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The use of acid hydrolysis for extracting minerals from shellfish for thermoluminescence detection of irradiation

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Abstract

Inorganic grits contained in shellfish can be used for the thermoluminescence detection of irradiation treatment. Acid hydrolysis of the flesh of the shellfish to leave the minerals behind has been explored as an alternative to the physical dissection method previously employed. The hydrolysis technique was developed and assessed using six species of shellfish (*Nephrops norvegicus*, brown shrimps, mediterranean crevettes, black tiger prawns, warm water shrimps and king scallops). It has also been evaluated under interlaboratory trial conditions. In all cases, excellent discrimination between irradiated and unirradiated products was obtained using hydrolysis extracts. The method produces results which are comparable or better than conventional physical extraction, and has some benefits in sample handling.

1. Introduction

The applications of radiation treatment have been shown to be of particular relevance to food processing [Advisory Committee on Irradiated and Novel Foods (ACINF), 1986; World Health Organisation (WHO), 1988). The safety of food can be improved through the reduction of pathogenic microorganisms and by reducing those organisms which cause food spoilage, the quality of the food can be improved and shelf-life extended. Ionising radiation can inhibit the metabolic processes leading to ripening and spoilage of high value products, such as exotic fruits and vegetables and shellfish, and dramatically reduce or eliminate some pathogenic organisms, using radiation doses below 10 kGy. Irradiation causes few of the changes to colour, texture, flavour and nutrients which may result from canning or freezing these products. Recent surveys have indicated that more than 60 commercial radiation processing plants exist in more than 30 countries, operating under a diverse set of national regulations [International Atomic Energy Authority (IAEA), 1987], and able to irradiate a range of foods and food ingredients, including shellfish.

In Europe and the United Kingdom, uptake of the technique has been inhibited by consumer acceptance (Jack & Sanderson, 1995). Current UK legislation [Ministry of Agriculture Fisheries and Food (MAFF), 1992, 1993; Statutory Instruments, 1997] and recent EU proposals (EN, 1997), embody both controls on the process and stringent requirements for labelling of irradiated produce at all stages of supply and marketing. Detection methods are required in support of such regulations. A range of physical, chemical and biological methods has been investigated over recent years with the aim of developing reliable tests to determine whether food has been irradiated (Johnston & Stevenson, 1990; Raffi & Belliardo, 1991). The recent publication of European standard procedures for chemical detection of radiolytic products from lipids (EN 1996a,b), for electron spin resonance detection of stable radiolytic radicals (EN, 1996c,d) and for thermoluminescence (TL) detection of irradiated silicates from foods (EN, 1997) reflects progress towards this goal.

Thermoluminescence (TL) is a radiation specific phenomenon which arises due to energy stored by trapped charge carriers following irradiation. The energy released following thermal stimulation is accompanied by detectable luminescence (Sanderson, Carmichael, Spencer & Naylor, 1996). It has been successfully demonstrated that thermoluminescence (TL) is associated with silicate

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minerals present as contaminants in most foodstuffs, and can be used for unambiguous detection of irradiated herbs and spices (Sanderson, Slater & Cairns, 1989). Following a European interlaboratory trial (Sanderson, Schreiber & Carmichael, 1991), this was adopted in the UK as the first validated method for detecting irradiated foods (MAFF, 1992, 1993). A European standard on this method has been prepared and adopted (EN, 1997).

Recent attention has been focused on extending the validated method to other food products, such as fruits, vegetables (Schreiber, Helle & Bogl, 1993) and shellfish (Pinnioja, 1993). Shellfish, which can pose significant food poisoning risks, are prime candidates for irradiation. Given the current UK legislation, development of quick, routine tests for irradiation is a priority.

TL analysis of shellfish can utilise both carbonates from the shell (Carmichael, Sanderson & NiRiain, 1994) and silicates trapped in the intestines or the shell (Autio & Pinnioja, 1990). Autio and Pinnioja (1990) were the first to show that extraction of the intestinal grits by hand picking (manual dissection) is possible, but it is an extremely laborious task requiring dissection of several shellfish for each analysis and the quantities recovered are very small. There is also a risk of cross contamination between samples. Pathogens produced by the rapid spoilage of shellfish may also pose a health hazard during analysis in some circumstances.

