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QUALITATIVE AND QUANTITATIVE CHANGES IN THE COMPONENTS OF IRRADIATED FOODSTUFFS. SUGGESTIONS FOR FURTHER ANALYTICAL STUDIES AS A CONTRIBUTION TO THE EVALUATION OF WHOLESOMENESS

J.F. DIEHL

Bundesforschungsanstalt für Ernährung
Karlsruhe

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Qualitative and Quantitative Changes in the Components of Irradiated Foodstuffs. Suggestions for Further Analytical Studies as a Contribution to the Evaluation of Wholesomeness.

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Between 1967 and 1973 the Commission of the European Communities was engaged in research in collaboration with five laboratories in Community countries, into the physical, chemical, biological and anatomical changes which occur in irradiated foodstuffs. The purpose of this research was to develop practical analytical control methods which could be used to identify irradiated foodstuffs.

As the scope of the above-mentioned research did not extend to the evaluation of the results from the point of view of health, the Commission requested the author of this report to study the specific changes observed, and the ways they affect wholesomeness, under a study contract.

(The Editor)

Qualitative and quantitative changes in the components of irradiated foodstuffs. Suggestions for further analytical studies as a contribution to the evaluation of wholesomeness

by J.F. Diehl

Summary:

The effects of ionizing radiation on the chemical composition of foodstuffs are reviewed, and the available information is examined for its suitability as a basis for judging the wholesomeness of irradiated foodstuffs. Suggestions are made for further chemical studies, with the aim of supplementing the information available from animal feeding tests, so as to permit a satisfactory evaluation of the wholesomeness of all irradiated foodstuffs, without continuing ad infinitum the feeding tests, which have been going on for some 25 years.

Contents

1. Purpose of study

The purpose of this study is to summarize the results of research done so far on the effects of ionizing radiation (X-rays, gamma rays and electron beams) on the chemical composition of foodstuffs, and to see how far these results are of use in evaluating the wholesomeness of irradiated foodstuffs. Where sufficient data are not yet available for the toxicological and nutritional evaluation of irradiated foodstuffs, suggestions are made for further chemical and analytical studies.

2. Introduction

The laws on foodstuffs in all the Member States of the European Communities contain clauses prohibiting the irradiation of foodstuffs. Exemption permits may be granted when it has been shown that the process is not harmful to health. The procedure for providing evidence of this is described in the recommendations of a Joint FAO/IAEA/WHO Expert Committee (WHO, 1965). The main requirements are animal feeding tests over a long period, using several different types of animal, similar to the tests required for acceptance of food additives. But equating irradiated foodstuffs with food additives, which has become standard practice as a result of U.S. legislation, is not satisfactory.

The toxicological significance of an additive is assessed by giving groups of experimental animals different amounts of the substance in their food. Usually, harmful effects are observed when the amount given is above a certain level. The particular level (per kg of body weight and per day) just below that at which harmful effects are noted is called the "no effect level". To make allowance for the uncertainty of transferring findings on animals to human beings, and the possibility of increased sensitivity in sick persons, a safety factor of 100 is incorporated, and one hundredth of the no effect level is taken as the acceptable daily intake (ADI).

How can this principle be applied to irradiated foodstuffs? So far, whenever harmful effects have been observed in animals fed with irradiated foodstuffs, these were not due to toxic effects of radiation-induced

substances but to deficiencies, usually an insufficient vitamin content in the experimental diet. In an effort to give the animals as much of the irradiated food as possible, they were often being fed a diet which did not suit their physiological requirements (e.g. 35 % onions or raisins when irradiated onions or raisins were being tested!). Regardless of whether the food was irradiated or not, the groups of animals compared in these experiments were often all unhealthy because they had been given the wrong kind of food. In the dose range used for irradiation in practice (max. 5 Mrad) no toxic effects which are unquestionably due to irradiation have been observed - even when 100 % of the food was irradiated. As we do not know the "effect level" we cannot tell what level should be taken as the "no effect level". Besides, the safety factor of 100 cannot be applied in most cases, since it is usually not possible, as with additives, to give an animal 100 times the amount (related to body weight) to be consumed by humans. Even when toxicologists have not observed any harmful effects from feeding irradiated food to animals, they still hesitate to classify such foodstuffs as "acceptable" as they do not know the relevant safety factor. Attempts to raise the safety factor by applying a higher radiation dose are only valid up to a point, as in many cases the reaction products formed at high radiation doses are completely different from those at low doses. In this respect irradiation can be compared with heating. In assessing the wholesomeness of a type of fat heated to 300°, it is not possible to arrive at a higher safety factor by testing the same "fat" heated to 1000° on experimental animals.

