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Fourier Transform Infrared Spectroscopic Analysis of Protein Secondary Structures Found in Egusi

Yekeen A. Fasasi[1], Nikhil Mirjankar[2], Ayuba Fasasi[2, *]

[1]The Oke-Ogun Polytechnic, Department of Mathematics and Statistics, Saki (TOPS), Nigeria
[2]Oklahoma State University, Department of Chemistry, OK, Stillwater, USA

Email address:
ayubafasasi@gmail.com (A. Fasasi)
[*]Corresponding author

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Abstract: FTIR spectroscopy was used to analyze secondary structural composition of protein in egusi. Spectral deconvolution using mathematical techniques such as fourier self deconvolution and second derivative was used to provide meaningful information about the molecular structure of proteins in egusi. The use of second derivative to deconvolute the structure of the amide band in the spectral region 1725 cm^{-1} to 1580 cm^{-1} revealed some features indicating valleys that can be associated to the secondary structure of amide I band.

Keywords: Egusi, Nigeria, FTIR, Protein, Derivative, Deconvolution, Fourier

1. Introduction

Egusi seeds, which belong to cucurbitaceous plant family, constitute of large biological molecules or macromolecules with one or more long chains of amino acid residues. They are known for their use as the main ingredients in a West African traditional delicacy, predominantly popular in the Nigerian local tribes [1, 2]. The seeds are often shelled and ground (see figure 1) prior to usage. The Canadian government came to the aid of Cameroon in the late 1980s when it funded a project intended to develop a machine to help Cameroonians shell egusi seeds [3]. Presently, a machine has been developed in Nigeria to shell egusi [4].

Infrared (IR) spectroscopy is one of the suitable methods in establishing experimental techniques for the analysis of secondary structure of polypeptides and proteins [5-7]. Since all infrared spectra are aligned to a He-Ne laser frequency, the spectra acquired using this technique is precise and reliable. This negates the likelihood of unnecessary overlapping peaks. This gives IR spectrometers the superior capability of usage in analysis of proteins, especially in egusi which typically has a complex matrix. There have been failed attempts to use Nuclear Magnetic Resonance (NMR) spectroscopy and X-ray crystallography to characterize proteins [8]. Vibrational states of molecules can be probed using infrared and Raman spectroscopy.

In this paper, Fourier Transform Infrared (FTIR) spectroscopy was used to measure the wavelength and absorption intensity of IR radiation of the protein components of egusi seeds and powder. IR spectra of high polymers are usually interpreted in terms of the vibrations of a structural repeat unit [3]. The polypeptide and protein repeat units give rise to nine characteristic vibrational bands, namely, amide A, B, and I-VII. The two most prominent vibrational bands of protein backbone are the amide I and II bands [5]. Amide I band spans the spectral region 1600-1700cm^{-1} which correspond to C=O stretch of peptide linkages. The amide II band arises mainly from in-plane NH bending and froms the CN stretching vibration [3]. Amide II band shows much less protein conformational sensitivity than its amide I counterpart [3]. Although there are other amide conformational bands, these can be very complex and difficult to rely on for interpretation. IR bands characteristic of the proteins and peptides have been published [9].

2. Experimental

The egusi seeds were shelled and pounded into a powder with particle size distribution in the order of a few microns in diameter. About 0.1 g by mass of the powder was then deposited on the internal reflection element of an FTIR spectrometer. Spectra were measured at 2 cm^{-1} resolution

with 164 scans per measured spectrum.

3. Results and Discussion

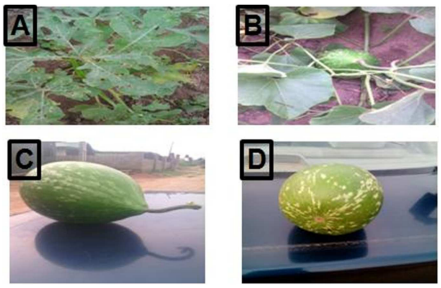

Figure 1. *Fat and protein-rich seeds of egusi in cucurbitaceous plant.*

Figure 2. *Egusi seeds shown in (A) and when dried, shelled, and ground into powder(B). The powdered form is cooked for consumption*