The development of a simple and less time consuming mineral extraction method would clearly be advantageous. Isolating minerals by dissolving the organic (flesh) portions of the shellfish, leaving the insoluble inorganics behind, is a promising alternative. Use of acid hydrolysis was investigated at SURRC and applied during collaborative trials in 1997 which confirmed that it has some benefits in comparison with physical extraction methods (Sanderson, Carmichael & Fisk, 1997; Sanderson, Carmichael, Spencer & Naylor, 1994). Acid hydrolysis is one of three principal methods for cleaving peptide bonds to remove organic material, each of which has advantages and disadvantages. The other methods are alkaline hydrolysis and enzymatic hydrolysis. Both these methods have been used in the detection of irradiated bone (Stevenson, Marchioni, Gray, Stewart, Bergaentzle & Kuntz, 1996). Alkaline hydrolysis proceeds rapidly and does not require prolonged heating, but it produces more vigorous reactions than acid and may reduce the percentage recovery of intestinal minerals, which are already in minimal quantities. There are several theoretical advantages to using enzyme hydrolysis. The enzymes are peptide specific and readily cleave bonds that are resistant to non-specific catalysts; they should also act rapidly and could complete the hydrolysis in minutes. In practice, however, enzyme-catalysed hydrolysis of peptide bonds does not proceed faster than the other methods, and more importantly, often does not proceed to completion. This reduces the utility of this method.

Acid hydrolysis, therefore, appears to be the most promising alternative to manual dissection. For the technique to be adopted, however, it must be established that the detection performance is at least as good as for the conventional method. The experiments described below quantitatively compare the two methods and present the case for validation of the alternative method.

2. Experimental

Two methods for the extraction of minerals from the intestines of shellfish for TL analysis were investigated. The first requires manual dissection, followed by separation of the remaining organic material, while the second method involves the dissolution of the flesh leaving minerals behind.

2.1. Isolation of minerals using physical extraction methods

Suitable minerals for TL analysis are found in the intestines of shellfish. The intestines can be seen through the shell (where present) on the convex side of the shellfish as a 1–2 mm dark tube. Once the tract has been identified the flesh can be cut carefully with a scalpel and the intestines removed with forceps. Several approaches have been developed including hand picking (Autio & Pinnioja, 1990; Pinnioja, 1993), and manual dissection plus density separation. The latter method was applied here. Intestines of several shellfish from the same sample batch were placed together in a centrifuge tube (20 ml) containing a small quantity of deionised water (5 ml) and then sliced with the scalpel. After centrifuging for 1 min at 1000 *g* the water was completely removed by decantation. Five millilitres of sodium polytungstate (density 2 g cm^{-3}) was added to the tube which was then placed in an ultrasonic bath for 5–10 min and then centrifuged (1 min at 1000 *g*). The tungstate was decanted off and the minerals were washed several times with deionised water, cleaned with 1M HCl, neutralised with ammonia, and washed several more times with deionised water followed by acetone. The minerals were suspended in 5 ml of acetone and poured into flat bottomed tubes (10 ml) containing clean, weighed stainless steel discs. These tubes were left overnight in an oven at 50°C to evaporate the acetone. Minerals sedimented onto the discs, which were then reweighed to determine sample mass.

2.2. Isolation of minerals using the acid hydrolysis technique

Ten to twenty grams of whole sample were placed into a round bottomed flask containing 200 ml of 6 M HCl. The mixture was then refluxed at 110°C for 2 h

during which time the liquid changes from a colourless to a clear brown solution. After cooling, the acid was removed either by rotary evaporation or by dilution with deionised water (400 ml) followed by settling and decanting of the dilute acid solution. The minerals remaining at the bottom of the flask were washed several times with deionised water and then rinsed several times in acetone. The mineral grains were dispensed onto clean, weighed stainless steel discs, as in the manual dissection method; yield is determined by reweighing.

2.3. Thermoluminescence measurements

TL measurements for this study were carried out on the SURRC PC TL reader with computer controlled temperature ramping, automatic reheat facility and background subtraction program, with a single photon counting light detector (EMI 9883QB photomultiplier tube with Corning 7/59 and Chance HA3 filtration). The TL glow curves (photon counts versus temperature) were recorded from each sample disc from ambient temperature to 400°C at a heating rate of $6°C\,s^{-1}$. The TL oven was purged with oxygen free nitrogen at a rate of 25 l h^{-1}. After the first glow (G1), each sample was irradiated to a standard dose of 1 kGy in a ^{60}Co gamma source, and preheated for 30 min in an oven at 50°C. The second TL glow (G2) was then recorded under the same conditions as G1. This procedure follows the validated methods (MAFF, 1992, 1993; EN, 1997).

