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Conformation analysis of egg white under thermal gelation and determination of protein secondary structures using Raman Spectroscopy

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ABSTRACT Egg white, which is predominantly protein, is widely utilized in foods for its high nutrient value and functional properties. Thermal denaturation and coagulation process of egg white is accompanied by conformational changes of protein structures. The gelation process reflects a change in the secondary structure of the protein molecules. Raman spectroscopy is used to study the heat induced gelation process by predicting the change in the secondary structure of protein indicated by formation of intermolecular β-sheet in the amide-I and amide-III region. Whole egg white samples were heated up to a temperature of 100 ºC for 5 and 15 minutes. The egg white samples were scanned using a Raman spectroscopic system and spectra measured between 500-1800 cm^{-1} were analyzed to qualitatively estimate the changes in the egg's secondary structure. Preliminary results indicate that the thermally induced conformational changes in egg white involve the secondary structure change from α-helix and random coil to β-sheet.

Keywords: Raman spectroscopy, egg white, protein, secondary structure, thermal denaturation.

INTRODUCTION In food processing, hen egg white is used as an excellent gelled ingredient to modify food texture. Apart from its nutrient value, egg white possesses excellent functional properties, e.g., gelation and foaming, upon heating. Hen egg white accounts for approximately 60% of the total egg weight. About 84%-89% of egg white is water and protein is the major component of egg white solid. Lipids content of egg white is 0.03% whereas total carbohydrate content is approximately 1%. Carbohydrates are partly bound to the protein and partly free (Belitz

et al. 2009). There are about 13 different types of proteins in the egg white. Ovalbumin is the most abundant protein constituting 54% of the total protein. Conalbumin and ovomucoid are the other two most abundant proteins accounting for approximately 12% and 11% of the total protein solids separately. The structure and properties of thermally induced ovalbumin gel depends on the temperature and duration of heating. Also, molecular structure determines the functional properties of proteins. Therefore, it is of interest to study the relationship between protein structures and functional properties upon thermally induced coagulation.

There are several ways to determine the protein structure using spectroscopy. Most commonly, circular dichroism (CD), infrared (IR), and Raman spectroscopy are used to study protein structure. Raman spectroscopy, in particular, has been extensively used to determine the polypeptide secondary structure in protein molecules. There are three major secondary structures in protein, viz., α-helix, β-sheet, and random coil. In the egg white proteins, ovalbumin polypeptide is irregular and ovomucoid has little ordered secondary structure. Only conalbumin has extensive α-helix structure. Upon thermal denaturation, ovalbumin and conalbumin form intermolecular β-sheets (Painter and Koenig 1976). Such shift in protein secondary structure is reflected in the Raman signal as a shift in wavenumber and changed intensities. In Raman spectroscopy, two regions, i.e., the amide I band (in the range of 1700–1600 cm^{-1}) and the amide III band (in the range of 1300–1190 cm^{-1}), have comparable strength. Therefore, these two spectral regions could be used to deduce protein secondary structure (Ismail et al. 2006).

Raman effect is a weak phenomenon that occurs when samples are irradiated with intense light using light source such as laser. Upon excitation, two types of scattering occurs, i.e., elastic (Rayleigh) scattering and inelastic (Raman) scattering. Only about 1 in 10^{10} photons will undergo Raman scattering and the rest will be elastically scattered (McCreery 2000). Ever since its renaissance, Raman spectroscopy has attracted the interest of food scientists for the past few decades. In the early 1990s, attempts were made to analyze trace components and study structural changes of protein in foodstuffs using Raman technique (Ozaki et al. 1992). The use of Raman spectroscopy to determine the iodine values in lipid-containing foods rather than purified materials has been demonstrated (Sadeghi-Jorabchi et al. 1990). Lately, the utilization of Raman spectroscopy was extended to the study of grain and cereal foods (Barron 2008, Sohn 2005, Archibald 1998). Keller et al. (1993) considered the practicality of utilizing near-infrared excited Raman technique for quality control of food. It concluded with many of the advantages of Raman spectroscopy, e.g., non-destructive analysis and little sample preparation, over conventional analysis methods as a quality control tool. In general, macro-components, i.e., proteins, lipids, carbohydrates and water, together with minor components such as pigments and microorganisms are all suitable analytes for Raman technique (Li-Chan 1996). The diversified applications of Raman in agri-food sector include food quality control, fundamental research, compositional analysis, and adulteration detection among many others (Kizil and Irudayaraj 2008).

