Asian Journal of Pharmaceutical and Health Sciences
www.ajphs.com

FTIR Spectral Analysis on Protein Powder

Shanthi K.[1], **Renugadevi T.S.**[2], **Gunasekaran S.**[3]

[1]Department of Physics, Quaid-E-Millath Govt.college, Chennai, India
[2]P.G Department of Physics, Women's Christian college, Chennai, India
[3]Registrar, Periyar university, salem India

ARTICLE HISTORY

Received: 20-Jun-2011

Accepted: 10-Jul-2011

Available online: 10-Nov-2011

Keywords:

FTIR, Obesity, Protein powder.

***Corresponding author:**

E-mail: devi_renuga@yahoo.com
Phone: 9884003856

ABSTRACT

Obesity is a physical condition that results from excessive storage of fat in the body. Weight more than 20% above the desired body mass index, which is calculated from an individual's age, height, and weight. The excess fat tissues result in obesity and causes health disorders. Poor diets and the lack of physical exercise are main factors in the increase of obesity. At present diet and physical exercise promote weight loss over short term, to achieve and maintain a healthy weight. To reduce the obesity protein powder is designed to be taken every day as nutritional supplement. By consuming protein throughout the day, to stabilize the blood sugar levels. Protein powder is mainly used for weight management to reducing body mass index. The present study aims to analyze the protein powder by FTIR spectroscopic technique, the pure protein powder was procured from a nutritional club and used as such. The FTIR spectra were recorded in the mid infrared region between 4000 – 400 cm^{-1}. A qualitative analysis of these protein powders has been carried out by making a satisfactory vibrational band assignment for the fundamental modes of vibration observed in the infrared spectrum. Some specific vibrations are identified; absorbance values are noted under different conditions of exposure. The ratios of absorbance among some specific modes of vibration are calculated, which represents the internal standards. The sets of the internal standards of the protein powder is compared with suitable storage conditions to check whether any change has taken place due to the environmental exposure.

INTRODUCTION

Individuals and groups at risk of developing obesity involves a range of long-term strategies. These include prevention, weight maintenance, management of co-morbidities and weight loss. They should be part of an integrated, multi-sectoral, population-based approach, which includes environmental support for healthy diets and regular physical activity. Effective weight management for obese person, who loss weight slowly and constantly, are more successful in keeping their weight down when they are intake of protein is recommended for regulating the weight loss. There are various other methods followed for reducing weight which includes using of drugs and supplements that decrease appetite, block fat absorption, or reduce stomach volume thus limiting the intake of food energy. FTIR spectroscopy has a unique place in the area of spectroscopy as a powerful tool to characterize the inorganic and organic compounds by chemists. Its application in biology for studying the structure and conformation of proteins, nucleic acids and lipids has been well documented in the literature [1-4]. The advances made in the instrumentation such as the introduction of FTIR spectroscopy have paved the way to its usefulness in weight management [5]. Protein plays an essential role in maintaining a healthy diet for human. Intake of protein helps to control food cravings, reduce stress levels, and boost energy throughout the day. The body breaks down the protein slowly, providing a steady, long lasting supply of energy [6] It also means avoiding blood sugar highs (or lows) associated with high carbohydrate consumption which can lead to energy fluctuations and weight gain. Protein powder delivers a wide range of amino acids to help tone muscles, reduce body fat and support the immune system. Quality proteins with a thermogenic (fat burning) agent such as l-carnitine or hydroxy citric acid (HCA) can increase fat loss and help to achieve faster results [7-8]. L-Carnitine which stimulates the process that converts fat into energy to help reduce body fat levels. Hydroxy Citric Acid which is a natural ingredient that helps to suppress appetite and inhibit fat production [9].

Bodybuilding supplements are dietary supplements commonly used by those involved in bodybuilding and athletics. They include protein branched-chain amino acids (BCAA), glutamine, essential fatty acids, meal replacement products, prohormones, creatine, thermogenic products and testosterone

boosters. There is no scientific consensus in favor of the usefulness of many bodybuilding supplements, and some have potentially harmful side-effects [10]. Protein powder is one of the best weight loss supplements. Studies have been done and all modern research shows that protein is much more filling than either fat or carbohydrates, and this makes it an extremely important factor in controlling the appetite and avoiding hunger cravings. It's one of the main reasons most weight loss diets are high in protein [11]. The aim of the study developed a simple, precise and accurate FTIR Spectroscopic analysis on the nutritional protein powder sample.

