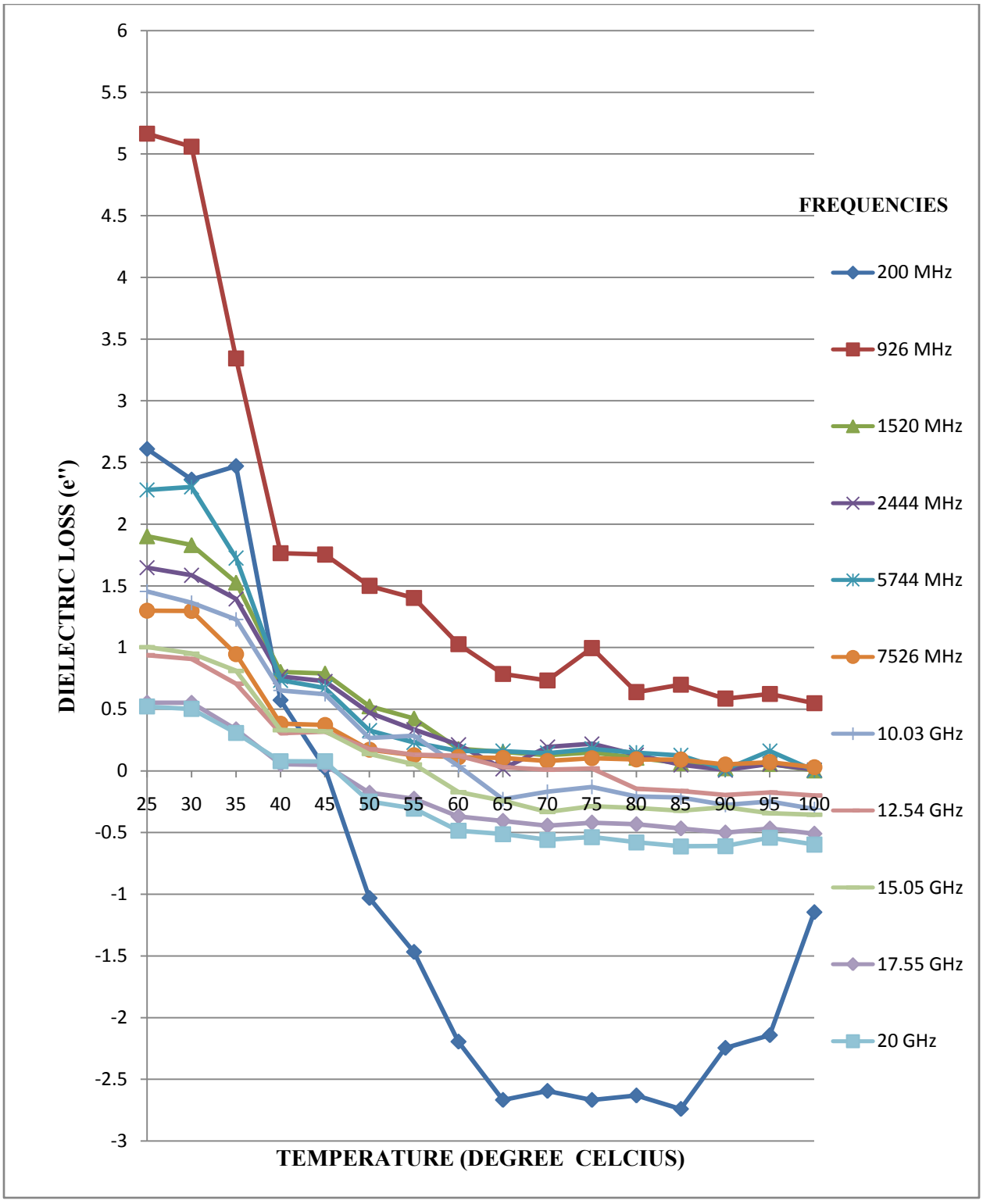

Figure 4.8 Dielectric Loss Vs Temperature for Eggshell Membrane for 11 Frequencies

Figure 4.9 DSC Thermogram for Eggshell Membrane

Effect of Frequency

Frequency of the waves has a significant effect on the dielectric properties of a material due to the frequency dependence of dipolar and ionic conduction mechanisms (Datta et al. 2005).

The dielectric constant and loss were more sensitive to change (protein denaturation) at lower frequencies than that at higher frequencies. Though the dielectric constant remained positive for all frequencies (Fig 4.10) the dielectric loss was mostly negative for higher frequencies (> 10 GHz).

The pattern for dielectric loss at 200 MHz was very different from those observed at other frequencies (Fig 4.8). The dielectric loss decreased from 25 ^{0}C to 65 ^{0}C, remained mostly uniform from 65 ^{0}C to 80 ^{0}C, and then increased between 80 ^{0}C and 100 ^{0}C.These differences in trend from other frequencies might be due to the failure of the dipoles to align themselves to changing electromagnetic field at lower frequencies. The dielectric values for constant and loss were maximum at 926 MHz for all temperatures except at 25 ^{0}C (maximum at 200 MHz), it was also the most sensitive to the changes occurred due to protein denaturation. The dielectric constant and loss increased for frequencies from 200 MHz to 926 MHz and decreased there forth.

No particular trend in dielectric loss or constant was observed with the changing frequency (Figs 4.10, 4.11). The dielectric constant and loss may increase or decrease with frequency. A similar observation was also reported by Datta and Nelson for a low moisture commodity such as wheat grain (Datta et al. 2005).

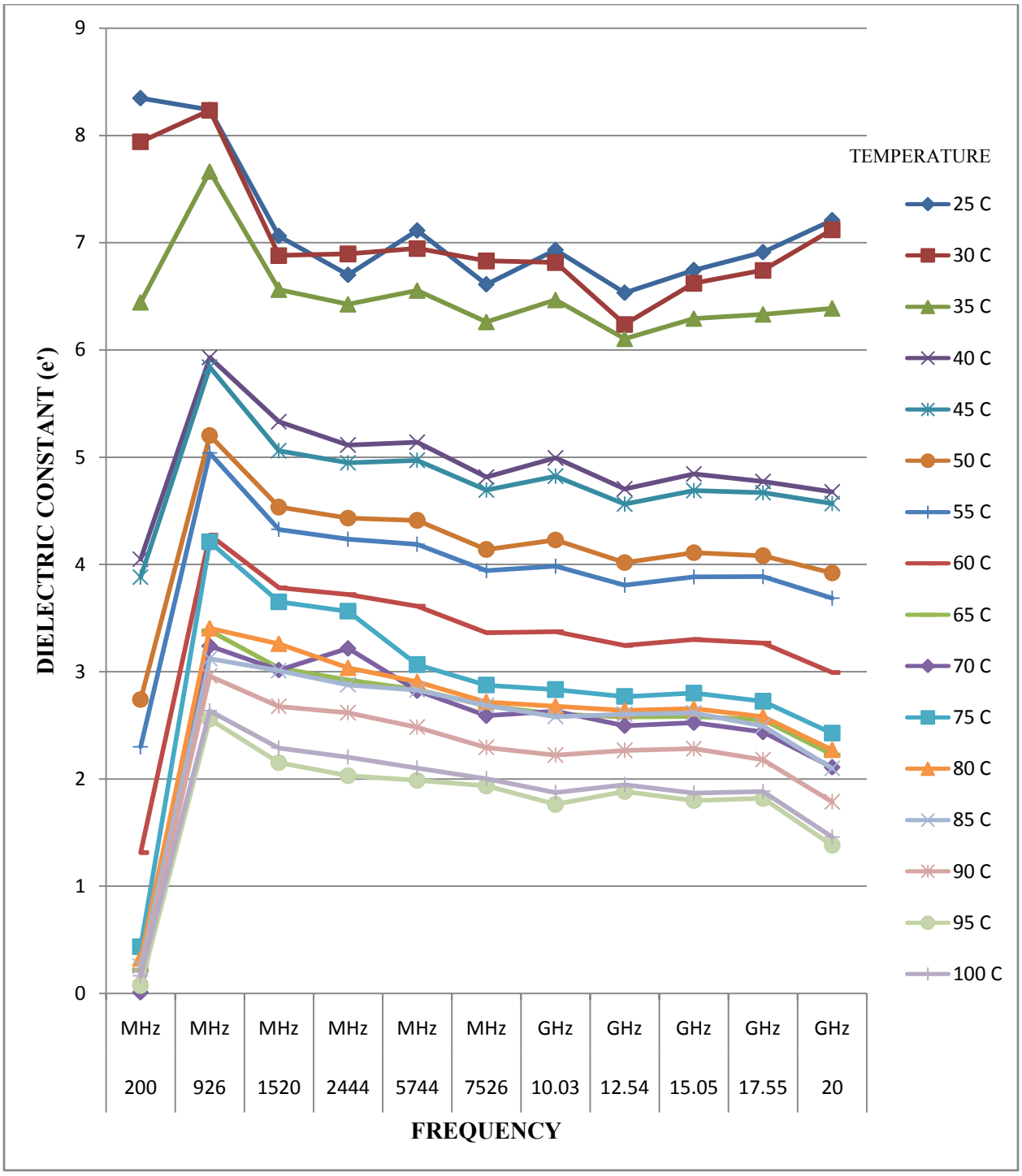

Figure 4.10 Dielectric Constant Vs Frequency for Eggshell Membrane

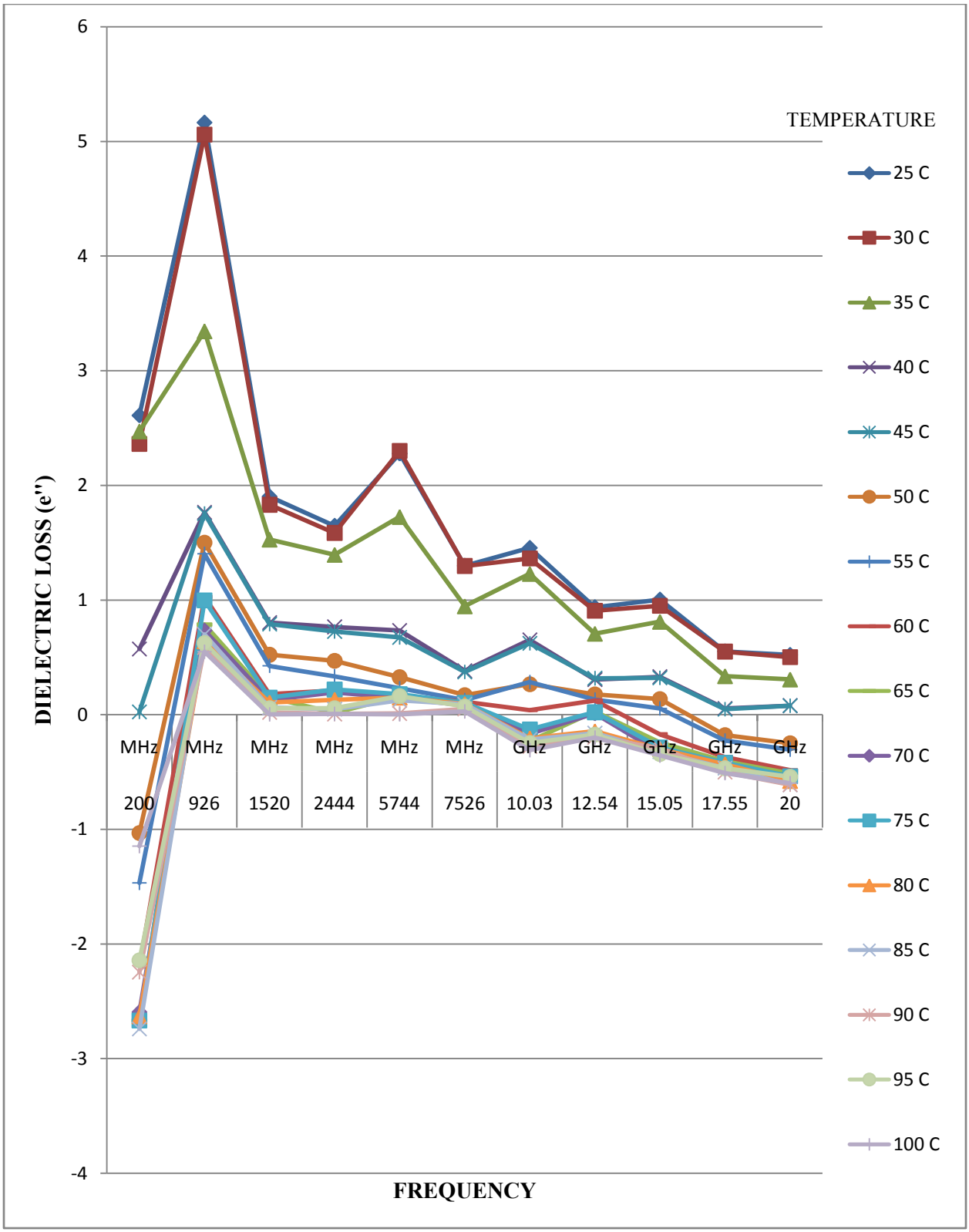

Figure 4.11 Dielectric Loss Vs Frequency for Eggshell Membrane

Reheated Sample

The dielectric constant and loss for reheated sample gradually decreased with temperature (Figs 4.12, 4.13). No change is dielectric loss or constant was observed at temperatures at which protein denatured in fresh samples (70 ^{0}C - 80 ^{0}C, 90 ^{0}C - 100 ^{0}C), certifying that the changes observed in dielectric constant and loss between 70 ^{0}C to 80 ^{0}C and 90 ^{0}C to 100 ^{0}C in the fresh sample was due to the denaturation of protein and not due to some other factors. It should be noted that the dielectric loss and constant values could not be measured at 200 MHz in the reheated sample.

