γ-Irradiated Seafoods: Identification and Dosimetry by Electron Paramagnetic Resonance Spectroscopy

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Electron paramagnetic resonance (EPR) spectroscopy was used to measure the production of free radicals induced by ^{60}Co γ-rays in shrimp exoskeleton, mussel shells, and fish bones. The EPR spectrum for irradiated shrimp shell was dose dependent and appeared to be derived from more than one radical. The major component of the radiation-induced spectrum resulted from radical formation in chitin, assigned by comparison with irradiated *N*-acetyl-D-glucosamine. Other measurements include the total yield of radicals formed as a function of dose and the longevity of the radiation-induced EPR signal. Similar measurements were made for mussel shells and fish bones, and the results are compared and discussed. It was concluded that irradiated shrimp (with shell attached) could definitely be identified by this technique; however, precise determination of absorbed dose was less straightforward. Positive identification of irradiated fish bones was also clearly distinguishable, and dosimetry by EPR appeared to be feasible.

The prospect of utilizing ionizing radiation for the purpose of sterilizing foods meant for human consumption has been extensively studied for the past 30 years (Goresline, 1983). Recent approval of food irradiation processing by the U.S. Food & Drug Administration (*Fed. Regist.*, 1986) have created concerns as to the regulatory aspects of this decision. The primary questions of importance are as follows: First, in order to monitor indiscriminate use of this technology for foods that have not been FDA approved, can radiation-processed foods be distinguished from the nonprocessed foods? Second, can the dose absorbed by irradiated foods be determined after the fact (i.e., postirradiation dosimetry (PID))?

PID of chicken thigh bones irradiated with or without the surrounding meat has previously been shown to be feasible (Desrosiers and Simic, 1988). It was demonstrated that the irradiated bone was clearly distinguishable from the nonirradiated bone by electron paramagnetic resonance (EPR) spectroscopy. In addition, the observed radiation-induced EPR signal was found to increase linearly with the dose absorbed (1–5 kGy). Therefore, it was concluded that EPR spectroscopy is a useful tool for PID for chicken meat that contains at least a minute piece of bone.

We sought to explore the use of EPR as a technique to examine irradiated shrimp, mollusks, and fish. The hard exoskeleton of shrimp should be able to serve as a dosimeter if the shrimp is irradiated with the shell attached. In fact, a radiation-induced EPR signal has been observed (Dodd et al., 1985) in shrimp shell exposed to ionizing radiation. However, in the course of extending their observations to establish a dose-dependent relationship, we obtained quite different spectra (vide infra). Thus, it is the focus of this study to describe these observations, along with those of fish bone and mussel shell, and derive from them conclusions as to the limitations of EPR as a technique for postirradiation examination of shrimp, mollusks, and fish.

EXPERIMENTAL SECTION

The shrimp used for this study was the pink variety, 40–50 count. The origin of the shrimp could have been from either the United States, Taiwan, or Argentina according to information supplied by the wholesale distributor (ESK Co.). Frozen shrimp were thawed, and the shell was removed. The shells were irradiated by a ^{60}Co source (absorbed dose rate 0.0683 kGy/min) at ambient temperature. ^{60}Co radiation source was calibrated by Fricke dosimetry. The shells were held vertically in Pyrex beakers for the irradiation step. The shell was stored at −20 °C overnight, cut (from the central portion of the shell), and weighed (typical sample weights were 20–50 mg).

Mussels (domestic) were irradiated live and whole at ambient temperature in the same ^{60}Co source. Force was then applied to fracture the shell, and a piece of about 200 mg was used. Fish bone from filleted fresh flounder (domestic) was irradiated at ambient temperature. The bone was then lyophilized and scraped clean of any external meat residue attached. Fish meat irradiated in its natural, wet state at ambient temperature was lyophilized and then ground with a mortar and pestle. Mussel and fish samples were desiccated at ambient temperature until analyzed.