MATERIAL AND METHODS

The protein powder sample was collected from the nutrition club, kolathur, Chennai, India. The protein powder should be stored in tightly closed, thin polythene cover. The behavior of these protein powder that were stored under the prescribed storage with those stored at altered conditions has been compared. The FTIR spectra of the samples have been recorded for the pure protein powder stored in well-sealed polythene cover, exposed to sunlight and at ice point .

By employing FTIR spectral techniques the change in the quality of the materials when stored in different conditions have been studied .FTIR is useful for identifying chemicals that are either organic or inorganic. it can be utilized for both qualitative and quantitative analysis of components of an unknown material. Today FTIR instruments are computerized which makes them faster and sensitive than the older dispersive instruments.

RESULT AND DISCUSSION

Material Identification

To identify the material being analyzed, the unknown IR absorption spectrum is compared with standard spectra in computer databases or with a spectrum obtained from a known material. Spectrum matches identify the protein or other constituent(s) in the sample. Absorption bands in the range of 4000 - 1500 wave numbers are typically due to functional groups (e.g., -OH, C=O, N-H, CH_3, etc.). The region from 1500 - 400 wave numbers is referred to as the fingerprint region. Absorption bands in this region are generally due to intermolecular phenomena and are highly specific to each material. The specificity of these bands allows computerized data searches within reference libraries to identify a material. The molecular structure of protein is shown in Fig.1.

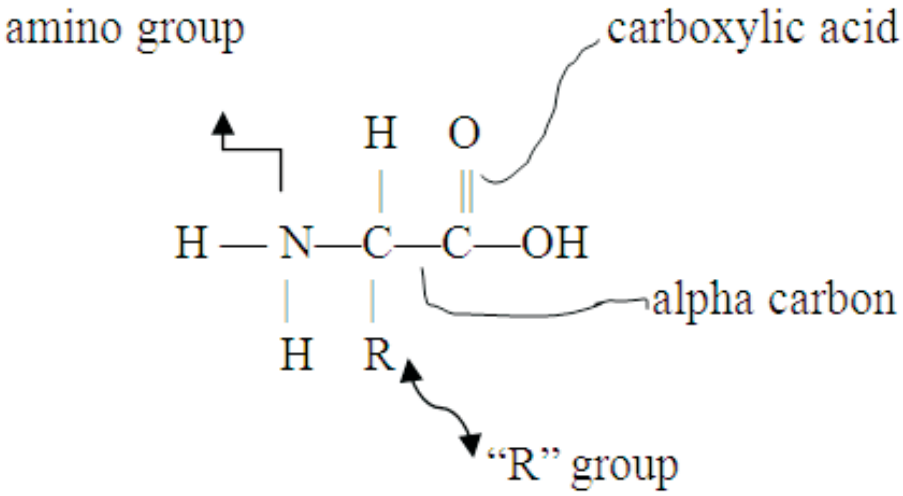

Fig. 1: The structure of an amino acid; the basic building block for making proteins

Qualitative Analysis

FTIR spectra were recorded in the mid region 400 – 4000 cm^{-1} using Perkin – Elmer spectrometer in KBr pellet technique at Sophisticated Analytical Instrumentation Facility, Indian Institute of Technology, Chennai, India. A qualitative analysis of this sample has been carried out by making a satisfactory vibrational band assignment for the fundamental modes of vibration observed in infrared spectroscopy. The FTIR spectra have been recorded for protein powder kept in suitable storage condition and exposed to various environmental hazards. Some specific modes of vibration are identified and the absorbance values are noted under different condition of exposure.

Spectral Qualitative data analysis

Spectral bands in vibrational spectra are molecule specific and provide direct information about the biochemical composition. FTIR peaks are relatively narrow and in many cases can be associated with the vibration of a particular chemical bond (or a single functional group) in the molecule. In this technique the molecular-level information allowing investigation of functional groups, bonding types, and molecular conformations is mainly carried out. The vibrational bands are relatively narrow, easy to resolve, and sensitive to molecular structure, conformation, and environment. The FTIR spectra of protein powder samples were recorded at suitable storage condition and different environmental exposure shown in Fig.2-4.