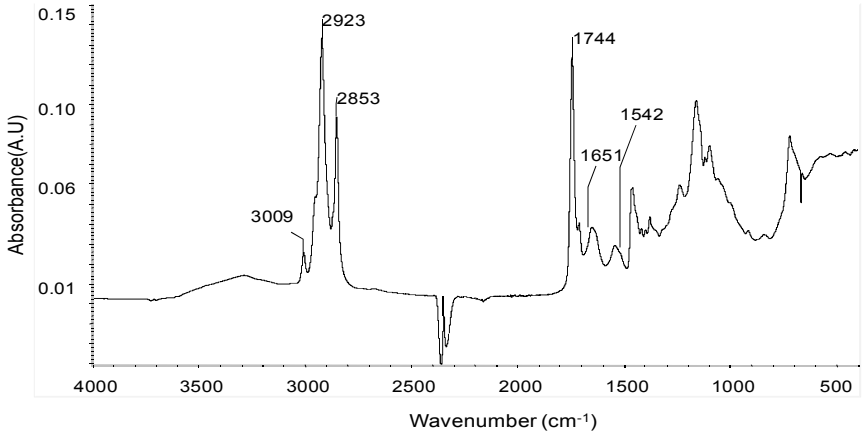

Figure 3. *FTIR Spectrum of egusi powder in its raw state over the entire mid-infrared range. The intense peaks at 2923-2853 cm-1 indicating C-H stretching vibrations. The peak at 3009 cm-1corresponds to C-H of C=C-H stretching vibration. The peak at 1744 cm-1 corresponds to C=O of lipids.*

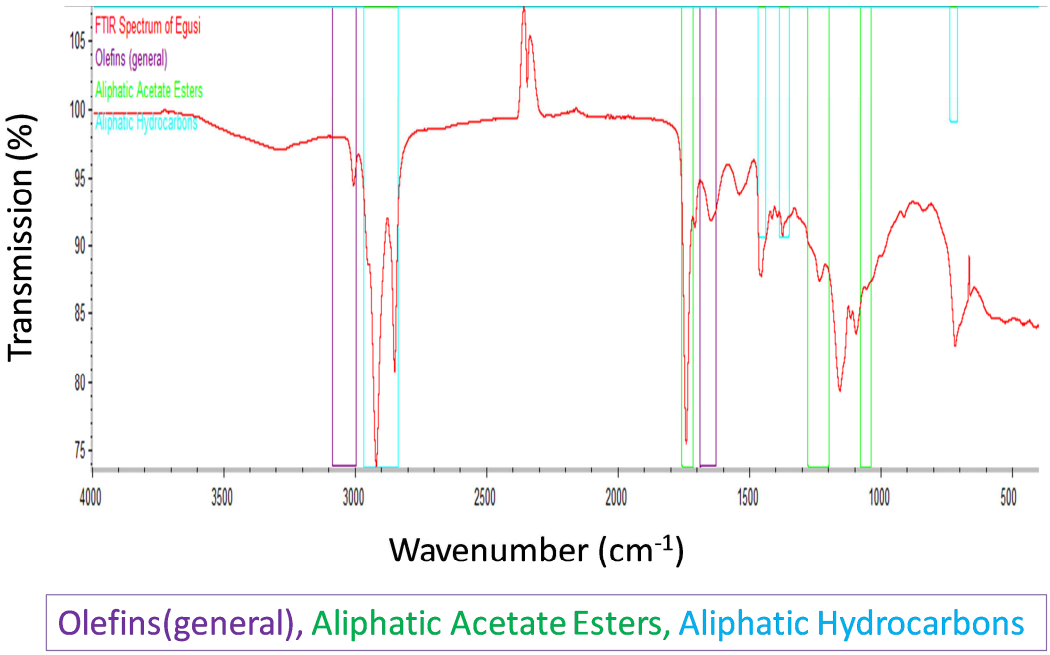

Figure 4. *The use of commercial software(OMNIC) to interpret spectrum of egusi over the entire mid-ir range suggests olefins, aliphatic acetate esters and aliphatic hydrocarbons could be present.*

Figure 1 shows pictures of the cucurbitaceous plant in which fat and protein-rich egusi seeds are contained. Figure 2(A) shows the harvested and dried egusi seeds. The seeds are often shelled, and ground into powder as shown in figure 2(B). The powdered form is cooked for consumption. Figure 3 shows the FTIR spectrum of egusi powder in its raw state

over the entire mid-infrared range of 400 - 4000 cm^{-1}. The intense absorption bands found at 2923-2853 cm^{-1} indicate C-H stretching vibrations. The peak at 3009 cm^{-1} corresponds to C-H of C=C-H stretching vibration. The peak at 1744 cm^{-1} corresponds to C=O of lipids. An attempt to use commercial software to interpret the spectrum for the presence of major functional groups gives an idea of the presence of some functional groups. Figure 4 shows the use of OMNIC to interpret spectrum of egusi over the entire mid-IR range. It suggests the presence of olefins, aliphatic acetate esters and aliphatic hydrocarbons. However, as it does not give a complete spectral breakdown. There are several methods that can deconvolute the spectra for molecular structural elucidation and understanding. The use of discrete wavelet transform (DWT), Fourier self deconvolution (FSD), and second derivative are feasible. In this study, second derivative and FSD were used to interpret the amide bands of proteins in egusi.

