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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. This study explores the possibility of separating the membrane from eggshell by the use of microwaves. Given that a material's response to electromagnetic radiation depends upon its dielectric properties, it was first necessary to determine these properties in eggshell and membrane over the range of 200 MHz to 20 GHz, at temperatures ranging from of 25 ^{0}C to 100 ^{0}C. The effectiveness of hot water and microwave treatments in reducing the total bond energy (mJ) to overcome in separating chicken egg membrane from its shell was then investigated. Microwave-treated eggs showed significantly lower ($p \leq 0.01$) bond energy than non-treated or hot-water-treated eggs. While increasing power density and soaking time both significantly ($p \leq 0.01$) reduced bond energy of microwave-treated eggs, neither treatment temperature nor the interaction between temperature and power density had a significant effect. A model for calculating the bond energy as a function of power density and soaking time is also presented.

Keywords: Dielectric properties, microwave, eggshell, egg membrane

1. INTRODUCTION

The recent and growing interest in separating membranes from eggshells is reflected by the growing number of patents describing/developing effective methods for their separation.

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 waste. However, there has been an exponential growth in the processed egg industry, and nowadays, 30% of eggs produced in United States are destined for the processed egg industry. United States Department of Agriculture estimates for 1984 indicate that in manufacturing liquid and dry egg products, the processed egg industry consumed 25.6 million cases of eggs, while by 1997 this consumption had risen to about 50 million cases of eggs. This produced over 109 Gg of unprocessed egg shell waste, with disposal costs of between \$25,000 and \$100,000 yr^{-1} (MacNeil 2001, 2006).

Keeping in mind such high disposal costs and their continued increase as a result of higher landfill taxes and increasing environmental concerns, one needs to find an alternative method by which to transform waste eggshells into a value added commodity, thus providing financial benefits to a competitive processed egg industry (MacNeil 2001, 2006).

There are a number of uses for eggshell and membrane when separated, but few when they remain attached. The eggshell and membrane represent a reserve of many bioactive components which can be used once an effective separation of the eggshell and membrane has occurred (MacNeil 2001).

The presence of collagen in eggshell membrane has drawn attention given its high economic and monetary value. Indeed, collagen (particularly types I, V and X) constitutes 10% of the total protein content of the egg membrane (MacNeil 2001, 2006; Wong et al. 1984; Arias et al. 1990). The 109 Gg of eggshell waste produced by the processed egg industry in 1997 would have yielded 55 Gg of eggshell and 9 Gg of membrane, capable of yielding 450 kg of collagen, presently priced at \$1000 g^{-1}(MacNeil 2001, 2006). Hyaluronic acid, another high value substance, is also naturally present in eggshell membrane, representing from 0.5% to 10% of the membrane (Long et al. 2005).

U.S. 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, and 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 tissues lost or damaged due to cuts, injuries, burns and ulcerations (Neuhauser 1965).

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

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

In this particular study microwave treatment was used for separation of eggshell and membrane. Given that the membrane has higher moisture content than the eggshell, we hypothesized that; compared to the shell, it would absorb more energy from electro-magnetic waves, resulting in differential heating of the shell and membrane. This would, in turn, lead to the expansion of the membrane, weakening the physical interaction between the shell and the membrane, thereby assisting in the separation of the membrane from the shell.

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 these parameters 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 while the dielectric loss is related to the energy absorption and dissipation of the electromagnetic energy from the field. Hence, the dielectric properties of eggshell and membrane were first studied in order to assess the response of eggshell and membrane to microwaves.

The present study was performed 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. To investigate the possibility of employing microwave treatment for separation of membrane from eggshell.

2. MATERIALS AND METHODS

Commercially available chicken 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. All measurements were performed in triplicate.

PART I. Dielectric Properties Measurement

Egg Membrane Sample

Membrane and shell were carefully separated by manual peeling (after discarding the egg white and yolk). The samples consisted of both the inner and outer membrane. The membranes were washed thoroughly with distilled water to remove any egg white sticking to the surface of the membrane. The membranes were then placed between two absorbent papers for 15 minutes to remove surface water and were allowed to air dry at room temperature for 15 minutes. The membranes of roughly 10 eggs were then carefully folded, to avoid trapping air between layers of membrane, into 20 mm diameter hemispheres, each representing one replicate. The samples were placed in air tight vials until use. All the measurements were performed on the same day of peeling.

Equipment and Procedure

Dielectric properties of the samples were measured using an open ended coaxial probe (Agilent 8722 ES s-parameter Network Analyzer, Santa Clara, USA) equipped with a high temperature probe (model 85070B). 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 was placed in a small cylindrical borosilicate glass test tube (20 mm diameter, 50 mm tall and 2 mm thick). The high temperature probe was mounted on the stand with the flange of the probe facing downwards and along with the cable, fixed so as not to move during sample measurement. Before each experiment, the flange and the aperture of the probe were cleaned with ethanol and then wiped with a paper towel. A 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 samples were heated using a metallic sample holder (Fisher scientific, USA). The dielectric properties were measured at 301 different frequencies from 200 MHz to 20 GHz, over a temperature range of 25 ^{0}C to 100 ^{0}C. The samples were heated at a rate of 0.5 ^{0}C/min. The sample temperature 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.