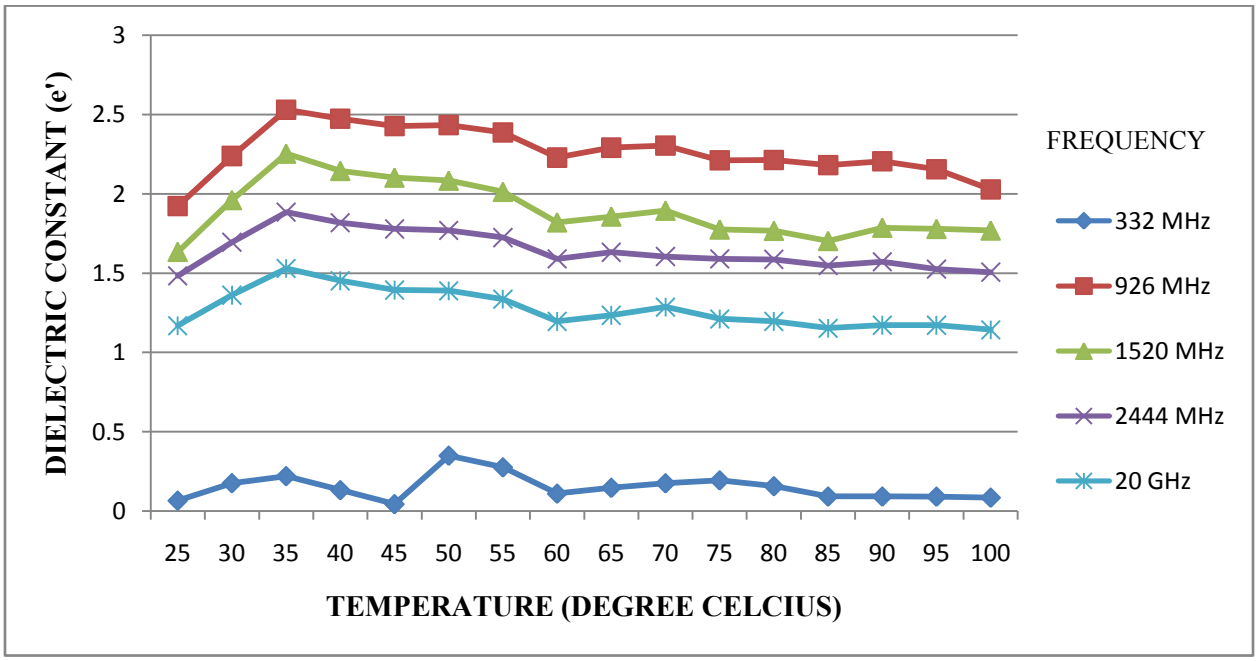

Figure 4.12 Dielectric Constant for Reheated Sample (Eggshell Membrane)

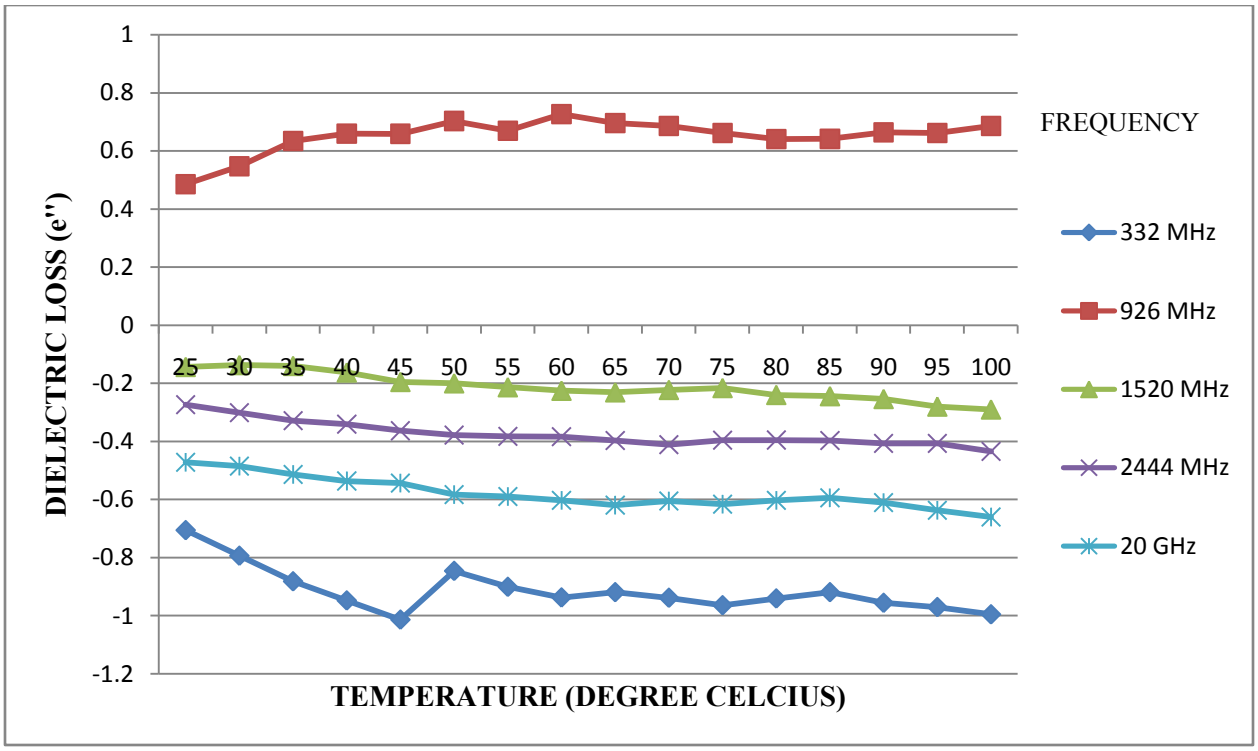

Figure 4.13 Dielectric loss for Reheated Sample (Eggshell Membrane)

4.3.2 Eggshell

The eggshell which is largely made up of calcium carbonate (95%) and only a minor amount of organic matrix which are incorporated in the calcite crystals and present on the cuticle (Stadelman and Cotterill 1996).

The cuticle which is the outermost water insoluble layer of the eggshell is largely an organic layer with protein content as high as 90%, with a high content of cystine, glycine, glutamic acid and tyrosine. Fucose, hexosamine, sialic acid are present as constinuents of polysaccharides (Stadelman and Cotterill 1996).

Dielectric Properties

The dielectric constant and loss decreased with temperature (Figs 4.14, 4.15). The dielectric constant of the eggshell decreased steeply for frequencies above 10.03 GHz and between the temperatures of 85 ^{0}C and 100 ^{0}C. The dielectric constant remained positive

for all frequencies and temperatures. In contrast, the dielectric loss value was negative for all frequencies except at 926 MHz, which possibly could be due to the low moisture of the eggshells and failure of the dipoles to align themselves with the changing electromagnetic field at very low and at higher frequencies (Datta et al. 2005). The decrease in dielectric constant and loss with temperature could again be due to the low moisture content of the eggshell and the effect of temperature on dielectric properties.

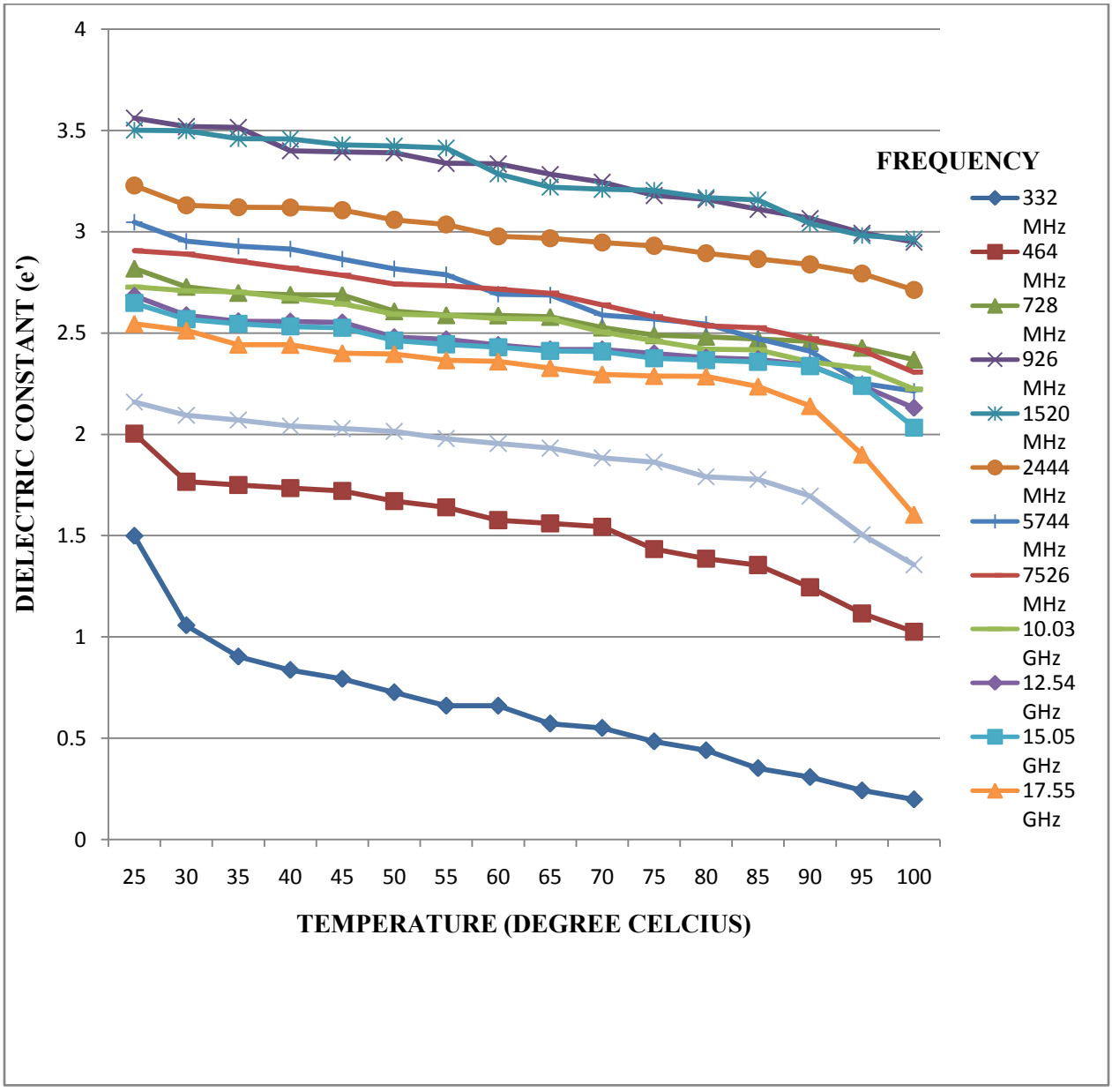

Figure 4.14 Dielectric Constant Vs Temperature for Eggshell at 13 Frequencies.

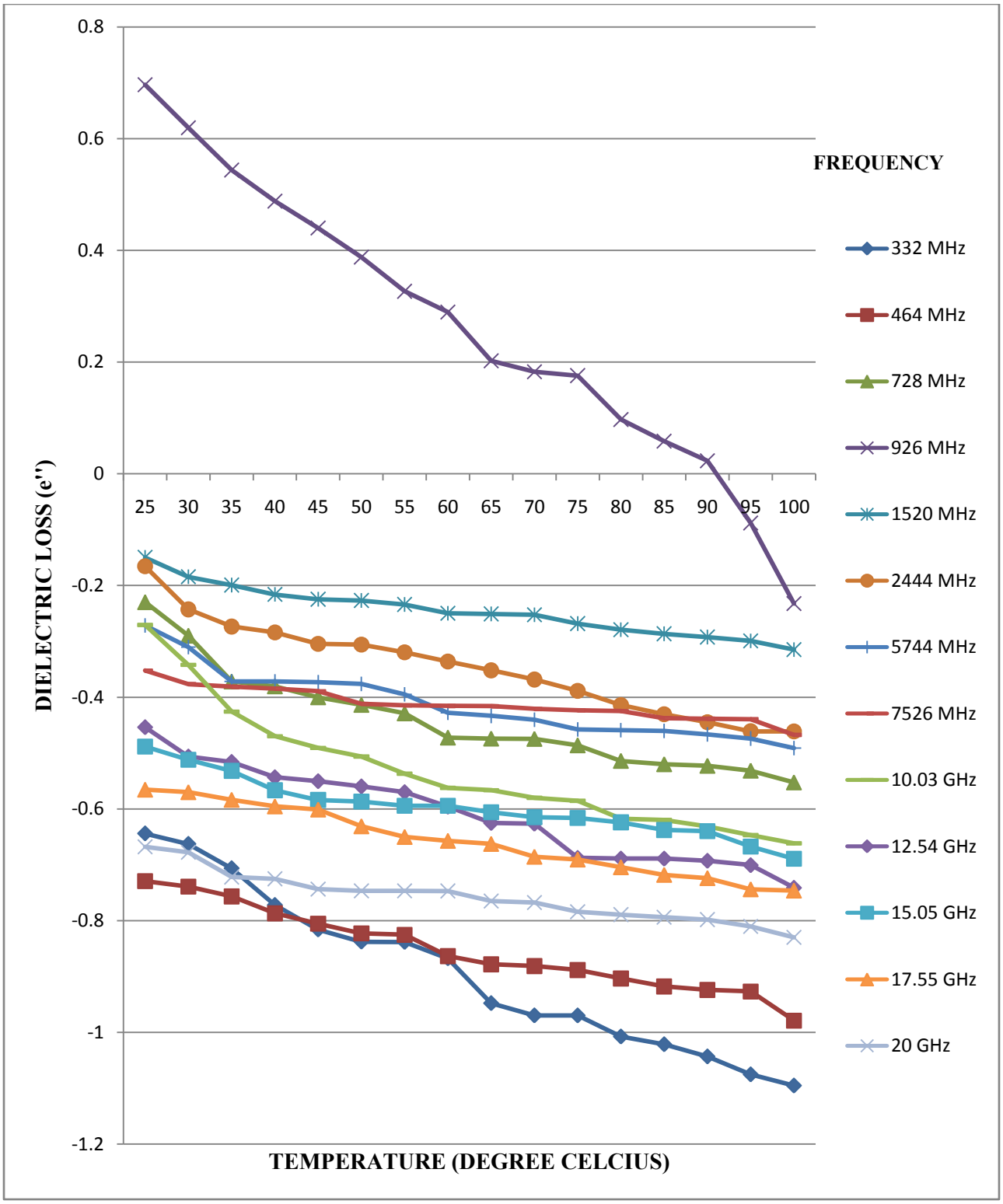

Figure 4.15 Dielectric Loss Vs Temperature for Eggshell at 13 Frequencies.

Effect of Frequency

As observed for eggshell membrane, the dielectric constant and loss for eggshell increased for frequencies from 332 MHz to 926 MHz and decreased there forth. No value for dielectric constant or loss could be detected at 200 MHz.

The deviation among the values for dielectric constant at frequencies from 332 MHz to 926 MHz was larger than those observed for frequencies there forth. However, the values for dielectric constant (Vs temperature) at 1520 MHz were comparable to those observed at 926 MHz.

Also, as observed for eggshell membrane, no particular trend in dielectric loss or constant was observed with changing frequencies (Figs 4.16, 4.17). The dielectric constant or loss may increase or decrease with frequency. A similar observation was also reported by Datta and Nelson for low moisture commodity such as wheat grain (Datta et al. 2005).

