Incidence of Irradiated Foods in the Distribution Network of Prague

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Abstract

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The samples, 29 in total, of poultry, rabbit meat, cheese and exotic fruits were taken from the distribution network of Prague. None of the samples was declared as irradiated according to the Decree of the Ministry of Health, CR, No. 133/2004 Sb. The check of their possible exposure to irradiation was made by means of two methods, i.e. the procedure according to EN 1784 (determination of hydrocarbons generated by irradiation using gas chromatography) and the determination of non-bonded *o*-tyrosine by means of HPLC with electrochemical detection. Neither method brought evidence for the exposure to irradiation, i.e. the purchased foodstuffs concerned were not labelled deceitfully.

Keywords: irradiated food; poultry; cheese, fruits; hydrocarbons; *o*-tyrosin

The initial information on the possibility to employ ionising radiation for the protection and preservation of foodstuffs dates as far back as the third decade of the last century. In 1950s, irradiation became the object of interest for food manufacturers and sellers as well as for food consumers. It was applied to the wide range of foodstuffs, including, e.g., meat including poultry and fish, seafood, cereals and vegetables or even nuts and spices. In the first place, its ability is utilised to reduce considerably the levels of pathogens, parasites and infestation by insects, resulting in the increase of food safety and significant shelf life extension. Thus it is also positively appreciated that the changes caused by irradiation in food chemical composition are generally much lower than those resulting from the application of other preservation methods.

Nevertheless, certain minor changes in composition can be observed in food after irradiation as compared to its original condition. Such changes are generally caused by the interactions of primary free radicals or ions generated by irradiation with various reactive groups of substances present in the biological material. The reactions may either result in quite new compounds, or in stabilisation of secondarily formed radicals in the solid or polymer areas of the sample material. The material changes occurring on foodstuff irradiation are thoroughly and in detail dealt with by Glidewel *et al.* (1993). At the same time, these authors describe various options for employing such changes in the development of analytical methods for the evidence of irradiation. The methods can be divided into five basic groups comprising EPR spectroscopy, luminescence methods, the measurement of physical parameters, chemical methods, and microbiological methods.

However, since the very beginning of the use of irradiation up to now, professionals' and laypersons' circles have disputed the problems of suit-

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ability and health safety of this method for food preservation. The main reservations are based on the fact that irradiation is accompanied with certain major physiological changes (e.g. inhibition of germination and ripening), damage to DNA, the loss of vitamins and persistence of toxins in the food even after the toxin producing microorganisms have been destroyed.

This inconsistence of views on irradiation manifested itself also by different conditions and requirements applied to the process in European and extra-European countries. That was why the European Union adopted, in 1999, two Directives No. 1999/2/ES and 1999/3/ES, laying down uniform conditions for food and food material irradiation in accordance with the EU legislation, to provide for the maximum information of the public on purchased products, the protection of consumer interests, and the improvement of the food market in general. In addition to the provision of technical and other basic conditions, the food manufacturers and distributors are required to label all the irradiated foodstuffs accordingly, including the foodstuff, in which irradiated components are used. In compliance with the above-mentioned Directives, the Decree of the Ministry of Agriculture No. 133/2004/Sb. was issued to govern the conditions for food irradiation in the CR.

Naturally, the adoption of the said Directives entails also the possibility to control the labelling of the irradiated foodstuffs in their distribution, which, in turn, shall rely on the availability of adequate analytical methods. Up to now, ten European Standards have been issued concerning the detection of irradiation of various food commodities, including four screening ones, which are gradually implemented in the Czech legislation as Czech Technical Standards. Delincée (2002) subjected the standards to a more detailed analysis and discussed the possibilities of making them more precise. Even though the standardised methods have been developed on the basis of the former research findings, there are a considerable number of other proposed methods that have not achieved the status of the European Standard, as their inter-laboratory validation has not been successfully completed up to now. Moreover, one can expect intensive developments in research activities to take place in this field, as the clear identification of an irradiated foodstuff will require the application of a few analytical methods in parallel.

The objective of the present work was to ascertain whether selected irradiated foodstuffs (poultry, cheese, exotic fruits) occurred within the distribution network of Prague without being properly labelled in compliance with the Decree of the Ministry of Agriculture No. 133/2004 Sb. The standardised analytical method according to EN 1784 (Anonymous 1966) (or ČSN 56 001 – Anonymous 1998) was used to check the possible irradiation of the samples. The method is based on the fact that, by irradiation, fatty acids split off triacyl-glycerols contained in fats. The fatty acids are transformed into hydrocarbons that have either one carbon atom less, or two carbon atoms less and one double bond more than the parent fatty acid. Thus, the composition and incidence of hydrocarbons generated by irradiation can be predicted from the ascertained relative abundance of particular fatty acids in the sample tested.