PART II. Microwave Separation of Eggshell and membrane

A total of 39 commercially available eggs were used in this part of the study and two heating systems (dipping in hot water *vs.* microwave treatment) were tested with respect to their effectiveness in separating the membrane from the eggshell.

Hot Water Treatment

For the hot water treatment, the eggs were dipped in hot water (75°C - 80°C) until they reached the desired internal temperature (40°C, 50°C or 60°C), as monitored by a K-type thermocouple, inserted through a small opening into the air cell at the large end of the egg. The eggs, arrayed large end up on a plastic stand, were placed inside a 1 L beaker which was then filled with tap water in such a manner that roughly 0.75 cm of the eggs remained above water level, so as to prevent the water from entering the egg through 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.

Microwave Treatment

The effectiveness of the microwave treatment in separating the membrane from the eggshell was investigated for the factorial combination of three treatment factors at three levels each (Table I). Each treatment combination was replicated three times.

Table I. Experimental Design for Microwave Treatment

	Factors	Levels
1.	Soaking Time (hours)	a) 0 b) 24 c) 48
2.	Temperature (^{0}C)	a) 40 b) 50 C) 60
3.	Power Density (W/g)	a) 1 b) 1.5 c) 2

The soaking treatment (0, 1 or 2 days) was done so as to increase the moisture content, in view of the dependency of microwave heating on dipolar rotation (water exhibits a strong permanent dipole moment).The microwave treatment was imposed 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 exposing it to microwave energy until it reached the desired air cell temperature, as measured by the same type of K-type thermocouple as employed in the hot water treatment experiments. The time taken to reach the desired temperature was determined in preliminary studies.

Shell Samples

Once the egg had received 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. Three 30 mm × 10 mm strips of eggshell were cut from each egg 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.

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 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. 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 effectiveness of a given treatment (hot water or microwave treatment) was judged in terms of the reduction in bond strength between the shell and membrane [hereafter termed **bond energy**, *i.e.*, the total energy (mJ) required to separate the membrane from the eggshell] after the particular treatment was imposed.

Control

For all statistical analyses the bond energy after a particular treatment was compared to that of non-treated (control) eggs. The bond energy of control eggs was measured in the same manner as that of treated eggs.

Data Analysis

MATLAB 7.8 was used for all data analysis. ANOVA was performed for all the treatments. A multiple comparison test based on the least significant difference (LSD) was performed for factors found to be significant in the ANOVA analysis. The process was optimized based on a mathematical relationship developed for membrane bond energy as a function of soaking time and power density.

3. RESULTS AND DISCUSSION

PART I. Dielectric Properties

3.1 Egg Membrane

The dielectric constant and dielectric loss of the eggshell membrane decreased as the temperature rose from 25°C to 70°C, increased sharply from 70°C to 75°C, then decreased sharply from 75°C to 80°C . The dielectric constant continued to decrease from 80°C to 95°C, but a slight increase was observed from 95°C to 100°C. In contrast, the dielectric loss remained mostly constant from 80°C to 100°C (Fig 1, 2).

The dielectric constant remained positive (positive value) for all temperatures and frequencies but at higher frequencies the dielectric loss value was negative for temperatures above 55°C. In case of frequencies above 15 GHz, a negative dielectric loss value was observed at 45°C, 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°C to 70°C could be due to the low moisture content and high ash content (Biova-ovacore, LLC, Ames, IA, USA) of the eggshell membrane. The constituents of the ash content are mostly salts and have been found to be negatively related to the dielectric constant, since the salts bind water, thereby decreasing their ability to orient themselves to changing electric field direction (Sipahioglu and Barringer 2003).

