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Interactions of Bovine Muscle Tissue with 2450 MHz Microwaves Studied in the Mid-Infrared Region

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The effects of microwaves at 2450 MHz at 600 W on bovine muscle tissue have been studied by means of Fourier transform infrared spectroscopy. Spectral analysis in the amide I region after microwave cooking showed that an increase in intensity occurred in the region around 1665 and 1695 cm^{-1} , that can be attributed to β -turns and β -sheet features, respectively. This result characterized disorder processes in the protein. In addition, CH_2 methylene and carbonyl band vibrations of samples under exposure to microwave heating appeared lower than vibrations of samples heated by conventional oven. This result demonstrated that the Maillard reaction occurs partially after cooking by microwave oven.

Keywords: Microwave oven, FTIR spectroscopy, Mid-infrared region, Amide I, Bovine meat, Maillard reaction.

INTRODUCTION

Physical and chemical reactions occur during food preparation as a result of the interaction between food components and environmental conditions like heat, light, air, and materials that are used during cooking process. Nutritional properties of meat products depend heavily on the method of cooking, determining its compositional attributes such as appearance, flavor, juiciness, fat, and texture of meat products.^[1] Meat proteins denature producing structural changes in the meat, such as the destruction of cell membranes, shrinkage of meat fibers, and the aggregation and formation of myofibrillar.^[2]

Cooking in a conventional oven consists of the physical processes of heat transfer by radiation from the source of heat to the metal wall at the base of the oven, by conduction from the base to the other walls and by convection through the heated air currents set up in the oven to the food at temperatures ranging from 120 to 240°C. In particular, the properties of meat depend upon the methods of cooking in a conventional oven. For instance, conventional cooking of meat in a oven can be done preheating the oven and maintaining the same one temperature or starting with a high temperature and then reducing to a lower temperature^[3,4] or cooking meat at low temperature and successively at high temperature for a short time.^[5]

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The use of the microwave-oven for cooking began during the 1970s as a result of Japanese technology transfer and global marketing,^[6] using microwave energy around 2450 MHz emitted by a power source, the magnetron, that is absorbed by the food and it is cooked in the microwave oven. The electromagnetic field (EMF) generated inside the microwave oven produces rotation and collision of polar molecules inside the food. Water molecules are the main molecular dipoles inside the food together with ionic compounds that rotate rapidly in food (about 2450 million times a second) following the frequency of microwaves, colliding with other molecules and disrupting hydrogen bonds with water generating frictions and heat, because water and salt are the two major ingredients that influence dielectric properties of food.^[7]

This mechanism produces heating of foods, whereas conventional heating in an oven transfers thermal energy from the food surface toward its center much slower, because the low conductivity of food materials.

Although microwave-oven is widely used as a means of food preparation, insufficient information is available on the consequences of microwave heating on the composition and nutritional quality of the food. Traditional thermal processing techniques can be beneficial to foods in such areas as preservation and flavor formation but detrimental in damaging other sensory and nutritional properties. Otherwise, some studies revealed that meat cooked in a microwave-oven can provide a product comparable to oven preparation.^[8] In contrast, other authors reported that the aroma and flavor of hot-air oven cooked products are better and more acceptable as compared to microwave oven cooked products.^[9] Furthermore, some authors reported that microwave heating affect fat oxidation and fatty acid isomer formations,^[10–12] and that the effects of heat processing on protein value, utilization, and availability are equivocal.^[13] The aim of this study was to investigate the effects of microwave-oven cooking on meat of bovine muscle tissue using Fourier transform infrared (FTIR) spectroscopy. Muscle tissue properties have been described in other articles.^[14–16] FTIR spectroscopy has just been used in studies regarding the analysis of components of milk, fat, meats protein, and carbohydrates.^[17–20]

MATERIALS AND METHODS

Meat Samples

Bovine meats from various muscle tissues were collected from five different commercial processing plants, packed on ice, transported to the laboratory, and maintained at the temperature of 4°C before treatment. Meat samples were minced using a plate with 6 mm holes to obtain a more uniform cooking inside the meat samples. Also, the minced meat was divided into portions of 150 g each with a thickness of about 5 mm to allow a quick cooking in the ovens. Meat samples were cooked immediately without any additional treatment.