Further, the incidence of irradiation of identical samples of poultry was tested in parallel by the determination of a marker substance, i.e. *o*-tyrosine formed by irradiation from phenylalanine, both non-bonded and bonded in proteins. This reaction has been known already since the half of the last century (Stein & Weiss 1948) and Karam and Simic (1988) proposed it for demonstrating the irradiation of foodstuffs rich in protein. In principle, two chromatographic methods were developed for the determination of *o*-tyrosine generated in this way, after releasing it by hydrolysis from proteins contained in the sample. The first method employed GC-MS (Karam & Simic 1990; Pedersen & Fuhlendorff 1991), while the other method was based on HPLC using either fluorescence (Chuaqui-Offermanns & McDougall 1991; Chuaqui-Offermanns *et al.* 1993; Helle *et al.* 1993; Meier *et al.* 1990; Ibe *et al.* 1991) or electrochemical (Bernwieser *et al.* 1995) detections. The analyses were carried out mostly with samples of chicken meat and eggs. Recently, methods were developed that employed, as the irradiation marker, *o*-tyrosine formed from non-protein-bonded phenylalanine. The equipment employed consisted of an HPLC instrument fitted with either electrochemical (Krach *et al.* 1997, 1999) or fluorescence (Hein *et al.* 2000) detectors. The two last mentioned methods are considered suitable for the purpose as the hydrolysis of proteins in the sample preparation, which was the critical step in the formerly applied procedures, is avoided in this case (Krach *et al.* 1997).

However, in spite of considerable research efforts, this method has not been successfully validated on the inter-laboratory level up to now and could not be included in the EU standardised methods. The main reason resides in the facts that the formation of tyrosine is dependent on the conditions of irradiation, and that foodstuffs contain small but widely fluctuating amounts of naturally occurring *o*-tyrosine as a result of the action of tyrosine hydroxylase (Ishimitsu *et al.* 1986; Hart *et al.* 1988).

MATERIALS AND METHODS

Samples checked. Eleven samples of poultry (goose, turkey and duck coming from France, Hungary and China), one sample of rabbit meat (France), 8 cheese samples of Camembert type (France) and 9 samples of exotic fruits (mango, avocado from Peru, Brazil and Spain) were purchased in Prague distribution network. None sample was labelled as irradiated. Parts of selected samples (2 samples of turkey, 1 of cheese, 2 of mango and 2 samples of avocado) were additionally irradiated by the doses of 7.0 or 2.0 kGy to verify the findings.

Determination of hydrocarbon by gas chromatography. Hydrocarbons were determined in accordance with EN 1784 (or ČSN 56 001) standard. That is why the basic data only are given here.

Chemicals: Hexane for org. trace analysis (Merck); Diethyl ether p.a., ACS, ISO (Merck); Florisil PR 60 to 100 mesh (Fluka); Sodium sulphate anhydr. calcinated at 650°C (Lachema).

Hydrocarbon standards: 1-dodecene 95% (Aldrich); *n*-tridecane, min. 99% (Sigma); 1-tetradecene, min. 99% (Sigma); *n*-pentadecane, min. 99% GC (Sigma); hexadecane, pure > 98% (Fluka); 1-hexadecane approx. 99% (Fluka); 1,7-hexadecadiene (Fluka); heptadecane, approx. 97% (Sigma); 8-heptadecene, > 97% (Fluka); octadecane, approx. 99% (Sigma); 1-octadecene, approx. 97% (Sigma); eicosane, approx. 99% (Sigma).

Apparatus: Ultra-turrax TP 18/10, Janke & Kunkel GmbH; Soxhlet extractor; NBC 800 Membrane Vacuum Pump, KNF Neuberger LABOPORT; Hewlett Packard 5890 Gas Chromatograph, series II, with HP 6890 Auto Sampler and FID Detector.

Fat extraction: Extraction of fat from the homogenised samples of poultry meat, rabbit meat and cheese was carried out by *n*-hexane in a Soxhlet extractor. Fat from the samples of avocado and mango seeds was obtained by the direct extraction of the homogenised samples with *n*-hexane for 2 min. The extraction agent was removed using a rotary vacuum evaporator at the temperature of 40°C and the pressure of 250 kPa, and the residue was dried in the flow of nitrogen.