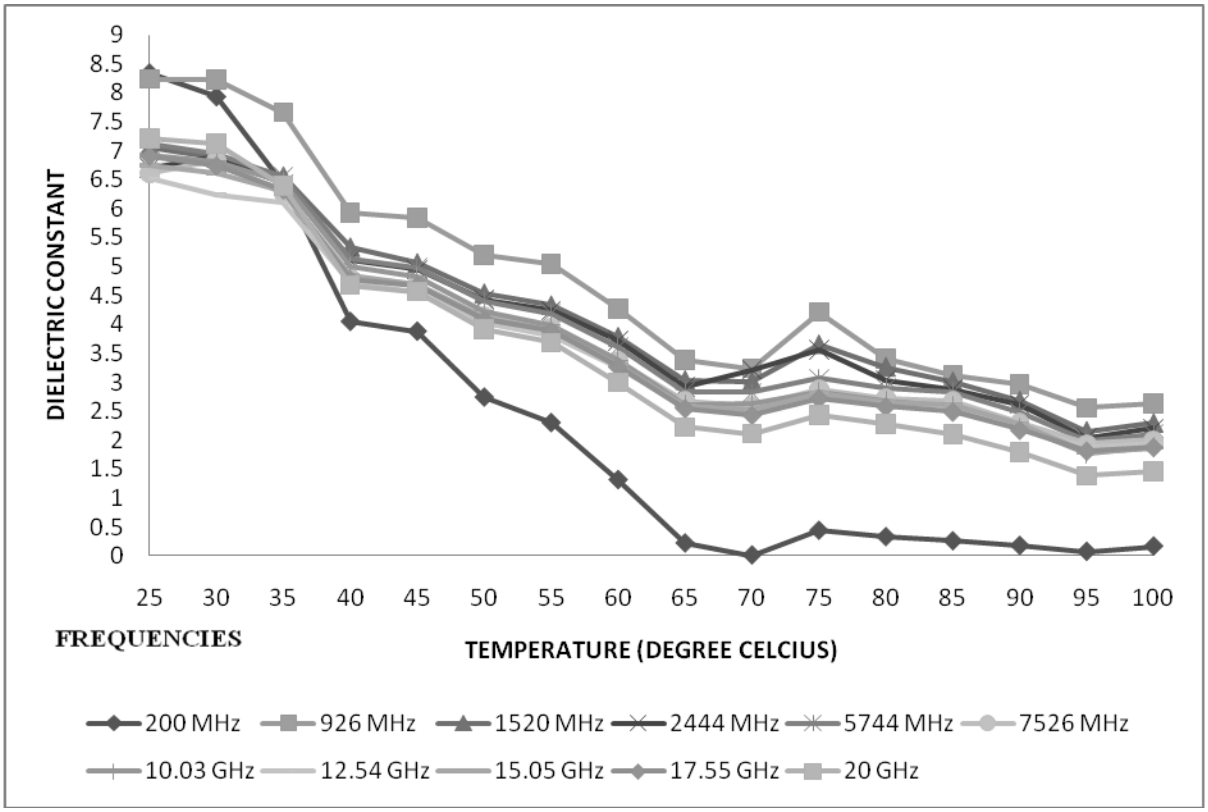

Figure 1 Dielectric Constant Vs Temperature for Eggshell Membrane at 11 frequencies

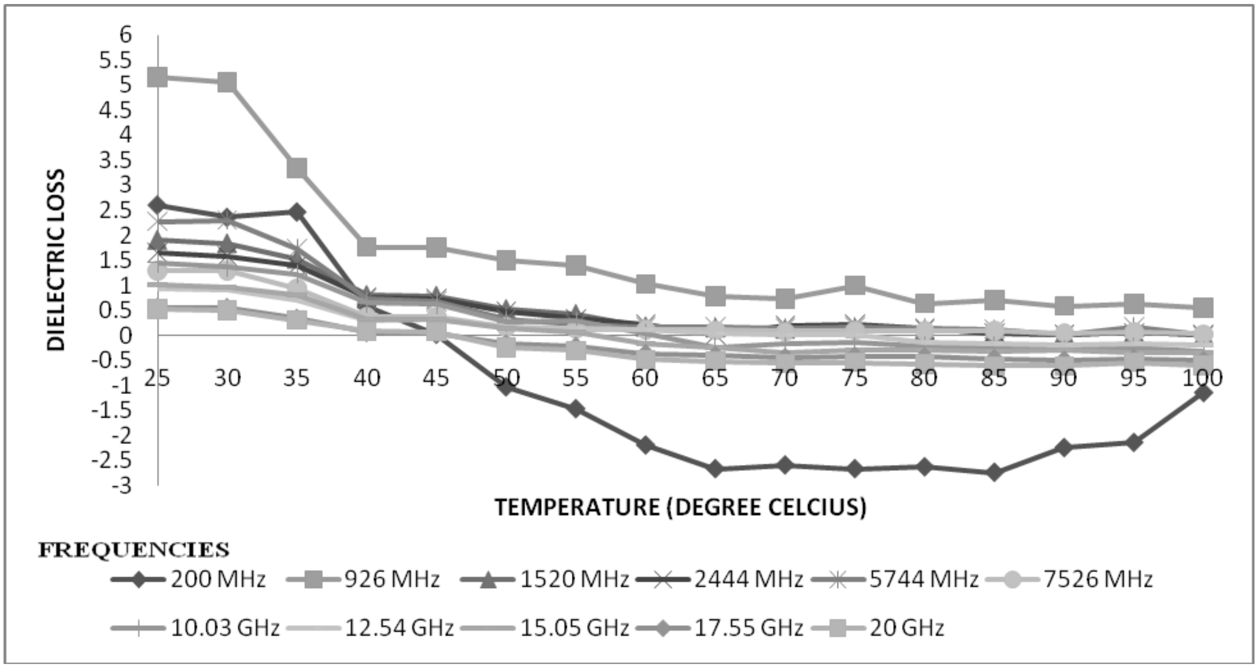

Figure 2 Dielectric Loss Vs Temperature for Eggshell Membrane for 11 Frequencies

Effect of Frequency (Egg Membrane)

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.

The dielectric constant remained positive for all frequencies whereas, 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: the dielectric loss decreased from 25°C to 65°C, remained mostly uniform from 65°C to 80°C, and then increased between 80°C and 100°C. These differences in trend from other frequencies might be due to the failure of the dipoles to align themselves to the changing electromagnetic field at lower frequencies. The dielectric constant and loss were greatest at 926 MHz for all temperatures except at 25°C (maximum at 200 MHz).The dielectric constant and loss increased for frequencies from 200 MHz to 926 MHz and decreased thereafter.