EPR spectra were recorded with a Varian E-109 X-band spectrometer at 20 °C. For these studies the microwave power used was 10 mW. The position of the radiation-induced EPR signal was compared with that of the standard 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Aldrich) for accurate measurement of the spectroscopic splitting factor, *g*. The measured *g* factors had a standard deviation of ±0.03% (1σ). The integral of the EPR spectrum was determined by the cut and weigh method; by repetitive measurements it was found that the integral of the EPR signal was accurate to within ±5%. *N*-Acetyl-α-D-glucosamine (Aldrich) was used as received.

RESULTS AND DISCUSSION

Nonirradiated shrimp shell, treated in the same manner as the irradiated sample (see the Experimental Section) only excluding the irradiation step, was examined by EPR spectroscopy. The resulting spectrum (Figure 1A) is dominated by the characteristic resonances of Mn^{2+} (Wertz and Bolton, 1972). Apparently in this particular lot (or species) of shrimp the Mn^{2+} ion has been incorporated into the exoskeleton matrix. The presence of Mn^{2+} may be common to all shrimp; however, to my knowledge this is the first such observation. Mn^{2+} was also found in nonirradiated mussel shell (Figure 1B) and was obviously in higher concentration (although quantitative data cannot be derived from these measurements) judging from the intense signal. No EPR signal of any kind was detected for nonirradiated fish bone.

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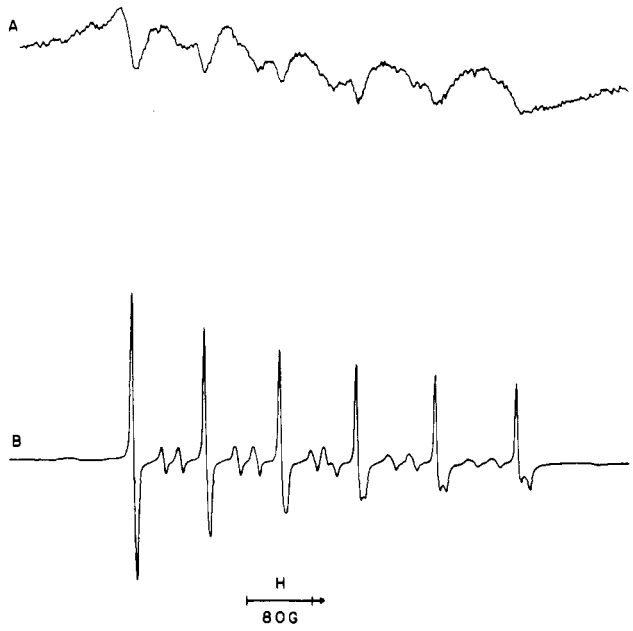

Figure 1. EPR spectra of nonirradiated (A) shrimp and (B) mussel shell. Horizontal scale is shown in gauss (G); the arrow is in the direction of increasing field.

Irradiated Shrimp. The radiation-induced signal in shrimp shell (Figure 2A) was measured at a modulation amplitude 10 times lower than that used for the endogenous signal intensity (thus minimizing any interference). At a dose of 0.25 kGy the radiation-induced signal is barely resolvable from the spectrometer noise (not shown) but is reproducible. The radiation-induced signal at a dose of 0.5 kGy is now clearly resolvable and is dominated by a sharp singlet at $g = 2.0050$ (signal to noise ratio of 12:1). At 1.0 kGy (Figure 2B) the radical that yields a sharp singlet (in center of spectrum) is still present and will be referred to in the text as radical A. A number of accompanying resonances that were merely suggested at the lower doses are now also apparent and will be referred to as radical B. However, this designation is for the sake of discussion only, and from these data it cannot be concluded that the resonances result from single or multiple radicals.

