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Effects of preservation by gamma-irradiation on the nutritional quality of Australian fish

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Gamma-irradiation preservation of two species of Australian marine fish (Black Bream and Redfish) resulted in no significant changes in their fatty-acid compositions, even when performed at up to three times the commonly recommended maximum dose for fish. Vitamin E loss was evident in some fillets but could not be correlated with the treatment dosage. All irradiated fillets were found to have vitamin E contents above the levels believed to be desirable for human consumption, relative to the amounts of accompanying polyunsaturated fatty acids.

INTRODUCTION

The consumption of diets rich in fish by various populations and by groups participating in clinical trials has been associated with a wide range of positive health effects. These include low incidences of cardiovascular diseases and mortality from atherosclerosis and heart disease, reduced inflammation associated with rheumatoid arthritis and psoriasis, and low blood pressure and viscosity (Höygaard, 1941; Brown, 1951; Ehrström, 1951; Rodahl, 1954; Bang & Dyerberg, 1972; Bang *et al.*, 1980; Kromhout *et al.*, 1985; Yamori *et al.*, 1985).

The lipids of fish and other seafood are distinctive because of their high proportions of n-3 polyunsaturated fatty acids (PUFAs). This feature should also make them extremely oxidatively labile. However, the highly polyunsaturated lipids are usually accompanied by high concentrations of the natural antioxidant vitamin E, which presumably protects them against this kind of attack. Vitamin E is the major lipid antioxidant in living organisms (Tappel, 1980), but its exact distribution within membranes and mode of action are not yet well understood. Because of its role in protecting fatty acids against oxidation, the dietary requirement for vitamin E is usually given in terms of the content of its most abundant and bioactive form, alpha-tocopherol (α-T), relative to that of the polyunsaturated fatty acids. Horwitt *et al.* (1961) suggested a level of 0·6 mg α-T/g PUFA, as well as a daily intake of 5·0 mg for adults. Draper (1980) stated that only 0·18–0·52 mg α-T/g PUFA was required. It should be noted that these values are all only rough estimates of the vitamin E requirements of the over-all diet. The actual amount of vitamin E needed depends on the fatty-acid composition of the tissues into which it will be incorporated, which in turn is determined in a complex way by that of the over-all diet (Witting, 1974).

Vitamin E levels in fish vary with sexual maturity, location and season of catch, and water temperature (Kinsella, 1982). They were reported to be within the range of 0·16–1·1 mg α-T/g PUFA for Finnish fish by Syväoja *et al.* (1985). Even populations with highly polyunsaturated diets (such as Greenland Eskimos) very rarely exhibit symptoms of vitamin E deficiency, which include cellular accumulation of age pigments, tissue lesions, and disorders of the reproductive, nervous, and cardiovascular systems. This is due to the presence of high levels of vitamin E accompanying highly polyunsaturated fats in natural foods.

Vitamin E deficiency, however, may become a concern when populations consume large amounts of fish that have been processed in ways that reduce their degrees of protection against fatty-acid oxidation. Little work has been published in this area since that of Bunnell (1965).

The exposure of foods to gamma-(γ-)irradiation is a preservation technique that may reduce the vitamin E protection of foods. Gamma-irradiation of foods with high moisture contents (such as fish, which are approximately 80% water) is known to generate free radicals within them. Free radicals are capable of causing extensive oxidative damage to the highly polyunsaturated lipids of fish.

Gamma-irradiation of fish has commonly been found to produce characteristic 'burnt' flavours and odours, the extent at a particular dose depending on

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the species and lipid content. Nickerson *et al.* (1983) suggested that the appropriate dose (providing a compromise between maintained eating quality and prolonged shelf-life) needs to be determined for each species. It is generally recommended that fish be treated at low doses (to a maximum of approximately 2–2·5 kGy) to avoid organoleptic deterioration.

This paper presents the results of a study of γ-irradiation at levels up to three times those recommended for preservation on two species of lean Australian fish. The fish were analysed for fatty-acid composition and vitamin E content before and after irradiation to enable the nutritional significance of their inclusion in the human diet, in terms of ingested fatty acids and antioxidant capacity of the vitamin E, to be evaluated.