Fat purification: Fat samples were purified by elution with *n*-hexane using a column charged with de-activated Florisil (17 g), employing *n*-eicosane as an internal standard added. Iso-octane (1.0 ml) was added to the resulting solution before its evaporation in a rotary vacuum evaporator at the temperature of 40°C and the pressure of 250 kPa.

Separation and detection of hydrocarbons

The mixture of hydrocarbons obtained was separated by means of gas chromatography under the following conditions.

Column: DB-5 HT, supplied by J & W, 30 m × 0.25 mm, 0.25 μm; Injector: Splitless, t = 360°C; Detector: FID, t = 360°C; Carrier gas: nitrogen 5.0, (flow rate 3.24 ml per min at 30°C), EPC system; Temperature/time programme: 50°C for 2 min; 10°C/min up to 70°C, 2.5°C per min upon to 180°C, 25°C/min up to 360°C, 360°C for 15 min.

Total time of analysis: 70 min; Detection limit: 0.5 mg of hydrocarbons per kg of fat; Repeatability expressed as standard deviation STD (n = 8); STD between 0.002–0.004 (max. and min. values for hydrocarbons tested).

Determination of fatty acids. Fatty acids were determined by gas chromatography as methyl esters after re-esterification in an alkaline medium (Bohačenko & Kopicová 1999).

Determination of o-tyrosine by HPLC using electrochemical detection. The determination was carried out using the modified method of Krach *et al.* (1999). Contrary to the original method the Coulochem II detector with a dual electrode was replaced with a classical single-electrode detector an independently operating GuardStat unit fitted upstream of the detector. This unit was replaced, to a large extent, the cleaning electrode of Coulochem II.

Chemicals: *p*-hydroxyphenyl-lactic acid, for HPLC, 98% (Sigma); trichloroacetic acid, 99.0% (Sigma); *o*-tyrosine, > 98% (NT) (BioChemika); *m*-tyrosine, > 98% (NT) (BioChemika); *p*-tyrosine (VEB Berlin-Chemie); methanol for chromatography (LiChrsolv – R); sodium hydroxide, min. 98% (Sigma); water, demineralised.

Apparatus: HPLC System: Agilent 1100 Series; Electrochemical detector: 10490A Hewlett Packard; GuardStat: Model 5020, ESA, Inc.; Ultraturrax TP 18/10, Janke & Kunkel GmbH & Co KG; Ultrasonic bath, UC 002 bm1, Tesla; Ultracentrifuge, MLW T 52.1.

Preparation of samples for HPLC

Internal standard solution (20 ml, containing *p*-hydroxyphenyl-lactic acid – 225 μg/l dissolved in 0.3M trichloracetic acid) was added to the meat sample (5 g, homogenised by Ultraturrax) in a beaker. The beaker was placed in ultrasonic bath, the mixture was stirred and then extracted at ambient temperature for 60 min. The liquid phase was separated by centrifugation (at 2800 g for 10 min) and after filtration through a micro-filter of 0.45 μm pore size, the sample was ready for feeding in to HPLC column.

Determination of o-tyrosine by HPLC. Column: Reprosil 100 C 18, 5 μm, 250 × 4 mm, Dr. Maisch GmbH; Elution agent A: Mixture of 0.05M trichloroacetic acid, glacial acetic acid and methanol in the ratio of 96:1:3 by volume. After adjusting the pH value to 3.75 using 2M NaOH, the mixture was filtered through a membrane micro-filter of 0.22 μm pore size; Elution agent B: Mixture of demineralised water and ethanol in the ratio of 20:80 by volume; Conditions of chromatography: Elution by agent A: Flow rate – 1 ml/min; temperature – 25°C; injection – 20 μl; Voltage – ECD: +600 mV; GuardStat: +410 mV; Stop time: 60 min. After completing the measurements, the column was washed by agent B for 30 min.

Detection limit: 0.005 mg *o*-tyrosine/100 g of sample; Repeatability expressed as standard deviation SDT (n = 12) – SDT = 0.002 (level: 0.01–0.02 mg *o*-tyrosine/100 g), SDT = 0.009 (level: 0.10–0.50 mg *o*-tyrosine/100 g).

