DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date: June 15, 2005

From: Division of Product Manufacture and Use, HFS-245

Subject: FAP 9M4697: Use of Ionizing Radiation to Reduce Microorganisms in Raw and Processed Food Including Multiple Ingredient Food Products

To: Division of Biotechnology and Gras Notification Review, HFS-255
Attn.: L. Highbarger Ph.D.

INTRODUCTION:

The National Food Processors Association (NFPA), on behalf of the Food Irradiation Coalition (FIC), is petitioning to amend 21 CFR 179.26 to include the use of ionizing radiation for the control of food-borne pathogens and infectious protozoa, and extension of shelf-life, in a variety of human foods. The NFPA asserts that ionizing radiation is a safe and effective technology for the control of food-borne pathogens in a variety of human foods. CFSAN microbiologists are evaluating the microbiological aspects associated with the radiation treatment of the foods requested in this petition.

During the review process for this petition, the Agency received an objection to the petition stating that irradiation treatment of food will lead to an increase in trans fat in the diet. This objection referenced an article by Brito et al. (Brito, M.S.; Lucia. A.; Villavicencio, C.H.; Mancini-filho, J. "Effects of irradiation on trans fatty acids formation in ground beef." Rad. Phys. Chem. 63, 337-340, 2002) that stated that there was in increase in the amount of trans fat found in irradiated ground beef. The Agency had several experts in trans fat analysis review this article and it was found to be deficient in several areas. Because of this conclusion, the Agency did not believe that the results and conclusions stated in this article were accurate. To verify this determination, the Agency investigated the unsubstantiated claim that radiation treatment of ground beef can increase the amount of trans fat present in food.

Several questions about the validity of the data and hence the conclusions of the above referenced article include, the irradiation procedures which were not consistent with good manufacturing practices, the gas chromatographic procedure, which was not described in sufficient detail and did not follow validated procedures for the analysis of trans fat, and the statistical analysis had many inconsistencies and flaws.

Several trans fatty acids occur naturally in the fatty tissue of beef and their distribution has been shown to be seasonal and dependent on the diet of the cattle (Wolff, R.L., J. Am. Oil Chem. Soc., 71, 277-283, 1994 and Wolff, R.L., J. Am. Oil Chem. Soc., 72, 259-272, 1995). It has been suggested that red meat could be a major natural dietary source of trans-18:1 fatty acids and that in the U.K., the daily consumption of trans isomers from meat would be approximately 1.5 g/person (Gurr, M.I., Bull. Int. Dairy Fed., 166, 5-18, 1983). For beef tissue trans-18:1 fatty acids generally tend to be present in higher amounts in adipose tissue (5-6%) than in muscles (2-3%). However, the distribution profiles of individual trans-18:1 isomers in meat lipids and tallow are practically identical, with vaccenic acid accounting for approximately one-half of the total isomers (Wolff, R.L., et al., in "Trans Fatty Acids in Human Nutrition." J.L. Sebedio and W.W. Christie, Eds., The Oily Press, 1998, pp. 1-33).

The analysis of trans fatty acids is not an easy task. Even with the longest and most efficient capillary columns (50-100 m columns coated with highly polar cyanoalkyl polysiloxane phases), a complete separation of trans- from cis-18:1 isomers is not achieved. Under the best conditions, chromatograms obtained with these columns show trans-18:1 isomers emerging under a peak that precedes that of oleic acid. Only unresolved peaks, corresponding to individual trans isomers, are distinguishable with poor resolution, which makes quantification questionable. Results obtained by GC alone, such as those reported in the reference cited in the objection letter, should be considered with caution.