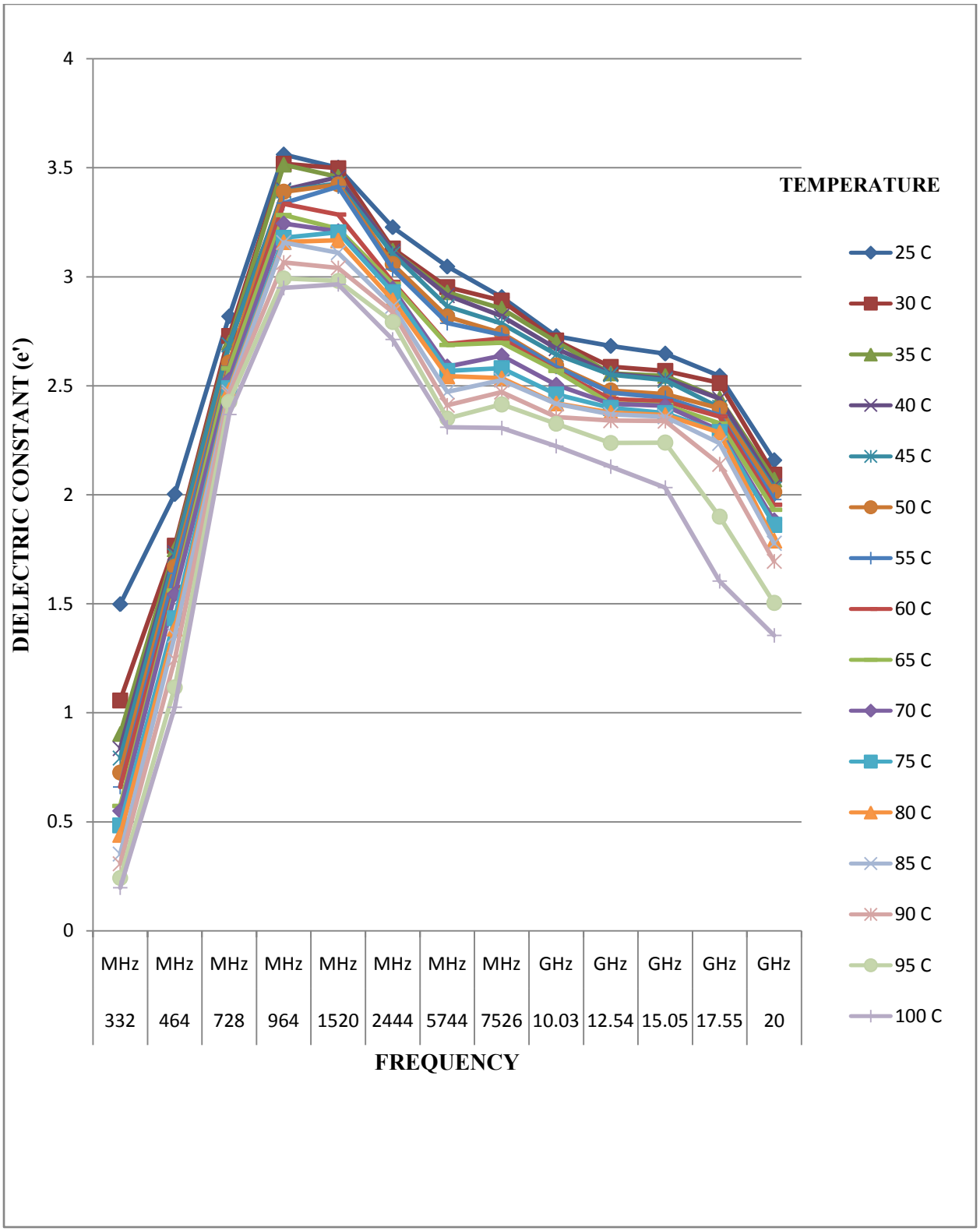

Figure 4.16 Dielectric Constant Vs Frequency for Eggshell

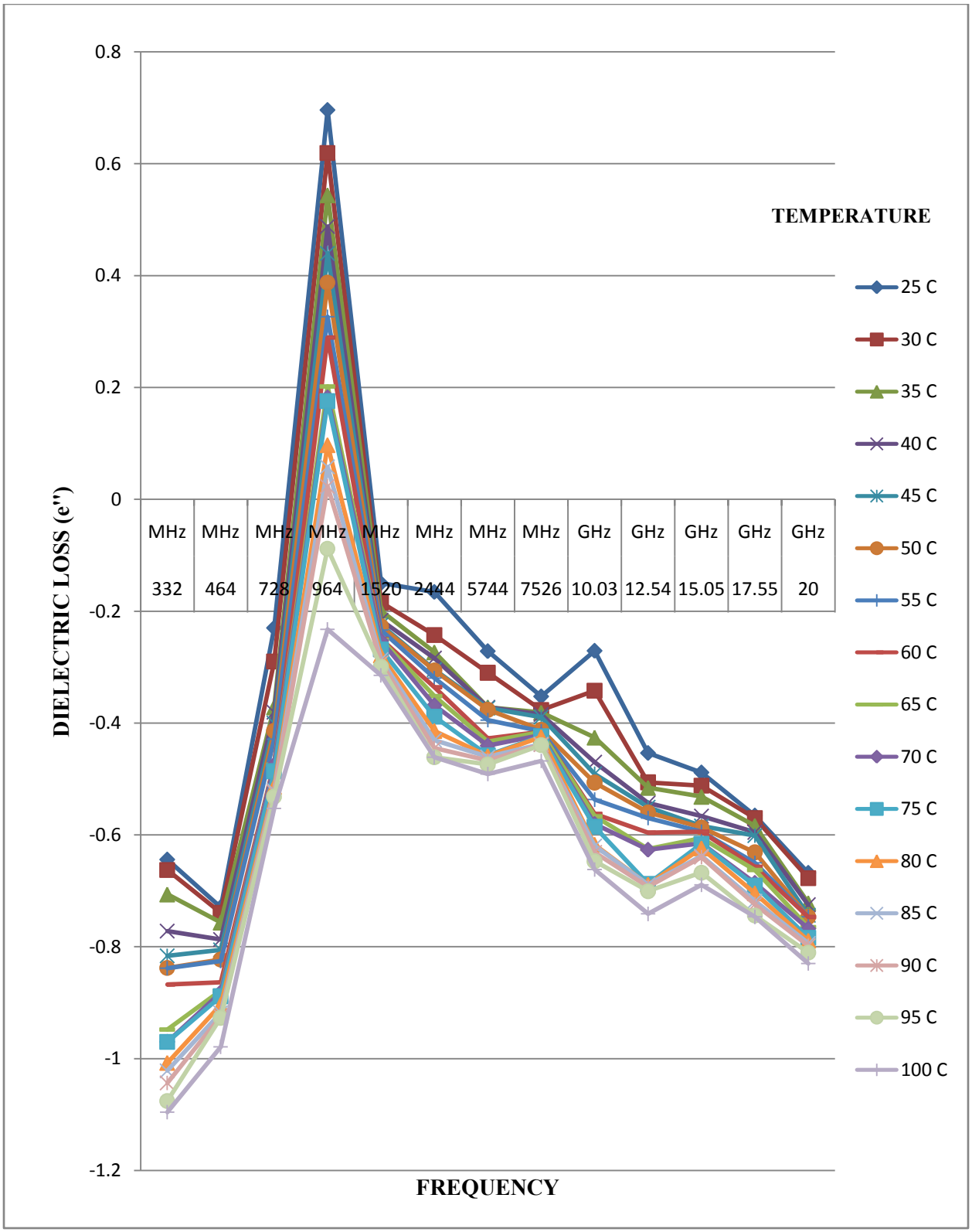

Figure 4.17 Dielectric Loss Vs Frequency for Eggshell

4.4 CONCLUSION

The dielectric properties can be used for detection of protein denaturation in egg membrane. For the protein undergoing denaturation between the temperature range of 70 ^{0}C and 80 ^{0}C, the dielectric constant and loss increased between 70 ^{0}C to 75 ^{0}C and later decreased between 75 ^{0}C to 80 ^{0}C. However, for the protein undergoing denaturation between 90 ^{0}C and 100 ^{0}C, a decrease in the dielectric constant was observed but the dielectric loss remained mostly uniform. Dielectric constant was observed to be more sensitive to protein denaturation than dielectric loss. For eggshell, the presence of trace amounts of proteins present in the calcified layers and in the cuticle could not be detected by the dielectric properties. The dielectric constant and loss decreased gradually with increase in temperature.

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CONNECTING TEXT

After studying the dielectric properties of eggshell and membrane, it was apparent that the dielectric properties of eggshell and membrane were in accordance to that hypothesized i.e., the egg membrane had higher values of dielectric constant and loss than eggshell and would thus behave/respond better to microwaves than eggshell leading to a differential heating between the two, thereby leading to the separation of eggshell and membrane.

CHAPTER 5

MICROWAVE ASSISTED SEPARATION OF EGGSHELL AND MEMBRANE

Abstract

The effect of hot water and microwave treatment on separation of eggshell and membrane was investigated in this study. The effectiveness of a treatment was analyzed in terms of reduction in the total energy required (expressed in milli Joules) to separate the eggshell and membrane and was termed as bond energy. A tensile testing machine was deployed to measure the bond energy for 30 mm X 10 mm eggshell strip. In all the statistical analyses the bond energy after a particular treatment was compared to the bond energy for non-treated eggs. There was no significant difference ($p > 0.01$) for bond energy between the hot water treated eggs and non-treated eggs. For microwave heating, three factors with three levels each were considered. All the microwave treated eggs had bond energy significantly different ($p < 0.01$) from non-treated and hot water treated eggs. It was determined that power density and soaking time played a significant role in reduction of bond energy for microwave treated eggs, with neither the temperature nor the interaction between temperature and power density playing any significant role. A Model for calculating the bond energy as a function of power density and soaking time is also presented.

Keywords: eggshell, egg membrane, microwave.

5.1 INTRODUCTION

In recent years there has been a growing interest in separation of eggshell and membrane which is clearly apparent by the growing number of patents describing/developing methods for efficient separation of the same.

Eggshell which forms the outer crust of an egg is a non-edible product with very limited use and value and is largely disposed of as a waste. There has been an exponential growth in the processed egg industry with 30% of egg produced in United States today is consumed by the processed egg industry. According to an estimate by the United States Department of Agriculture, the egg processing industry consumed 25.6 million cases of egg in 1984 to manufacture liquid and dry egg products. In 1997 the same industry

consumed about 50 million cases of egg, producing more than 120,000 tons of unprocessed egg shell waste with disposal costs between $ 25,000 and $ 100,000 per year (MacNeil 2006, 2001).

Keeping in mind the high disposal costs which continue to increase due to increase in landfill taxes and increasing environmental concerns, it is necessary to find an alternative method/solution which would transform the waste eggshells into a valuable item, giving financial benefits to the competitive egg processing industry. Apart from giving manufacturers a new profit stream it would help overcome the high disposal costs and environmental concerns (MacNeil 2006, 2001).

There are many uses of separated eggshell and membrane but not many when they are attached. It is established that the eggshell and membrane are a reserve of many bioactive components which can be utilized by efficient separation of the eggshell and the membrane (MacNeil 2001).

Collagen constitutes 10% of the total protein content of the egg membrane (MacNeil 2006, 2001). Collagens of type I, V and X have been identified in the eggshell membrane (Arias et al. 1990; Wong et al. 1984a). A lot of emphasis has been given to the presence of collagen in eggshell membrane due to its high economic and monetary value. Keeping in mind the 1997 estimates, 120,000 tons of eggshell waste would yield 110,000 tons of eggshell and 10,000 tons of membrane. Considering that 10% of membrane is collagen, it would yield 1,000 pounds of collagen, which is presently priced at $ 1000 per gram or about $ 454,000 per pound (MacNeil 2006, 2001). Also, Hyaluronic acid which is another substance of high monetary value is naturally present in and is a constituent of eggshell membrane. The total hyaluronic content of eggshell membrane is estimated to be between 0.5 – 10% (Long et al. 2005).

A number of proteins have been found to be novel and specific to the eggshell. Ovocleidin- 17, Ovocleidin-16 a 80 ka protein (742 amino acids) containing two N-

glycosylation and two disulphide bonds, Ovocalyxin – 32 and 25, Ovocalyxin 36 , are localized in various layers of the calcified shell (Gautron and Nys 2007).

Much importance has been given to the presence of various therapeutic and cosmetically active components such as collagen, hyaluronic acid, glucosamine, chondroitin sulphate present in eggshell membrane having potential applications in cosmetic and pharmaceutical industries. The following components when extracted from other natural resources, demands for significant processing cost due to the presence of these compounds in low quantity or due to the additional costs levied to obtain these compounds in the desired purity. Therefore, the extraction of these compounds from egg membrane, which is typically a waste product, is expected to reduce the cost considerably. Also, depending upon the targeted application the composition / percentage of the compounds can be altered to serve the purpose (Long et al. 2004).

US patent no. 2007/0178170 held by Devore et al. (2007) discusses the anti-inflammatory properties of eggshell membrane and processed eggshell membrane preparations. Eggshell membrane was reported to be an ideal split- thickness skin graft (STSG) donor site dressing. It exhibited properties of pain relief, wound protection, promotion of healing (Yang et al. 2000). Also, dried non-fibrous egg membrane products assisted and stimulated healing process in damaged mammalian tissues such as the tissues lost or damaged due to cuts, injuries, burns and ulcerations (Neuhauser 1965).

Various other uses of eggshell membrane such as the use of exterior layers of the egg (cuticle, shell and shell membranes) as a support for growth of bacterial culture (Lifshitz et al. 1965), removal of heavy metals and gold from industrial waste water using greatly swollen eggshell membrane- conjugated chitosan beads have been reported (Shoji et al. 2004).

A major problem with profitable utilization of the waste eggshell is ensuring the complete separation of the shell and the membrane, as when separated both items can have significant value.

The presence of high density continuous distribution of mammillary knobs at the outer shell membrane provides an optimal interface for the establishment of a firm attachment/bond between the shell and the membrane. The membranes which are a matrix of interwoven protein fibres, act as a structural reinforcement contributing significantly to shell strength. This strong physical attachment between the shell and the membrane makes their separation extremely difficult (MacNeil 2005; Orberg 1990). In this particular study microwave treatment was used for separation of eggshell and membrane, based upon the hypothesis:

The separation of the eggshell and membrane by microwaves would depend upon the fact that the membrane has higher moisture content than the eggshell which would lead to more absorption of the electro-magnetic waves by the membrane than the shell. The difference in the moisture content of the eggshell and membrane would result in a differential heating of the shell and the membrane leading to the expansion of the membrane, which would weaken the physical interaction between the shell and the membrane; thereby, assisting the separation of the membrane and the shell. Also the membrane is a protein matrix with relatively high concentration of polar amino acids which would also respond further to the electro-magnetic waves.

In the previous study of dielectric properties of eggshell and membrane, it was apparent that membrane had higher dielectric properties than the eggshell, again suggesting that membrane would respond better to microwaves than the eggshell.

The present study was performed with the following **objectives**:-

1. To investigate the possibility/efficiency of microwave treatment for separation of eggshell and membrane.

2. To investigate the effect of moisture content, varying temperature, power density on the separation of eggshell and membrane.

5.2 MATERIAL AND METHODS

A total of 39 commercially available eggs were used in this study. All eggs were of large size, with an average weight of 58 g each. The eggs were stored at 4 ^{0}C until used. All measurements were performed in triplicates.

Two system of heating were considered and the efficiency of each in separating the eggshell and membrane analyzed.

1. Dipping the eggs in hot water (75 ^{0}C – 80 ^{0}C).
2. Microwave treatment of eggs.

5.2.1 Hot Water Treatment

For the hot water treatment, the eggs were dipped in hot water (75 ^{0}C - 80 ^{0}C) until they reached the desired temperature. Three temperatures were studied 40 ^{0}C, 50 ^{0}C and 60 ^{0}C. The temperature was monitored using a K type thermocouple, which was inserted by making a small opening at the larger end of the egg containing the air cell (Fig 5.1).

The eggs were heated by first adjusting them on a plastic stand with the larger end of the egg facing upwards (Fig 5.1). After which the egg along with the stand was placed inside a 1000 ml cylindrical beaker. The cylindrical beaker was then filled with tap water in such a way that the top few inches of the egg (approximately 0.3 inches) remained above water level. It was done so as to prevent the water from entering the egg from the opening created to insert the thermocouple. The beaker was then heated using an electric hot plate (Fisher scientific, USA) and the temperature of the water monitored using an alcohol thermometer (Fig 5.1).

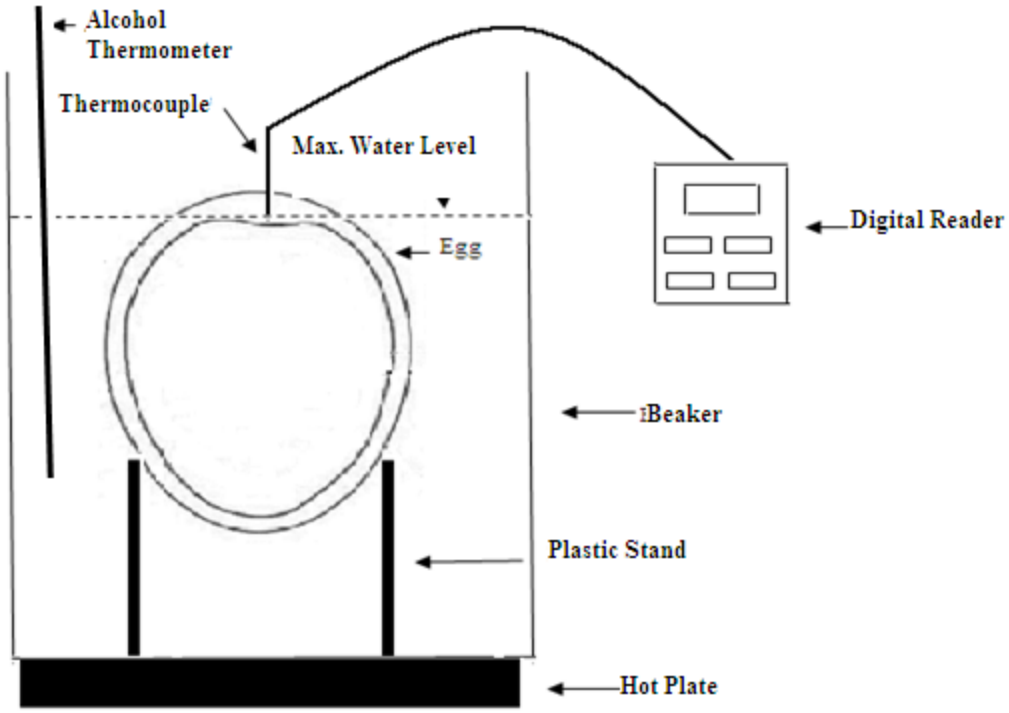


Figure 5.1 Experimental Setup for hot water treatment

5.2.2 Microwave Treatment

The efficiency of microwave treatment in separating the eggshell and membrane was extensively studied. For the analysis of the same, three factors with three levels each were considered. The factors and levels are summarized in the table below. All the measurements were performed in triplicates.