MATERIALS AND METHODS

Samples

Nine normal adult-sized Black Bream (*Acanthopagrus australis*) and nine Redfish (*Centroberyx affinis*) were obtained from catches off New South Wales during Autumn, 1990.

Both fillets were removed from each fish (skin intact), individually sealed in plastics bags, and stored at −22°C. All fillets were placed on ice 24 h prior to irradiation, which was performed on one fillet from each fish. All fillets were returned to frozen storage within 2 h of the completion of the irradiation procedure.

Triplicate samples of both species were irradiated at each of three doses (1, 2, and 6 kGy). Ice was packed around each fillet before irradiation and was still present at the completion of the exposure. Gamma-irradiation at a dose rate of 0·999 Gy/s was performed in the Underwater Calibration Facility (UCF) at the Australian Nuclear Science and Technology Organisation (ANSTO) facility at Lucas Heights (Sydney).

Lipid extraction and analysis

Extraction of the total lipid from the white flesh (skin removed) was by the method of Bligh and Dyer (1959), as adapted by Kinsella (1977). This was performed on a sample (5 g) of the minced white flesh of each fillet.

Methyl esters (FAMEs) of the total bound fatty acids (triglycerides plus phospholipids) were prepared by reaction of the chloroform extract (plus trinonadecanoin as internal standard) with methanolic sodium methoxide (0·5M) at room temperature (Shehata *et al.*, 1970).

The phospholipids were isolated by solid-phase extraction on aminopropyl bonded-phase cartridges (500 mg sorbent, Waters, MA) by the method of Kaluzny *et al.* (1985). FAMEs of these fatty acids were then prepared as for the total-lipid extract, after neutralising with methanolic sodium hydroxide.

FAMEs from both fractions were analysed by gas–liquid chromatography on a Hewlett-Packard 5890A GC, identified by mass spectrometry (Armstrong, 1992) and quantified as described previously (Armstrong *et al.*, 1991).

Vitamin E extraction and analysis

A portion of the minced fillet (10 g) was also removed for vitamin E extraction. This was done by the method of Syväoja *et al.* (1985), which involves saponification followed by extraction into hexane. Care was taken to avoid oxidation of vitamin E by blanketing the mixture in nitrogen gas where possible, excluding light, and keeping the temperature below 40°C even during solvent evaporation (Söderhjelm & Andersson, 1978).

γ-Tocopherol (95% pure, Eastman–Kodak) was used as the internal standard and was added at the beginning of saponification.

Concentrated extracts (in hexane) were analysed by normal-phase high-performance liquid chromatography (HPLC). A silica DYN Microsorb column (5-μm particles, 100-Å pore size, 4·6 × 250 mm, Rainin Instruments Inc., MA) was used, with 0·40% 2-propanol/hexane as mobile phase. Detection was by UV, at 295 nm. Analysis time was approximately 30 min. Vitamin E levels were determined as the amount of the most abundant and bioactive isomer, α-tocopherol.

Both HPLC and GC data were recorded and integrated by using the DAPA computing integrator package (DAPA Scientific Software, Perth, WA, Australia).

Lipid and water-content determination

The lipid content of all fillets (%*L*) was determined gravimetrically from duplicate aliquots (2 ml) of the lipid extract after evaporation under a stream of nitrogen.

Water contents were determined by drying in a Mettler LP12 infrared moisture balance (setting 6).

Statistical analysis

Fatty acid percentage compositions (by mass) were calculated for each FAME chromatogram. Comparisons were then made between selected fatty acid ratios of control and irradiated fillets for both the total bound and the phospholipid fatty-acid fractions by ANOVA (parametric) and the Mann–Whitney test (non-parametric). The null hypothesis in each case was that means were the same at the 95% confidence level ($\alpha = 0{\cdot}05$). ANOVA (only) was performed at each dose level (there being insufficient data available for Mann–Whitney testing).

Multivariate comparisons were made by using principal components analysis (PCA). Data matrices were constructed from all fatty acids (variables) present at above 0·5–1·0% of the total (depending on the number of fish (observations) and memory constraints). Raw matrices were standardised.

