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A simple chemical method for identification of irradiated industrial processed food

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Abstract

The dependence of *o*- and *m*-tyrosine levels in protein rich products upon the absorbed radiation dose offers the possibility for identification of radiation processed foodstuffs. A simple and fast method was applied for determining irradiation of industrial processed food. Free amino acids of irradiated and unirradiated liquid egg samples (liquid egg, liquid egg yolk) and homogenized turkey sausage samples were extracted with trichloroacetic acid. After centrifugation and filtration aliquots of the crude extract were applied for HPLC analysis using a RP-C18 column and trichloroacetic acid as ion-pairing agent in combination with an electrochemical dual cell detector. *o*- and *m*-Tyrosine were identified by relative retention times and their peak height ratios at different potentials in comparison to those of a standard mixture and quantified by calibration curves. The commercial products investigated were unirradiated.

Keywords: Irradiation; Industrial processed protein rich food; *o*- and *m*-Tyrosine; HPLC; Amperometric detection

1. Introduction

Food irradiation is increasingly recognized as a method for preserving various kinds of food (Satin, 1996). Results show that meat, seafood, vegetables and fruits, are suitable for radiation processing. Using low radiation doses only small chemical changes occur.

In food containing phenylalanine as a consequence of irradiation treatment the formation of *o*-, *m*- and *p*-tyrosine is observed, where their yield is proportional to the absorbed radiation dose. Hence, *o*- and *m*-tyrosine are suggested as markers for irradiated protein rich food. Usually *o*-tyrosine is determined after acid hydrolysis to identify irradiated food (Bernwieser et al., 1995; Chuaqui-Offermanns & McDougall, 1991; Hein, Simat, & Steinhart, 1997; Karam & Simic, 1990; Meier, Bürgin & Fröhlich, 1989). The reported data of these investigations are difficult to compare, because different background levels and under comparable irradiation conditions different yields of *o*-tyrosine were found. The critical step in the applied procedure is the acid hydrolysis of the freeze-dried samples.

Recently, we have published a simple, time saving method based on the irradiation induced tyrosine yield, which avoids the acid hydrolysis (Krach & Sontag, 1997; Krach, Sontag, Solar & Getoff, 1997). The analytical method is founded on the fact, that free amino acids are present in low concentrations in protein rich food (Lück & Pavlik, 1963; Vazquez-Ortiz, Caire, Higuera-Ciapara & Hernandez, 1995). During irradiation the tyrosine isomers result from free phenylalanine as well as from phenylalanine bound in proteins. After extraction of the free amino acids the compounds were separated by ion-pair chromatography and detected in a coulometric electrode array detector. Since the interest for identification of irradiated industrial processed food (Hartmann, Ammon & Berg, 1997; Helle et al., 1993) is increasing a study was undertaken in this respect. The goal of this work was, therefore, to examine the suitability of *o*- and *m*-tyrosine as markers for processed food. In the frame of these studies a special attention has been given to the replacement of the electrode array detector by a coulometric/amperometric dual cell detector. The advantage of this detector is based on the fact that it is more frequently applied in routine laboratories than the electrode array detector. As model samples for this study turkey sausages and industrial processed liquid egg

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products, which are of importance in manufacturing baker's ware and noodles, were used.

2. Materials and methods

2.1. Apparatus

Merck Hitachi System D-6000, consisting of AS-2000A Autosampler, L-6200 Intelligent Pump and D-6000 Interface (Hitachi, Tokyo, Japan) equipped with an Inertsil ODS-2 column, 250×2.1 mm, particle size: 5 μm (GL-Sciences Inc., Tokyo, Japan) and a Phase Separation ODS-2 precolumn, 10×2 mm, particle size: 5 μm (Upchurch Scientific, Oak Harbor, WA, USA). The system is controlled by an IBM PC/AT compatible computer provided with a Merck D-6000 chromatographic software.