3.2 Eggshell

The dielectric constant and loss decreased with temperature (Figs 3, 4). The dielectric constant of the eggshell decreased steeply for frequencies above 10.03 GHz and between the temperatures of 85°C and 100°C. The dielectric constant remained positive for all frequencies and temperatures. In contrast, the dielectric loss value was negative for all frequencies 926 MHz, which might be attributable to the low moisture of the eggshells and failure of 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.

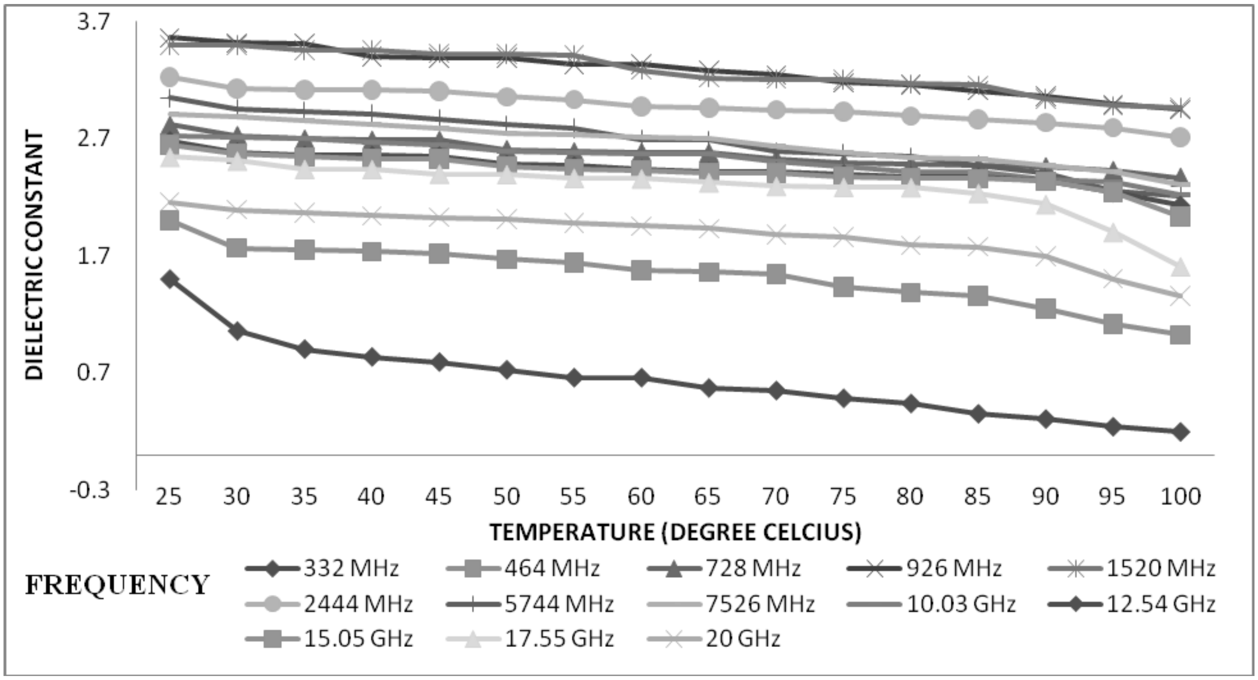

Figure 3.Dielectric Constant Vs Temperature for Eggshell at 13 Frequencies.