Several reliable analytical methods are available for the determination of trans fatty acids including recognized Official Methods (AOCS Press, Mossoba et al., 2003). The procedures for trans fatty acid analyses are based on techniques generally used in the lipid field, namely, capillary gas chromatography (GC), thin-layer chromatography impregnated with silver-nitrate (AgNO3-TLC), silver-ion liquid chromatography (Ag-LC), Fourier transform infrared spectroscopy (FTIR), and mass spectroscopy (MS). These techniques, when used in combination, are more than adequate for full characterization of isomeric trans fatty acids, including complete identification and quantitation. The choice of one technique or combination of techniques over another depends on several factors, for example, the complexity of the sample, sample amount, and the purpose of the analysis.

EXPERIMENTAL RESULTS:

To investigate the possible increase in the levels of trans fat in ground beef after radiation treatment, the Division purchased fresh ground beef at a local grocery store. The ground beef was repackaged in smaller size packages and irradiated at source temperature (app. 30 °C), as was done in the article of question, and frozen on dry ice. Under good manufacturing practices the ground beef will be irradiated either fresh, at source temperature, about 30 °C, refrigerated at about 5 °C, or frozen.

The analysis of trans fat in the untreated and the irradiated ground beef was performed using "Official Methods for the Determination of Trans Fat" (AOCS Press, Mossoba et al., 2003). Two methods were used, the first was a GC/MS method to analyze the individual fatty acids after conversion to their methyl esters, and the second method involved the analysis of the extracted fat by FTIR spectroscopy.

Capillary GC coupled with flame ionization detection (FID) is the most convenient and inexpensive analytical tool for the determination of fatty acids. The fatty acids are always determined as their methyl esters (FAMEs) and are very easily prepared by transesterification. The use of mass spectroscopy (MS) as the detection method instead of FID provides the scientist with a more reliable identification of the fatty acid compared to only using the retention time as is the case with FID. The mass spectral characterization provides a unique identification of the analyte and does not rely on the need for unique standards for each analyte; however, not every laboratory has this detection technique. Gas chromatography coupled with mass spectroscopy is the most convenient method for the determination of the double-bond position in isomeric fatty acids, as 4,4-dimethoxyoxazoline (DMOX) derivatives.

The GC region between 18:0 and 18:2n-6 shows a complex mixture of many overlapping trans-18-1, cis/trans-18:2isomers and saturated fatty acids which at best can only be partially resolved using a highly polar 100 m capillary column such as CP-Sil 88. Three factors greatly influence the quality of the separation of the FAMEs in this region; the temperature program used, the sample load, and the relative abundance of the FAMEs. When the 18:1 region is analyzed, the sample load should be reduced sufficiently to give a partial resolution of the unresolved 13t-/14t-18:1 peaks from the major oleic acid (9c-18:1) peak. However, reducing the sample load to that extent often makes identification and quantitation of minor FAMEs more difficult. Therefore, to identify all of the FAMEs with confidence it is advisable to analyze the samples at two sample loads. If the amounts of the minor fatty acids in the 18:1 region are much less than that for oleic acid, the analysis of all of the FAMEs by GC is not possible. Due to the large amount of oleic acid, in comparison to the trans-18:1 fatty acids, it has been suggested that the trans- and cis- fatty acids should first be separated from one another using Ag-LC (AOCS Recommended Practice Ce 1g-96 "Trans fatty acids by silver-ion exchange HPLC".). The trans-18:1 fatty acids can then be analyzed by GC separately from the cis- fatty acids. This separation was not performed during the course of these experiments since it was determined by FTIR (see below) that there was no increase in trans fat after treatment with ionizing radiation.

The results obtained in the Divisions laboratories for the determination of trans fats in control and irradiated ground beef using the GC procedure with mass spectral detection were inconclusive. The amount of oleic acid present in the fat extracted from these samples exceeded that for the minor trans fats such that it was not possible to resolve the trans-18:1 fatty acids from the oleic acid. Dilution of the sample resulted in trans FAME peaks that were undistinguishable from the baseline noise. We were however able to identify the trans FAMEs in the 18:2 region of the chromatogram (see figure 1). It can be seen from these data that there was no increase in the amount of trans-18:2 found in the ground beef even after a radiation dose of 10 kGy. The same results were obtained for the ground beef whether it was irradiated at source temperature, refirgerated or frozen.