RESULTS AND DISCUSSION

As there is no operator in the Czech Republic engaged in the irradiation of foodstuffs of concern, imported products only were purchased for the purpose of this check. However, none of them was labelled as irradiated. To demonstrate the irradiation using the method according to EN 1784, the contents of the selected (recommended) fatty acids were determined in all samples (Table 1). The results show that both the saturated (palmitic, stearic and myristic) and unsaturated (oleic, linoleic) fatty acids were found in all samples tested. By irradiation, the above acids may generate the corresponding hydrocarbons of C_{n-1} and C_{n-2}:1 types. In the spectrum of acids, small amounts of linoleic acid were found in cheeses only, while myristic acid was present in relatively high proportions; small amounts of stearic acid were found in the samples of avocado.

The survey of the hydrocarbon amounts which were found in the samples coming from the distribution network is shown in Table 2.

From the results it ensues that:

– Hydrocarbons 17:1 and 16:2, or 17:2 and 16:3, which should be generated by the irradiation of oleic or linoleic acids were not found in any sample tested.
– As to the hydrocarbons derived from saturated fatty acids (palmitic, stearic and myristic), only small amounts of saturated 17:0 substances (0.50–3.15 mg/kg of fat) were found in most of the poultry and cheese samples, while the respective unsaturated hydrocarbons were not found.

Table 1. Mean distribution of selected fatty acids (% in the fat) in the samples of poultry, rabbit, fruits and cheese

Fatty acid	Turkey	Duck	Goose	Rabbit	Mango	Avocado	Cheese
Myristic	nd	nd	nd	nd	nd	nd	11.0
Palmitic	21.57	22.66	22.51	29.59	8.9	13.8	33.0
Stearic	7.25	6.34	6.42	6.92	32.8	0.7	9.8
Oleic	30.28	53.26	47.76	28.34	46.1	62.9	21.7
Linoleic	27.68	9.20	13.60	13.02	7.4	11.7	2.3

nd – not detected

Table 2. Survey of the hydrocarbons detected in the samples within Prague distribution Market

Sample	No.	Hydrocarbon content (mg/kg of fat)		
		15:0	16:0	17:0
Turkey	1	nd	nd	nd
	2	nd	0.67	2.10
	3	nd	nd	0.51
	4	nd	1.50	3.15
	5	nd	nd	nd
	6	nd	nd	nd
Goose	1	nd	nd	nd
	2	nd	nd	nd
Duck	1	nd	nd	nd
	2	nd	nd	nd
	3	nd	nd	nd
Rabbit		nd	nd	0.50
Cheese	1	nd	nd	0.84
	2	nd	0.73	1.35
	3	nd	nd	0.50
	4	nd	nd	0.52
	5	nd	nd	1.19
	6	nd	nd	0.76
	7	nd	nd	0.58
	8	nd	nd	0.50
Mango	1	9.13	nd	nd
	2	16.82	nd	nd
	3	1.89	nd	nd
	4	7.70	nd	nd
	5	18.75	nd	nd
	6	34.47	nd	nd
Avocado	1	1.09	nd	nd
	2	nd	nd	nd
	3	2.07	nd	nd

nd – amount < 0.5 mg/kg of fat (detection limit)

– Hydrocarbon 15:0 only was found in the samples of mango and avocado (in the range 1.09–34.47 mg per kg of fat).

According to the above-cited standard only those samples should be deemed conclusively irradiated in which hydrocarbons generated by unsaturated fatty acids were found and whose ratio would serve for the calculation of the proportions of hydrocarbons generated from saturated fatty acids (paragraphs 8.1 and 8.3 of the standard). In addition, the standard states (see paragraph 9 thereof) that an isolated incidence of saturated hydrocarbons (in this case 15:0 and 17:0) cannot be concidered as an evidence of irradiation, as such substances are often present either as contaminants (due to the contact of the foodstuff with its plastic package) or as naturally occurring substances.

Taking this presumption and the above-mentioned results into account, one can state that none of the checked samples of foodstuffs taken form the distribution network was treated by irradiation.

This statement was confirmed by a reference experiment in which the hydrocarbons of concern were determined in selected, additionally irradiated samples. As follows from the data of Table 3, after irradiation, appreciable amounts were found of generated hydrocarbons. In the poultry samples were detected: unsaturated 14:1 (generated from palmitic acid), 16:1 (from stearic acid), 17:1 and 16:2 (from oleic acid) and 17:2 and 16:3 (products of linoleic acid) that had not been found in the samples before irradiation. Similar changes occurred in the samples of cheese, in which hydrocarbons 13:0 and 12:1, generated from myristic acid, were found in addition. As to the fruit, in the irradiated samples of mango were found unsaturated hydrocarbons 16:1 (from stearic acid), 17:1 and 16:2 (generated from oleic acid). The unsaturated hydrocarbons generated from palmitic acid and linoleic acid, i.e. 14:1 and 17:2 or 16:3, respectively, were not detected, obviously because of the lower initial levels of the fatty acids and lower radiation doses employed. In the samples of avocado, after irradiation, were found unsaturated hydrocarbons: 14:1 (product of palmitic acid), 17:1 and 16:2 (products of oleic acid), 17:2 and 16:3 (formed from linoleic acid). For the same reasons as in mango, the product of stearic acid, 16:1, was not detected.