The vibrational spectrum of a compound is the super position of vibrational band of the various functional groups present in it. By observing the nature, position, shape & relative intensity of the vibrational bands and comparing them with that of structurally and chemically related compounds, asatisfactory frequency assignment of these functional groups present has been done. The vibrational band assignment for protein power is given in Table 1.

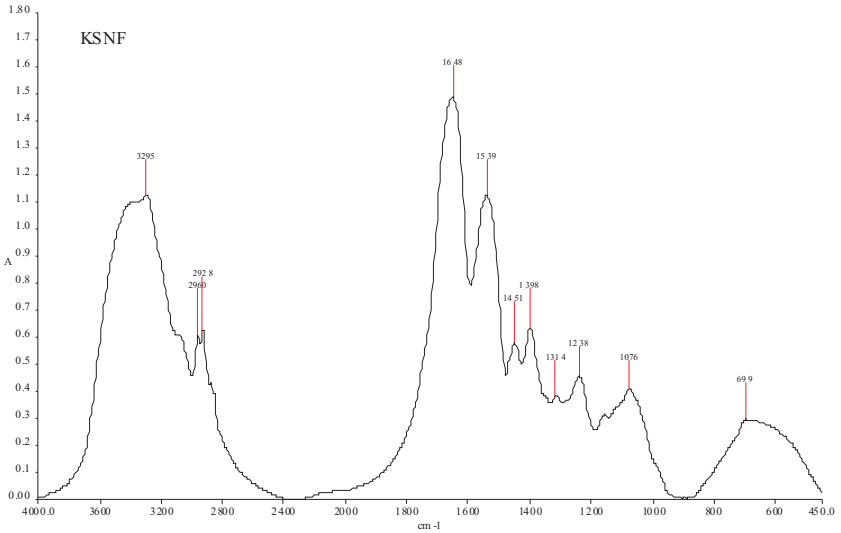

Fig. 2: FTIR spectrum of Protein powder at ideal strorage condition

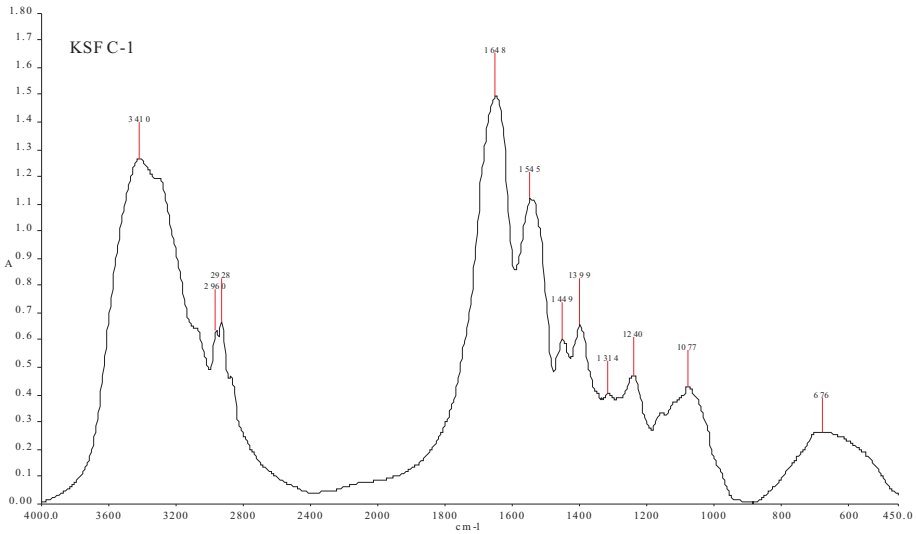

Fig. 3: FTIR spectrum of Protein powder kept at cold condition

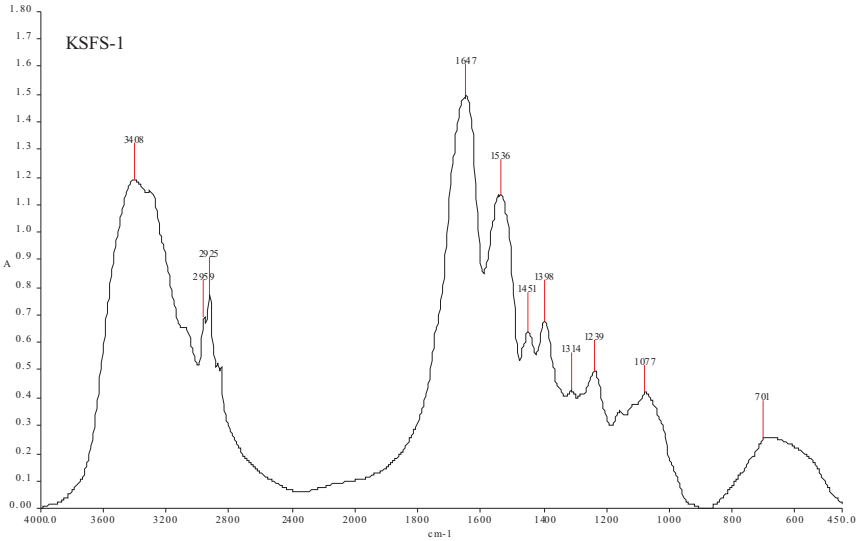

Fig. 4: FTIR spectrum of Protein powder exposed to sunlight radiation

Table No.1: FTIR Vibrational band assignment for Protein powder

Frequency (cm^{-1})	Vibrational band assignment
3295(vvs)	N-H stretching of proteins
2960 (w)	CH3 asymmetric stretching
2928 (v)	CH3/CH2 , C-H stretching
1648 (vvs)	C=O stretching
1539(s)	C-N stretching/N-H bending
1451(s)	CH2 bending
1398 (s)	COO- symmetric stretching
1314(w)	O-H deformation
1238 (w)	C-N symmetric stretching
1076 (vs)	C-O symmetric stretching
699(vw)	C-H out of plane /C-C=O deformation.

s-strong, m-medium, w-weak, vs-very strong, vvs-very very strong , vw-very weak

Table No.2: Absorbance for specific modes of vibration of Protein powder under different Storage conditions

Frequency (cm^{-1})	Absorbance		
	Labeled condition	Exposed to sunlight	At ice point
2960	0.609071	0.691652	0.634041
2928	0.629684	0.769731	0.666464
1648	1.494531	1.493608	1.494799
1539	1.129026	1.134321	1.114839
1451	0.581104	0.641476	0.601062
1398	0.637324	0.679632	0.65513
1314	0.382252	0.427543	0.405991
1238	0.457259	0.496491	0.470796
1076	0.412084	0.4201	0.427649
699	0.297542	0.257526	0.26006