Two-dimensional scatterplots were produced from the first two principal components in each case and were used to determine the existence of clusters.

ANOVA and PCA were carried out by using a statistical software package (Statgraphics, Statistical Graphics Corporation, MD, USA).

RESULTS AND DISCUSSION

An initial γ-irradiation trial (at the same doses as were later used) was carried out on fillets that had been vacuum-packed. A pink discoloration was observed at all dose levels. Subsequent exposures, performed on fillets packaged in air, alleviated this problem. The fish analysed (Redfish and Black Bream) had very low tissue-lipid contents (1·02 ± 0·64 (SD) and 1·37 ± 0·35 g/100 g, respectively), as is common to most Australian marine fish. Although the exclusion of oxygen from the irradiation packages has been generally recommended to prevent oxidation, it may not be necessary for very lean fish and may actually be undesirable, as was found here and was also noted by Delincée (1983).

Fillets irradiated in air were analysed for their α-tocopherol contents, and the compositions of their total bound fatty acids (triglycerides plus phospholipids) as well as that of their phospholipid fatty acids.

Principal-components analysis of total bound fatty acid FAME profiles revealed no clusters in the two-dimensional scatterplots (data not shown) (the presence of which would have indicated differences between control and irradiated fillets). Univariate analyses of nutritionally important fatty-acid ratios (i.e. P/S, n3/n6, and % monounsaturates) from the total lipids of the irradiated and control fillets revealed no significant changes on irradiation. ANOVA comparisons at each dose gave a similar result for all except one Redfish (p = 0·038), treated at 1 kGy. These results are consistent with those of Rosinvalli *et al.* (1971), in which the irradiation of Cod and Haddock at 2 kGy was reported to produce only small changes in the fatty-acid profiles, which were probably within the error of the method.