The amounts of unsaturated hydrocarbons generated were also in good correlations with the proportions of fatty acids in the individual original samples.

The evidence for irradiation in the same set of poultry samples taken from the distribution network was obtained also by the determination of non-bonded tyrosine using HPLC method with electrochemical detection. The content of non-

Table 3. Survey of the hydrocarbons (mg/kg of fat) detected in additionally irradiated samples

Hydrocarbon	Turkey 4	Turkey 6	Cheese	Mango 1	Mango 2	Avocado 1	Avocado 2
12:1	nd	nd	1.33	nd	nd	nd	nd
13:0	nd	nd	0.64	nd	nd	nd	nd
14:1	2.91	4.19	3.48	nd	nd	0.50	0.74
15:0	4.98	4.24	6.89	15.17	13.27	1.28	1.54
16:3	5.42	4.93	1.19	nd	nd	0.50	0.50
16:2	4.76	6.54	2.22	3.27	0.50	2.29	2.43
16:1	1.24	1.71	0.81	1.62	1.07	nd	nd
16:0	1.01	0.50	nd	nd	nd	nd	nd
17:2	4.49	4.97	1.57	nd	nd	0.79	0.72
17:1	4.99	4.77	2.92	3.90	0.50	1.59	1.59
17:0	3.61	2.41	2.61	7.90	1.01	nd	nd

nd – amount < 0.5mg/kg of fat (detection limit)

Table 4. Survey of free (non-protein-bonded) *o*-tyrosine content (mg/100g) in poultry

Sample	No.	Determination		Mean
		1	2	
Turkey	1	0.010	0.009	0.0095
	2	0.016	0.017	0.0165
	4	0.020	0.022	0.0210
	6	0.010	0.014	0.0120
Goose	1	0.009	0.008	0.0085
	2	nd	nd	nd
Duck	1	0.020	0.019	0.0195
	2	0.010	0.011	0.0105
	3	0.015	0.017	0.0160
Additionally irradiated samples				
Turkey	4	0.446	0.406	0.4260
	6	0.223	0.220	0.2215

nd – amount < 0.005 mg/100 g of sample (detection limit)

bonded tyrosine, found in all the samples, fluctuated within the range of 0.009–0.021 mg/100 g of sample (Table 4) that corresponded well with the natural levels found in 12 samples of demonstrably non-irradiated chicken, analysed in the course of the implementation of this method (in Výroční zpráva řešení projektu QC 1111 za rok 2003, VÚP Praha 2004). Correctness of this evaluation was proved again by determining the content of non-bonded tyrosine in two samples of turkey (No. 4 and 6) irradiated additionally by the dose of 7 kGy. About twenty times higher levels of non-bonded o-tyrosine were found in the samples after irradiation (Table 4).

In conclusion, it can be stated that in the check of the selected foodstuffs taken from the distri-

bution network of Prague (the first one of its type made in the CR), the two methods did not provide evidence for any previous irradiation of the samples tested. Therefore, the foodstuffs of concern were not labelled deceitfully. We believe that the evidence for food irradiation by the determination of non-bonded tyrosine could be used as a screening method in laboratories equipped with a HPLC instrument using electrochemical detection.

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Souhrn

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V tržní síti byl odebrán soubor 29 vzorků drůbeže, králičího masa, sýrů a exotického ovoce, přičemž ani jeden z nich nebyl deklarován, v souladu s vyhláškou MZd ČR č. 133/2004 Sb., jako ozářený. Kontrola jejich možného

předchozího ošetření ozářením byla provedena dvěma metodami, a to podle EN 1784 (stanovení uhlovodíků generovaných ozářením plynovou chromatografií) a dále stanovením volného *o*-tyrosinu pomocí HPLC s elektrochemickou detekcí. V žádném případě nebylo ani jednou z těchto metod ozáření prokázáno, takže nedošlo ani ke klamavému značení nakoupených potravin.

Klíčová slova: ozářené potraviny; drůbež; sýry; ovoce; uhlovodíky; *o*-tyrosin

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