An increase in dose from 5 to 20 kGy (Figure 2C–E) yields varying intensity changes as well as the disappearance and appearance of various spectral features. It is also apparent from these spectra (Figure 2B–E) that the peak-to-peak intensity of A does not change linearly as a function of dose. Analysis of the intensity changes observed for B is additionally complicated by dose-dependent changes in the spectral features. Assuming the chemical composition of the shrimp exoskeleton is the same among all the samples, the observed changes could arise from the combination of a number of many different radicals with differing dose response (given the obvious chemical complexity of the shrimp shell matrix), combining to yield different overall spectra as a function of dose. EPR spectra were also obtained for shrimp shell irradiated at 30 and 40 kGy (not shown) and had the same general features as Figure 2C–E, varying only in intensity.

The shrimp exoskeleton matrix is composed of chitin, a structural polysaccharide. A major component of chitin is the amino sugar D-glucosamine. In order to determine whether the observed radiation-induced EPR spectrum of shrimp shell is derived from the glucosamine component of the exoskeleton matrix, *N*-acetyl-D-glucosamine was irradiated (5 kGy) in the powder form at ambient temperature. Approximately 12 h later the EPR spectrum was measured. The resulting EPR spectrum (Figure 2G) is

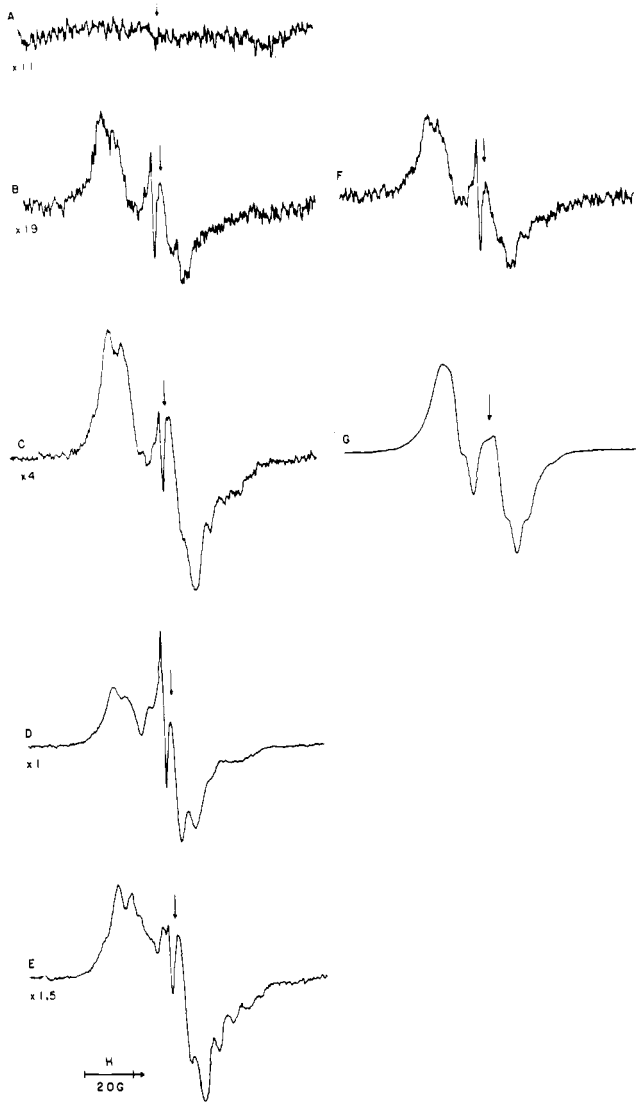

Figure 2. EPR spectra of shrimp shell exposed to various doses of γ radiation: (A) 0 kGy; (B) 1 kGy; (C) 5 kGy; (D) 10 kGy; (E) 20 kGy. Normalized (with respect to sample weights and instrumental parameters) scaling factors are shown by each spectrum. Spectrum F is the 1-kGy irradiated shell (B) measured again 9 days later. Spectrum G is *N*-acetyl-D-glucosamine irradiated at a dose of 5 kGy. Horizontal scale is shown in gauss (G); the arrow is in the direction of increasing field. The inserted vertical arrow defines the position of the DPPH resonance ($g = 2.0036$).