Table I. Experimental Design for Microwave Treatment

Factors	Level
1. Soaking Time	1. O day
	2. 1 day (Microwave Treatment after soaking the egg in tap water for 24 hours)
	3. 2 days (Microwave Treatment after soaking the egg in tap water for 48 hours)
2. Temperature	1. 40 ^{0}C
	2. 50 ^{0}C
	3. 60 ^{0}C
3. Power Density	1. 1 W/g
	2. 1.5 W/g
	3. 2 W/g

The microwave treatment was given by placing the egg (with a small opening at the larger end of the egg containing the air cell) inside the microwave cavity of a conventional 1250 W, 2450 MHz microwave oven (Panasonic, Canada) and treated until it reached the desired temperature (Fig 5.2). The time taken to reach the desired temperature was predetermined during the preliminary studies. The temperature was measured using a K type thermocouple.

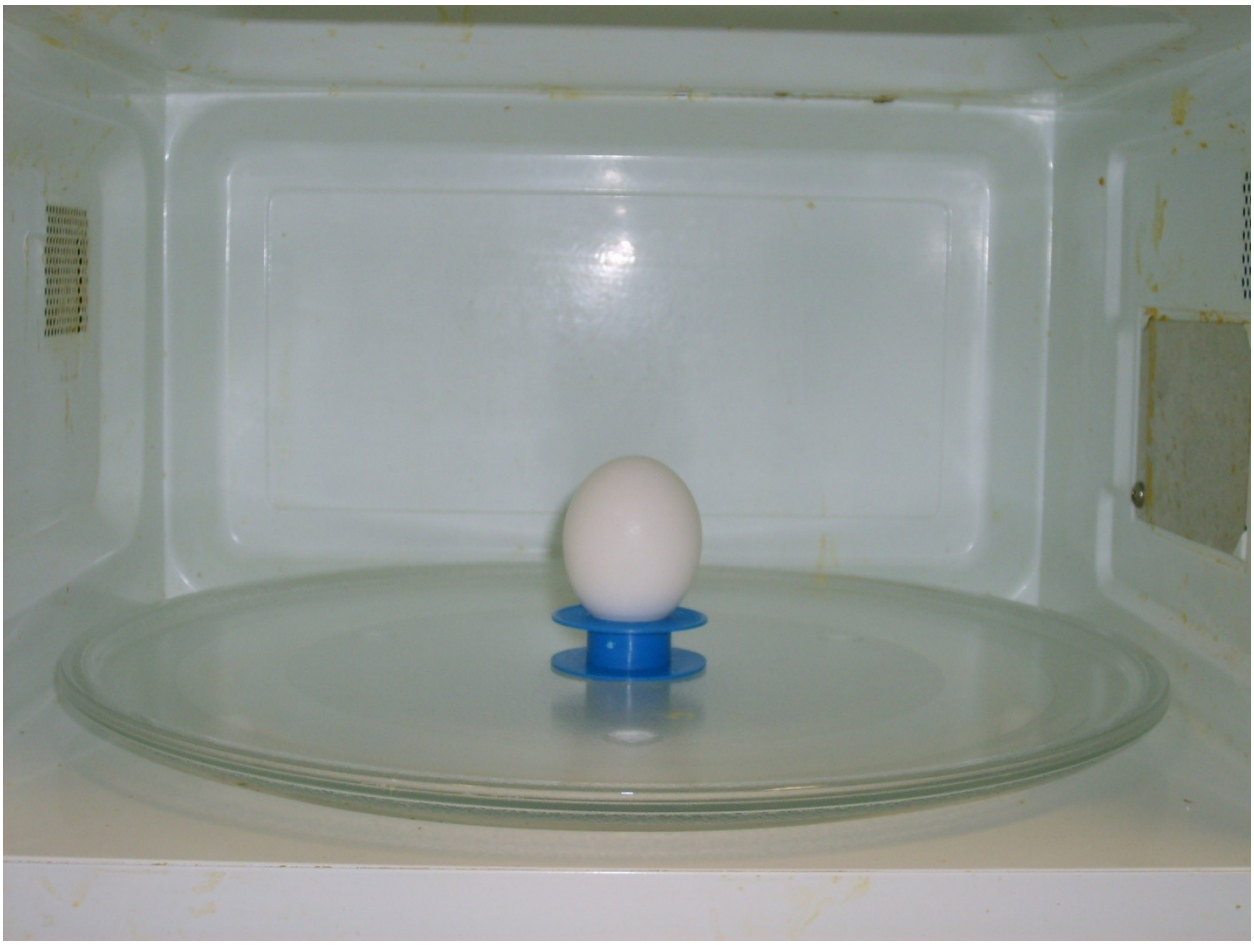


Figure 5.2 Microwave treatment of egg

5.2.3 Shell Samples

Once the egg was given the desired treatment (hot water or microwave treatment), the albumin and yolk were discarded by making a small opening at the larger end of the egg. After which, three strips of eggshell (from each egg) 30 mm X 10 mm in dimension was cut along the equator of the egg (to maintain uniformity in the samples) using a dremel (Dremel experts, US). Three strips of eggshell from one egg formed one replicate. Therefore, for three replicates (three eggs) nine strips of eggshell were considered.

5.2.4 Equipment

Measurement of bond strength between the shell and the membrane was done by using a tensile testing machine (Instron – 4502, Instron Corporation, USA) controlled by a computer software (Instron series IX, version 8.25).

The shell samples to be analyzed were glued to a custom made shell sample holder mounted on the tensile testing machine (Fig 4.3). About 5 mm of the membrane (from the shell strip) was manually separated and attached to the clip connected to a 50 N load cell. As the clip moved upwards at a constant rate of 10 mm/min, the membrane was separated from the shell and the energy required to do so was recorded in terms of mJ.

The efficiency of the given treatment (hot water or microwave treatment) was judged/analyzed by reduction in bond strength between the shell and membrane after the particular treatment was given. The reduction in bond strength between the shell and the membrane was measured in terms of reduction in the total energy required (expressed in mJ) to separate the membrane from the eggshell (for the particular eggshell strip) and hereby, referred to as **bond energy**.

5.2.5 Control

For all the statistical analysis the bond energy after a particular treatment was compared to the bond energy of non treated eggs, i.e., the eggs for which no treatment was given and hereby referred to as control. The bond energy for control eggs was measured in the same way as for other treated eggs (section 5.2.4) with the only difference being that the eggs did not undergo any treatment.

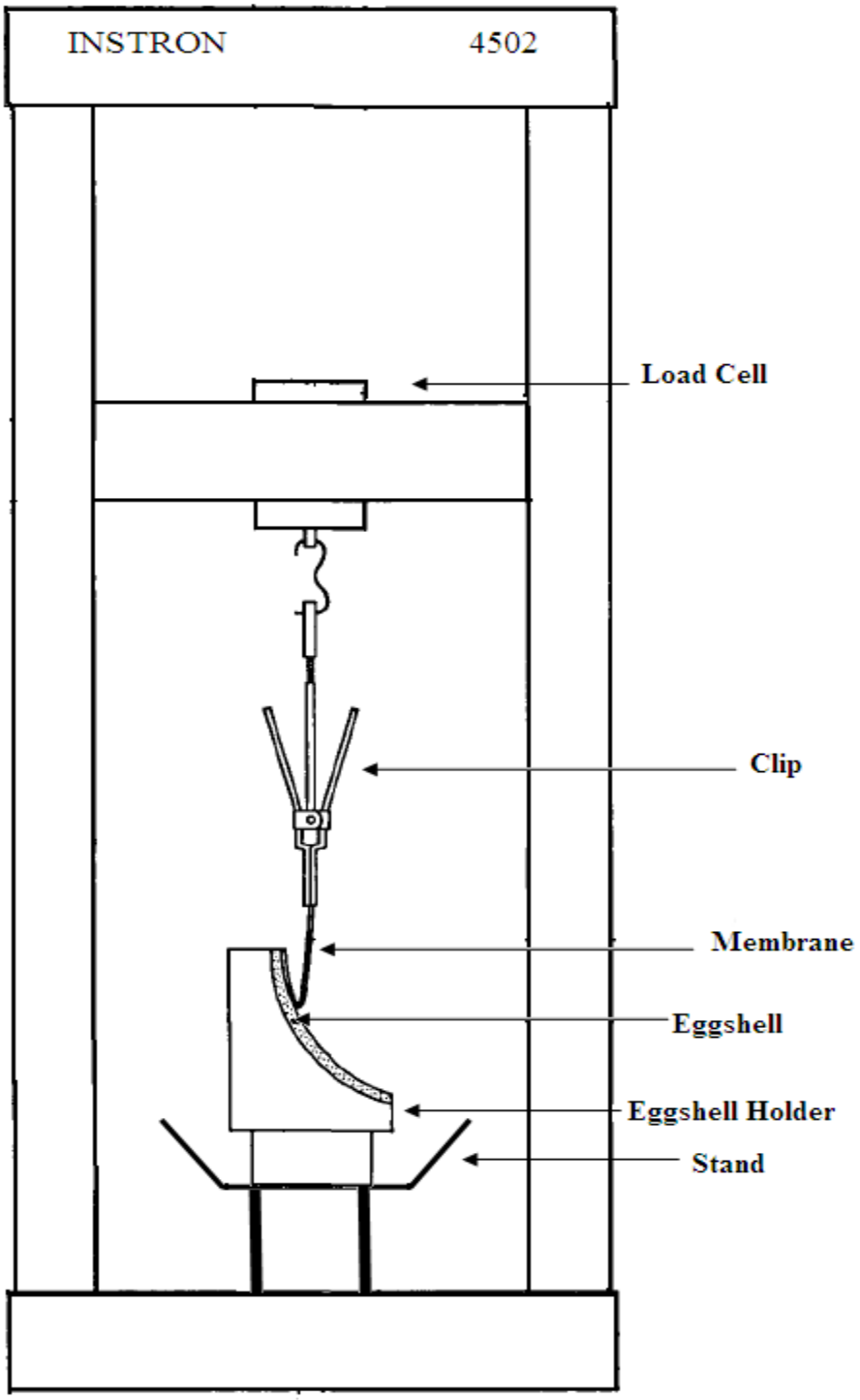


Figure 5.3 Experimental Setup for measurement of Bond Energy (Adapted from: Orberg 1990)

5.2.6 Data Analysis

MATLAB 7.8 was used for all data analysis. ANOVA was performed for all the treatments. Multiple comparison test based on least significant difference was performed for factors found to be significant from ANOVA analysis. Also, the process was optimized depending upon the mathematical relationship developed for microwave treatment as a function of soaking time and power density.

5.3 RESULTS AND DISCUSSION

Two different systems of heating were considered and the effect of each on the reduction of bond energy between eggshell and membrane was studied.

5.3.1 Bond Energy for Non-Treated Eggs

For all the statistical analysis, the bond energy after a particular treatment was compared to the bond energy of non-treated eggs (control). Figure 5.4 presents the bond energy for non-treated eggs, where each bar represents one strip and each series represents one replicate i.e. one egg. As stated earlier, three strips from one single egg formed one replicate.

There was no significant difference for bond energy among the three replicates for non-treated eggs ($p > 0.01$), which also certifies the uniformity and correctness in measuring technique for bond energy. The mean bond energy (mean for all three replicates) for non-treated eggs was found to be 7.772 mJ.

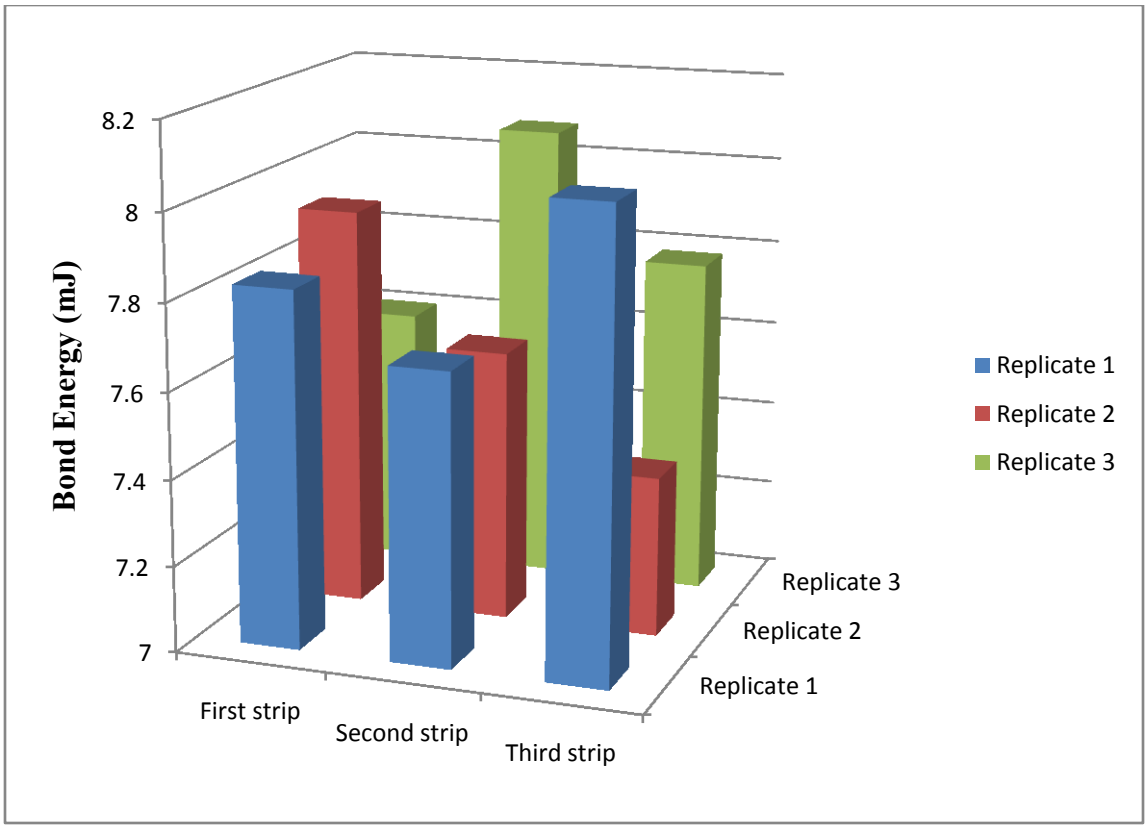


Figure 5.4 Bond Energy for Non-Treated Eggs (Control)

5.3.2 Hot Water Treatment

The hot water treatment was given by dipping the egg in hot water (75 ^{0}C- 80 ^{0}C) until it reached the desired temperature. Three temperatures 40 ^{0}C, 50 ^{0}C and 60 ^{0}C were studied. The purpose of giving hot water treatment to the eggs was to analyze the effect of application of heat on the bond energy between the eggshell and membrane. Figure 5.5 represents the bond energy after hot water treatment of eggs at 40 ^{0}C, 50 ^{0}C and 60 ^{0}C. Each bar represents the mean value of one replicate at that particular temperature i.e. the mean value of three eggshell strips.