3. Results

A series of controlled experiments was conducted to examine the possibility of extracting silicate minerals from the intestines of shellfish using acid hydrolysis and to compare the results of TL analysis of these extracts with those minerals extracted by the conventional manual dissection method.

Six shellfish species (*Nephrops norvegicus*, brown shrimps, mediterranean crevettes, black tiger prawns, warm water shrimps and king scallops), were chosen and purchased from a retailer. The samples were split into two portions, one portion kept as a control and the other irradiated to 1 kGy in the SURRC ^{60}Co source. The samples were stored frozen for 68 days (retailers recommended maximum storage time) prior to extraction of the silicate minerals using both methods. All preparation and handling of samples was carried out under subdued safelight to minimise bleaching of the TL signal. Samples were analysed as paired irradiated and unirradiated control samples with four fold replication. For both methods the irradiated samples of all species can be readily distinguished from the unirradiated control samples.

The amount of silicate material extracted using either method will vary considerably from species to species and also within species. However, overall the acid hydrolysis method does give slightly higher quantities of minerals. An example of this is the results from twelve discs of *Nephrops norvegicus*. The acid hydolysis method gave 1.97 ± 1.04 mg and the physical extraction method gave 0.92 ± 0.23 mg. A similar trend was also observed for brown shrimps, black tiger prawns, mussels, shrimps and king scallops.

Examples of glow curves from unirradiated and irradiated *Nephrops norvegicus* using acid hydrolysis and physical extraction are shown in Figs. 1 and 2. It is notable that the glow curves using the acid hydrolysis method are more reproducible in shape than those for the physical extraction method and this was also observed for the other species. Fig. 3 shows first glow versus second glow for both methods for all six species. Again, the separation between unirradiated and irradiated samples is better for silicates extracted by acid

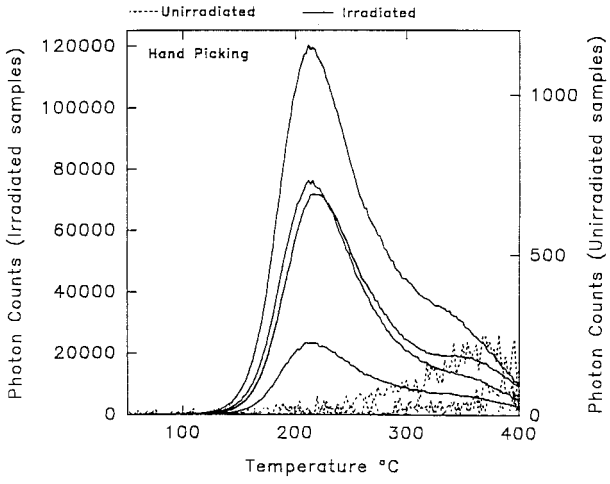

Fig. 1. Glow curves for *Nephrops norvegicus* using manual extraction method.

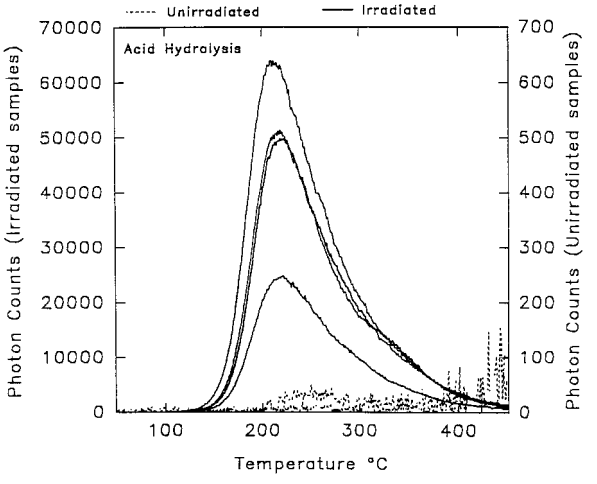

Fig. 2. Glow curves for *Nephrops norvegicus* using acid hydrolysis method.

hydrolysis and the glow ratios (G1/G2) for each category are more tightly grouped.

A set of six unknown samples from two species of prawns (Vietnam cat tiger prawns and China Reds) obtained for a German interlaboratory blind trial (Schreiber et al., 1995) were also analysed using silicates extracted by acid hydrolysis and by physical extraction. The samples were separated and analysed in duplicate under the same laboratory and instrumental conditions as before. Fig. 4 shows the results obtained for both separation methods; the unirradiated and irradiated products were correctly identified. It is again noticeable that the duplicate aliquots for both unirradiated and irradiated samples, separated using acid hydrolysis, are more tightly grouped together than those using the physical extraction method.