comparable to that obtained for shrimp shell irradiated at the same dose (Figure 2C). The similarity of the spectral features due to irradiated shrimp shell and glucosamine suggests that radical B may be derived from the chitin component of the shell matrix. However, contributions from other polysaccharide components and/or other unknown shell matrix components cannot be discounted from these data. The major difference between the two spectra is the presence of the sharp singlet at $g = 2.0050$, radical A, in the shrimp shell. The origin of radical A is unknown: Initially one may surmise the signal is derived from a calcified component of the matrix, but the spectral characteristics (g factor, line width, hyperfine splitting pattern) do not match those obtained for irradiated bone of different origins (vide infra).

The longevity of the radiation-induced signal was monitored. After the first measurement the samples were again stored at −20 °C (approximate time the samples were left at room temperature during the measurement period was 6–10 h). Nine days later the samples were again

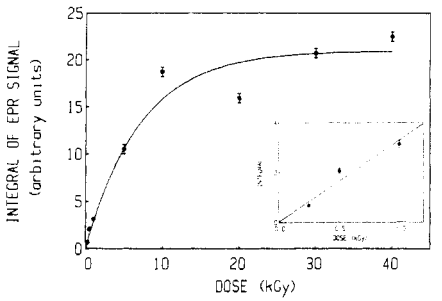

Figure 3. Integral of the shrimp shell EPR spectra (divided by the sample weight) plotted as a function of the absorbed dose. Error bars represent the standard deviation of different measured EPR integrals. The inset is an enlargement of the low-dose measurements.

brought to room temperature, and the EPR spectrum was measured. In Figure 2F it is shown that there was no decay of the radiation-induced EPR signal during the storage period and also demonstrates that the signal survived two freeze–thaw cycles. Continued monitoring of the EPR signal revealed no decay of the signal (at 1 kGy) as long as 43 days later (again surviving two additional freeze–thaw cycles during this period). At the longer measurement times (26–43 days), the integral of the EPR signal at higher doses was reduced but the general shape of the dose–yield relationship as shown in Figure 3 was maintained. No changes in the control sample were observed with time. The EPR signal present in irradiated glucosamine was still present when measured after nearly 5 months (142 days).

Examination of the tail portion of the shrimp irradiated at 30 kGy revealed that a similar spectrum to that of the central shell is obtained (not shown). Although the relative intensities are different, the basic features are present in both parts of the shrimp.

The integral of the total EPR spectrum obtained for irradiated shrimp shells was correlated with the dose absorbed (Figure 3). The integral begins to plateau at doses greater than 10 kGy, which is similar to data obtained for irradiated bone samples (Stachowicz et al., 1972). The deviation from the curve calculated to fit the data probably reflects the complexity of the shell matrix and thus the difficulty in the measurements. This plateau region is well above the proposed dose range to be used to irradiate meats and shrimp (1–3 kGy). According to our data a correlation between dose and the EPR integral could be determined, and in fact linearity at lower doses is suggested from the data (Figure 3, inset). However, due to the complexity of the signal in this dose range it may be advantageous to develop a method by which one or more of the interfering signals can be eliminated to leave a single quantifiable signal that can be better correlated with the absorbed dose.

The radiation-induced EPR spectra reported here differ markedly from those observed by Dodd and co-workers. This observation most likely results from the different type of shrimp used for each study. These results may indicate the difficulty in the universal application of this technique to the examination of irradiated shrimp. If EPR is to be utilized for this purpose, each type of shrimp may have to be categorized as to its interactions with ionizing radiation.