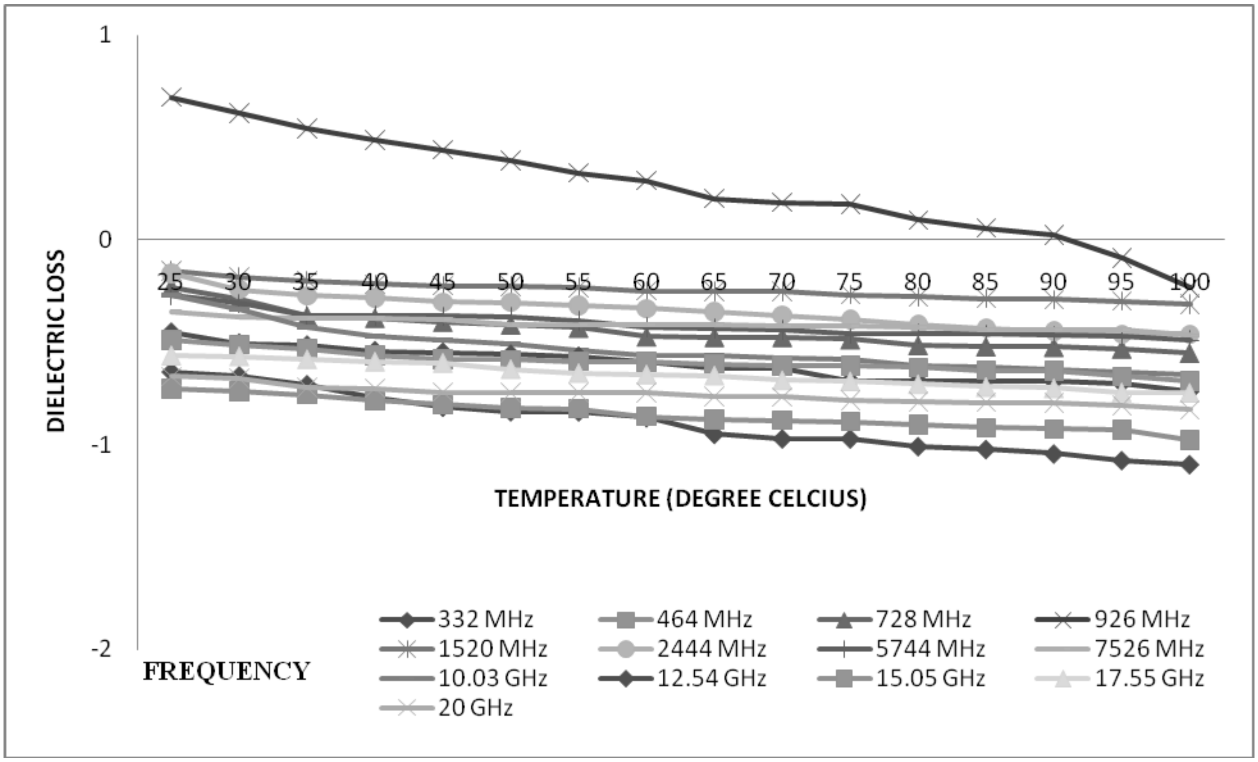

Figure 4 Dielectric Loss Vs Temperature for Eggshell at 13 Frequencies.

Effect of Frequency (Eggshell)

As observed for eggshell membrane, the dielectric constant and loss for eggshell increased for frequencies from 332 MHz to 926 MHz and decreased thereafter. 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 thereafter; however, the values for dielectric constant (*vs*. temperature) at 1520 MHz were comparable to those observed at 926 MHz.

PART II. Microwave Assisted Separation of Eggshell and Membrane

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 (due to higher moisture content) than eggshell and would thus behave/respond better to microwaves than eggshell. Also, as no abrupt changes in dielectric properties was observed up to 60°C; the maximum treatment temperature employed for limited to 60°C, in order to maintain the fitness of purpose for either constituents (eggshell and membrane).

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

3.4 Hot Water Treatment

To investigate the effect of the application of heat on the bond energy between the eggshell and membrane, a heat treatment was applied by dipping the egg in hot water (75°C- 80°C) until it reached the desired internal temperature of 40°C, 50°C or 60°C. While there appears to be a trend towards slightly greater bond energy as the treatment temperature rises, these differences were not significant ($p>0.01$). The overall mean bond energy for treatment temperatures of 40°C, 50°C and 60°C were 7.35 mJ, 7.443 mJ and 7.575 mJ respectively. Also, there was no significant difference ($p > 0.01$) in bond energy between hot water treated and non-treated eggs.

3.5 Microwave Treatments

3.5.1 Microwave Treatment without presoaking (MC_0)

Eggs received microwave treatment without being presoaked in water. As the power density increased, the bond energy between the shell and membrane decreased, with the lowest bond energy being observed at 60°C and a power density of 2 W/g. While power density had a significant effect ($p \leq 0.01$) on bond energy, neither temperature nor its interaction with power density had any significant effect ($p > 0.01$) on bond energy. A multiple comparison test based on the least significant difference showed all power densities levels to be significantly different among themselves.