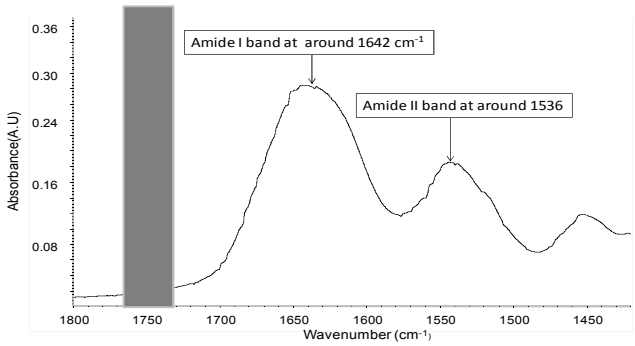

Figure 5. *FT-IR spectrum of Protein in egusie. Note the presence of amide I and II bands at 1642 cm-1 and 1536 cm-1 respectively. The C=O peak at 1744 cm-1 was blanked for clarit.*

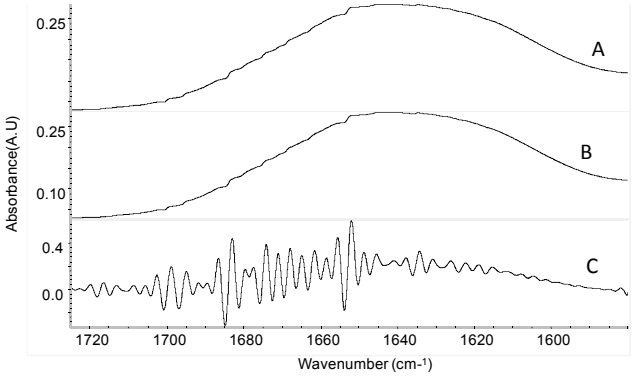

Figure 6. *Amide I Region: A- Original spectrum; B- Fourier Self-Deconvoluted (FSD) Spectrum; C – Second Derivative (Revealing valleys associated with secondary structures).*

Figure 5 shows the IR spectrum of protein in egusi. The presence of amide I and II bands at 1642 cm^{-1} and 1536 cm^{-1} are demonstrated, respectively. The C=O peak at 1744 cm^{-1} was blanked for clarity. The spectral range between 1800 cm^{-1} to 1435 cm^{-1} reveals the amide I and II bands at 1642 cm^{-1} and 1536 cm^{-1}, respectively. Vibrational modes of C=O indicative of the presence of lipids are observed at 1748 cm^{-1}. Amide I band is due to C=O stretch and some N-H stretching characteristics. Amide II band is due to N-H in plane bending and C-N stretching vibrations. Each spectrum was baseline corrected using OMNIC. Second derivative was calculated for the spectral region from 1725 cm^{-1} to 1580 cm^{-1} as they are informative in illustrating changes that occur in the secondary structure of proteins. Second derivative was performed using the Savitzky-Golay derivative routine. Parameter values used for the routine were 7 data points with polynomial order 3 as this condition proved to be more informative. Fourier self-deconvolution of the spectra was calculated for the region 1725 cm^{-1} to 1580 cm^{-1} with an enhancement factor of 1.3 and bandwidth of 30. The second derivative deconvoluted structure of the amide band in the spectral region 1725 cm^{-1} to 1580 cm^{-1} revealed some features indicating valleys that can be associated to the secondary structure of amide I band (figure 6). Table 1 shows assignments of deconvoluted amide I band frequencies to secondary protein structure. More characteristic features have been investigated in many studies [9-11].

Table 1. *Assignment of deconvoluted amide I band frequencies to secondary protein structure.*

Frequency/ cm^{-1}	Assignment
1698	β-sheets
1682	β-sheets
1644	β-sheets
1649	Random coils and helices
1632	Intramolecular β-sheets
1613	Extended hydrated chains/intermolecular β-sheets
1594	Glutamine side chain

4. Conclusion

FTIR spectroscopy can be used for studying secondary structural composition of protein in egusi. Spectral deconvolution using mathematical techniques can provide molecular structure. The use of second derivative to deconvolute the structure of the amide band in the spectral region 1725 cm^{-1} to 1580 cm^{-1} revealed some features indicating valleys that can be associated to the secondary structure of amide I band. The second derivative worked better than the use of FSD.

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