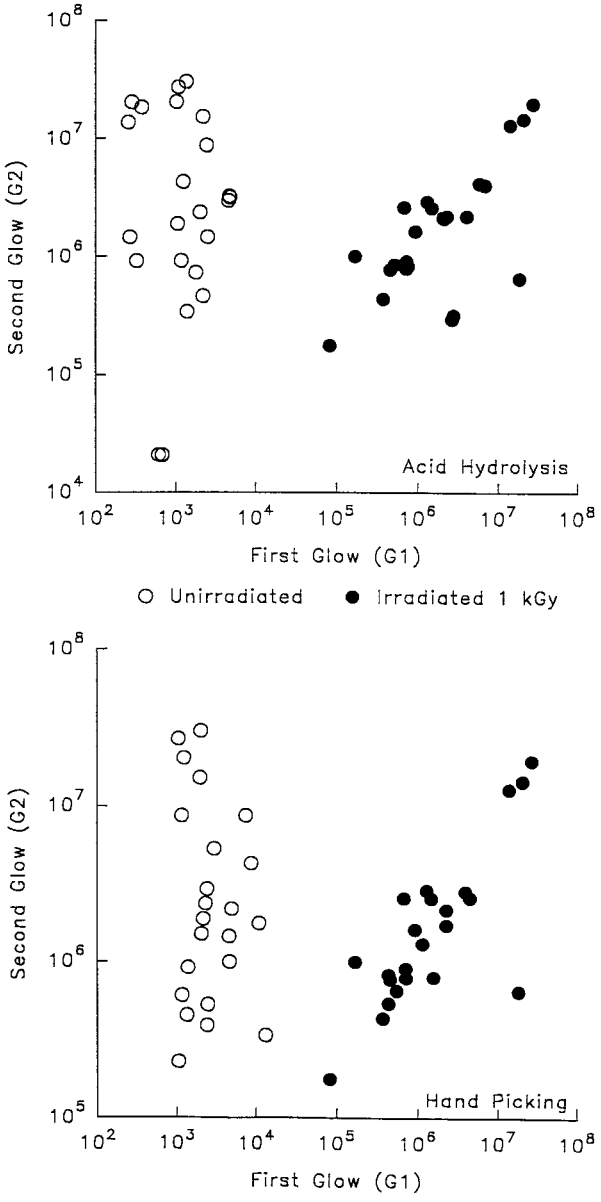

Fig. 3. First glow versus second glow plot for the six species of shellfish stored frozen for 68 days.

A further opportunity to assess the validity of the hydrolysis method arose during recent interlaboratory trials organised by SURRC. The protocol called for

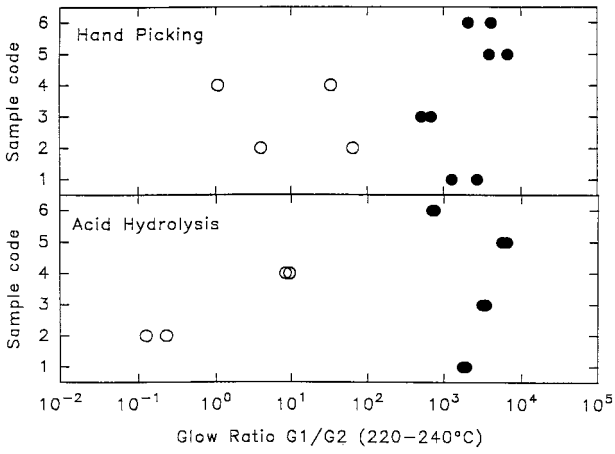

Fig. 4. Glow ratio plot for the six blind samples of two species of prawns — German interlaboratory trial.