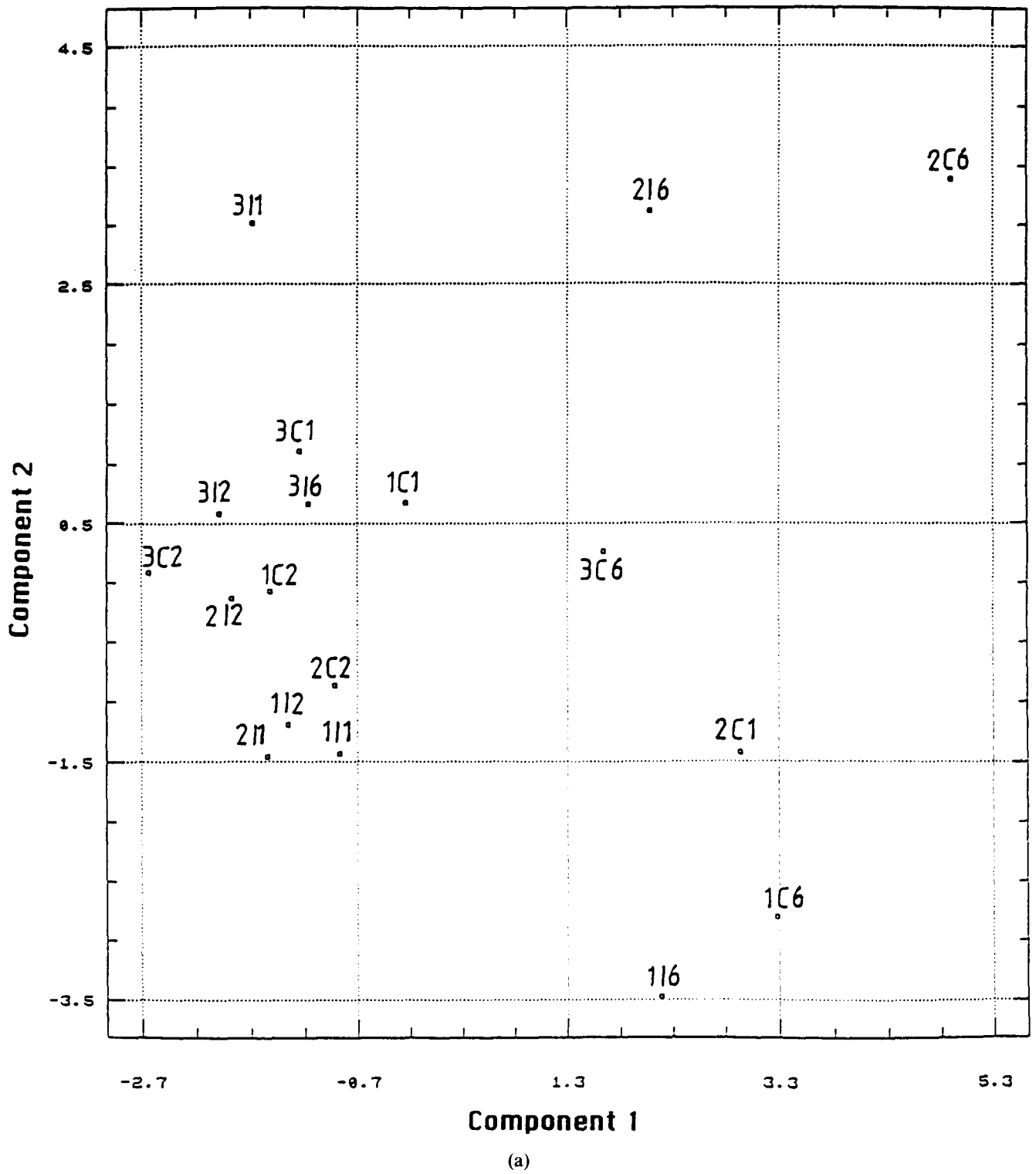
No significant differences were observed between the phospholipid fatty acid chromatograms of control and irradiated fillets. Principal components analysis of the phospholipid fatty acid compositions for the two species also showed no evident clustering (Fig. 1(a) and 1(b)). Both species did, however, exhibit altered correlations with one of the displayed components between the fillets irradiated at 6 kGy and their controls. In both cases, this was found to signify a poorer correlation between irradiated samples and saturated and monounsaturated fatty acids than with the controls. (This was shown by the decreased correlation of Black Bream with its first and of Redfish with its second principal component.) This correlation was not expected, since it would be predicted that the PUFAs would be susceptible to oxidative attack. However, it is consistent with the findings of Dubravcic and Nawar (1969) for volatile lipid-oxidation products in Mackerel oil irradiated at up to 60 kGy. They reported the major components produced to be those known to originate from saturated and monounsaturated fatty acids (14 : 0, 18 : 1, 20 : 1, and 22 : 1), while insignificant quantities of PUFA products (from 18 : 4, 20 : 5, and 22 : 6) were detected. PUFAs are believed to be intimately associated with the phytyl side-chains of antioxidant tocopherol molecules when bound in membranes. In our irradiated fish, the loss of saturated and monounsaturated fatty acids may have occurred because of the poorer protection against free-radical attack afforded to these molecules than to PUFAs. It may also have been contributed to by a selectivity of free radical attack for more saturated fatty acids, due to resonance stabilisation of PUFA chains, as was put forward by Dubravcic and Nawar (1969) to explain their result for irradiated oil.

Univariate analyses of the phospholipid fatty acid P/S and n3/n6 ratios of all controls compared with those of irradiated fillets indicated no significant changes. A similar result was obtained when the data were analysed at each dose level.

Vitamin E contents of fillets, as well as their percentage retention on irradiation, are presented in Tables 1 and 2. More than half of the fillets irradiated exhibited lower vitamin E levels than their controls. This was probably due to radiation-induced tocopherol oxidation. The extent of losses, however, could not be correlated with dose. The variability in the α-tocopherol losses may be due to variations in the extent of protection by other antioxidants, such a vitamin C, in each fillet. Some fillets actually gave increased α-tocopherol levels on γ-irradiation, which may have been due to the

Table 1. Water content and vitamin E (alpha-tocopherol): Retention on irradiation of Redfish