Experimental Design

A set of different meat samples, prepared as explained above, was inserted in a conventional electric oven Indesit Mod. KG 8414 XES/I. Cooking began with preheating the oven at the temperature of 180°C; meat was cooked for 15 min at this temperature and a thermocouple was used for temperature control. Other meat samples were subjected to heating in a domestic microwave oven model Whirlpool AVM 541/WP/WH. Measurements of the power density emitted during microwave oven cooking were performed using a Narda SRM 3000. Two peaks around 2435 and 2480 MHz appeared in the spectrum analysis during microwave oven cooking (see Fig. 1). The integrated value of the power density in the range 2350–2550 MHz amounted to 227 mW/m². A set of samples were heated in the same microwave oven at the power level of 600 W for

SRM-TS Remote & Data Analysis

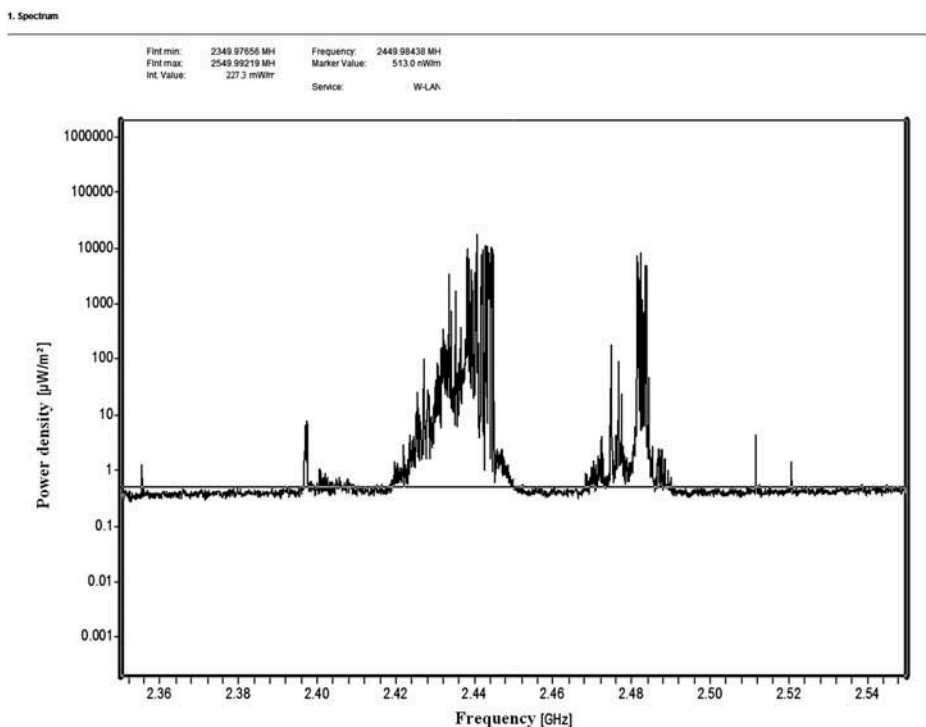


FIGURE 1 The spectrum analysis performed by a Narda SRM 3000 in the range of 2350–2550 MHz of the power density levels related to microwaves generated by a microwave oven Whirlpool Model AVM 541/WP/WH during bovine meat cooking, acquired at 30 cm from the door of the oven.

180 s. After cooking meat samples were removed from the ovens, homogenized, stored in sterile jars, and immediately subjected to the following assays.

Infrared (IR) Spectroscopy

FTIR absorption spectra were recorded at room temperature of 20°C by a spectrometer Vertex 80v of Bruker Optics. Meat samples of 12 mg (cooked as above exposed) were placed between a pair of CaF₂ windows. For each spectrum 64 interferograms were collected and co-added by Fourier transformed employing a Happ-Genzel apodization function to generate a spectrum with a spectral resolution of 4 cm⁻¹ in the range from 7000 to 1000 cm⁻¹. Each measure was performed under vacuum to eliminated minor spectral contributions due to residual water vapor. However, smoothing correction for atmospheric water background was performed and IR spectra were baseline corrected and area normalized. In addition, vector normalization was used, calculating the average value of the spectrum and subtracting from the spectrum decreasing the mid-spectrum. The sum of the squares, of all values, was calculated and the spectrum was divided by the square root of this sum.

