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A FOURIER TRANSFORM INFRARED SPECTROSCOPIC STUDY ON SKELETAL MUSCLE OF SHEEP

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Abstract: The paper reports FTIR spectroscopic data on sheep skeletal muscle. The study reveals the presence of contractile proteins, phospholipids, triglycerides and cholesterol along with phosphates in the skeletal muscle. Conformations of proteins are also evident from the spectral study.
Keywords: Skeletal muscle, Sheep, FTIR spectroscopy.

1. Introduction

Fourier transform infrared (FTIR) spectroscopy is a potential tool to extract vital information in the study of biological system. Extensive research work is being done on biological apatite and other biological inorganic materials.

Jean – Louis Damez and Sylvie Clevjon [1] discussed various biophysical methods that can be used to understand meat structure. Kelly L pearce, et. al., [2] analysed, using low field nuclear magnetic resonance spectrometer, the distribution and mobility of water; postmortem changes in muscle; and the factors affecting these in relation to fresh meat quality parameters like water holding capacity, tenderness and juiciness. Isabel Campos, et. al., [3] used near infrared spectroscopy (NIRS) as an online analytical technique to predict the sodium content in dry – cured ham slices. The results of the study showed that NIR measurements can be utilized in giving accurate sodium content information of packaged dry – cured ham slices. Ana Carilona, et. al., [4] used near infrared spectroscopy and multivariate calibration for simultaneous determination of glucose, triglycerides and high density lipoprotein in animal plasma.

Parvel Kaspar, et. al., [5] studied angular absorption of light for evaluation of structural damage to porcine meat caused by ageing, drying and freezing. They showed that the measurement of optical angular dependency of absorption in relation to muscle fiber can be utilized for detecting the structural damage to the sample for meat quality control purpose.

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Ebru Deniz, et. al., [6] studied to detect the meat types at different concentrations in the mixed raw meat samples by using FT-IR spectroscopy. Mixtures of chicken meat and beef were prepared by adding chicken meat at 0, 20, 40 and 100% (wt/wt) concentrations to beef as the main meat type. They reported that IR spectra were promising and indicating especially five bands (wave numbers between 2917-2920 cm^{-1}, 2849-2850 cm^{-1}, 1740-1742 cm^{-1}, 1196-1197 cm^{-1}, 1176-1177 cm^{-1}) and concluded that FTIR could be used in identifying species in the beef and chicken meat mixtures.

Gangadhar, et. al., [7] reported, based on FTIR spectroscopy, that the constituents of ovine scapular cartilage are mainly collagen and proteoglycans; Carbonate ions and Phosphate ions are in a very small quantity.

Nazima Siddiqui and Adeel Ahmad [8] presented IR spectroscopic data of edible and medicinal oils of plant origin. For IR analysis, Ten edible oils and fifteen medicinal oils were selected. FTIR spectra were recorded. The FT – IR spectra of edible and medicinal oils showed a series of bands with different intensities and revealed the composition of fatty acids and degree of saturation of the selected oils. The study suggested that IR spectroscopy could be considered as a vital technique for identification, analysis, determination of degree of saturation of fatty acids and detection of adulteration of oils of plant origin.

Syed Ismail Ahmed, et. al., [9] studied physical properties such as specific gravity, viscosity, surface tension, refractive index and electrical conductivity of human urine of healthy donors and of patients suffering from chronic kidney disease (CKD) for possible early detection of proteinuria. The decrease in surface tension of CKD urines was observed due to high albumin excretion and increased blood urea nitrogen. Fourier transform infrared (FTIR) spectra in the mid IR region were recorded for normal and albumin treated urine. It was observed that peaks at 1641 cm^{-1} and 1450 cm^{-1} in IR spectra were the most specific peaks for urea and albumin, respectively. They concluded that FTIR method of detecting proteinuria is quick and cheaper.

Syed Ismail Ahmed, et. al., [10] quantitated the amount of urea in urine by using FTIR Spectroscopy. FT-IR spectra of urine of healthy persons were recorded in the region 1500 - 700 cm^{-1} by adding urea of concentrations of1.25, 2.5, 5 and 10 μg/mL. The spectra revealed primary peak at 3400cm^{-1} and a secondary peak at 1641cm^{-1}related to urea. A graph between concentration of urea and intensity of IR absorption showed a linear relationship. They reported an increase in the intensity of absorption at wave number 1641cm^{-1}, which confirmed the specific peak for Urea.

Vijaya Ushasree, et. al., [11] made FTIR analysis of whole blood, plasma and serum and reported characteristic spectral bands pertaining to fibrinogen, hemoglobin, erythrocyte membrane, lipids and other plasma proteins.

The present study is an attempt to analyse molecular composition of sheep skeletal muscle through FTIR spectroscopy.

2. Material and Methods

The muscle samples from thigh, attached to femur bone, of sheep were collected from meat shop after 4 to 6 hours of slaughtering. The samples were cleaned and dried in a microwave oven at a temperature of around 50 ^{O}C in about twenty microwave drying session of 50 sec with a gap of 20 – 30 sec. The dry samples were powdered using an agate mortar. Infrared spectrum of the powder was recorded in FTIR spectrometer (Shimadzu FTIR – 8400S (Fig. 1) in the range of 4000 cm^{-1} to 400 cm^{-1}. For the spectral recording, a small quantity of muscle powder was mixed with Potassium Bromide (KBr) in the ratio of 1:4 and pressed in a stainless steel dye to produce thin KBr wafer, containing a relatively high concentration of the sample in IR transparent KBr matrix.