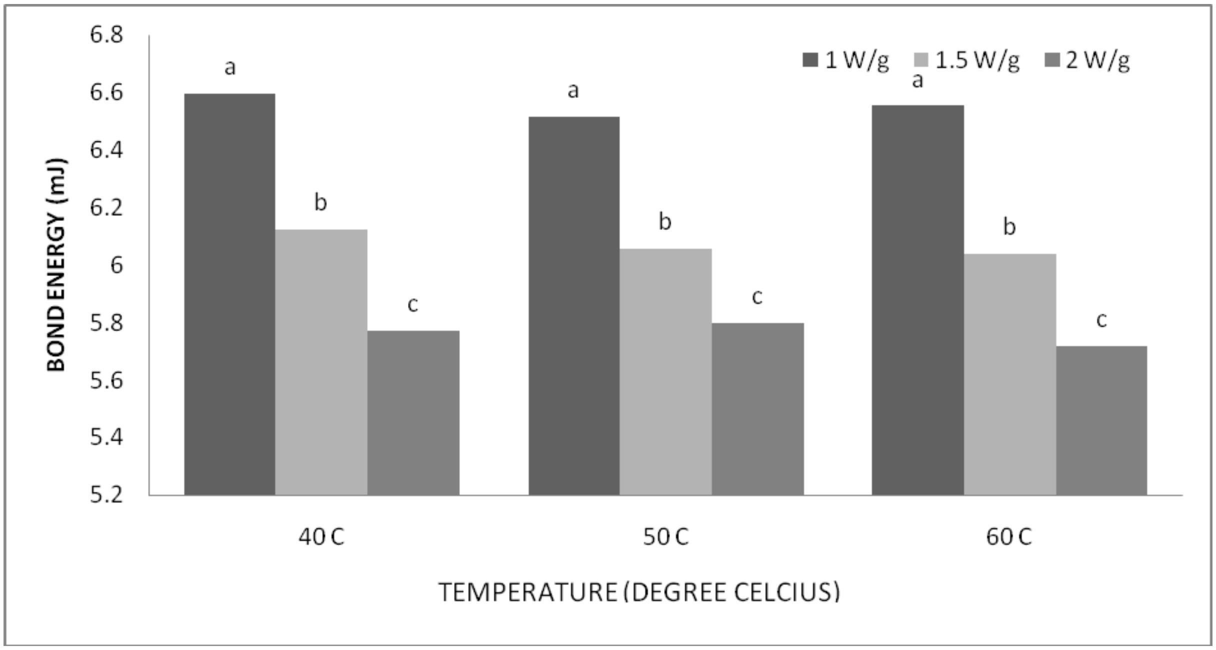

Figure 5 Bond Energy at different power densities for MC_0. For each temperature and power density combination the bar represents the mean bond energy (*n* = 9, three shell strips × three eggs). * Values with different letters are significantly different (p <0.01)

3.5.2 Microwave Treatment after soaking the eggs for 1 day (hereby referred to as MC_1)

Soaking eggs in water would increase the moisture content of the eggshell and membranes, making them respond more strongly to microwaves (dipolar rotation). The minimum bond energy for MC_1 was obtained at a power level of 2 W/g. As the power density increased the bond energy decreased. Only power density (p <0.01) had significant effect on bond energy, whereas neither temperature nor its interaction with power density had any significant effect (p >0.01). A multiple comparison test based on least significant difference performed on power density (significant factor) demonstrated that all the power levels were significantly different from one another.

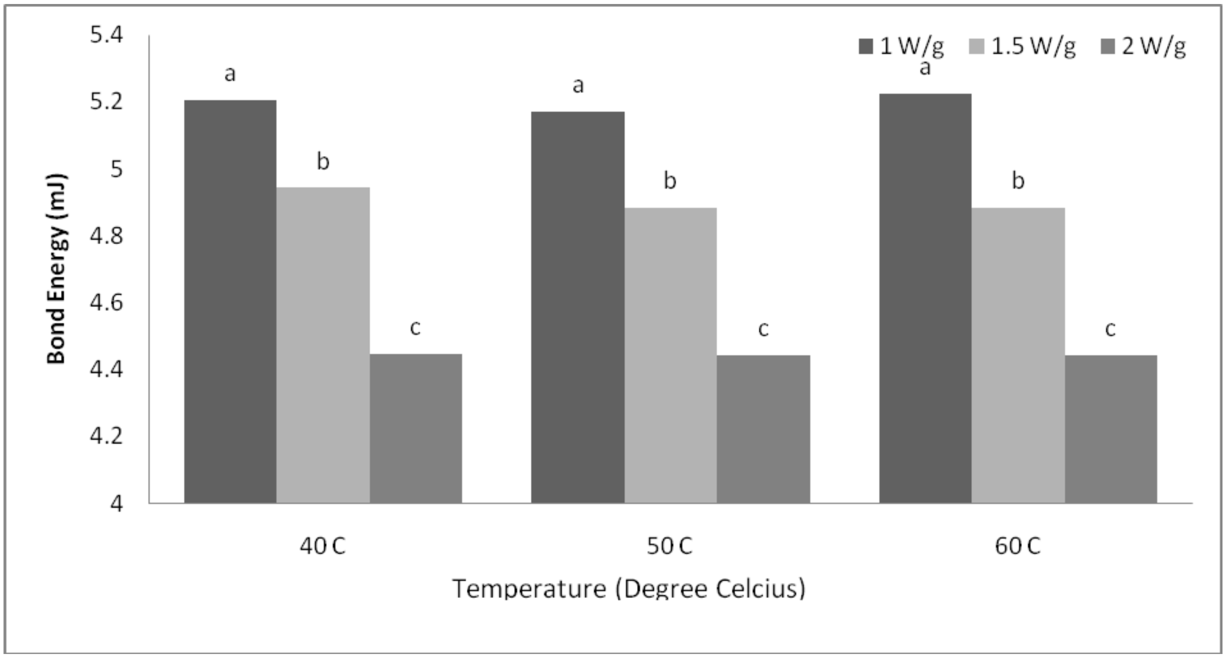

Figure 6 Bond Energy at different power densities for MC_1. For each temperature and power density combination the bar represents the mean bond energy ($n = 9$, three shell strips × three eggs) * Values with different letters are significantly different ($p < 0.01$)

3.5.3 Microwave Treatment after soaking the eggs for 2 days (hereby referred to as MC_2)

On the theory that a commodity's higher moisture content would confer a greater response to microwaves (due to permanent dipole moment of water), eggs were given the microwave treatment after presoaking them in tap water for 48 hours. The bond energy for MC_2 decreased with an increase in power density to a minimum bond energy of 3.43 mJ (mean value) observed at a temperature of 40°C and power density of 2 W/g. Also, the bond energy is 55.7 % lesser than that measured for un-treated eggs.

Only power density had a significant effect on bond energy ($p \leq 0.01$), whereas neither temperature nor the interactions having any significant effect on bond energy ($p > 0.01$). A multiple comparison test based on least significant difference performed on power density (significant factor) revealed that all the power densities were significantly different from one another.