The present work, aims to analyse the protein powder which is consumed in the form of milk shake by nutrition club members by fourier transforms infrared spectroscopy (FTIR) techniques and determines spectral changes in different storage condition and to characterize the presence of all specific chemical groups in this sample. FTIR spectra were collected in the mid infrared (MIR) region between 4000-400cm^{-1}. The FTIR spectra recorded changes in the absorption patter. The relative intensities of the vibration band assignments has been made. The general regions of the infrared spectrum in which various kinds of vibrational bands are observed Amide I and amide II bands are two major bands of the protein infrared spectrum. The amide I band (between 1600 and 1700 cm^{-1}) is mainly associated with the C=O stretching vibration and is directly related to the backbone conformation. Amide II results from the N-H bending vibration and from the C-N stretching vibration. This band is conformationally sensitive. Amide III and IV are very complex bands resulting from a mixture of several coordinate displacements. The out-of-plane motions are found in amide V, VI and VIII.

The infrared spectra of protein powder are characterized by a set of absorption regions known as the amide region and the C-H region. The most widely used modes in protein structure studies in the amide region are amide I, amide II has been thoroughly investigated by and amide III already discussed by Gunasekaran & co-workers et al [12]. The amide I band arises principally from the C=O stretching vibration of the peptide group. The amide II band arises primarily N-H bending with a contribution from C-N stretching vibrations. The amide III absorption is normally weak and arises primarily from N-H bending and C-N stretching vibrations. The amide absorptions are considered sensitive to protein conformation.

The band due to N-H stretching of proteins observed at 3295 cm^{-1} and the band exhibit in the region at 2060 cm^{-1} can be immediately assigned to be due to CH_3 asymmetric stretching mainly lipids. The vibrational band present at 2928 cm^{-1} in FTIR spectra band assigned to the due to aromatic CH_3 / CH_2 , C-H stretch mainly for lipids. A strong absorption band at 1648 cm^{-1} is assigned to C=O stretching of proteins.