Detection system: Coulochem II with high sensitivity analytical cell, model 5011 (ESA, Chelmsford, MA, USA).

Irradiation source: A ^{60}Co-γ-source (Gammacell 220, MDS Nordion International. Inc., Kanata, ON, Canada) providing a dose rate of 125 Gy/min was used.

2.2. Chemicals

DL-*o*-Tyrosine, DL-*m*-tyrosine and DL-*p*-hydroxyphenyllactic acid (Sigma Chemical Co., St. Louis, USA); trichloroacetic acid, methanol, glacial acetic acid, sodium hydroxide, (Merck, Darmstadt, Germany); all reagents applied were analytical grade.

2.3. Solutions and samples

Standard solutions: An internal standard solution containing 225 μg l^{-1} *p*-hydroxyphenyl lactic acid in 0.3 M trichloroacetic acid was prepared. A stock solution of o-tyrosine (65 mg l^{-1}) and *m*-tyrosine (30 mg l^{-1}) in internal standard solution was made and it was further diluted with internal standard solution to the appropriate concentration of *o*- and *m*-tyrosine.

Eluent: 0.05 M trichloroacetic acid, glacial acetic acid and methanol (96:1:3; v:v:v) were mixed and adjusted from pH 2.5 to 3.5 with 2 M sodium hydroxide and the eluent was filtered through a 0.22 μm membrane filter.

Samples: Commercial available turkey sausages, liquid egg, liquid egg yolk and fresh eggs were used for the experiments.

2.4. Irradiation procedure

On liquid egg and liquid egg yolk, both stored in 150 ml polyethylene bottles and on turkey sausages, packed in 1 l polyethylene bags, doses of 0.5, 1, 2, 4 and 6 kGy were applied in presence of air at room temperature (20°C). Irradiated and unirradiated liquid egg, liquid egg yolk and the sausages after homogenizing were stored in a refrigerator at −18°C.

2.5. Analytical method

For determination of *o*- and *m*-tyrosine in unirradiated and irradiated samples 250 mg of sausages and 400 mg of liquid egg, egg yolk and fresh eggs, respectively, were weighed in microtest tubes. After adding 1 ml internal standard solution (225 μg l^{-1} *p*-hydroxyphenyl lactic acid in 0.3 M trichloroacetic acid) and mixing, the samples were placed in an ultrasonic bath (Bandelin electronic, Sonorex SuperRK 103H, Berlin, Germany) at room temperature for 60 min (sausage samples) and for 15 min (egg samples), respectively. The liquid phase was separated by centrifugation (10 min at 2800 *g*) and filtration through a Millex-GW 13 0.22 μm filter (Millipore Corporation, Bedford, MA, USA) and finally 5 μl of the crude sausage- or 20 μl of the egg extracts were injected into chromatographic system (flow: 0.25 ml/min). The separation of the compounds from the matrix on the reversed phase column were optimized using pH values between 2.5 and 3.5 for the eluent. *o*- and *m*-Tyrosine were then detected in a dual cell detector. The potential of the first electrode (coulometric mode) was fixed at +410 mV, and that of the second electrode (amperometric mode) was set to +600 mV against modified palladium reference electrodes. The response of the second electrode was stored and evaluated by calibration curve. Between samples standard solutions were injected to control the response of the detector. Additionally for peak identification and to ascertain the peak purity from the first sample of a series a two step analysis at different potentials of the second electrode (+470 and +600 mV) were made. In those samples *o*- and *m*- tyrosine were identified by comparing their relative retention times and their peak height ratios at different potentials with those of a standard mixture.

2.6. Statistical analysis

Linear regression analyis (*o*- and *m*-tyrosine in turkey sausages) and second order polynomial regression analysis (*m*-tyrosine in liquid egg products) were applied to seven replicate samples of each unirradiated and irradiated (0.5, 1, 2, 4 and 6 kGy) product.