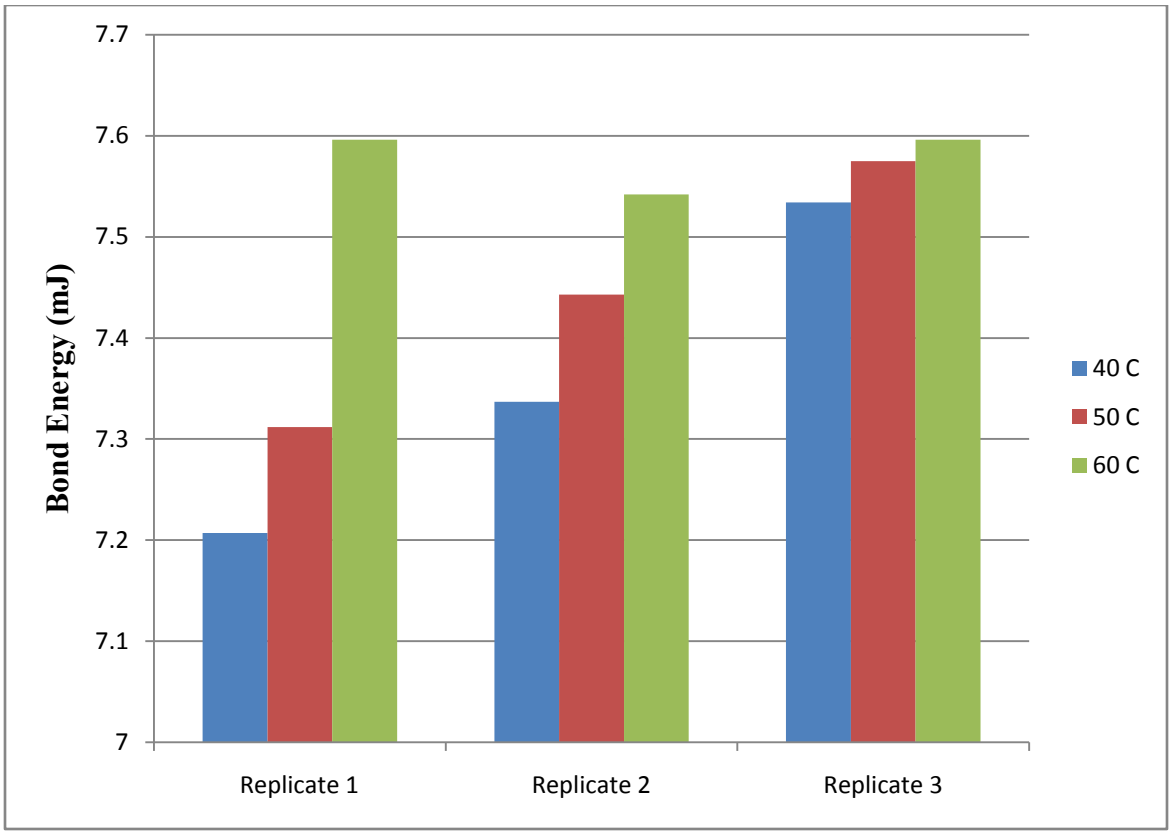
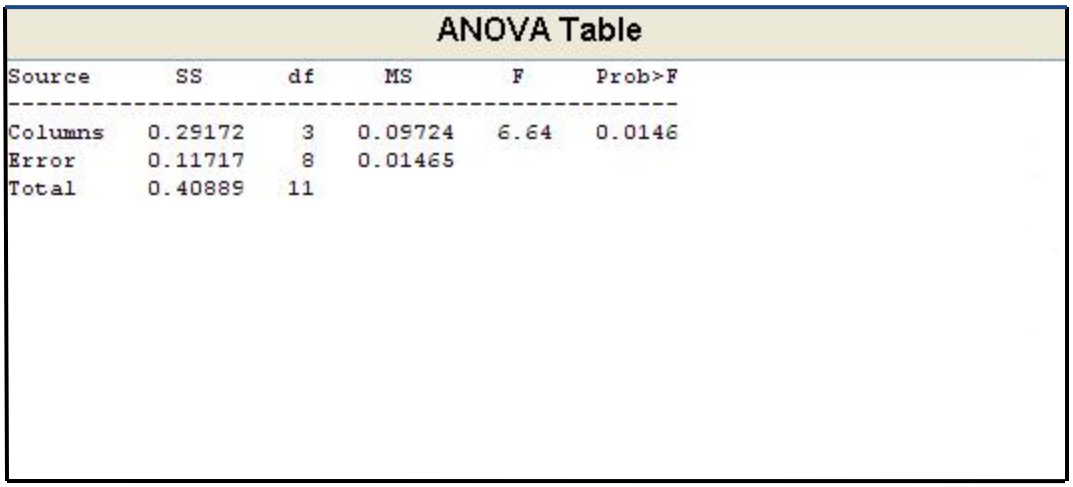


Figure 5.5 Bond Energy after Hot Water Treatment at different temperatures

There was no significant difference for bond energy among the three replicates for hot water treatment ($p > 0.01$). The mean bond energy for three replicates at 40 ^{0}C, 50 ^{0}C and 60 ^{0}C were 7.35 mJ, 7.443 mJ and 7.575 mJ respectively. Also, there was no significant difference for bond energy when compared with non-treated eggs ($p > 0.01$).

From the analysis of the results, it was clearly apparent that the mere application of heat had no effect on the bond energy between the eggshell and membrane and for the same reason hot water treatment was not extensively studied.



ANOVA Table

Source	SS	df	MS	F	Prob>F
Columns	0.29172	3	0.09724	6.64	0.0146
Error	0.11717	8	0.01465		
Total	0.40889	11			

Figure 5.6 ANOVA for Hot Water Treatment (where: Columns represents control and hot water treatment at various temperatures).

5.3.3 Microwave Treatment

The effect of microwaves on bond energy between the shell and membrane was extensively studied. Three factors were considered while studying the effect of the same. The first factor studied was soaking time. The eggs were dipped in tap water (room temperature) for the desired period (Table I), which was done so as to increase the moisture content, in view of the dependency of microwave heating on dipolar rotation (water exhibits strong permanent dipole moment). The other factors considered were temperature and power density. Three temperatures which were studied are 40 ^{0}C, 50 ^{0}C and 60 ^{0}C at power densities of 1 W/g, 1.5 W/g and 2 W/g.

In the following sections, each microwave treatment depending upon the soaking time is separately discussed, with a comparative study among the microwave treatments and between the microwave and control discussed towards the end.

Microwave Treatment without Soaking (hereby referred to as MC0)

The eggs were given microwave treatment without being soaked in water. Figures 5.7 and 5.8 present the bond energy at different power levels and temperature respectively,

where each bar represents the mean of all the replicates at that particular temperature and power density respectively.

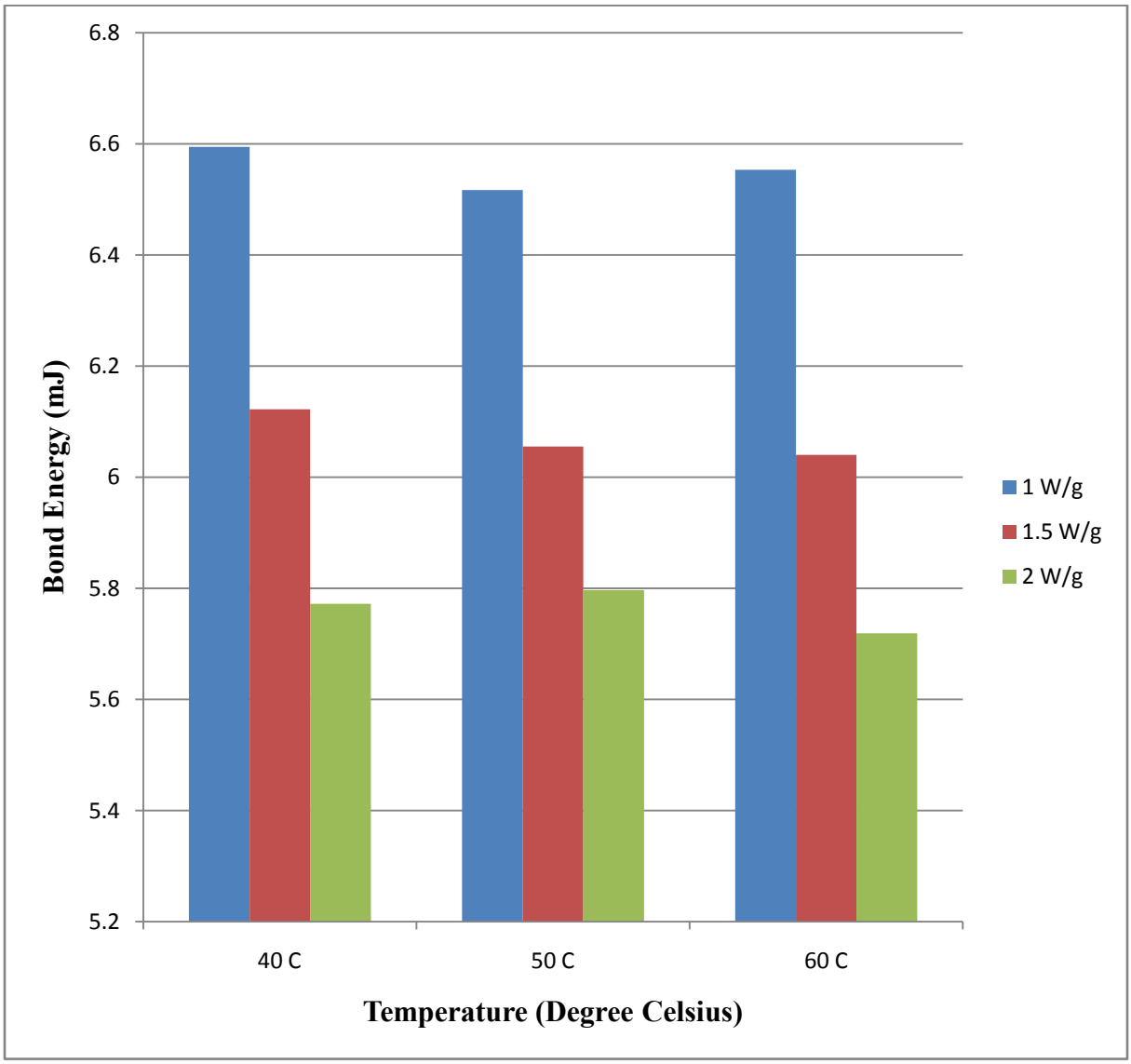


Figure 5.7 Bond Energy at different power densities for MC0

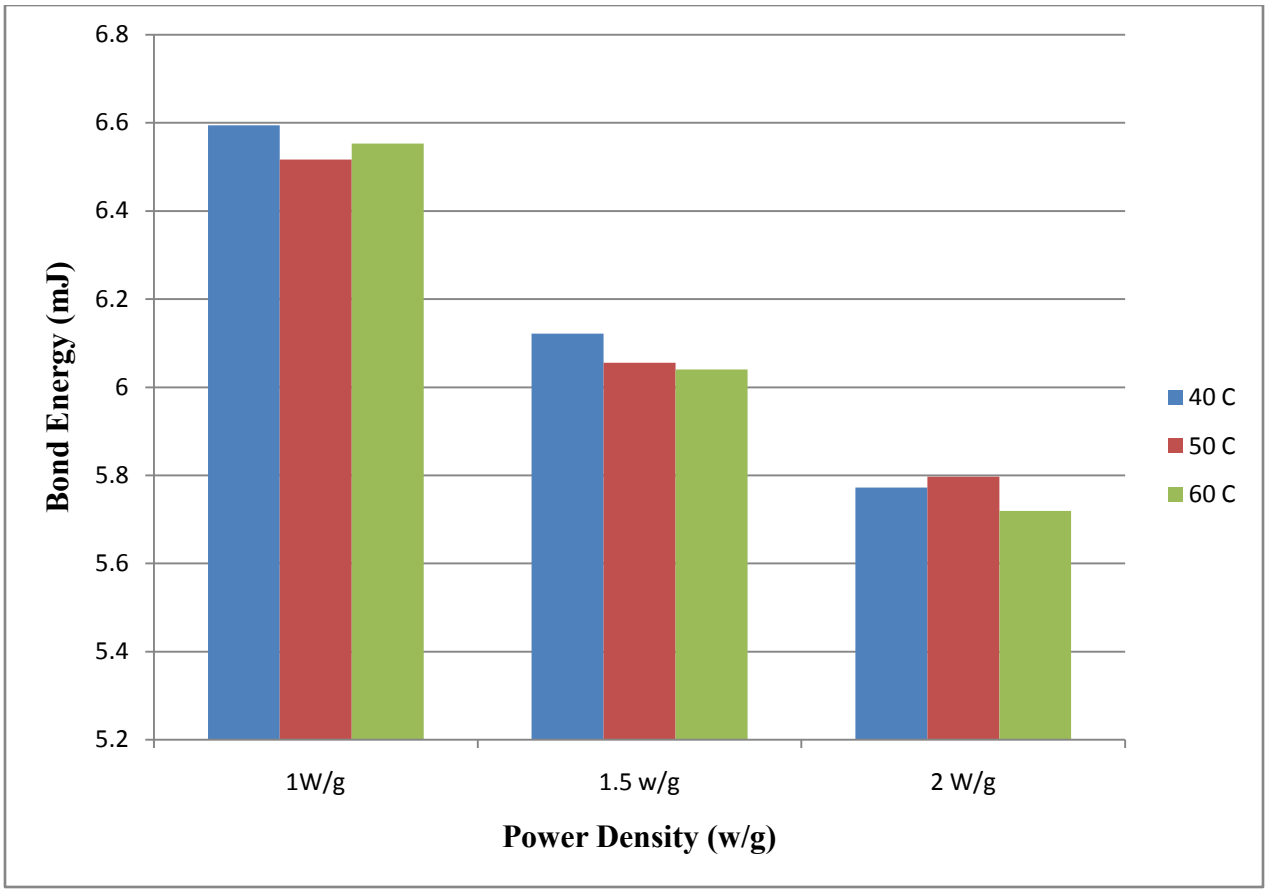


Figure 5.8 Bond Energy at different temperatures for MC0

From the figures it is clearly apparent that microwaves do have an effect on bond energy. As the power density increased, the bond energy between the shell and membrane decreased with the lowest bond energy being observed at 60 ^{0}C at a power density of 2 W/g. However, from the statistical analysis (ANOVA), the temperature nor did the interaction between the power density and temperature had any significant effect (p >0.01) on bond energy with only power density having a significant effect ($p < 0.01$) on bond energy (Fig 5.9). Multiple comparison test based on least significant difference performed on power density (significant factor) showed that all the power densities lied in different groups and were significantly different from each other (Fig 5.10).