The bands at 1539 cm-1 are assigned due to C-N stretching vibration of proteins and N-H bending. The CH_2 bending gives rise to absorption band at 1451 cm^{-1} mainly due to lipids. And the band at 1621 cm^{-1} in IR is assigned to COO^- symmetric stretching for fatty acid and amino acid. A band at 1314 cm^{-1} has been assigned to O-H deformation.

The amide I band are primarily associated with the stretching motion of the C=O group. This C=O band is sensitive to the environments of the peptide linkage and also depends on the protein's overall secondary structure.

Table No.3. Internal ratio parameters for Protein powder for different storage conditions

Storage Conditions	Internal Standard of specific modes of vibration								
	$A_{2960}/_{2928}$	$A_{2928}/_{1648}$	$A_{1648}/_{1539}$	$A_{1539}/_{1451}$	$A_{1451}/_{1398}$	$A_{1398}/_{1314}$	$A_{1314}/_{1238}$	$A_{1238}/_{1076}$	$A_{1076}/_{699}$
Labeled condition	0.967264	0.421325	1.323734	1.942898	0.911787	1.667287	0.835963	1.109625	1.384960
At ice point	0.951350	0.445855	1.340820	1.856146	0.916795	1.613656	0.862350	1.100893	1.64424
Exposed to sunlight	0.89856	0.51535	1.31674	1.76829	0.94385	1.58962	0.861129	1.181840	1.631291

The medium intensity band observed at 1238 cm^{-1} in the FTIR spectrum assigned due to that of the C-N symmetric stretching vibration and 1076 cm- 1 is assigned to that of the C-O symmetric stretching vibration. Finally the band at 699 cm^{-1} has been assigned to the C-H out of plane /C-C=O deformation.

CONCLUSIONS

Fourier Transform Infrared (FTIR) spectroscopic techniques have been effectively employed for the qualitative and quantitative analyses on Protein powder. The advantage of FTIR over other techniques is convenience. IR spectra can be obtained for protein powder in a wide range of environments with a small amount of sample. A satisfactory vibrational band assignment of the protein powder has been done for FTIR spectra which confirms the basic functional groups present in the compound. The intensity ratio parameter among specific peaks were calculated and the results clearly show that some vibrational bands are more altered due to sunlight exposure and storage at ice point. This clearly denotes that a change in the quality of the Protein powder has taken place due to the change in storage condition.

REFERENCE

1. Roeges NPG, A Guide to the complete interpretation of infrared spectra of organic structures, UK : John Wiley & Sons, (1994).

2. Cooper EA, Knutson K. Fourier transform infrared spectroscopy investigation of protein structure. Pharm Biotechnol 7, 101-1433,(1995).

3. Mantsch HH, Chapman D, Infrared spectroscopy of biomolecules, New York : Wiley-Liss, (1996).

4. Brandenburg K, Seydel U. Infrared spectroscopy of glycolipids. Chem Phys Lipids; 96, 23-40, (1998).

5. Diem M, Boydston-White S, Chiriboga L. Infrared spectroscopy of cells and tissues: Shining light onto a novel subject. Applied Spectroscopy, 53, 148-161, (1999).

6. Cavalu, Simona,Simona Cinta Pnzaru, Qualitative and quantitative aspects in analysis of ginseng pharmaceuticals using vibrational spectroscopy, Romanian. J. Biophys., 15, 61–66, (2005).

7. Mohan. J, Organic Spectroscopy – Principle and Applications, Nasrosa Publishers, New Delhi, (2001).

8. Chopra, R.N Nayar,S.L. Chopra.IC, In: Glossary of Medicinal Plants, CSIR Publication, New Delhi, p. 104,(1996).

9. Venyamiov & Kalnin 1990 , Biopolymers 30, (13-14), 1243-57,(1990).

10. Kichgessner,M., P. Roth, R.Spoerl, A.Schnegg, R.J.Kellner.E. Weigand, A comparative view of trace elements and growth, Nutrition Metabolism, 21, 119–143, (1977).

11. Khanna, V.G.Kannabiran.K, Larvicidal effect at Hemidesmus indicus, Gymnema sylvestre and Eclipta prostrata against Culex qinquifaciatus mosquito larvae, African Journal of Biotechnology, 6(3), 307–311,(2007).

12. S.Gunasekaran et al./Rec Res Sci Tech 2, 20-31, (2010) .