Irradiated Mussel. Very intense EPR signals are produced in mussel shells exposed to ionizing radiation (Figure 4B–D). A rough estimate was made (based on peak heights) of the relative signal to noise ratios of the radiation-induced EPR signals present in shell, shrimp exoskeleton, and fish bone. It was found that the signal

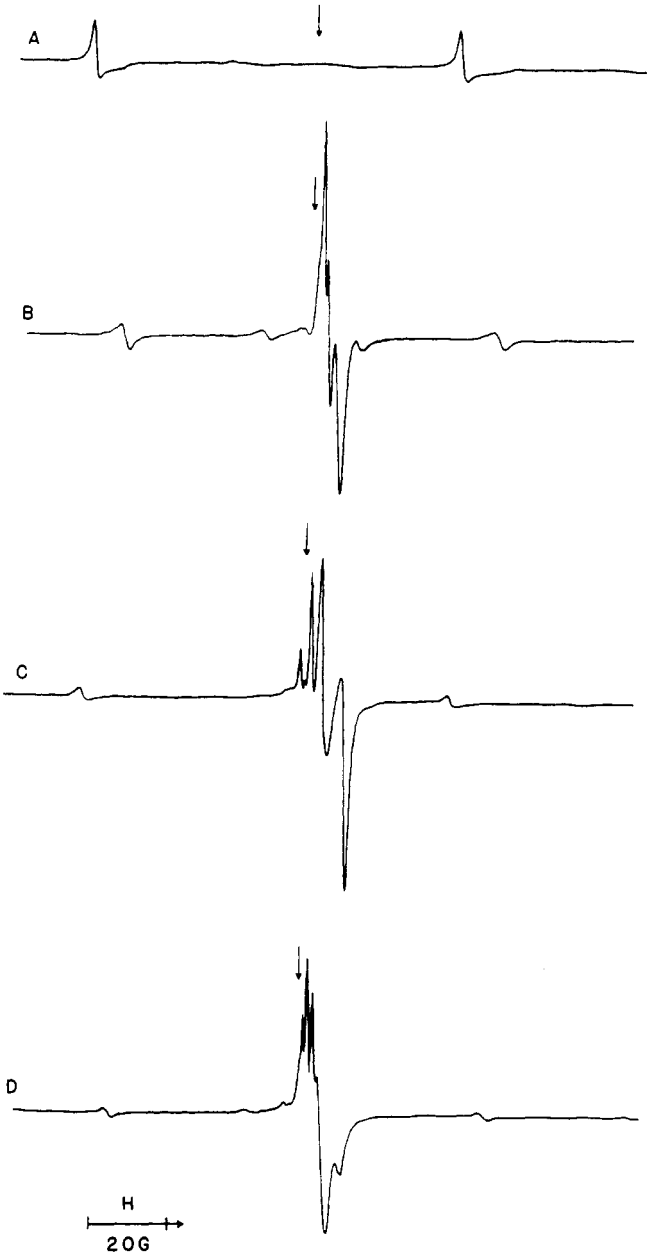

Figure 4. EPR spectra of mussel shell exposed to various doses of γ radiation: (A) 0 kGy; (B) 1 kGy; (C) 2 kGy; (D) 3 kGy. Horizontal scale is shown in gauss (G); the arrow is in the direction of increasing field. The inserted vertical arrow defines the position of the DPPH resonance (g = 2.0036).

present in mussel shell was nearly 1000 times greater than that found in shrimp and about 300 times greater than fish bone. The estimate was made from samples irradiated at the same dose (1 kGy), and the peak heights were normalized with respect to instrumental parameters and sample weights.

The observed radiation-induced EPR spectrum (measured approximately 36 h after irradiation) is a composite of a number of anisotropic resonances, and the origins of all the signals have not been assigned. The signals are present in the g = 1.997–2.004 region, and close examination reveals that they contain resonances at g = 2.002 and 1.997, which correlates with radiation-induced signals previously observed in shell (Ikeya et al., 1984) and have been assigned, in the case of dental enamel, to CO_2^- (Bacquet et al., 1981). Radiation dosimetry using this resonance has been demonstrated previously (Brady et al., 1968; Caracelli et al., 1986; Desrosiers and Simic, 1988;