ANOVA Table - MW Treatment only
(Rows represent Temperatures(degC) - 40, 50 & 60)
(Columns represent power density (W/g)- 1.0,1.5 & 2.0)

Source	SS	df	MS	F	Prob>F
Columns	2.86973	2	1.43486	360.92	0*
Rows	0.01622	2	0.00811	2.04	0.159
Interaction	0.01377	4	0.00344	0.87	0.5032
Error	0.07156	18	0.00398		
Total	2.97128	26			

Figure 5.9 ANOVA for MC0 (* P values less than 0.01 were displayed as 0)

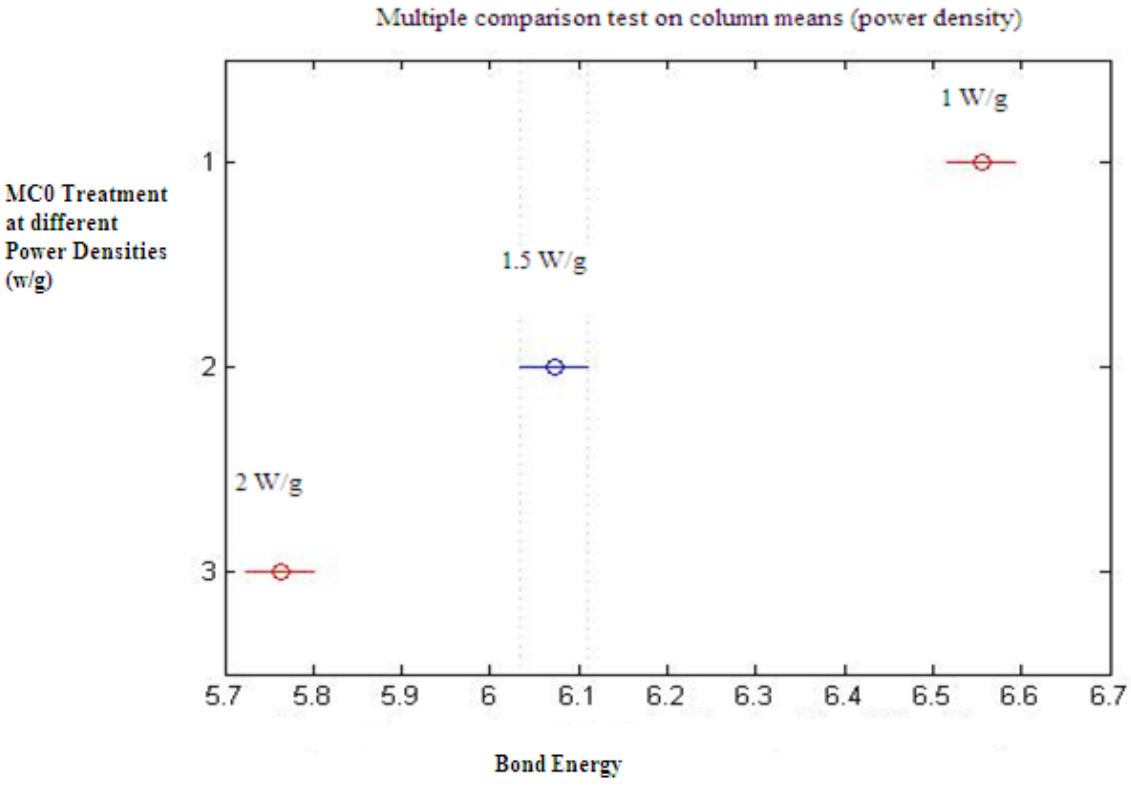


Figure 5.10 Multiple comparison tests for MC0

Microwave Treatment after soaking the eggs for 1 day (hereby referred to as MC1)

The eggs were microwave treated after being soaked in tap water for 24 hours. Soaking the eggs in water would increase the moisture content of eggshell and membranes making them respond better to microwaves (dipolar rotation). Figures 5.11 and 5.12 illustrate the bond energy for MC1 at different applied power densities and temperatures, where each bar represents mean of all the replicates at that particular temperature and power density respectively.

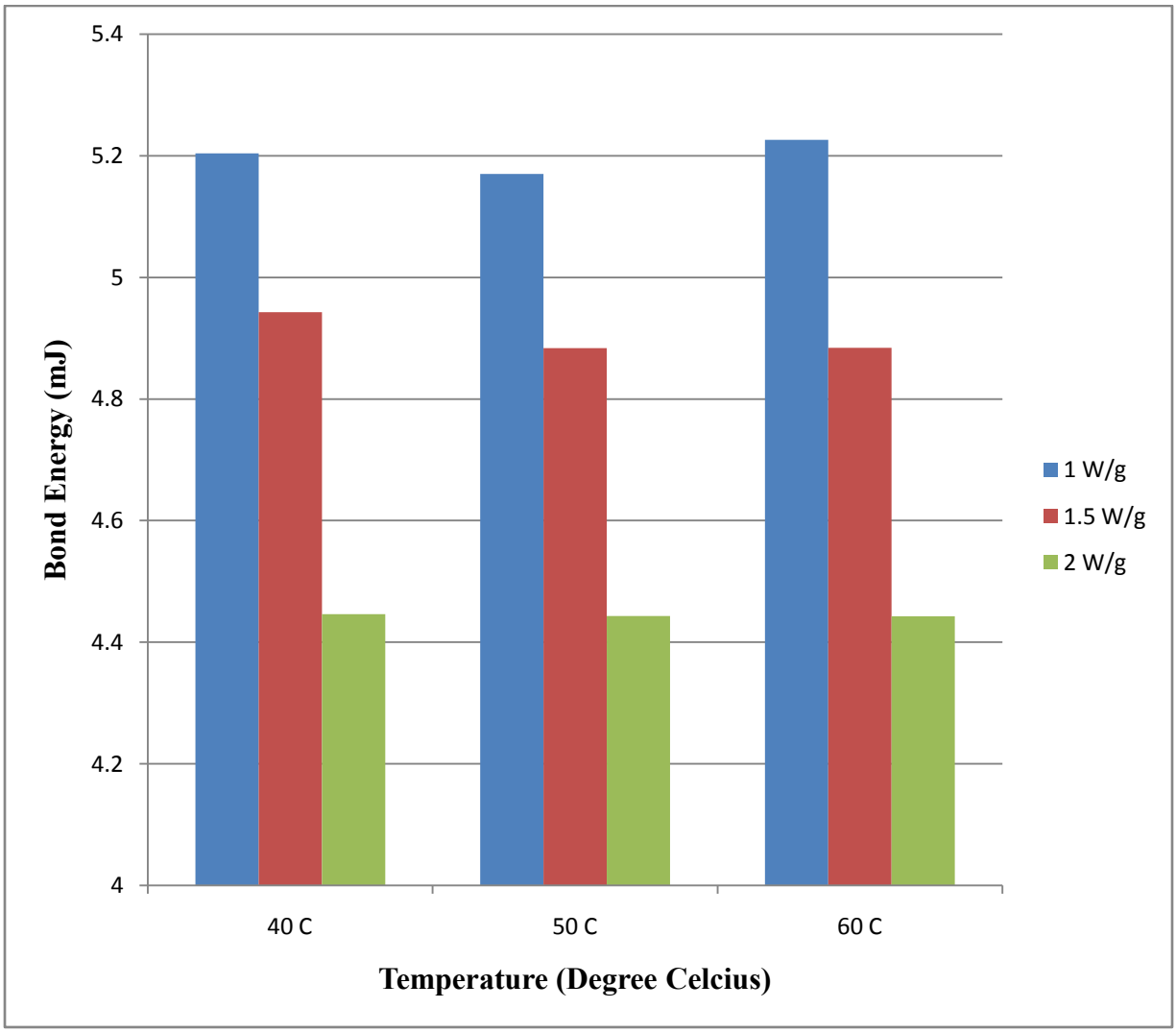


Figure 5.11 Bond Energy at different power densities for MC1

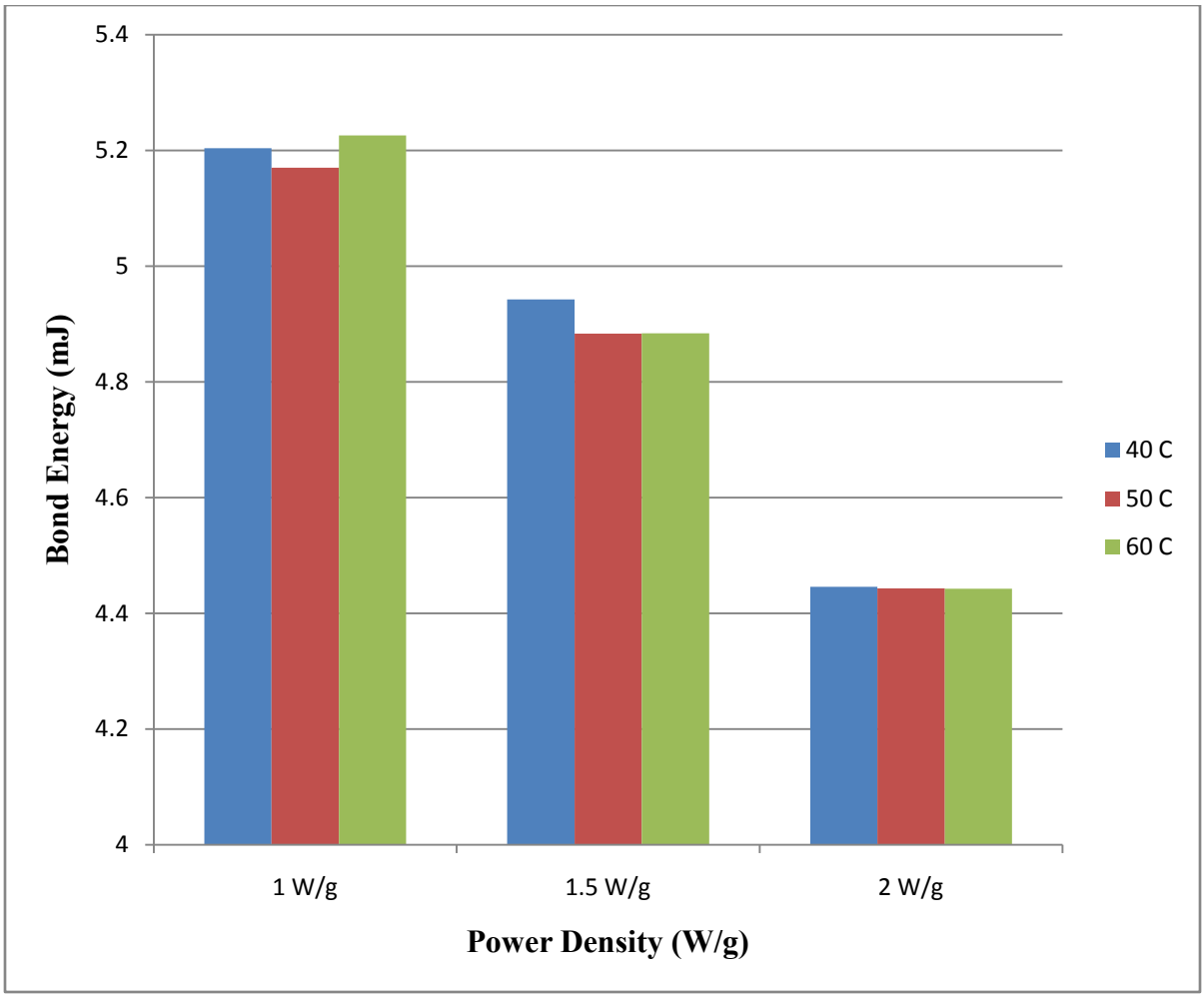


Figure 5.12 Bond Energy at different temperatures for MC1

Again, from looking at the figures it can be said that minimum bond energy for MC1 was obtained at power level of 2 W/g, with temperature having very little or no effect. As the power density increased the bond energy decreased. ANOVA performed (Fig 5.13) on collected data established that only power density ($p < 0.01$) had significant effect on bond energy, with neither temperatures nor the interactions having any significant effect ($p > 0.01$). Multiple comparison test based on least significant difference performed on power density (significant factor) demonstrated that all the power levels lied in different groups and were significantly different from each other (Fig 5.14).

ANOVA Table - MW Treatment after 24Hrs Soaking
(Rows represent Temperatures (degC) - 40, 50 & 60)
(Columns represent Power density (W/g) - 1.0, 1.5 and 2.0)

Source	SS	df	MS	F	Prob>F
Columns	2.61241	2	1.30621	1319.45	0*
Rows	0.00458	2	0.00229	2.31	0.1274
Interaction	0.00707	4	0.00177	1.79	0.1755
Error	0.01782	18	0.00099		
Total	2.64189	26			

Figure 5.13 ANOVA for MC1 (* P values less than 0.01 were displayed as 0)

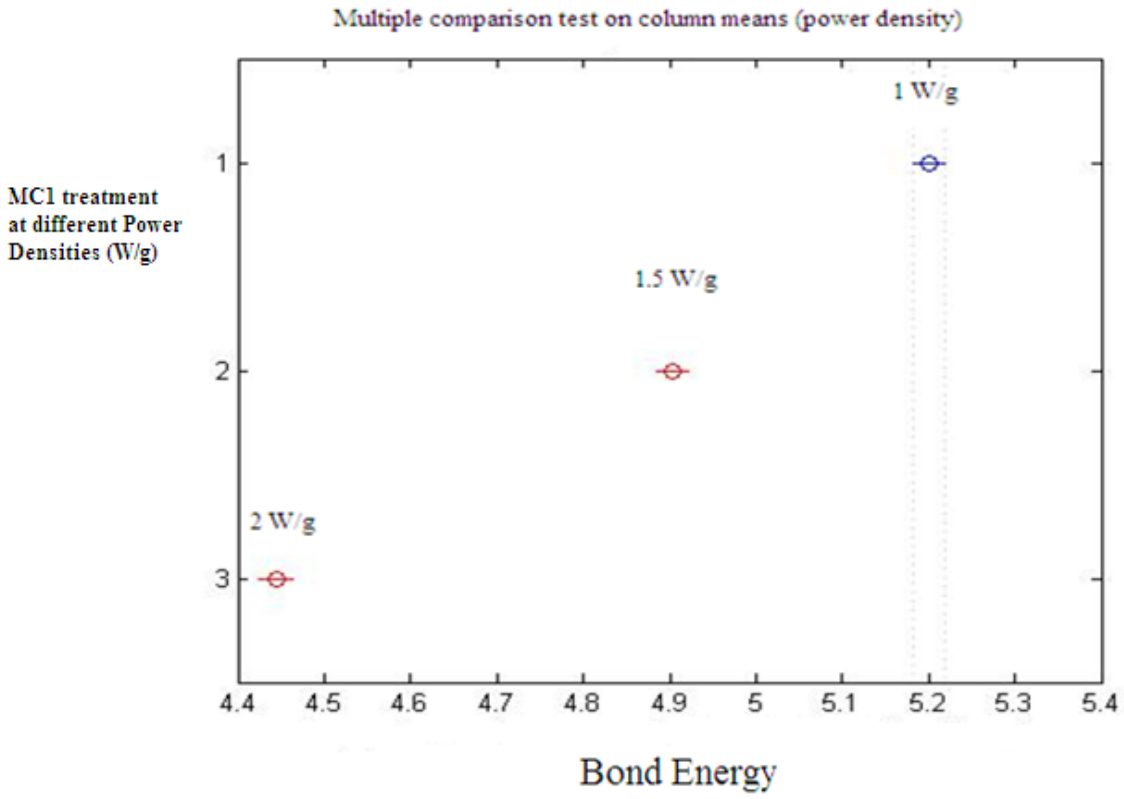


Figure 5.14 Multiple Comparison tests for MC1

Microwave Treatment after soaking the eggs for 2 days (hereby referred to as MC2)

The eggs were given microwave treatment after soaking them in tap water for 48 hours. Higher the moisture content of a commodity, the better it would respond to microwaves (due to permanent dipole moment of water). Figures 5.15 and 5.16 illustrates the bond energy for MC 2 at different applied power densities and temperatures, where each bar represents mean of all the replicates at that particular power density or temperature. The bond energy decreased with increase in power density with minimum bond energy of 3.43 mJ (mean value) observed at a temperature of 40 ^{0}C and power density 2 W/g. Also, the bond energy is 55.7 % lesser than that measured for un-treated eggs.