In this presentation, we plan to investigate the feasibility of using Raman spectroscopy on frozen egg white samples, both raw and thermally denaturized. By comparing the Raman spectra of both raw and thermally denaturized egg white protein, the reported Raman shift pattern is expected to be corroborated.

MATERIAL AND METHODS The experiment is conducted in two steps. Egg white samples were collected and prepared. Within two days of sample preparation, Raman measurement of egg white samples was taken.

Egg white samples Hen eggs were purchased from a local grocery store. Eggshells were cracked to allow egg white to seep through. Raw egg white fluid was collected in a petri dish and put into the freezer immediately after collection. To thermally denaturize egg white samples, another portion of egg white was collected and put into boiling water. Half of the boiled egg white

sample was taken out after 5 minutes of boiling and immediately frozen. The remaining boiled egg white sample was allowed to boil for an additional 10 minutes and frozen immediately after that. All egg white samples were frozen for 48 hours before being taken for Raman measurement. During transportation all samples were kept frozen.

The Raman microscope system A LabRamHR confocal Raman microscpectrometer system (Horiba Jobin Yvon, Edision, NJ, USA) was used to acquire Raman spectra in this experiment. The width of spectrograph entrance slit is 100 μm and the confocal hole size was set at 800 μm. The microscope (OlympusBX41) was equipped with a ×10 (Nikon) microscope objective. A near-infrared laser (Lynx series TEC 100 diode laser, Sacher Lasertechnik GmbH, Marburg, Germany) with 830 nm wavelength and 100 milliwatts output at the sample was used as the excitation. The spectral resolution is 4 cm^{-1}. Scanning spectral range was from 525 to 1800 cm^{-1}. Integration time is set at 10 s. For each spectra, 9 accumulations were acquired during each scan. Raman data were automatically saved. Background signal was also corrected.

Each sample, i.e., raw, 5 minute boiling time, 15 minutes boiling time, was made into triplicates. In each Raman spectra, the Raman peaks were manually identified and labeled.

RESULTS AND DISCUSSION The Raman spectra for raw and boiled egg white samples are shown in Fig. 1~Fig. 3. In Fig. 1, spectral lines are observed in the conformation-sensitive amide III region from 1300–1190 cm^{-1}. These spectral lines are resolved at 1235, 1255, and 1281 cm^{-1}. The spectral line near 1281cm^{-1} could be assigned to α-helix structures. The lines between 1230 and 1240 cm^{-1} in the spectrum could be attributed to the existence of antiparallel β-sheet conformation. Therefore the weak feature at 1235 cm^{-1} could indicate the existence of a small amount of β-sheet structure. Lines fall within the spectral region between 1243 and 1249 cm^{-1} are assigned to random coil conformation. Proteins with no regular ordered structure all have their amide III lines found at 1254 cm^{-1}. Therefore, the spectral line at 1255 cm^{-1} could be assigned to random coil structure of the egg white protein.

