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Discrimination of pork content in mixtures with raw minced camel and buffalo meat using FTIR spectroscopic technique

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Abstract

The structural characterization of meats such as camel, buffalo, sheep and pork was studied using Fourier transform infrared spectroscopy (FTIR) in the range (4000 cm^{-1} - 400 cm^{-1}). Also the structural characterization of 10%, 30%, 50% and 70% (W/W) pork-in-camel and pork-in-buffalo mixtures meats were studied using this technique. All the samples were homogenized and dried using phosphorous penta-oxide. The structural assignments of the characteristic absorption bands of the samples under investigation were discussed. The relative content of protein to lipid in the samples was determined using the absorbencies ratios of C=O stretching band (amide I) at 1654 cm^{-1} and N-H bending band at 1540 cm^{-1} (amide II) to C-H stretching at 2924 cm^{-1}. A comparison between these ratios for any given samples was carried out. The deconvolution of the FTIR spectra in the region 2000 cm^{-1}-1000 cm^{-1} was used for more characterization of the samples using the area under the peak. The data showed that the camel meat has the highest protein content and this content decreased in the following trend, buffalo > sheep > pork. In case of pork-in-camel and pork-in-buffalo mixtures, the relative protein to lipid content of them decreased with increasing of pork content in these mixtures.

Introduction

The authentication of food is an important issue for both consumer and the food industry. The determination of food authenticity and the detection of adulteration are attracting an increasing amount of attention. With regard to meat and meat products, major authenticity issues concern the substitution of high value raw materials with less costly cuts from the same or different animal species, offal or other proteins of animal or vegetable origin. In some countries the consumption of certain meats (e.g. pork) is proscribed for religious reasons. Therefore, arrange of methods for species identification have been investigated, most of which are based on the examination of muscle extracts. These include electrophoretic procedures (Barai *et al*., 1992; McCormick *et al*., 1992; Skrokk and Horni, 1994) immunological techniques (Pickering *et al.*, 1995) and DNA-based procedures (Ebbehoj and Thomsen, 1991a and b). None of these methods is rapid and they all require sophisticated laboratory procedures.

FTIR spectroscopy is becoming an attractive alternative to the existing analytical techniques in food analysis because it is rapid, low cost, and noninvasive. FTIR spectroscopy region (4000 - 400 cm^{-1}) in particular provides information on very large number components, and the absorption bands are sensitive to the physical and chemical states of individual constituents. Transmission FTIR spectrometry has been used for determination of protein and fat in meat (Dion, 1994). A feasibility study on the application of MIR to determine the freshness and speciation in pork, chicken and turkey meats has been reported (Al-Jowder *et al*., 1997). The discrimination of raw chicken, pork and turkey meats using visible, near and mid-infrared spectroscopic techniques has also been studied (Rannou and Downey, 1997). Some workers (Al-Jowder *et al.*, 1999) reported that MIR spectroscopy is useful for a variety of different analysis of minced beef, ox kidney and ox liver. It is readily able to distinguish between the muscle and offal tissue types. Other authors (Yang and Irudayaraj, 2001) studied the characterization of beef and pork using Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS). They reported that FTIR-PAS technique can be used to analyse meat products nondestructively and without complicated sample preparation, compared with IR attenuated total reflection (ATR) spectra the obscuring effects of moisture were reduced in the PAS spectra. Sahilah *et al.* (2011), used PCR amplification of mitochondrial DNA for determining the authentication of raw meats.

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The aim of this study is the identification of the species type of meat, differentiation between them and the detection of pork in camel or buffalo mixtures meats using FTIR spectroscopy as a simple and rapid detection method.

Materials and Method

Samples preparation

All samples were purchased from local market and had the same cut from the animals. All the observable fatty parts of the samples were removed. The meats were minced and homogenized. The mixtures were prepared by adding an amount of pork meat in the range of 10-70% with either camel or buffalo meat and mixing well. All samples were mixed well and dried as a very thin layer in Petri dishes using phosphorous penta-oxide till constant weight. The dried samples were ground to very fine powder for FTIR spectroscopic studies.

FTIR measurement

The KBr disk method was used for FTIR spectroscopic studies of the samples. For each sample the spectra were recorded 3 times with 16 scan from 400 to 4000 cm^{-1} for each spectra. Fourier transform infrared spectrometer Jasco 430 interfaced to a personal computer operating under windows-based Jassco software was used to record the Fourier transform infrared spectra.