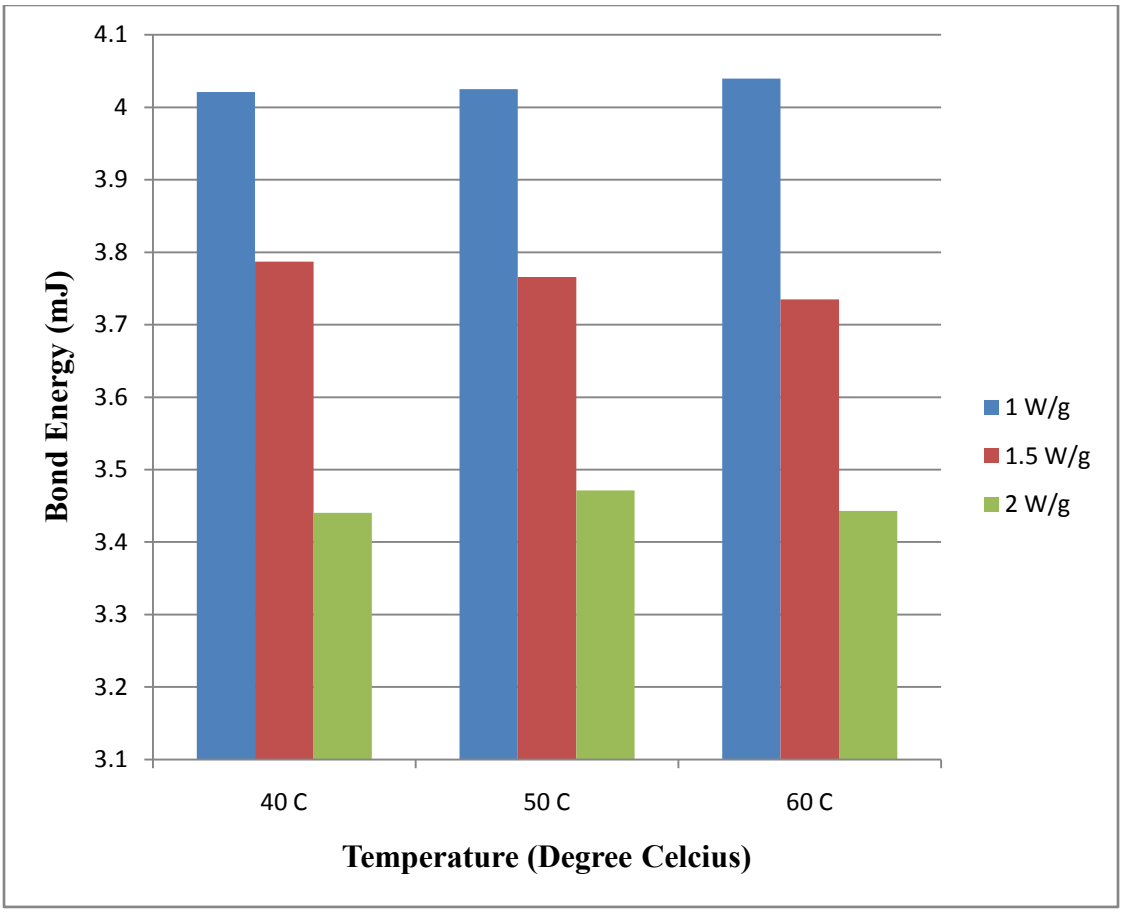


Figure 5.15 Bond Energy at different power densities for MC 2

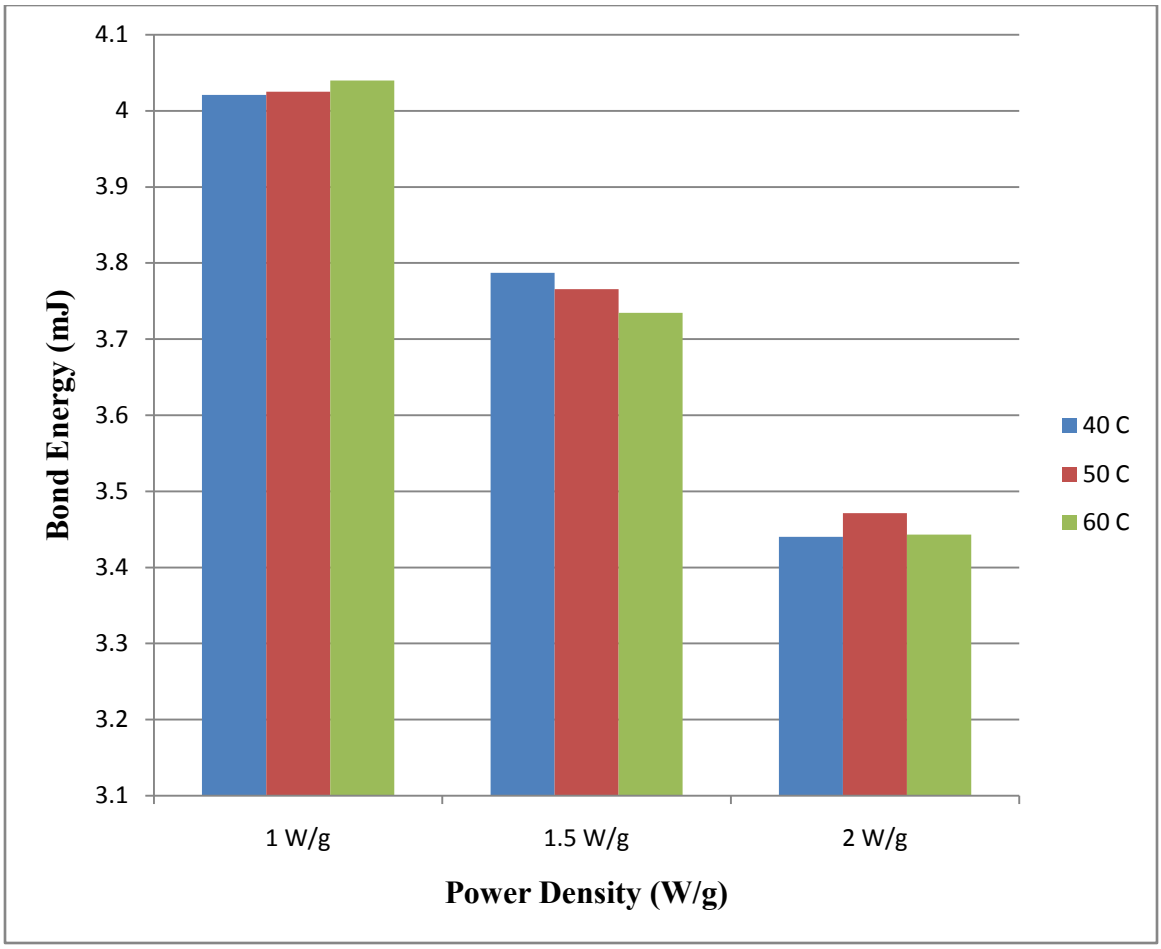


Figure 5.16 Bond Energy at different temperatures for MC 2

From the statistical analysis (Fig 5.17), only power density had a significant effect on bond energy ($p < 0.01$), with neither temperature nor the interactions having any significant effect on bond energy ($p > 0.01$). Also, multiple comparison test based on least significant difference performed on power density (significant factor) revealed that all the power densities were significantly different from each other (Fig 5.18).

ANOVA- Microwave Treatment after 48 hrs of soaking

(Rows represent Temperatures (degC) - 40, 50 & 60)
(Columns represent Power density (W/g) - 1.0, 1.5 and 2.0)

Source	SS	df	MS	F	Prob>F
Columns	1.50119	2	0.75059	1390.08	0*
Rows	0.00105	2	0.00052	0.97	0.398
Interaction	0.00546	4	0.00136	2.53	0.0767
Error	0.00972	18	0.00054		
Total	1.51741	26			

Figure 5.17 ANOVA for MC 2 (* P values less than 0.01 were displayed as 0)

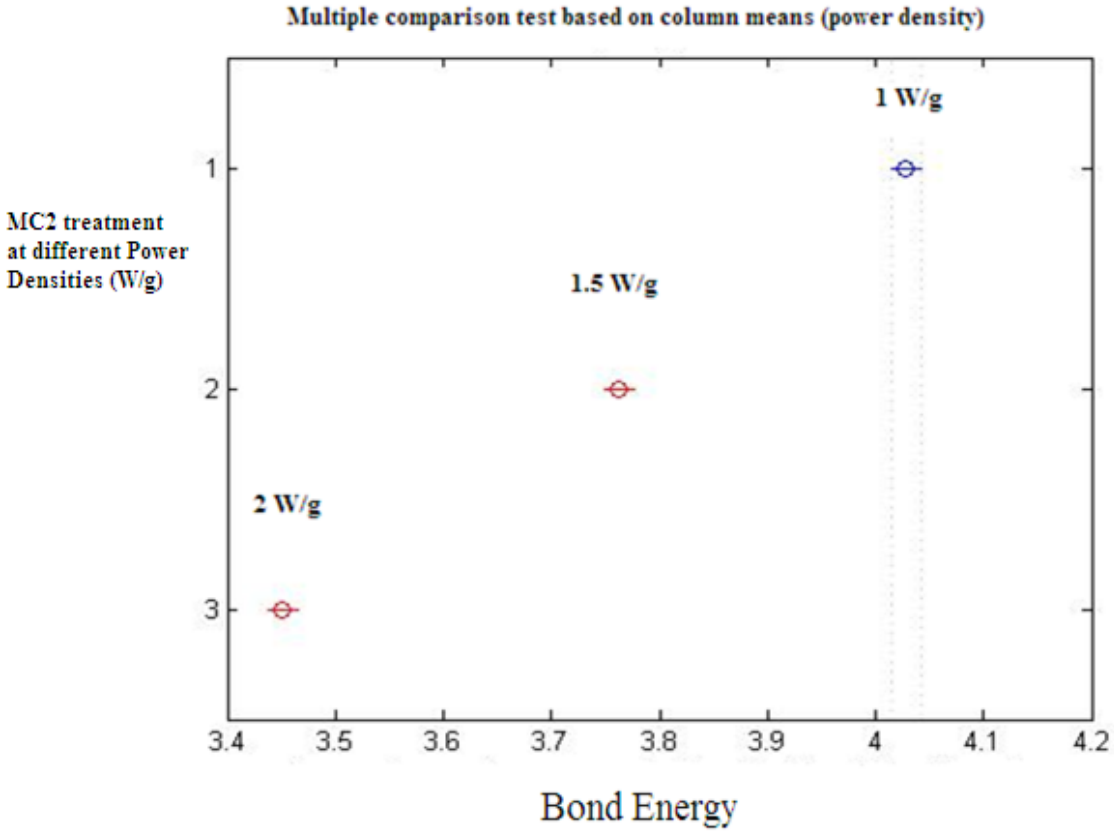


Figure 5.18 Multiple comparison tests for MC 2

5.3.4 Comparative Study of Microwave Treatments

From the analysis of results for all the microwave treatments (discussed in previous sections), it is clearly apparent that microwaves do have an effect on the bond energy between the eggshell and membrane, with microwave treatment of eggs reducing the required bond energy to separate eggshell and membrane. In all the microwave treatments MC 0, MC 1, MC 2, the bond energy decreased as the power density increased, with minimum bond energy being observed at 2 W/g for all the microwave treatments (Fig 5.19). Neither the temperature, nor the interactions between the temperature and power density had any significant effect on the reduction of bond energy.

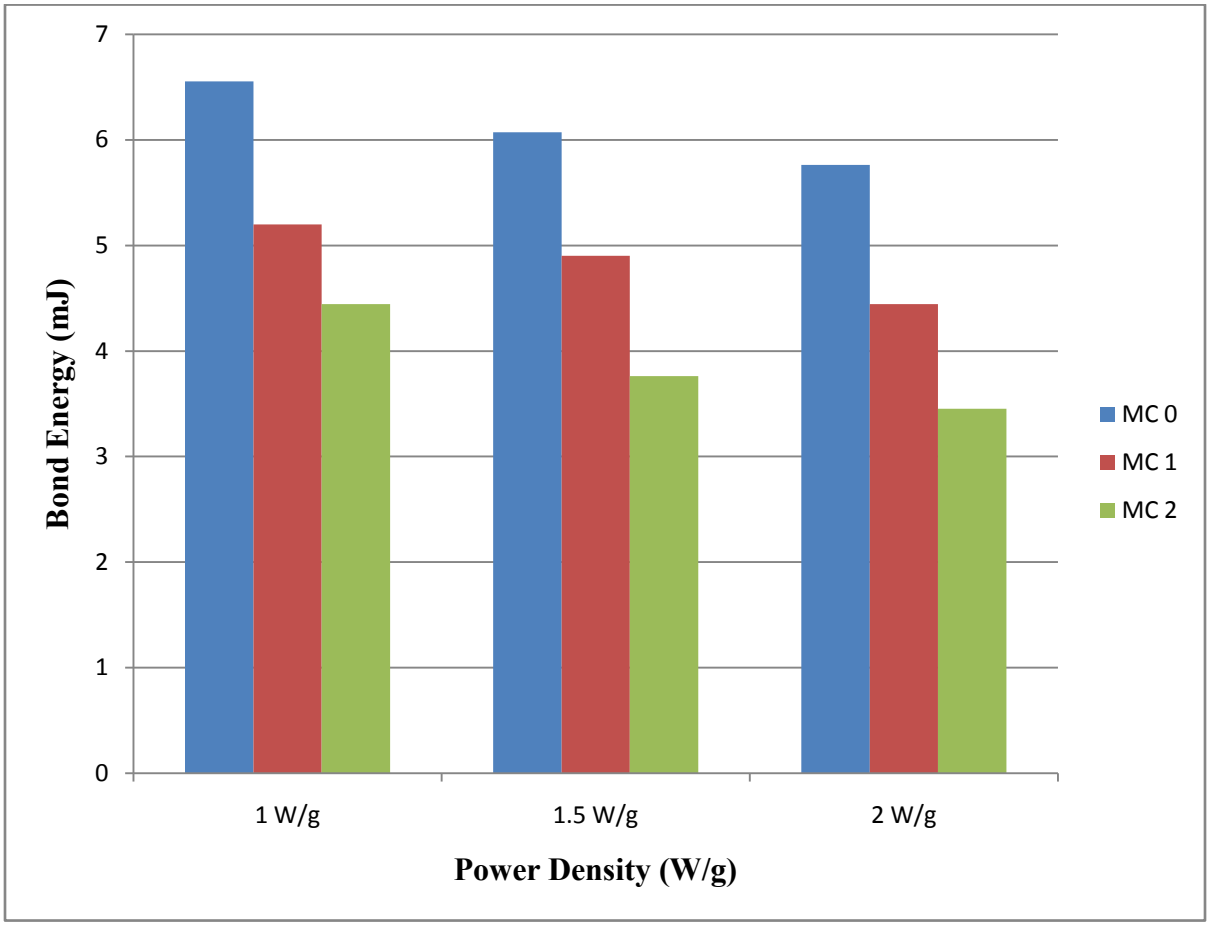


Figure 5.19 Bond Energy at different power densities for all microwave treatments

Analysis of variance performed for all microwave treatments (Fig 5.20) showed that power density ($p < 0.01$) , soaking time ($p < 0.01$) and the interactions between them ($p < 0.01$) played significant role affecting the bond energy, which can clearly be observed from figures 5.21 to 5.23 which represent the bond energy at a particular power density for all temperature and treatments. As the soaking time and power density increases the bond energy decreases which again might be due to the dependence of microwave heating on dipolar rotation. As the moisture content of food system increases, the dielectric constant and dielectric loss increases due to increased polarization (Orsat et al. 2005; Datta et al. 2005). Also, as observed for individual microwave treatments, neither temperature nor the interactions between density and temperature was a significant factor ($p > 0.01$). The minimum bond energy among all the microwave treatments was obtained for MC 2 at power density of 2 W/g and temperature of 40 ^{0}C.

Multiple comparison test based on least significant difference performed for all column means (power density) showed that all the microwave treatments lied in different groups and were all significantly different from one another (Fig 5.24).