The concentration of *o*- and *m*-tyrosine in unirradiated samples (intercept) and the dependence of *o*- and *m*-tyrosine concentration from the applied dose (slope) as well as standard deviation for both values (neglecting the quadratic term of the polynomial regression analysis of egg products, see results) were estimated.

3. Results and discussion

3.1. Chromatographic separation

Halogenated and specially trihalogenated acetic acid (TCA) can be used as an ion-pair agent for small cationic hydrophilic substances (MacCrehan & Shea, 1988). *o*- and *m*-Tyrosine form in a wide acid pH-range such ion-pairs, having a distinct retention time for both isomers, which increases with decreasing pH-values. Hence, for each kind of sample an optimal pH-value for separation of *o*- and *m*-tyrosine from matrix compounds can be achieved.

3.2. Electrochemical detection

A commercially available dual cell detector was applied for detection of the markers. It consists of one coulometric and one amperometric electrode in series. In this way a lot of interfering substances can be oxidized in the first cell at +470 mV, without oxidizing the tyrosine isomeres. These are then detected at the second electrode at +600 mV amperometrically with enhanced sensitivity (*o*-tyrosine: 12.4 nA ng^{-1}, *m*-tyrosine: 22.3 nA ng^{-1}) in comparison with two coulometric electrodes in series. A linear relationship between response and concentration range under investigation was found for *o*- (1.2 to 160 μg l^{-1}) and *m*-tyrosine (1.9 to 150 μg l^{-1}). Injecting 20 μl of the standard solution the detection limit (S/N = 3) for *o*-tyrosine is 1.2 μg l^{-1} and that for *m*-tyrosine 1.9 μg l^{-1}. The higher detection limit of *m*-tyrosine is due to its position in the decreasing part of the chromatogram (Fig. 1).

3.3. Determination of o- and m-tyrosine in turkey sausages

To find the time for complete extraction of the amino acids, samples were extracted 30, 60 and 90

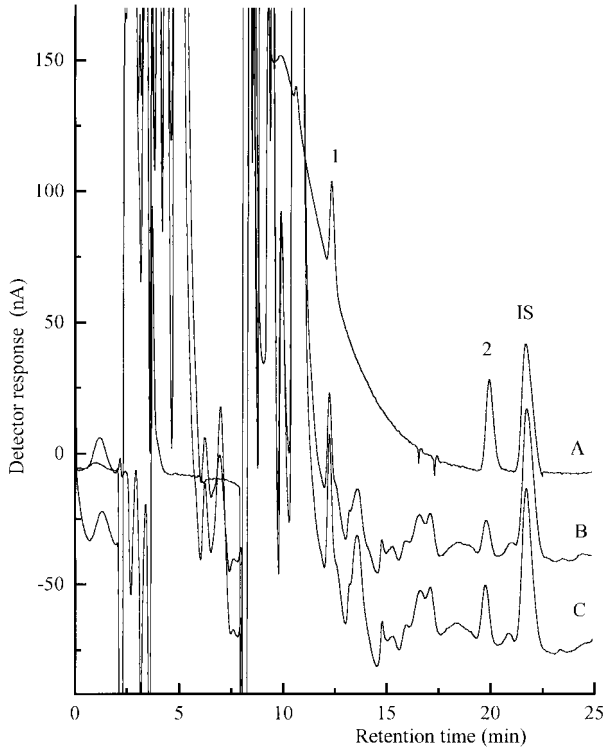

Fig. 1. Chromatograms of turkey sausages, irradiated with 2 kGy (B) and 4 kGy (C). Standard solution (A): *m*-tyrosine (1), *o*-tyrosine (2) and *p*-hydoxyphenyl lactic acid (IS).