Form the FTIR spectra of the samples under investigation, the absorbances ratios of the bands at 1654 cm^{-1}, 1540 cm^{-1} and 1395 cm^{-1} to the band at 2924 cm^{-1} and that of the band at 1745 cm^{-1} to the band at 1240 cm^{-1} were calculated using the base line method. The deconvolution of FTIR spectra of the meats under study in the region 2000 cm^{-1}-1000 cm^{-1} were used for more characterization. The ratio of the area under the bands at 1464 cm^{-1} to that at 1448 cm^{-1} was measured for the samples under study.

Results and Discussion

FTIR spectra of control meats

FTIR spectra of the different meats and their deconvoluted spectra for the range 2000-1000 cm^{-1} are shown in Figure 1. Visual examination of the spectra (Figure 1A) revealed that all the spectra of the samples have the absorption peaks at 2924 (2928 for camel spectra), 1654, 1540 (1546 for sheep) 1456, 1395, 1306 (1308 for camel) 1240, 1170 (1165 for pork), 1117 (1113 for camel) cm^{-1}. A small band at 3009 cm^{-1} was observed in the spectrum of pork meat. This band is characteristic for CH of stretching

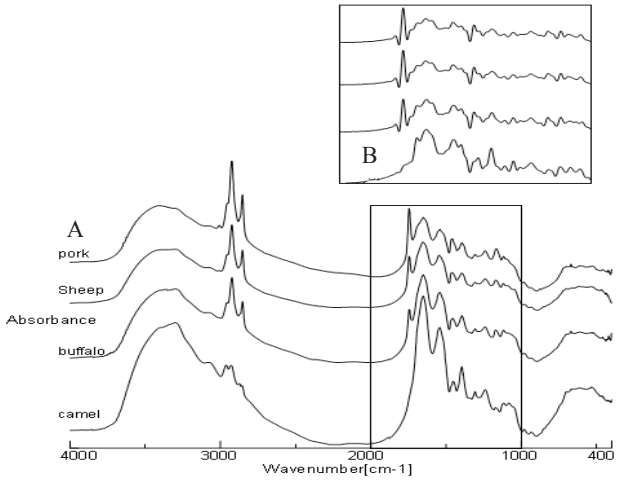

Figure 1. The raw and deconvoluted (for the region 2000-1000 cm^{-1}) FTIR spectra of meats

vibration mode of C=CH group. This may reflect that the pork meat contains a large amount of unsaturated fats.

The spectra of buffalo, sheep and pork exhibit absorption bands at 2853 cm^{-1} (C-H stretching of CH_3) and 1745 cm^{-1} (C=O of ester). These two absorption bands are diagnostic for lipids and are the most intense in case of pork spectrum then sheep and buffalo whereas the first one appears as a shoulder and the second one disappeared in case of camel spectrum. This result indicated that the camel meat has the lowest content of lipids and fatty acids esters. The major characteristic bands of protein at 1654 cm^{-1} and 1540 cm^{-1} are more intense in case of camel spectrum than that of the other spectra. These observations show that the protein content of meats under analysis decreases in the following consequence, camel > buffalo > sheep > pork meat. These results are confirmed with that obtained from literatures where it is reported that the protein content of camel, sheep and pork meats is 21.63%, 15.7% and 11.9% and that of lipid is 1.43%, 27.7% and 45.0% respectively (Babiker *et al*., 1990; Norman, 1978).

A brief summary of the structural assignment for the infrared absorption bands of meats as reported in the literatures is given below: The broad band in the region 3600-3200 cm^{-1} is assigned to O-H and N-H stretching vibration of water and amide. The absorption band at 3008 cm-1 is assigned to stretching vibration of =C-H (cis) of lipids. The two absorption bands at 2924 and 2853 cm^{-1} are assigned to the asymmetric and symmetric CH_2 and CH_3 stretching vibration of lipids, respectively. The band at 1745 cm^{-1} is assigned for C=O of esters of fatty acids. The bands at 1654 and 1545 cm^{-1} are assigned to the C=O stretching vibration (amid I) and N-H bending vibration (amid II), respectively. The band at 1467 cm^{-1} is assigned to C-H bending vibration (CH_2) of

lipids. The absorption bands at about 1450 and 1395 cm^{-1} are assigned to the asymmetric and symmetric C-H (CH_2) bending vibration of proteins. The band in near 1170-1154 cm^{-1} assigned to stretching vibration of C-O of proteins. The absorption bands at 1240 cm^{-1} and 1083 cm^{-1} are due to the antisymmetric (υ_{as} PO_2-) and symmetric (υ_s PO_2-) stretching vibrations of PO_2- respectively. PO_2- groups are present in both nucleic acids and phospholipids (Wilson and Kemsley, 1993; Hector and Henry, 1984, Manule *et al*., 1998, Parker, 1971 and 1983).