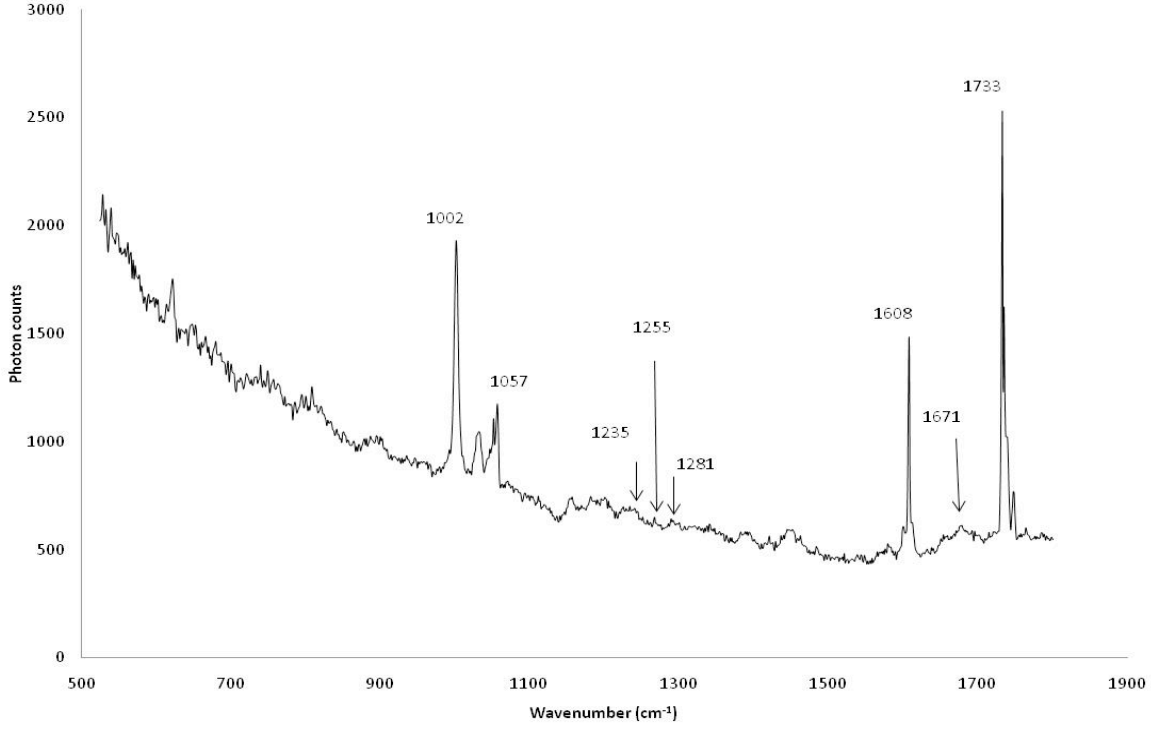

Figure 1. Raman spectra of thawed raw whole egg white liquid.

The amide I mode in the spectra, unlike the amide III region, did not resolve into separate lines that could be assigned to different secondary structures. Therefore, amide I around 1671 cm^{-1} did not provide as much information as the amide III in regards of protein structure. Intense Raman features at 1002, 1057, 1608, and 1733 cm^{-1} are due to either plastic container or the optics.

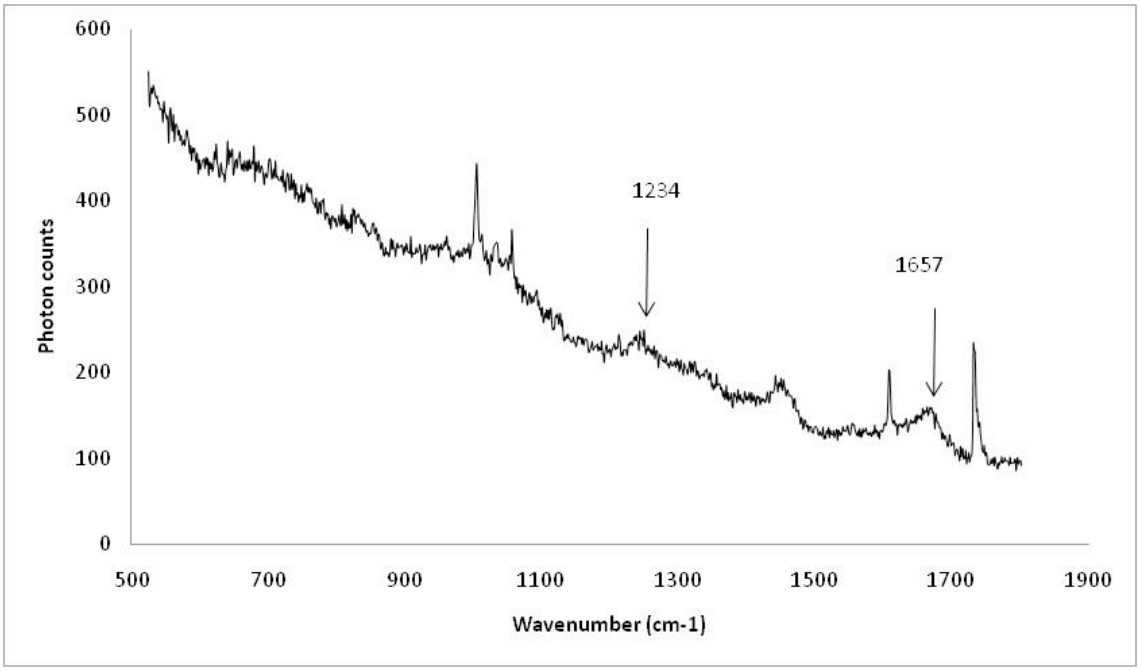

Figure 2. Raman spectra of frozen whole egg white boiled for 5 min.