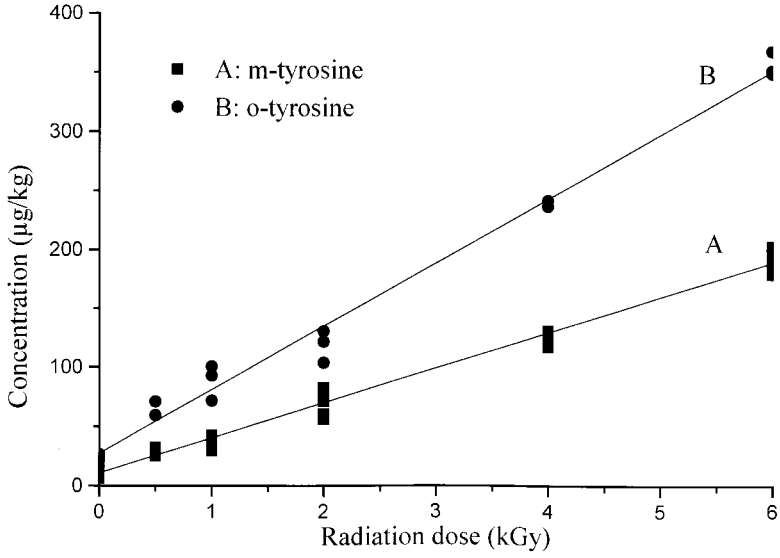

Fig. 2. Radiation induced yield (μg kg^{-1}) of *o*-tyrosine and *m*-tyrosine in turkey sausages as function of the absorbed dose (kGy). Each assay was repeated seven times.

min. After 60 min the concentration of the tyrosine isomers in the extracts did not increase further. The chromatographic separation of the tyrosine isomers from matrix compounds was optimized using different pH values of the eluent. At pH 2.5 optimal separation was obtained and the chromatograms of the individual samples irradiated in our laboratory are shown in Fig. 1.

A linear correlation ($n=42$) between *o*- ($r=0.994$) and *m*- ($r=0.993$) tyrosine concentration and the radiation dose was found (Fig. 2). The concentration in the non-irradiated samples (ground level) evaluated from seven replicate determinations was for *o*-tyrosine 27.3 ± 5.3 μg kg^{-1} and for *m*-tyrosine 10.7 ± 2.1 μg kg^{-1}. The increase of *o*- (53.9 ± 1.65 μg kg^{-1} kGy^{-1}) and *m*-tyrosine (29.7 ± 0.7 μg kg^{-1} kGy^{-1}) is sufficient to detect a radiation dose down to 0.5 kGy.

3.4. Determination of o-tyrosine in industrial processed liquid egg products

In liquid egg products only *o*-tyrosine could be separated from the matrix compounds (Fig. 3) and quantified at pH 3.5 in a short time.

It is remarkable that in none of the processed egg samples and in fresh eggs o-tyrosine could be detected. This is in contrast to chicken (Krach & Sontag, 1997) or turkey meat, which show a significant ground level.

Samples irradiated with different doses (0.5, 1, 2, 4, 6 kGy) were analyzed seven times. The evaluation of chromatograms leads to a nonlinear correlation ($y=A_0+A_1x+A_2x^2$) between the *o*-tyrosine concentration of liquid egg yolk ($r=0.996$, $r^2=0.991$) and liquid egg ($r=0.993$, $r^2=0.99$), respectively, and the radiation dose (Fig. 4). By neglecting the quadratic term for low irradiation doses (up to 2 kGy) a different increase of the *o*-tyrosine concentration for liquid egg yolk (87.5 ± 4.7 μg kg^{-1} kGy^{-1}) and liquid egg (21.3 ± 2.1 μg kg^{-1} kGy^{-1}) was found.