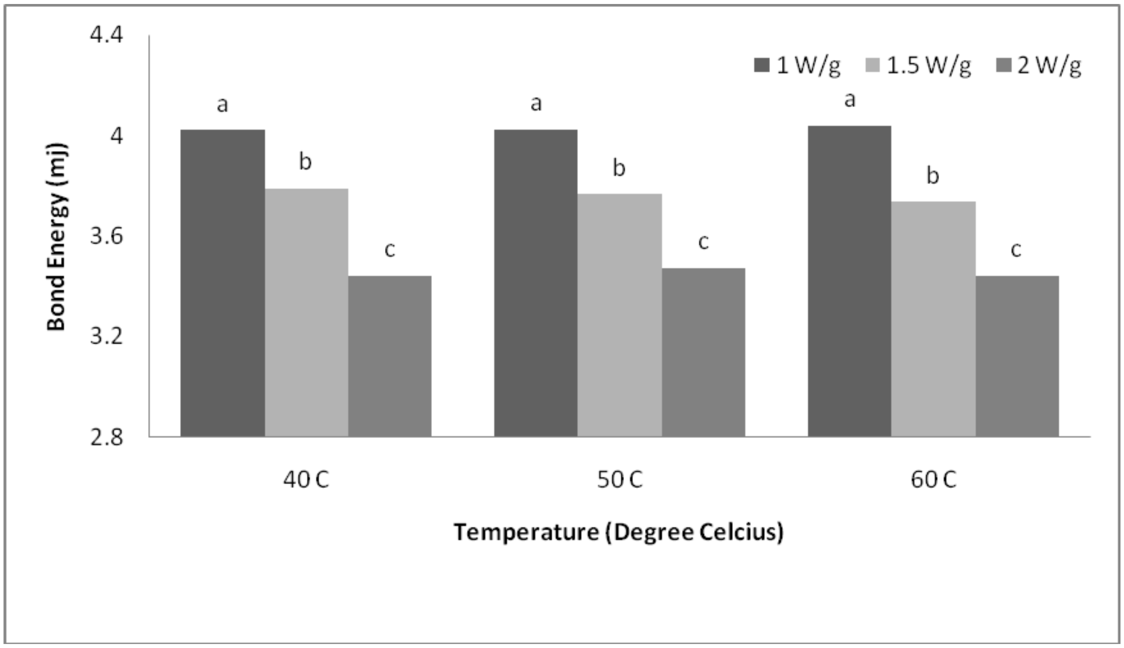

Figure 7 Bond Energy at different power densities for MC_2. For each temperature and power density combination the bar represents the mean bond energy ($n = 9$, three shell strips × three eggs) * Values with different letters are significantly different ($p < 0.01$)

3.5.4 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. Neither the temperature, nor the interactions between the temperature and power density had any significant effect on the reduction of bond energy.

Analysis of variance performed for all microwave treatments showed that power density ($p < 0.01$), soaking time ($p < 0.01$) and the interactions between them ($p < 0.01$) had a significant effect on bond energy. As the soaking time and power density increases the bond energy decreases which might again 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. Also, as observed for individual microwave treatments, neither temperature nor the interactions between density and temperature were significant ($p > 0.01$). The minimum bond energy among all the microwave treatments was obtained for MC2 at power density of 2 W/g and temperature of 40°C.