Sample	Vitamin E (mg/100 g muscle)	% Vitamin E retained	% Water
1 kGy			
Control 1	0·798		78·4
Irradiated 1	0·936	117%	77·7
Control 2	0·368		79·8
Irradiated 2	0·301	82%	80·2
Control 3	0·786		78·9
Irradiated 3	0·501	65%	79·3
2 kGy			
Control 1	0·470		78·1
Irradiated 1	0·512	109%	80·2
Control 2	0·882		80·6
Irradiated 2	1·143	130%	79·8
Control 3	0·765		79·5
Irradiated 3	0·512	67%	80·5
6 kGy			
Control 1	0·495		78·1
Irradiated 1	0·510	103%	79·2
Control 2	0·988		77·3
Irradiated 2	0·565	57%	78·1
Control 3	0·751		78·9
Irradiated 3	0·371	49%	79·0

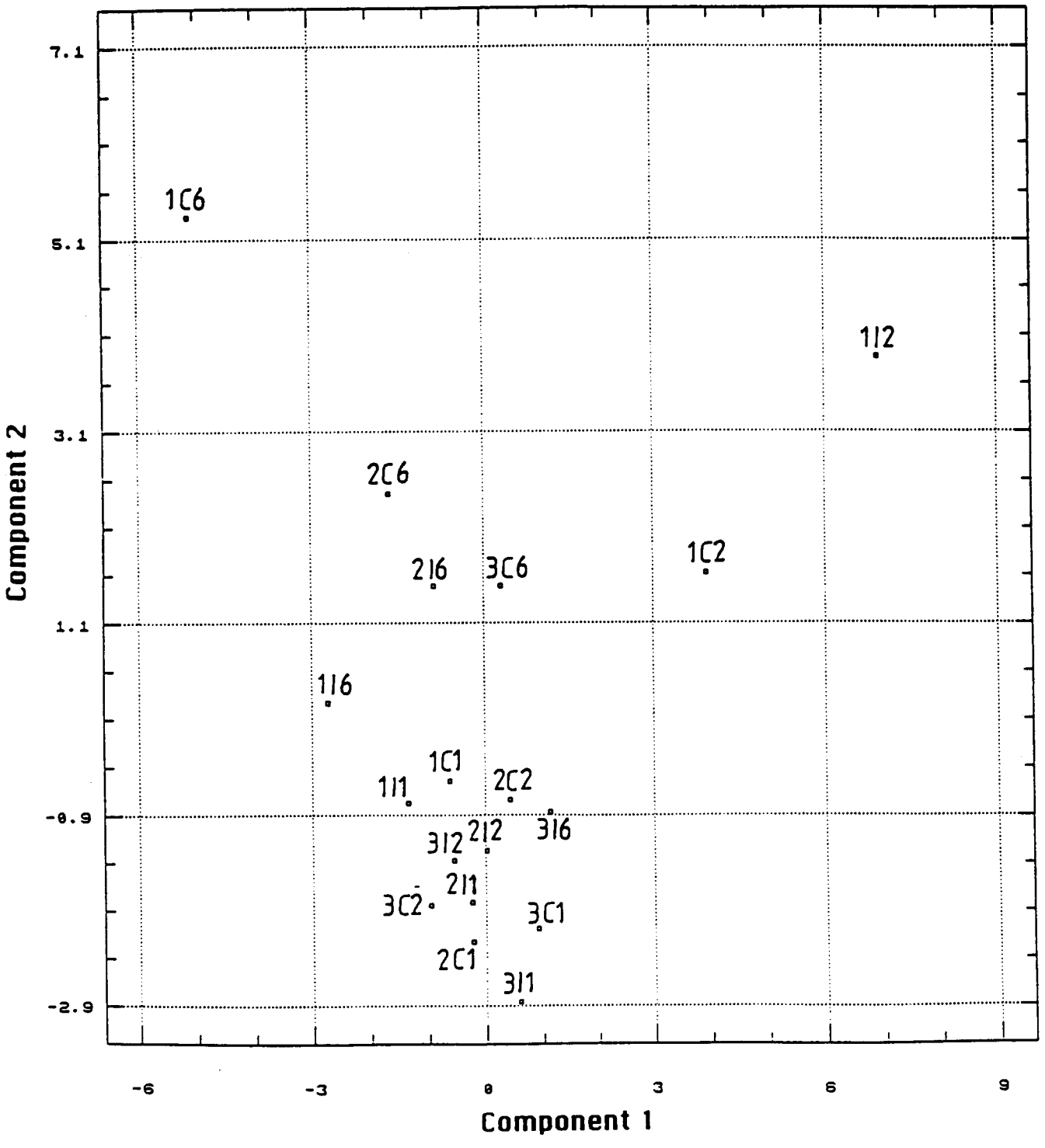


(a)

Fig. 1. PCA scatterplots of phospholipid fatty acids for irradiated and control fish: (a) Black Bream (38·7% of total variance displayed); (b) Redfish (49·4% of total variance displayed). *x*Iy = fillet number × irradiated at *y* kGy; C = Control.

loss of other components in the drip causing a concentration effect on the vitamin E. Water contents of the Redfish fillets were determined (Table 1) (insufficient Black Bream being available) and found, however, to be quite constant (± 2·7%) on γ-irradiation, which indicated that this was not an important factor. The variability in vitamin E contents between the control fillets was larger than that determined for the analytical method.

The nutritional effect of the vitamin E destruction needs to be assessed in terms of the dietary requirement for this component relative to ingested PUFAs, reflecting its role in protection against PUFA oxidation. If the value of 0·60 mg α-T/g PUFA suggested by Horwitt *et al.* (1961) is to be accepted, then it can be seen that all fillets analysed, whether fresh or irradiated, exceeded this requirement (Table 3). The differences in this ratio between control and irradiated fillets was determined not to be significant by univariate analyses.



(b)

Fig. 1.—*contd.*