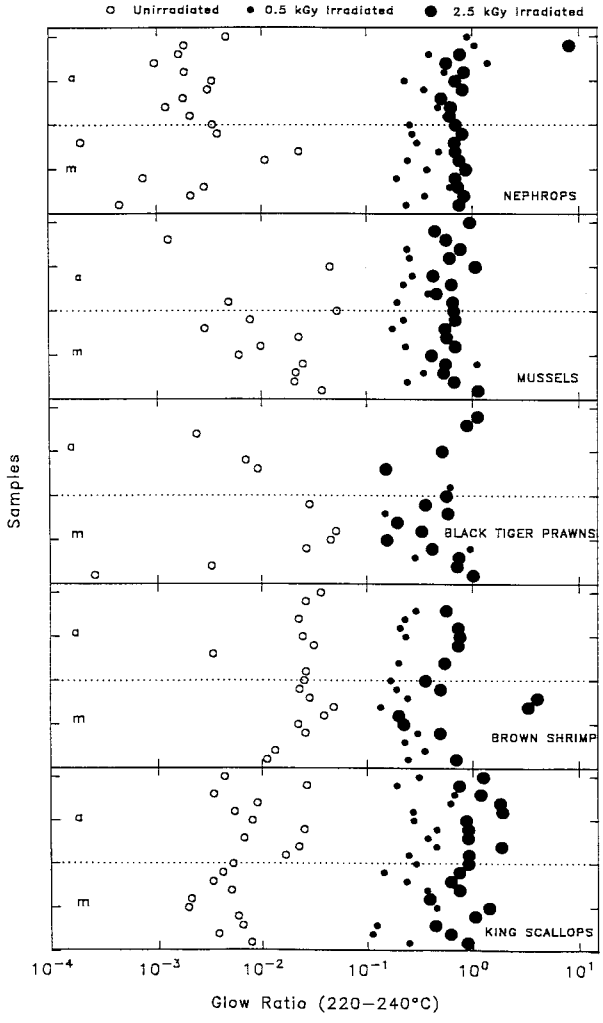

Fig. 5. Homogeneity testing results for five species of shellfish — MAFF interlaboratory trial — for manual dissection (m) and acid hydrolysis (a) methods.

both acid hydrolysis and manual dissection methods to be carried out on five species of shellfish (*Nephrops norvegicus*, mussels, black tiger prawns, brown shrimps and king scallops), presented as unirradiated, and irradiated to 0.5 and 2.5 kGy. Participants received one sample of each in each category. Homogeneity testing was carried out on the samples and Fig. 5 shows the results for both methods. Participants data for both methods were similar to the results seen from the homogeneity testing. Again both methods were able to distinguish between unirradiated and irradiated samples, although participants were not asked to distinguish between the two doses. Overall acid hydrolysis led to slightly better discrimination between unirradiated and irradiated samples, than the physical extraction method.

4. Conclusions

The analyses described above have demonstrated that it is possible to extract silicate minerals from shellfish by acid hydrolysis and distinguish between unirradiated and irradiated samples, at least as reliably as with the manual dissection method.

The hydrolysis technique is very simple, removing the need to select intestines from large quantities of individual shellfish and allowing several analyses to be carried out in parallel which is essential for speedy routine analysis. In cases where the material is in an advanced state of decay, it may be the only practical method.

All the species of shellfish examined under controlled experimental conditions show TL characteristics in keeping with their irradiation status; glow curves from acid hydrolysis preparations are cleaner with better reproducibility than those obtained for the physical extraction method. Sensitivity varied from aliquot to aliquot with both methods as expected from samples with heterogeneous mineralogy; the filtering and other instrumental conditions were optimised for feldspars. Density separation to obtain pure samples could be carried out, but would negate the speed advantage of the separation method and might not produce sufficient yields.

The use of heat during the acid hydrolysis separation might be expected to result in thermal shift observable in the TL glow curves. Examination of the peak maxima (T_{max}) and half peak maximum temperatures ($T_{1/2}$), for glow curves from all the samples analysed, did not show significant peak shifts in those extracted using acid hydrolysis in comparison with the hand picked separates. This may be a function of the greater variance of T_{max} in analyses of hand picked minerals or of the heterogeneous mineralogy. It may also be due to the presence of a thin organic coating on the surface of the minerals, inhibiting luminescence, which has been removed by the use of stronger acid treatment (6 M in comparison to 1 M). Further investigation in the use of stronger acid after the density separation stage of hand picked minerals is needed to fully assess the effect of the two hour heat at 110°C.

In recent interlaboratory trials aimed at validating TL detection of irradiated shellfish, where both methods of mineral extraction were used, reliable qualitative results were obtained from either method. Quantitative discrimination, however, between irradiated and unirradiated samples, and the reproducibility of quantitative results were generally better for extracts prepared by hydrolysis. The technique can clearly be used without compromising quality, has some practical benefits, and has further potential for extracting silicates from other food matrices.

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