The absorbances ratios of the bands at 1654 cm^{-1} (C=O stretching of amide I) 1540 cm^{-1} (N-H bending of amide II) and 1395 cm^{-1} (C-H bending) to the band at 2924 cm^{-1} (C-H stretching) were calculated and are used as a measurement of the ratios of proteins to lipids contents of the samples under investigation. These ratios are represented as histogram (Figure 2). As can be seen from Figure 2 the pork meat has the lowest value for the three ratios of protein to lipid. These results mean that the pork meat has the highest lipid content as compared with the other meats samples. The lipids content of the other meats decrease in the following consequence, sheep, flowed by buffalo and then camel meat. This result confirms that is obtained from the visual examination of the FTIR spectra of these meats and that reported in literatures (Babiker *et al*., 1990; Norman, 1978).

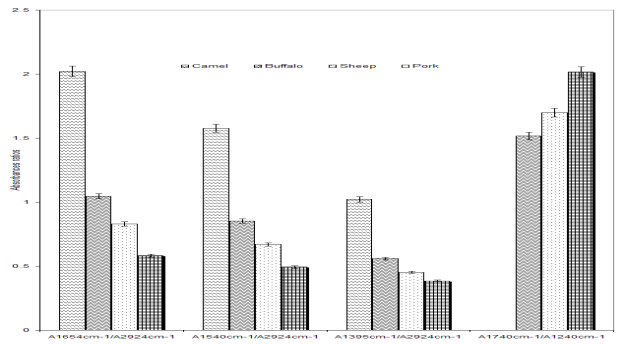

Figure 2. The absorbances ratios A1654cm^{-1}/A2924cm^{-1}, A1540cm^{-1}/A2924cm^{-1},A1395cm^{-1}/A2924$cm^{-1}$and A1740cm^{-1}/A1240cm^{-1} of camel, buffalo, sheep and pork meats

The absorption bands at 1240 cm^{-1} and 1083 cm^{-1} are due to the antisymmetric (υ_{as} PO_2^-) and symmetric (υ_s PO_2^-) stretching vibrations of PO_2^- respectively. PO_2- groups are present in both nucleic acids and phospholipids. However it was found that the ratio of the peak intensity between the υ_s C=O band at 1740 cm^{-1} and the υ_{as} PO_2^- band at 1240 cm^{-1} of phospholipids is in the rang of 1.9 -2.3 (Wong, 1991).

The absorbances ratio A 1745 cm^{-1} /A 1240 cm^{-1} of the samples under investigation was illustrated as histograms (Figure 2) . It appears from Figure 2 that the absorbances ratio A 1745 cm^{-1}/ A 1240 cm^{-1} for pork meat equals to 2.002 while it is about 1.516 and 1.698 for buffalo and sheep, respectively. This means that the absorption band at 1240 cm^{-1} in case of pork is associated with PO_2 groups of phospholipids, whereas it is represented by PO_2 groups in nucleic acids in case of buffalo and sheep meats. This result means that pork meat contains a large amount of phospholipids.

To evaluate the conformational proteins structure of amides, the amides bands were resolved into subpeaks through the deconvolution of the original spectra. The deconvolution of FTIR spectra of the meats under study in the region 2000 cm^{-1}-1000 cm^{-1} were used for more characterization and these are shown in Figure 1B.

It is observed from Figure 1B that the region of the band of amide I (1654 cm^{-1}) of camel meat spectrum (Figure 1A) is resolved into two strong subpeaks (Figure 1B) at 1693 cm^{-1} and 1653 cm^{-1} in addition to these subpeaks a third one is observed at 1630 cm^{-1} in the case of the other meats spectra (Figure 1B). In the region of the amide II band, two strong bands are observed at 1545 cm^{-1} and 1515 cm^{-1} in all deconvoluted spectra of the samples, whereas a band at 1575 cm^{-1} is noticed in buffalo and sheep meats spectra while a band at 1495 cm^{-1} is observed in that of pork meat.

The absorption bands at about 1690 cm^{-1} and 1650 cm^{-1} were assigned to turn and α helix protein structures respectively, while the band at 1630 cm^{-1} to β sheet protein structure (Byler and Susi, 1986) and those at about 1545 cm^{-1} and 1515 cm^{-1} to α helix protein structure (Kohei, 1992). The observed results indicated that the major proteins structure content of camel meat are α helix while buffalo, sheep and pork meats have a high content of this protein structure beside the β protein structure.