In conclusion, variations in fatty-acid composition and vitamin E content between individual samples were found to be greater than any irradiation-induced changes. No trends were detected in fatty acid composition, whereas a loss of vitamin E on γ-irradiation treatment was common.

These results suggest that the replacement of fresh lean Australian fish in the diet with the same species that have undergone preservation by treatment with γ-irradiation at normal radurisation doses, would maintain safe levels of vitamin E for protection against oxidation of the ingested PUFAs. The therapeutic effects believed to stem from the PUFA compositions of the fish would also be maintained.

The implications of these findings for Australian fish in general (which are commonly very lean) are that they can be treated at normal radurisation doses of γ-irradiation without the need for vacuum-packing, while retaining the nutritionally beneficial characteristics of their lipids.

Table 2. Vitamin E (alpha-tocopherol) retention on irradiation of Black Bream

Sample	Vitamin E (mg/100 g muscle)	% Vitamin E retained
1 kGy		
Control 1	0·332	
Irradiated 1	0·246	74%
Control 2	0·538	
Irradiated 2	0·502	93%
2 kGy		
Control 1	0·329	
Irradiated 1	0·274	83%
Control 2	0·334	
Irradiated 2	0·381	114%
Control 3	0·326	
Irradiated 3	0·203	62%
6 kGy		
Control 1	0·437	
Irradiated 1	0·253	58%
Control 2	0·372	
Irradiated 2	0·314	84%
Control 3	0·495	
Irradiated 3	0·511	103%

Table 3. Vitamin E protection of PUFAs (mg alpha-tocopherol/g PUFA)

Sample	Bream	Redfish
1 kGy		
Control 1	3·00	7·16
Irradiated 1	3·44	9·36
Control 2	0·89	14·3
Irradiated 2	NA	5·36
Control 3	1·84	9·31
Irradiated 3	1·61	5·23
2 kGy		
Control 1	1·25	5·71
Irradiated 1	2·45	3·41
Control 2	15·5	6·49
Irradiated 2	8·50	16·1
Control 3	1·65	9·38
Irradiated 3	2·38	6·67
6 kGy		
Control 1	1·22	4·93
Irradiated 1	1·16	8·73
Control 2	1·76	6·99
Irradiated 2	0·62	7·18
Control 3	2·62	6·40
Irradiated 3	1·11	3·00

NA, data not available.

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