The automatic baseline scattering correction function was used to subtract baselines from spectra, which allows to get spectra with band edges of up to the theoretical baseline.

Interactive baseline rubberband correction was used. This method also uses a rubberband which is stretched from one spectrum end to the other, and the band is pressed onto the spectrum from the bottom up with varying intensity. This method performs iteratively, depending on the number of iterations in the algorithm and the baseline as a frequency polygon consisting of n baseline points. The result spectrum will be the original spectrum less the baselines points manually set and a subsequent concave rubberband correction. We used the default value of $n = 64$ baseline points and 50 iterations. FTIR spectra were smoothed by Loess algorithm and the deconvolved spectra, fitted with Gaussian band profiles. Initial values for the peak heights and widths were estimated from the deconvolved spectra. In order to enhance the fine spectral structure, the Fourier self-deconvolution (FSD) technique was used. The concept of FSD is based on the assumption that a spectrum of single narrow bands is broadened in the liquid or solid state and cannot be distinguished in the amide envelope. A curve-fitting procedure can be applied to estimate quantitatively the area of each component representing a type of secondary structure.^[21]

RESULTS

Similar FTIR spectra were obtained using different samples from various bovine muscle tissues. In order to enhance the fine spectral structure of amide I region, the FSD technique was applied using a Lorentzian shape, with bandwidth = 11.45, deconvolution factor = 2, noise reduction factor = 0.5.

Two typical FTIR spectra in the range 1750–1500 cm^{-1} are shown in Fig. 2a, where the spectra of samples cooked in a conventional electric oven at the temperature of 180°C and in a microwave oven at the power of 600 W are represented in blue and red color, respectively. Comparison of the spectra reveals that the major changes upon conventional heat treatment occurred at 1742 cm^{-1} , where a broad band can be observed in the spectrum from meat cooked in the conventional oven, whereas this band from meat samples cooked in the microwave oven appeared to decrease intensity. This band can be assigned to the C=O mode of the first alkyl chain with a trans-conformation in the C–C bond adjacent to the ester grouping, representative of triglycerides content.^[22]

Two intense bands in the bovine meat spectrum are the amide I band, centered at 1648 cm^{-1} , which corresponds to an α -helix structure content due to C=O stretching vibration and a N–H bending mode, and the amide II, coupling of the N–H bending, and C–N stretching modes. These bands are related to proteins content, whose most representative component in meat is myoglobin. Indeed, it is an iron-oxygen-binding protein found in the muscle tissue of vertebrates which represents their myofiber. The analysis of the spectra represented in Fig. 2a showed that microwave oven cooking altered the amide I region. Previous literature showed that alterations of proteins secondary structure occur after exposure to mobile phone microwaves.^[23–27]

An increase in intensity around 1665 cm^{-1} (indicated by arrow in Fig. 2a) can be observed in the spectrum of bovine meat sample heated in microwave oven, that can be attributed to β -turns, characteristic of disorder processes in the protein.^[28,29] A relative increase of β -sheet component at 1695 cm^{-1} with respect to the α -helix structure was observed after microwave heating in comparison to conventional heating (see Fig. 2a). These results were significant ($p < 0.01$) as reported in Table 1. In addition, a shift of about 1.5 cm^{-1} toward lower frequency of the β -sheet content at 1635 cm^{-1} was observed comparing the spectra of samples heated by microwave oven and by conventional oven.

The shift toward lower wavenumbers after microwave heating and the increase in intensity of β -sheet and β -turns features can be attributed to an increasing transition dipole coupling due to a