3. Results and Discussion

For the IR analysis of a sample, IR spectrum is divided into *three* broad regions. Region I is from 4000 to 3000 cm^{-1}. It reveals the nature of hydrogen bonding. Region II is from 3000 to 1500 cm^{-1}. In this region bands pertaining to functional groups are present. Region III is from 1500 to 400 cm^{-1}, wherein information about most of the bio - minerals and their combinations, phases and substitutions is available.

Fig. 1. Shows FTIR spectrum of sheep skeletal muscle recorded in the range of 4000 cm^{-1} to 400 cm^{-1}. It reveals a series of bands with different intensities. Table 1 presents data on wave numbers and corresponding Transmission (%) obtained from FTIR spectrum along with characteristic vibrations of functional groups.

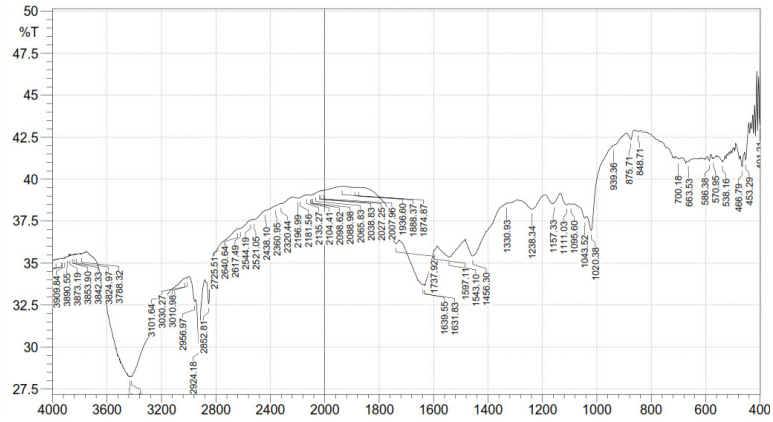

Fig. 1. FTIR spectra of skeletal muscle of sheep

The IR spectrum of muscle powder indicates the bands concerned with amides of proteins; triglycerides and phospholipids; phosphate and carbonate ions.

A broad band 3420 cm^{-1} in the region 3500 cm^{-1} to 3000 cm^{-1} is observed. This can be assigned as due to hydroxyl ions involved in hydrogen bonding. The broad band of hydrogen bonded hydroxyl ions in the muscle sample can be attributed to the presence of several hydrogen bonds of different energy.

The major bands observed at 1631cm^{-1} (C-H stretch), 1543cm^{-1}(N-H stretch and C-O- anti symmetric stretch)and 1238cm^{-1}(C-H stretch with N-H bend) related to Amide I, Amide II and Amide III respectively in the spectral region3000 cm^{-1} to 1500 cm^{-1} can be attributed to the organic component of the bone, which are essentially proteins, mainly collagen, actin and myosin. Specifically the band at 1639 cm^{-1} (C=O stretch) is of collagen protein.

Table 1. FTIR data on sheep skeletal muscle

Wave Number (cm^{-1})	Transmission (%)	Characteristic vibrations of functional groups
3420	28.23	H-O-H stretching
2924	30.15	Asymmetric stretching vibrations of CH_2 of acyl chains (lipids), cholesterol
2853	32.57	C-H stretching vibrations of fatty acids
1737	36.16	C=O stretching vibrations of phospholipids
1639	33.70	C=O stretching vibrations of collagen
1631	33.65	Amide I band of pleated sheath structures of proteins
1543	35.36	Amide II band of proteins, N-H stretching, Proteoglycans C-O-anti symmetric stretch
1456	35.43	P-O- anti symmetric stretch
1339	38.56	O-C-O stretching
1238	38.21	Amide III band of proteins; C-H stretch with N-H bend

1157	38.57	C-C vibration
1111	38.48	Triglycerides
1043	37.67	
1020	36.94	Glycogen
939	41.99	P-O- symmetric stretch
876	42.36	C-O out of plane bending
700	41.20	C-O-C deformation
663	41.03	C-S stretch
571	41.21	P-O- anti symmetric bend
466	40.74	P-O- symmetric bend

The band 2924 cm^{-1}(H-C-H asymmetric stretch) is of acyl chains, specially cholesterol. The bands at 2853 cm^{-1}(C-H stretch), 1737 cm^{-1}(C=O stretch), and (1111 cm^{-1}& 1043 cm^{-1}) are mainly concerned with fatty acids, phospholipids and triglycerides respectively.

The bands 466 cm^{-1} (P-O- symmetric bend), 571 cm^{-1} (P-O- anti symmetric bend), 939 cm^{-1} (P-O- symmetric stretch), 1456 cm^{-1} (P-O- anti symmetric stretch) are pertaining to phosphate ion (PO_4^{-3}). The bands 876 cm^{-1} (C-O out of plane bending); 1456 cm^{-1}, 1543 cm^{-1} (C-O-anti symmetric stretch) are characteristic of carbonate ion (CO_3^{-2})

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