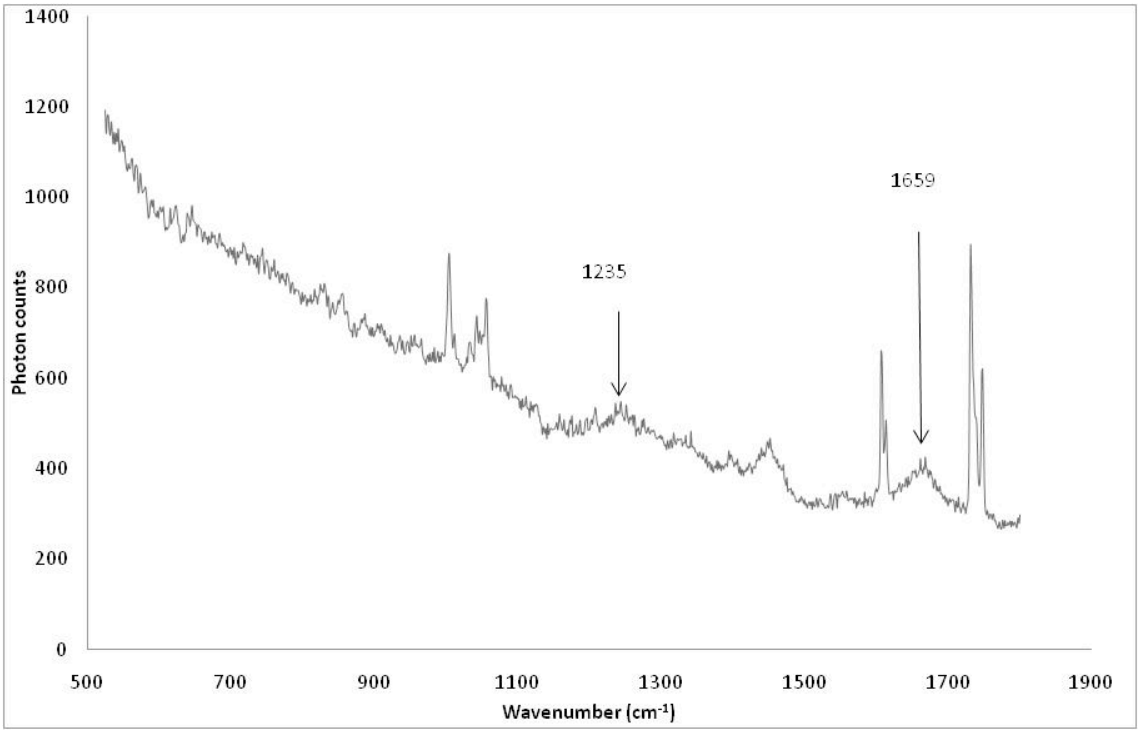

Figure 3. Raman spectra of frozen whole egg white boiled for 15 min.

Upon thermal denaturation by boiling egg white for 5 min, the Raman spectrum is shown in Fig. 2. As is evident in the spectra, an intense Raman line appears at 1234 cm^{-1}, demonstrating the formation of intermolecular β-sheet structure. There is also a wavenumber shift in the amide I region from 1671 cm^{-1} to 1657 cm^{-1}. The spectral changes indicated extensive secondary structure modification of protein molecules. Such change is explained as the formation of antiparallel β-sheet among ovalbumin molecules. In Fig. 3, where the egg white was boiled for 15 min, broadened Raman signal around 1234 cm^{-1}, which might indicate a more extensive formation of the intermolecular β-sheet structure. Other than that, spectral features are quite similar.

CONCLUSION Egg white samples were prepared to study the effect of thermal denaturation on protein secondary structures. Raman spectroscopy was used to demonstrate the conformational changes as indicated by the changing spectral patterns. It was found that extensive formation of intermolecular antiparallel β-sheet caused Raman shift from 1671 cm^{-1} to 1657 cm^{-1} in the amide I region. Intensified spectral line at 1234 cm^{-1} in the amide III region is also a strong indication of the structural change during thermal denaturation of egg white protein.

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