Because of the interest in accurate and rapid analytical methods for quantifying total trans fatty acids with isolated double bonds, many infrared spectroscopic procedures and official methods have been developed. The determination of total trans fatty acids by different IR spectroscopic procedures is based on the C-H out-of-plane deformation band observed at 966 cm^{-1} that is uniquely characteristic of isolated double bonds with trans configuration. These double bonds are found primarily in trans-monoenes, and usually at much lower levels in minor hydrogenation products. This IR methodology has been extensively used in the fats and oils industry and found to be extremely useful to determine the triacylglycerols or fatty acid methyl esters. However, samples consisting of free fatty acids must first be esterified, particularly at low trans levels (less than 15%). Transmission and internal reflection FTIR succeeded in improving the accuracy of this determination.

Using internal reflection, also known as attenuated total reflection (ATR), an official method was developed to rapidly measure the 966 cm^{-1} trans band as a symmetric feature on a horizontal baseline. The experimental aspects of the ATR infrared official method are far less complex than those involving transmission measurements. This approach uses "ratioing" the trans test sample single-beam spectrum against that of a reference material consisting of a trans-free oil and applies the ATR sampling technique to melted fats. This avoids the need for weighing test portions and their quantitative dilution with the volatile CS_2 solvent.

The results obtained in the Divisions laboratories for the determination of trans fats in control and irradiated ground beef using the FTIR procedure provided further evidence that the irradiation treatment of ground beef, even up to a dose of 10 kGy, did not increase the amount of trans fat that is present in the ground beef (see figure 2). The same results were obtained for the ground beef whether it was irradiated at source temperature, refrigerated or frozen.

CONCLUSIONS:

From these experiments, the Division of Chemistry Research and Environmental Review concludes that the radiation treatment of ground beef will not result in an increase in the amount of trans fat that is present in the untreated ground beef.

Kim M. Morehouse, Ph.D.

HFS-245 (Diachenko, Perfitti, file); HFS-255; HFS-265 (Chen)
HFS-245 (KMorehouse)
File: 9M4697_trans

Figure 1: Gas Chromatograms for the fatty acid methyl esters, showing the relevant region of the chromatogram, displaying the total ion current from the mass spectrometer on the y axis (Agilent 6890 gas chromatograph with a 5973N mass selective detector). The top trace is for a fatty acid methyl ester standard which contains trans-18:1 and trans, trans-18:2. The middle trace is for non-irradiated ground beef. The lower trace is for ground beef that was irradiated at source temperature.

Figure 2: Fourier-transform infrared spectral traces for the fat portion of non-irradiated ground beef, and ground beef irradiated at 2, 4, 6 and 8 kGy, irradiated at source temperature, ratioed against air. The peak at 970 wavenumbers is associated with the C-H out-of-plane deformation band that is uniquely characteristic of isolated double bonds with trans configuration.

Figure 3: Fourier-transform infrared spectral traces for the fat portion of ground beef irradiated at 2, 4, 6 and 8 kGy, irradiated at source temperature, ratioed against the fat from non-irradiated ground beef.