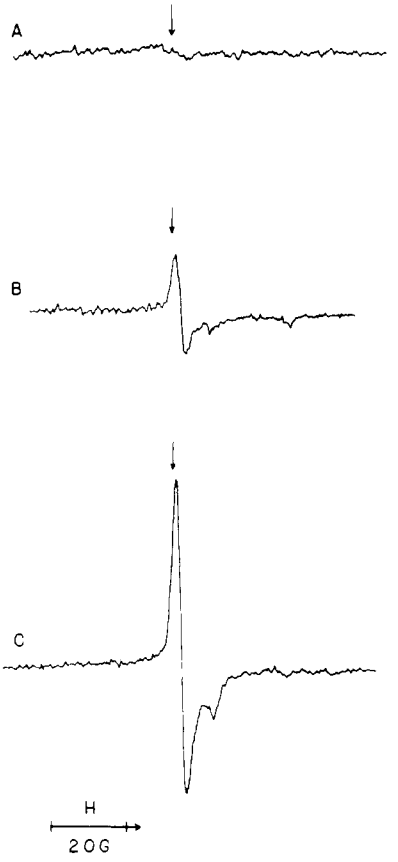

Figure 5. EPR spectra of fish bone exposed to various doses of γ radiation: (A) 0 kGy; (B) 1 kGy; (C) 3 kGy. Horizontal scale is shown in gauss (G); the arrow is in the direction of increasing field. The inserted vertical arrow defines the position of the DPPH resonance (g = 2.0036).

Ikeya et al., 1985; Pass and Aldrich, 1985) but was not possible here due to other overlapping resonances. Ikeya et al. found the EPR signal intensity of irradiated shells increased linearly with the absorbed dose in the region 1–8 Gy. The radiation-induced signal does not increase linearly with dose (1–3 kGy), and it may be that in this dose range the signal intensity is at or near the saturation (plateau) level.

The irradiated mussel shell EPR spectra were again measured 142 days later. Some of the observed resonances had either disappeared or diminished in intensity; however, the irradiated shell was still unequivocally distinguishable from the nonirradiated shell (for which no changes were observed with time).

Irradiated Fish Bone. Irradiated fish bone gave EPR spectra identical with that observed for all other types of bone measured previously (Caracelli et al., 1986; Fisher et al., 1971; Gordy et al., 1955; Houben, 1971; Stachowicz et al., 1970; Swartz, 1965). The radiation-induced spectrum (measured approximately 36 h after irradiation) produced in fish bone (Figure 5B,C) is comprised of an anisotropic signal with $g_{\perp}$ = 2.0025 and $g_{\parallel}$ = 1.9980.

In a previous study (Desrosiers and Simic, 1988), PID of chicken meat was shown to be feasible by EPR spectroscopy of irradiated bones (the absorbed dose in bone was calculated to be 3.4% less than that of the surrounding meat). The technique should also be applicable for irradiated fish containing bone. The peak-to-peak amplitude of the signal at g = 2.0025 was found to increase linearly with absorbed dose (1–4 kGy). The signal intensity was dependent on the orientation of the bone (when inverted 180°; typically a difference of 10–25%) in the EPR cavity, and the error bars for the data in Figure 6 reflect this

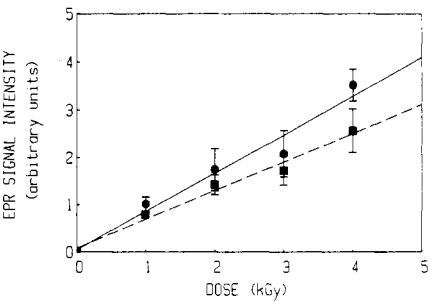

Figure 6. EPR peak-to-peak signal amplitude of the radiation-induced signal plotted as a function of the absorbed dose. Two sets of data are shown: EPR signal intensities measured at (●) 1.5 days (solid line) and (■) 142 days (broken line). Error bars represent the standard deviation of the EPR signal intensities of different bone samples as well as different sample orientations. Data points represent the calculated mean value for the data at each dose measurement.

variation along with sample-to-sample dependent variation in signal intensity.