3 Way Analysis of Variance for all Microwave treatments

Source	Sum Sq.	d.f.	Mean Sq.	F	Prob>F
Soak_time	76.7709	2	38.3855	20916.82	0*
PD	6.7769	2	3.3884	1846.41	0*
T	0.0115	2	0.0058	3.15	0.051
Soak_time*PD	0.2064	4	0.0516	28.12	0*
Soak_time*T	0.0103	4	0.0026	1.4	0.2451
PD*T	0.0221	4	0.0055	3.02	0.0256
Soak_time*PD*T	0.0042	8	0.0005	0.28	0.9689
Error	0.0991	54	0.0018		
Total	83.9015	80			

Figure 5.20 ANOVA for all microwave treatments (* P values less than 0.01 were displayed as 0)

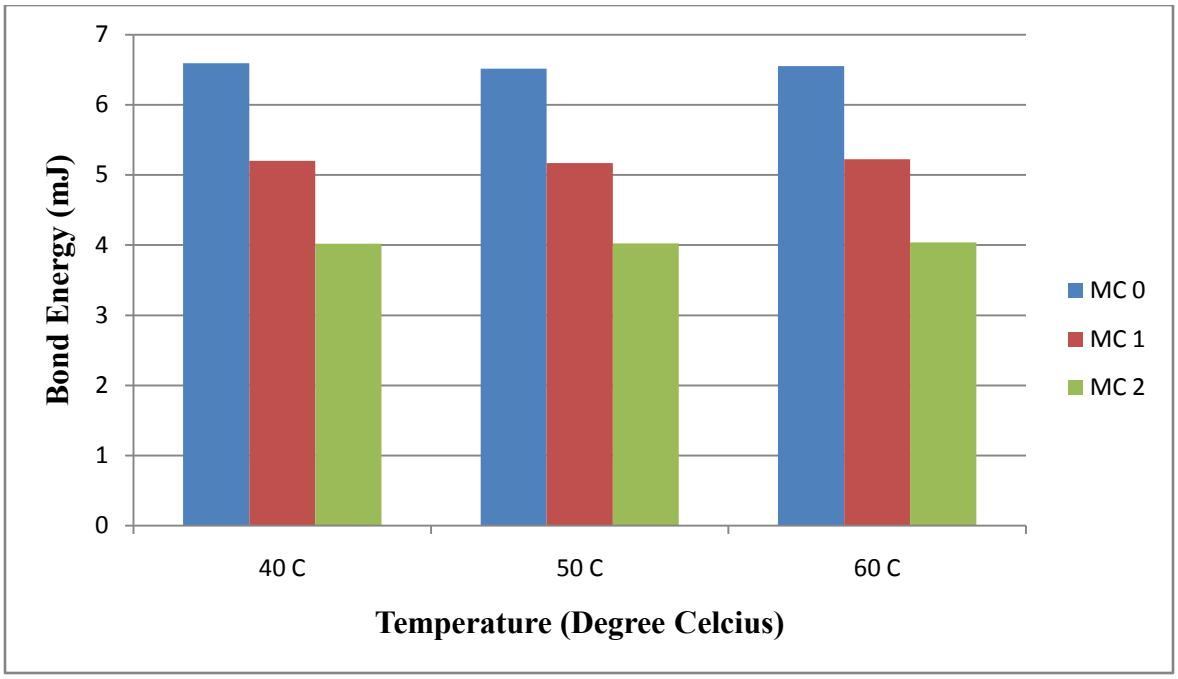


Figure 5.21 Bond Energy for all microwave treatments and temperatures at power density of 1 W/g

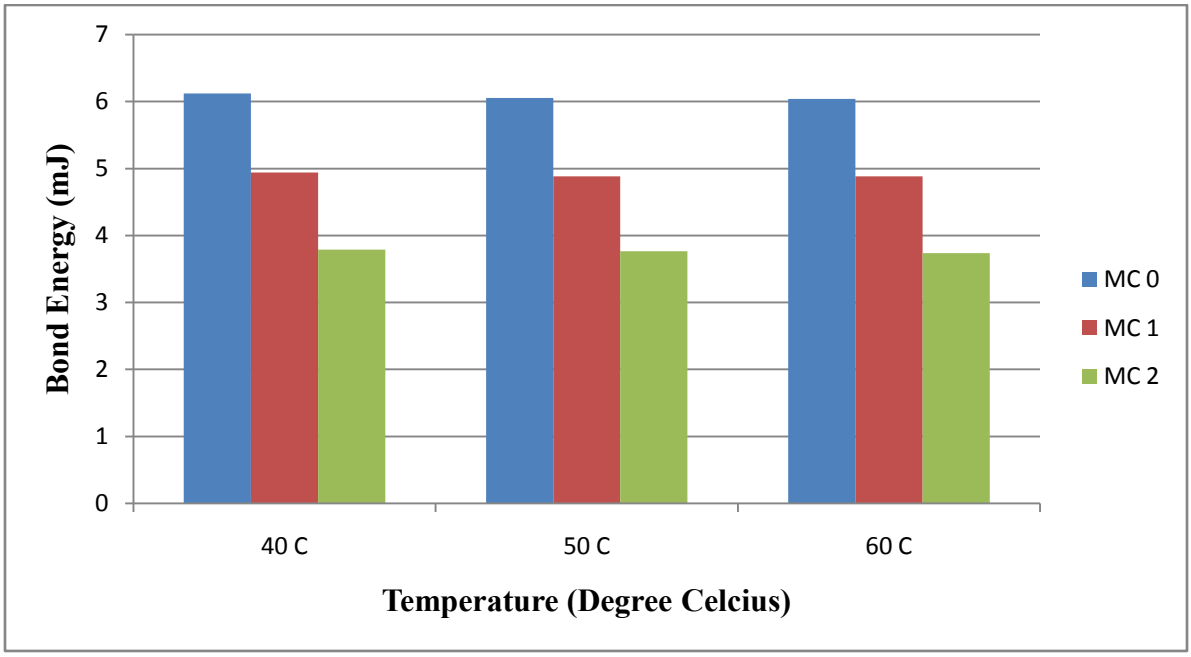


Figure 5.22 Bond Energy for all microwave treatments and temperatures at power density of 1.5 W/g

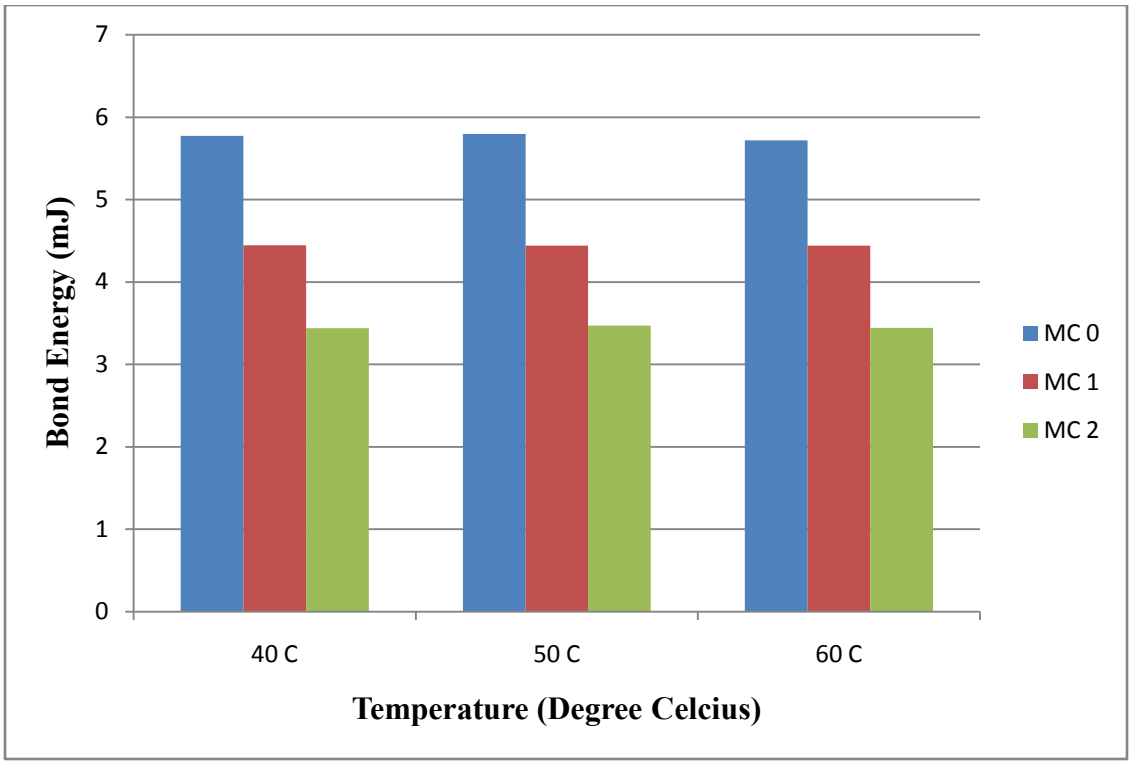


Figure 5.23 Bond Energy for all microwave treatments and temperatures at power density of 2 W/g

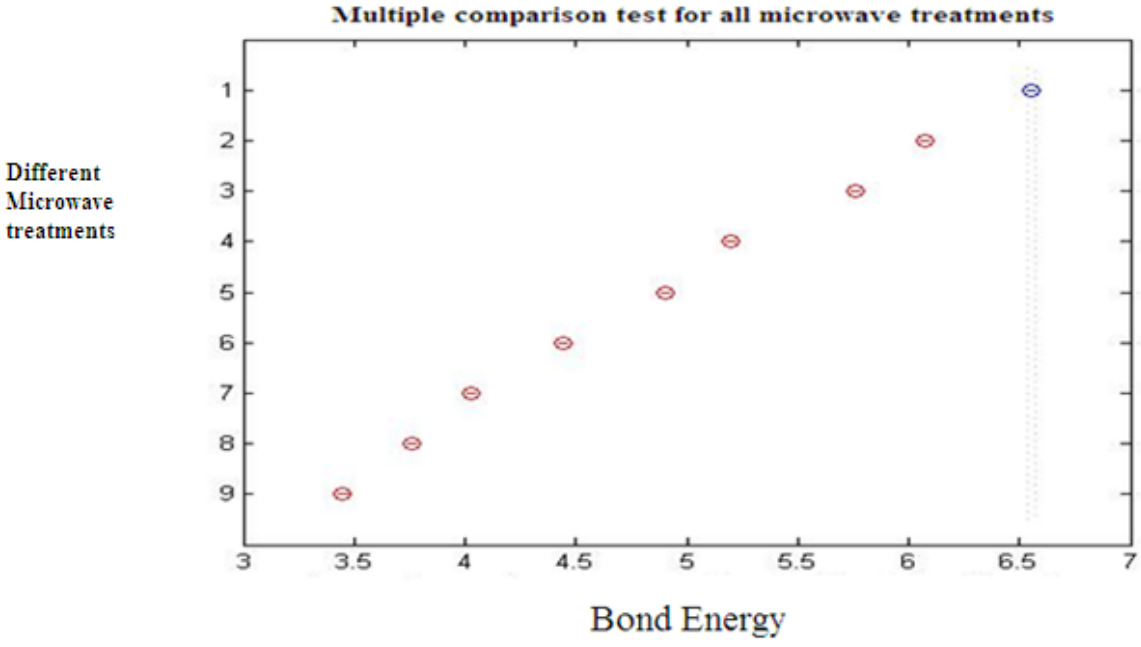


Figure 5.24 Multiple comparison tests for all microwave treatments

Bond energy at all microwave treatments appeared to be linearly related to soaking time and power density. As soaking time and power density increased the bond energy decreased. Multiple linear regressions based on linear programming approach was used to relate bond energy to soaking time and power density. Regression performed for the collected data yielded the following relationships:

$$\mathbf{BE = 7.162 - 1.19 \cdot S - 0.70 \cdot P} \qquad \mathbf{(R^2 = 0.9958)} \qquad \mathbf{(P < 0.01)}$$

Where: BE is the bond energy in **mJ** (for microwave treatment only)
S is the soaking time in **days**
P is the power density in **W/g**

The value for regression coefficient was very close to 1 indicating that the model had excellent predictability. Temperature was not included in the model as it did not have significant effect on bond energy for all microwave treatments.

The process was optimized to determine the minimum bond energy possible/required to separate the eggshell and membrane. Optimization was performed depending upon the model developed. Following the present set of conditions and parameters considered, the minimum bond energy possible is 0.1343 mJ when the egg is soaked for 4.35 days and a power density of 2.5 W/g is applied. The bond energy in that case would be 98% lesser than that for un-treated eggs.

From the analysis of variance and multiple comparison tests performed for all microwave treatments, hot water and control (Fig 5.25), it was determined that microwave treatments were significantly different ($p < 0.01$) from hot water and control with each microwave treatment being significantly different from one another.

The effect of microwaves on reduction of bond energy between the eggshell and membrane can again be due to the factors suggested in the hypothesis i.e. the differential

heating between the eggshell and membrane due to the difference in the moisture content of the two.

ANOVA Table (Where: Column represents different treatments)

Source	SS	df	MS	F	Prob>F
Columns	85.6417	12	7.13681	1392.88	0*
Error	0.1332	26	0.00512		
Total	85.7749	38			

Figure 5.25 ANOVA for all treatments (* P values less than 0.01 were displayed as 0)

5.4 CONCLUSION

Microwave treatment of eggs significantly reduces the bond energy/bond strength between the eggshell and membrane. Not only is the process faster is terms of treatment time but also much cleaner with minimum losses. The mere application of heat during the hot water treatment had no effect on bond energy between the eggshell and membrane. Also, during microwave treatment, temperature had no significant effect on reduction of bond energy. Power density and soaking time played significant role in bond energy reduction. The efficient separation of eggshell and membrane would not only act as a source of revenue for egg processing industries but also have a significant impact on the environmental and disposal costs associated with waste eggshells.

The optimization of the process based on the model developed might reduce the bond energy between the eggshell and membrane by 98 % compared to the untreated eggs.

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CHAPTER 6

SUMMARY AND CONCLUSION

Eggshell and Membrane as such find very little use and value in food, pharmaceutical or any other processed industry and are largely disposed of as a waste, with disposal costs between $ 25,000 and $ 100,000 per year.

It is established that eggshell and membrane are a reserve of many bioactive compounds of high economic and monetary value, which can be extracted by efficient separation of eggshell and membrane. The extraction of the many bioactive compounds present in the egg membrane would not only benefit the egg processing industry by giving them a new source of revenue but also the cosmetic and pharmaceutical industry by reducing the processing cost significantly, making the product cheaper and hence affordable for a wider section of society.

The strong interaction of the calcium carbonate crystals with the organic matrix has made the separation of eggshell and membrane very difficult and hence limiting the value of the waste eggshells. Many methods have being tried in order to separate the eggshell and membrane but with minimal results. The recent inventions in this regard lead to the generation of moist shells and membranes which have to be dried before they can be put to use, which increases the production cost and also the losses. The difficulties in handling of the moist membrane pieces have to be kept in mind.

There had been very little work done in the use of microwaves for separation of eggshell and membrane. The study was carried out in order to develop an alternative method for separation of eggshell and membrane by using microwaves.

The study consisted of first measuring the dielectric properties of eggshell and membrane in order to analyze the suitability and response of eggshell and membrane to microwave

treatment. Also, the possibility of using dielectric properties as an alternative method to detect protein denaturation was investigated.

The measurement of dielectric properties of eggshell and membrane gave a better understanding of the behavior of eggshell and membrane in a microwave environment and suggested/certified that microwaves could be deployed for separation of eggshell and membrane. Also, the denaturation of proteins present in the egg membrane could be detected by the dielectric properties, suggesting the feasibility of the process as an alternative method for the detection of protein denaturation.

The effect of hot water and microwave treatment on separation of eggshell and membrane was investigated in the latter part of the study. For all microwave treatments three factors with three levels each were considered. Microwave treatment greatly assisted the separation of eggshell and membrane. Not only was the process faster but also much cleaner with minimum losses. The mere application of heat during hot water treatment had no effect on the separation of eggshell and membrane. Also, for microwave treatments, neither the temperature nor the interactions between the temperature and power density had any effect on separation of eggshell and membrane with only power density and soaking time being the significant factors.

The effect of microwaves on separation of eggshell and membrane can be further investigated by increasing the soaking time and power density. Also, changes in the quality of egg membrane if any, due to the application of microwave treatment needs to be investigated. The values of dielectric constant and loss of eggshell and membrane were observed to be highest in the frequency range 926 MHz – 1520 MHz. The effect of microwave treatment on separation of eggshell and membrane in this particular range can be further investigated.

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