The nonlinear correlation of liquid egg yolk corresponds with the data of Krajnik, Ouint, Solar, Getoff,

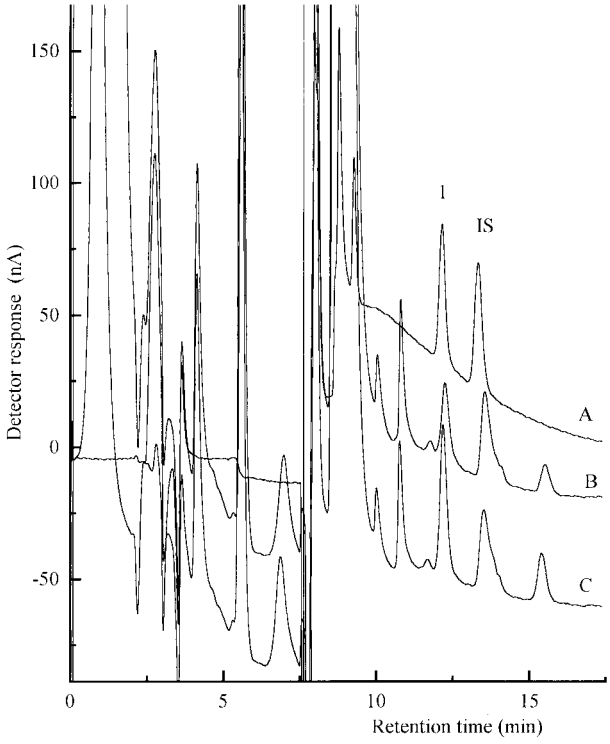

Fig. 3. Chromatograms of industrial processed liquid egg yolk irradiated with 2 kGy (B) and 6 kGy (C). Standard solution (A): *o*-tyrosine (1) and *p*-hydroxyphenyl lactic acid (IS).

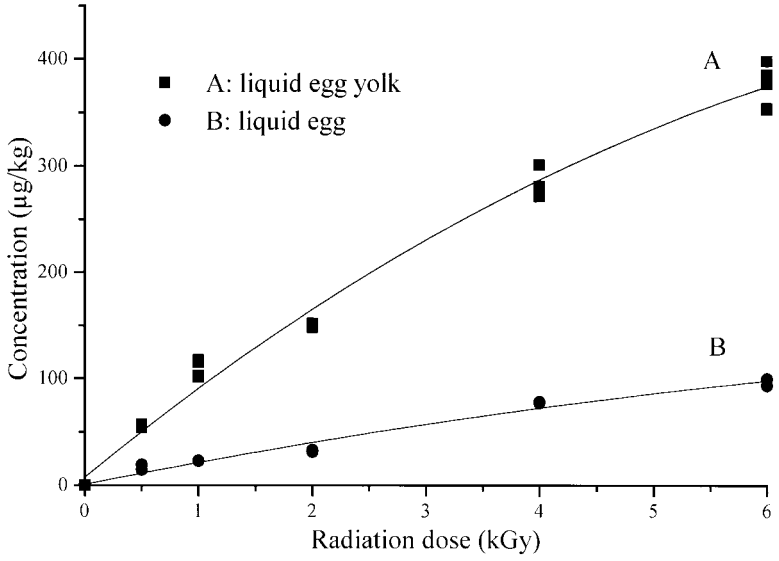

Fig. 4. Radiation induced yield (μg kg^{-1}) of *o*-tyrosine in liquid egg yolk and liquid egg as function of the absorbed dose (kGy). Each assay was repeated seven times.

and Sontag (1995), who found a similar behaviour when air saturated aqueous phenylalanine solutions were irradiated. Obviously in liquid samples the isomers are converted faster by the irradiation than in solid samples.

The amount of o-tyrosine formed by irradiation depends on the concentration of free phenylalanine. In albumin this concentration is very low, but in egg yolk much higher (Lück & Pavlik, 1963). This means that in liquid eggs, which show a weight distribution of approximately 36% egg yolk and 64% albumine (Eisenbrand & Schreier, 1995) the free *o*-tyrosine formed during irradiation has to be smaller than in egg yolk.

The fact that the ground level of *o*-tyrosine can be neglected (below detection limit) and the increase of the *o*-tyrosine concentration with the radiation dose in egg yolk is about 90 μg kg^{-1} kGy^{-1} makes it possible to detect a radiation dose down to 0.2 kGy.

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