The same bone samples were again measured 142 days after irradiation, and the results are also shown in Figure 6. The slope of the dose–yield relationship decreased by 25%, and the EPR signal amplitude for all measurements decreased. However, for the lower dose measurements (1–3 kGy; the proposed working region for meat irradiation) the data overlapped, demonstrating that within experimental error there is no change in response even 5 months (stored at room temperature) after irradiation.

Fish meat was also irradiated (1 kGy) in its natural wet state at ambient temperature, lyophilized, and ground to a powder. A very weak EPR signal was detected in both the irradiated and nonirradiated meat powder (g = 2.0045). Therefore, EPR spectroscopy cannot be used for PID of irradiated fish meat.

It was determined that EPR spectroscopy is, at the very least, certainly able to distinguish between irradiated and nonirradiated shrimp husk, shells, and fish bone. The requirement is that some portion of the exoskeleton or bone must be present at the time of irradiation and be available for analysis. The EPR signals induced by ionizing radiation can be detected months later, are stable at room temperature for bones and shell, and in the case of shrimp husk are able to withstand repeated freeze–thaw cycles.

For the shrimp used in this study, the origin of the radiation-induced EPR signal is predominately derived from the chitin component of the exoskeleton; however, other signals are observed and further studies are necessary to deduce the nature of these signals. It is important to note that the radiation-induced EPR signals observed in our study differ greatly from those previously reported elsewhere (Dodd et al., 1985). This is most likely a result of different shrimp exoskeleton matrices in the two sample types. From these data we may conclude that PID of irradiated shrimp by EPR may be further complicated by the fact that the signal observed is dependent on the origin/type of shrimp being examined.

The measured EPR signal intensity in bone as a function of the dose absorbed is linear in the approved dose range for meat irradiation, and PID of fish meat by this technique should be simple and feasible. PID of shells and shrimp husks in this dose range is not as straightforward, and more studies are necessary to develop this technique for this purpose. However, the technique does offer some promise, and at least initially it is clear that EPR spectroscopy could be useful in at least qualitatively distinguishing (i.e., as a screening technique) irradiated shrimp

and shell fish from the nonirradiated, provided some (a few milligrams) portion of the exoskeleton is present for analysis.

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Registry No. *N*-Acetyl-D-glucosamine, 3416-24-8; manganese, 7439-96-5.

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Simple Method for Preparing Bone-Free Ash from Fishery Products Analyzed for Mineral Content

Fuad M. Teeny

A simple method was developed for preparing bone-free ash from samples of canned and fresh fish products. The technique employs deionized distilled water to remove bits of bone from the sample ash prior to mineral determination. The addition of bone to the flesh prior to ashing had minimal effect upon the mineral level of the flesh.

A substantial amount of information has been published on the mineral composition of seafoods—raw and processed (Adams, 1975; Sidwell et al., 1973, 1977, 1978; Stansby and Hall, 1967; Thurston, 1958, 1960, 1961a–c). Among these and other published data, there appeared to be a certain amount of conflict regarding the levels of a few elements such as calcium (Ca) and phosphorus (P). Sidwell et al. (1973), in their analysis of several fresh and canned finfish, crustaceans, and mollusks, found great variability in the amounts of Ca and P in the raw flesh. They suggested that is probably due to the method of filleting the fish wherein the smaller fish retained more bone than larger fish. It is generally recognized that Ca and P are the two major minerals found in bone. Gordon and Roberts (1977) reported Ca levels of 5.5 and 252 mg/100 g of the edible portion of fresh and canned sockeye salmon, respectively, and P levels of 175 and 293 mg/100 g. It is likely that the higher levels of Ca and P in the canned samples were due to increased bone level in the canned samples over the fresh. It is rather difficult and time consuming to pick out bone by hand. The inclusion of even very small pieces of bone in the analytical samples can greatly influence the accuracy and precision of elements such as Ca and P.

During the course of our routine mineral analyses of several thousand samples of fresh and canned fishery

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