FTIR spectra of mixtures meats

The raw FTIR spectra for different concentrations of pork in camel and pork in buffalo the mixtures meats and their deconvoluted spectra over the region 1000 - 2000 cm^{-1} are shown in Figure 3. Visual examination of the raw spectra of pork in camel meat (Figure 3A) and pork in buffalo meat (Figure 3B) revealed that the appearance of a small band at about 3008 cm^{-1} in the spectra of 50% and 70% pork in camel meat and 30% pork in buffalo meat. This band is the most intense in control pork meat, which means that the unsaturated fats increased with addition of pork to either camel (pork concentration ≥50%) or buffalo meats (pork concentration ≥30%)

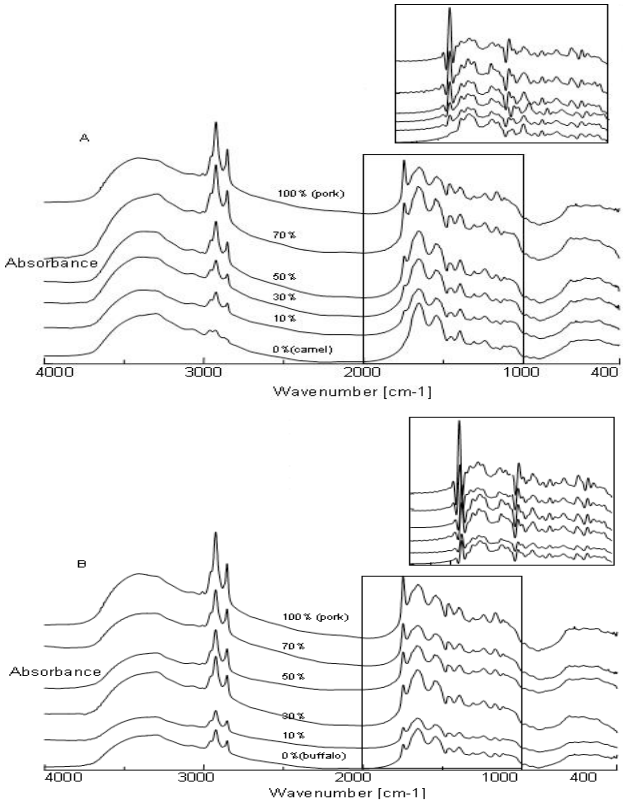

Figure 3. The raw and deconvoluted FTIR spectra of different concentrations of pork in camel (A) and buffalo (B) mixture meats

in high concentration.

Figure 3A shows that an absorption band at 2853 cm^{-1} (C-H stretching of CH_3) is observed in the spectrum of 10% pork in camel mixture. Also a shoulder at 1745 cm^{-1} (C=O of ester) is noticed in the spectra of concentrations 10% and 30% pork in camel meat mixtures and this shoulder becomes a band in the high concentrations of pork. The intensity of these two bands increases with increasing the pork concentration in camel meat. Comparing the two absorption bands at 1456 and 1395 cm^{-1} relative to each other it is found that the intensity of the absorption band at 1456 cm^{-1} is lower than that of the band at 1395 cm^{-1} in case of control camel meat spectrum and the intensity of this band increases with increasing the pork content in the mixtures till become higher than the band at 1395 cm^{-1} in the 70% pork concentration and control pork meat spectra. These results indicate that the lipid content of the mixtures increases with increasing of the pork content in the mixtures.

From Figure 3B it can be seen that the intensity of the absorption band at 1745 cm^{-1} with respect to that of the characteristic bands of protein at 1654 cm^{-1} and 1540 cm^{-1} increases with increasing pork content in buffalo meat till became the highest in 70% and the control pork spectra. In the spectra of the control

Table 1. The absorbances ratios A1654 cm^{-1}/A2924 cm^{-1} and A1540 cm^{-1}/A2924 cm^{-1} of the different concentrations of pork in camel and pork in buffalo mixtures meats

% of pork	A1654cm-1/A2924cm-1		A1540cm-1/A2924cm-1	
	camel	buffalo	camel	buffalo
0	2.01	1.0765	1.5656	0.8649
10	1.5321	0.9334	1.3027	0.755
30	1.1916	0.8607	0.9749	0.703
50	0.9985	0.7654	0.8230	0.6173
70	0.7715	0.6746	0.6366	0.5675
100	0.5933	0.5933	0.4964	0.4964

buffalo meat and pork concentration 10% and 30% the absorption band at 1456 cm^{-1} appears lower than that at 1395 cm^{-1}, while the intensity of this band is higher than that of the second one in 70% pork in buffalo and the control pork meats. These results reveal that the lipid content increases with increasing the percentage of pork in buffalo meat mixtures.