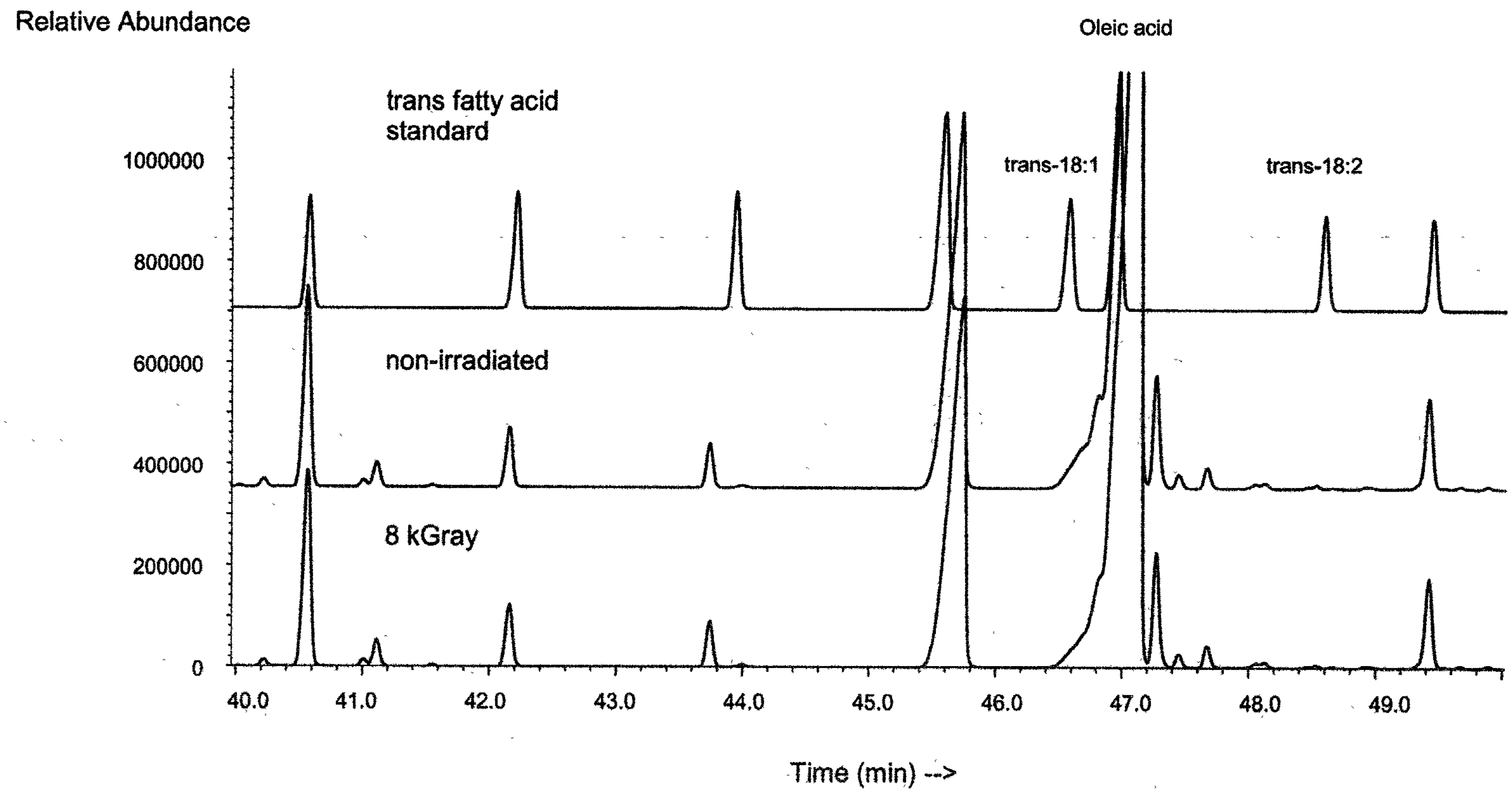
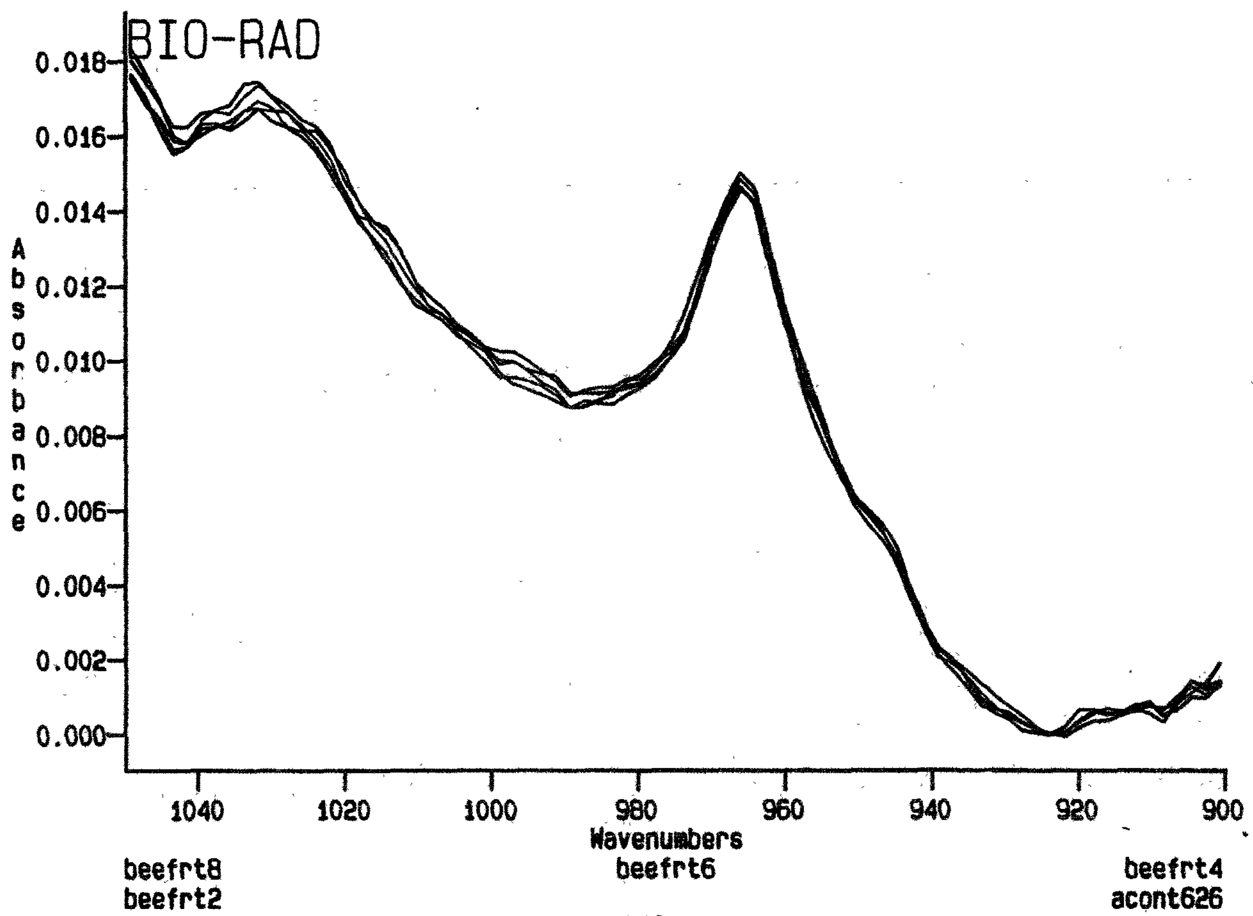
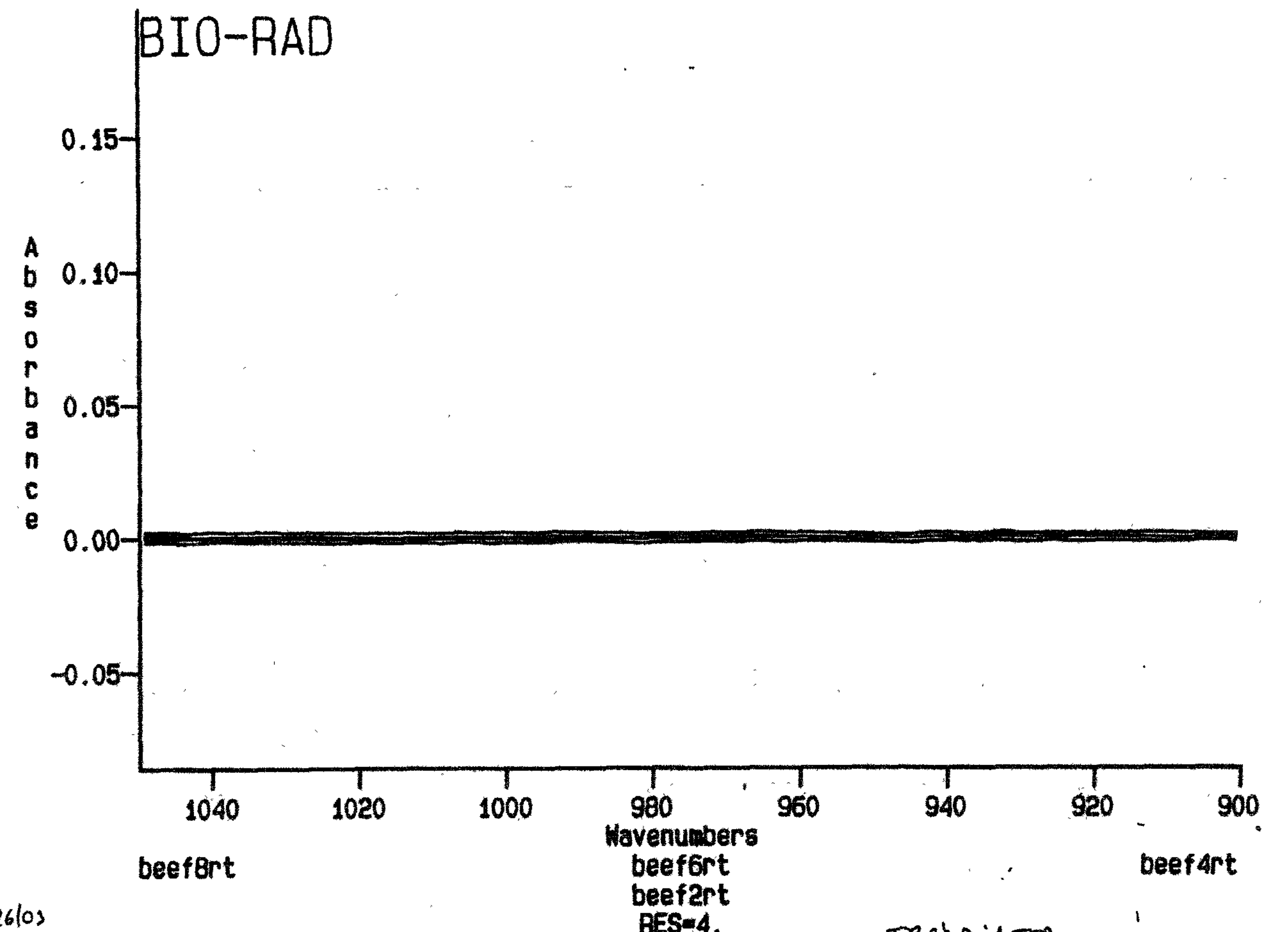

Relative Abundance
Oleic acid
trans fatty acid standard
1000000
trans-18:1
trans-18:2
800000
600000
non-irradiated
400000
8 kGray
200000
0
40.0
41.0
42.0
43.0
44.0
45.0
46.0
47.0
48.0
49.0
Time (min) -->

Figure 1



6/26/03
2,4,6,8 KGy Beef RATIOED AGAINST AIR (IRRADIATED AT ROOM TEMPERATURE)

Figure 2



6/26/03
2,4,6, 8KGy Beef RATIOED AGAINST UNIRRADIATED BEEF (AT ROOM TEMPERATURE) [IRRADIATED written above]

Figure 3