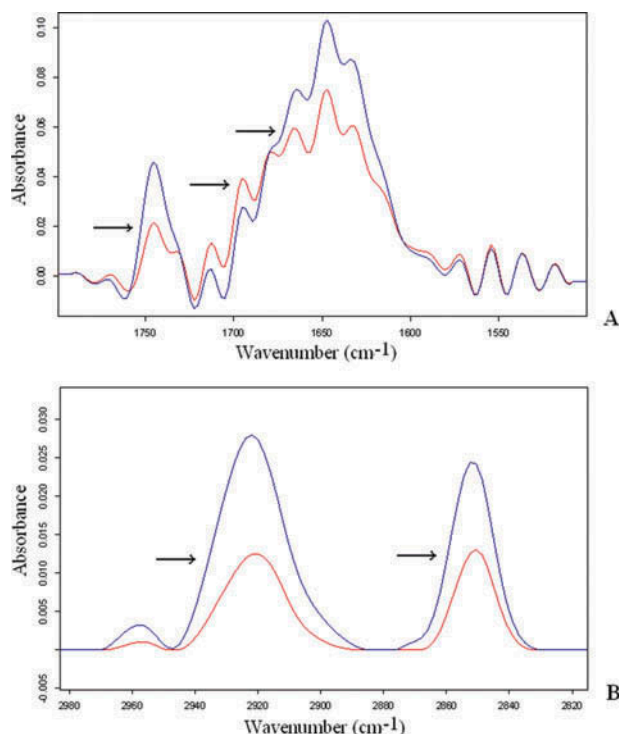


FIGURE 2 A: Two representative FTIR spectra in the range 1750–1500 cm^{-1} from muscle tissue of bovine meat cooked in a conventional electric oven at the temperature of 180°C and in a microwave oven at the power of 600 W, in blue and red colors, respectively. The decrease of the carbonyl band intensity at 1742 cm^{-1} occurred after microwave oven cooking. Fourier self-deconvolution analysis evidenced a relevant increase in intensity of β -turns content at 1665 cm^{-1} in Amide I, and a significant increase of β -sheet component at 1695 cm^{-1} with respect to the α -helix structure, comparing microwave oven and conventional oven cooking spectra; B: Representative spectra in the infrared region from 3000 to 2800 cm^{-1} . The band close to 2960 cm^{-1} originate from the asymmetric stretching $\nu_{\text{as}}\text{CH}_3$ of methyl groups, and the vibration bands at 2921 and 2853 cm^{-1} are assigned to symmetric and asymmetric bending $\nu_{\text{s}}\text{CH}_2$ and $\nu_{\text{as}}\text{CH}_2$ of the methylene group, respectively. The intensities of both bands of the methylene group were observed to decrease significantly after microwave oven cooking. The spectrum in red color corresponds to microwave cooking of bovine meat.

higher content in aggregated β -sheets structures. Such an increase was explained for thermally aggregated proteins indicating intermolecular β -sheet structure with very strong hydrogen bonds in comparison to that observed from β -sheets in native proteins.^[30]

The other intense vibrations in the IR spectrum of bovine meat are the CH_2 stretching vibrations appearing in the region from 3000 to 2800 cm^{-1} region (see Fig. 2b). In this region symmetric and asymmetric CH_2 and CH_3 stretching vibrations are observed, assigned to methylene and methyl group. The band close to 2960 originate from the asymmetric stretching $\nu_{\text{as}}\text{CH}_3$ of methyl groups, whereas vibration bands around 2921 and 2853 cm^{-1} are assigned to symmetric and asymmetric bending $\nu_{\text{s}}\text{CH}_2$ and $\nu_{\text{as}}\text{CH}_2$ of methylene, respectively.^[22,31] Both bands of the methylene group were clearly observed in spectra after cooking using the conventional electric oven, whereas these bands decreased in intensity in samples heated by microwave oven.

TABLE 1
Average integrated area ratios of bovine meat heated by conventional oven and by microwave oven. Each value reported represents the mean \pm SEM of 18 samples

Vibration band (cm^{-1})	Integrated area ratios of conventional oven cooking and microwave oven cooking
2921	1.95 \pm 0.08**
2853	2.00 \pm 0.09**
1742	2.03 \pm 0.08**
1695	0.62 \pm 0.06**
1665	0.59 \pm 0.06**
1648	0.97 \pm 0.07
1635	0.88 \pm 0.06*

Significant differences are pointed out (** $p < 0.01$; * $p < 0.05$).

Statistical Analysis

A statistical analysis (t -test) was applied to the integrated area of the vibration bands of amide I region and methylene group using 18 samples, considering significant the differences from control with $p < 0.05$. The vibration integrated area ratios of samples heated by conventional oven and by microwave oven have been calculated and expressed as mean \pm standard error of the mean (SEM) and reported in Table 1.