Table 1 shows the absorbances ratios of the bands at 1654 cm^{-1} (C=O stretching of amide I) and 1540 cm^{-1} (N-H bending of amide II) to the band at 2924 cm^{-1} (C-H stretching) of different concentrations of pork in camel and pork in buffalo meats. From Table 1 it is found that these ratios for the two groups decrease with increasing the percentage of pork in either of them. These results indicated that the addition of pork meat to either camel or buffalo meat decreases the proteins content of the mixtures.

The deconvolution of FTIR spectra in the region 2000 cm^{-1}-1000 cm^{-1} of the samples under investigation were studied. The deconvoluted FTIR spectra of different concentrations of pork in camel and pork in buffalo meats are located in the upper rectangular on right side over their raw spectra in Figure 3. A peak at 1746 cm^{-1} (C=O of lipids) is observed in the deconvoluted spectra of pork in camel mixtures (whatever the percentage of pork content in the mixture) and control pork meats (100%) as compared to the deconvoluted spectrum of the control camel meat (0%) in which this band is absent. The absorbance of this band increases with increasing the percentage of the pork in the mixtures till reach its maximum in the spectrum of control pork. The deconvoluted the control camel spectra has a shoulder at about 1464 cm^{-1} (δ CH_2 of lipid), whereas it is observed as a band in the other spectra as the amount of pork content in camel meat increases. In case of the deconvoluted spectra of pork in buffalo, the intensity of the absorbance band at 1446 cm^{-1} with respect to that at 1448 cm^{-1} increases with increasing the pork content in the buffalo meat mixtures. All the above results show that the addition of pork either to camel or buffalo meats causes an

increase in the lipid content of the mixtures.

The ratio of the area under the bands was measured for the absorption bands at 1464 cm^{-1} (δ CH_2 of lipid) to that at 1448 cm^{-1} (δ CH_3 of protein) for the samples under study. Figure 4 represents the relationship between the ratio of the area under the bands 1464 cm^{-1}/1448 cm^{-1} of the samples and the different percentage of pork in camel and pork in buffalo meats. From Figure 4 it is found that the ratio for the samples of camel group has the same trend for that of buffalo group as it is increase as the pork content in the mixtures increases. This relationship has a correlation coefficient r^2 = 0.9174 and 0.942 for pork in camel and in buffalo, respectively. These results indicate that the addition of pork meat with any amount to either camel or buffalo meats results in an increase in the lipid content of the mixtures. These results is confirmed with that obtained form other ratios in this study.

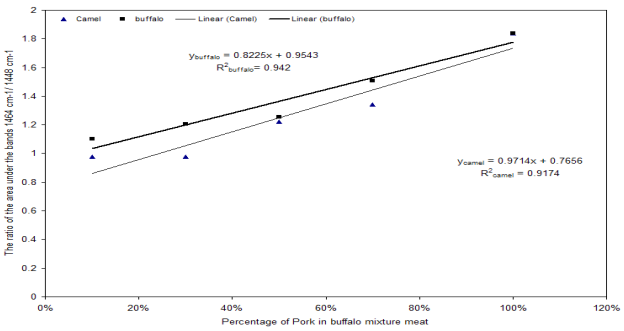

Figure 4. The relationship between the ratio of the area under the bands 1464 cm^{-1} /1448 cm^{-1} for the different percentage of pork in camel and in buffalo mixtures meats

Conclusion

It can be concluded that the FTIR spectra (4000 cm^{-1} - 400 cm^{-1}) of camel, buffalo, sheep and pork meat have strong and well separated bands arising from fat and protein. The spectrum of camel meat is the most different one among the others. Pork meat spectrum has the highest strong bands characterization for lipid relative to that of protein this referred to the highest amount of its lipid content. The conformation structure of protein in meats can be determined using the deconvolution of the original FTIR spectra. FTIR spectroscopy can be characterized the changes in camel or buffalo meats as a result of adding pork meat in different concentrations to either of them. The characterization can be made directly by visual examination for example the intensity of the absorption bands of lipid relative to that of protein or by determining the relative content of the proteins to lipids in the samples with taking the absorbances ratios or the ratio of the area under the bands characteristic of these components. This study showed that the FTIR spectroscopy can be used as a simple and a rapid potential tool to give an important information for a quick determination of relative content of protein to lipid and the conformation structure of proteins content of meat from different species and mixtures of two different types of meats.

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