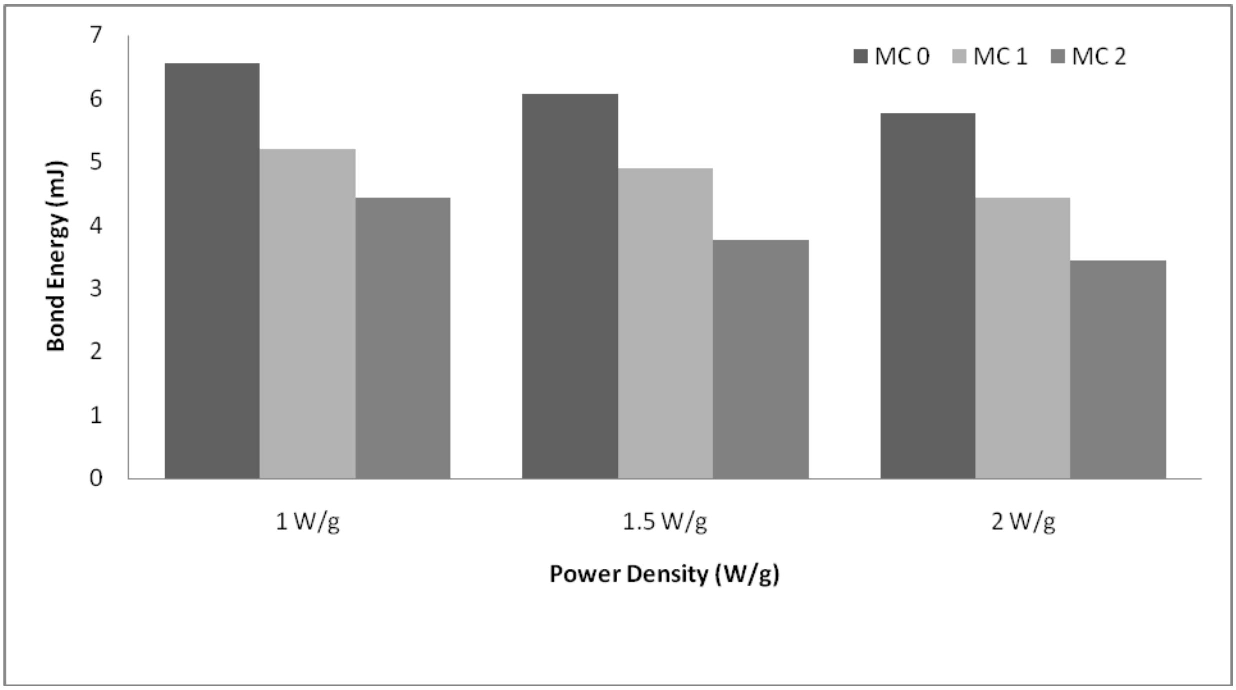

Figure 8 Bond Energy at different power densities for all microwave treatments. For each power density and microwave treatment combination the bar represents the mean bond energy (n = 27, three shell strips × three eggs × three temperatures)

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

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 were used to relate bond energy to soaking time and power density. Regression performed for the collected data yielded the following relationships:

$BE = 7.162 - 1.19\ S - 0.70\ P$ ($R^2 = 0.9958$) ($P < 0.01$)

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 predictive ability. Temperature was not included in the model as it did not have a 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 based upon the model developed.

Given the present set of conditions and parameters considered, the minimum bond energy possible is.1343 mJ for an egg soaked for 4.35 days and a power density of 2.5 W/g.

From the analysis of variance and multiple comparison tests performed for all microwave treatments, hot water and control, 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.

4. CONCLUSIONS

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 confirmed that microwaves could be used for separation of eggshell and membrane.

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 would further reduce the bond energy between the eggshell and membrane. Also, 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.

References:

A. Lifshitz, B.R.C.Baker., H. B. Naylor, 1965. The Exterior Structures of the Egg as Nutrients for Bacteria. Journal of Food Science, **30**: 516-519.

Arias , J.L., Fernandez, M.S., Dennis, J.E., and Caplan, A.I. 1990. Collagens of the chicken eggshell membranes. Connective Tissue Research, **26**: 37-45.

Datta, A.K., Sumnu, G., and Raghavan, G.S.V., 2005. Dielectric Properties of Food. In: Rao, M.A., Rizvi, S.S.H., and Datta, A.K. eds., Engineering Properties of Foods. CRC.

De Vore, D.P., Long , F.D., Osborne, P.M., Adams, G.R., and Franklin, M.R., 2007. Anti-inflammatory activity of eggshell membrane and processed eggshell membrane preparations. New Life Resources, LLC, US.

Gautron, J., and Nys, Y., 2007. Eggshell Matrix Proteins. In: Huopalahti, R., Lopez-Fandino, R., Anton, M., and Schade , R. eds., Bioactive Egg Compounds. Springer.

Long , F.D., Adams, G.R., and De Vore, D.P., 2005. Preparation of hyaluronic acid from eggshell membrane. New Life Resources LLC, US.

MacNeil , J.H., 2001. Method and appatus for separating a protein membrane and shell material in waste egg shells. The Penn State Research Foundation, US PATENTS.

MacNeil , J.H., 2005. Hatchery eggshell waste processing method and device. The Penn State Research Foundation US.

MacNeil , J.H., 2006. Method and apparatus for separating proein membrane and shell material in waste egg shells. In: Patent, U.S. ed. The Penn State Research Foundation, US.

Nys, Y., and Gautron, J., 2007. Structure and Formation of the Eggshell. In: Huopalahti, R., Lopez-Fandino, R., Anton, M., and Schade , R. eds., Bioactive Egg Compounds. Springer.

Neuhauser, I, 1965. Assisting healing of skin-denuded areas of the body with dried non-fibrous eggshell membrane products and compositions.

Orberg, J. 1990. Relationship between the shell membrane-shell bond and shell deformaton n hen's egg. British Poultry Science, **32**: 249-254.

Orsat, V., Raghavan, G.S.V., and Meda, V., 2005. Microwave technology for food processing: an overview. In: Schubert, H., and Regier, M. eds., The Microwave Processing of Foods. CRC.

Shoji, R., Miyazaki, T., Niinou, T., Kato, M., and Ishii, H. 2004. Recovery of gold by chicken egg shell membrane-conjugated chitosan beads. Journal of Material Cycles and Waste Management, **6**: 142-146.

Sipahioglu, O., and Barringer, S.A. 2003. Dielectric Properties of Vegetables and Fruits as a Function of Temperature, Ash, and Moisture Content. Journal of Food Science, **68**: 234-239.

Wong, M., Hendrix, M.J.C., Klaus, M.V.D., Little, C., and Stern, R. 1984. Collagen in the Egg Shell Membranes of the Hen. Developmental Biology, **104**: 28-36.

Yang, J.-Y., Chuang, S.-S., Yang, W.-G., and Tsay, P.-K., 2000. Egg Membrane as a New Biological Dressing in Split-thickness Skin Graft Donor Sites: A Preliminary Clinical Evaluation. 3rd Asian-Pacific Burns Conference. Department of plastic and reconstructive surgery, Chang Gung Memorial Hospital, Taipei, Taiwan.