DISCUSSION

The increase in intensity of the carbonyl band at 1742 cm^{-1} and of the methylene group after meat cooking in the conventional oven can be attributed to Maillard reaction. This reaction is one of the important consequences of the thermal processing of foods represented by a series of reactions which are initiated by the interaction between the carbonyl group of a reducing sugar and a free amino group of an aminoacid or a protein, producing the formation of brown pigments and numerous compounds responsible for browning, texture, and flavor during baking and roasting by means of complex reactions.^[32]

Maillard reaction follows the formation of the initial intermediates, including Amadori and Heyn's products among which there are CH_2 and $\text{C}=\text{O}$ compounds,^[33,34] explaining the relevant presence of their vibration bands in the spectra of meat sample cooked in the conventional oven. Conversely, the symmetric and asymmetric CH_2 vibrations and the $\text{C}=\text{O}$ carbonyl band at 1742 cm^{-1} were lower in intensity after meat cooking by microwave oven, showing that the Maillard reaction occurs partially using this type of cooking. Otherwise, this result is in agreement with the hypothesis that lipid oxidation occurs during microwave cooking, a result that was evidenced by other authors^[10-12] which showed that heating of oil samples in microwave oven enhances lipid oxidation. Also this result was confirmed by Herzallah^[35] who observed that microwave heating milk causes a significant increase in the level of the cholesterol oxidation products.

The results that the symmetric and asymmetric CH_2 vibrations and the $\text{C}=\text{O}$ carbonyl band at 1742 cm^{-1} after microwave oven heating were less intense than after conventional heating, can be considered a proof that the Maillard reaction does not occur completely after cooking in a microwave oven. Moreover, previous literature showed the higher the temperature, the greater the Maillard browning reaction.^[36] Therefore, spectroscopic results reported, highlighted above, that the temperature of the ground beef during cooking with the microwave oven was less than that

induced by cooking with the conventional oven at 180°C. Indeed, direct measurement of the temperature inside the food during cooking with a microwave oven cannot be carried out by using conventional methods, since either a thermocouple or a mercury thermometer would interact with microwaves altering the measure.

As regards the result concerning the increase in intensity of the β -sheet to α -helix ratio and the shift toward lower energy, that can be associated to protein aggregation processes more relevant in comparison with conventional heating, it can produce low access to intestinal digestive enzymes, resulting in low protein value. Indeed, if tissue contain the same protein content, their nutritive value may be different if their α -helix and β -sheet ratios in the protein secondary structures are different. Furthermore, the broad band around 1650 cm^{-1} assigned to α -helical structures, shows reduced band intensity upon increasing microwaves exposure indicating that the content in α -helical structures is reduced during microwave oven cooking.

Furthermore, regarding a relative increase in β -sheet structure which resulted after microwave heating of bovine meat, it was shown that proteins nutritive effectiveness and digestive behavior can be influenced by their α -helix and β -sheet ratios. High β -sheet to α -helix ratio can induce low access to gastrointestinal digestive enzymes, resulting in a low protein value and availability.^[37–39] This result could lead to a potential impact in carcinogenesis. Indeed, previous research demonstrated a correlation between meat processing and colon carcinogenesis.^[40]

CONCLUSIONS

The effects of microwaves at 2450 MHz on muscle tissue of bovine meat were studied in the mid-IR region by FTIR spectroscopy comparing the effects of microwave oven cooking at 600 W for 180 s with heating in a conventional electric oven at the temperature of 180°C for 15 min. Increases in intensity of the carbonyl band at 1742 cm^{-1} and of the methylene group at 1921 and 1853 cm^{-1} after meat cooking were observed, that can be attributed to the Maillard reaction. These effects were reduced using microwave oven with respect to conventional oven cooking, showing that Maillard reaction occurs partially using microwave oven. Microwave cooking at 600 W for 180 s produced an increase in intensity of the vibration band around 1665 cm^{-1} , attributed to β -turns that is characteristic of disorder processes in the protein. Also, a significant increase in intensity of β -sheet component at 1695 cm^{-1} occurred after exposure to microwaves, that can be attributed to aggregation of β -sheets structures. This result demonstrated that aggregation processes in protein's secondary structure of bovine muscle tissue occur after heating using microwave oven much more than in bovine meat cooked using conventional oven.

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