This uncertainty about the margin of safety is presumably the main reason why health authorities in most countries are very hesitant to grant permits for irradiated foodstuffs (or have not granted any, as in Belgium). For some 25 years more and more extensive animal feeding tests have been carried out, with increasingly exacting techniques (including mutagenicity and teratogenicity tests), to cover all remaining cases of doubt. In the United States a project is under way to investigate the wholesomeness of radiation-sterilized beef; it began in 1971 and will not be completed until 1976. This project alone will cost some 5 million dollars and will involve tests on 1 500 dogs, 27 000 rats and 20 000 mice (Josephson et al., 1974). Such experiments are now beyond the means of small or developing countries, and 22 states have joined together under an international scheme sponsored by the OECD and IAEA to finance further research jointly. It is most

unsatisfactory that in spite of the vast sums spent on animal feeding tests for more than two decades, there is as yet no prospect of a final decision on the wholesomeness of these foods. Although irradiated wheat has been tested in many experiments, irradiated rice will not be accepted until it has been tested in animal feeding experiments over several years. When it has been satisfactorily shown that irradiated beef is not harmful, yet another series of feeding experiments will probably be necessary before irradiated pork is approved, and so on.

The problem of evaluating varying irradiation condition has not yet been solved. For example, once animal feeding tests have been conducted with beef irradiated at room temperature, will it still be necessary, if beef for human consumption is irradiated at minus 30°, to carry out long-term tests on meat irradiated at minus 30°? If there have been animal feeding tests with whole, unpackaged irradiated fish but irradiated fish is to be marketed for human consumption as packaged fillets, will another 3-4 years of tests be necessary? Once fish irradiated with 5 Mrad has been adequately tested, will fish irradiated with 0.1 Mrad be approved for human consumption? (Cf. Diehl, 1973a).

Obviously it is impossible to answer these and related questions on the basis of animal feeding tests alone. Toxicological evaluation of irradiated foodstuffs must now make much greater use than hitherto of the findings of chemical research. It should be possible to answer the question of whether the results of tests on fish irradiated with 5 Mrad can be extrapolated to fish irradiated with 0.1 Mrad by adducing facts known from radiation chemistry. Chemical analysis should show us whether there are any basic differences between radiation-induced changes in pork and those in beef, and again, the role of temperature during irradiation and of packaging could be more quickly and cheaply established by analysis than by animal feeding tests.

Finally, the question of the safety factor can <u>only</u> be answered on the basis of chemical research. An irradiated food could conceivably contain certain radiation-induced compounds at a concentration <u>just</u> low enough not to produce harmful effects in the tests on animals. This would be just on the "no effect level" and the particular food should not be accepted for consumption (even though there were no harmful effects in the feeding

tests!), as the safety margin would be too small. On the other hand, it is just as likely that the concentration of radiation-induced substances present in certain irradiated foodstuffs is not just 100th but possibly as low as 1000th of the level necessary to cause harmful effects in the tests. In this case it would be quite absurd to speak of an insufficient safety margin.

These examples will have shown how important considerations of radiation chemistry are for evaluating the wholesomeness of irradiated foodstuffs. The following will investigate whether available findings are sufficient to form a basis for assessing health risks. Here it will of course be impossible to take account of all the literature which has been produced in some 25 years of intensive research on food irradiation. References have been selected primarily to give the reader easier access to further publications on this topic.

Although the title also mentions qualitative changes, this study will deal mainly with quantitative changes. The literature on qualitative tests on irradiated foodstuffs is far more extensive than the quantitative data. But only the latter will enable us to draw reliable conclusions for the assessment of health risks.

In describing the effect of radiation on the composition of foodstuffs, a basic distinction must be drawn between two phenomena:

a) radiation-chemical changes in composition, which take place during and shortly after irradiation and whose effect can only be measured at relatively high radiation doses (from about 100 krad up), and

b) radiobiological changes in composition, not usually occurring until several hours or days after irradiation, which can be produced even by low radiation doses (approx. 10 krad). Effects of this kind are of course only observed in living tissue, in this case actively metabolizing plant material (e.g. fresh fruit and vegetables).

The effects described in a) are discussed in chapter 3 of this report and those in b) in chapter 4.

3. Radiation-chemical changes in the composition of foodstuffs

The main components of most foodstuffs are water, carbohydrates, proteins and fats, with smaller amounts of pigments, vitamins, mineral salts, flavourings, etc. The next section (3.1) shows how these individual components react to irradiation. An attempt will also be made to predict, from the changes observed in individual components, what changes would take place in complex foods, and the extent of such changes. A subsequent section (3.2) describes the results of quantitative analysis of irradiated foodstuffs and correlates them with the theoretical predictions in section 3.1.

For ease of comparison, the values will be recorded for a radiation dose of 500 krad and a model food consisting of 80 % water and 6.6 % each of carbohydrates, proteins and fats.

3.1 The components of foodstuffs and the chemical effects of irradiation

In the energy transfer which occurs when matter is irradiated with ionizing radiation, electronically excited or ionized molecules are produced which immediately begin to react further, forming new products, especially free radicals, which can in turn take part in further reactions. The activated molecules continue to react with each other or with the surrounding matter. Dissociation, fragmentation, exchange and isomerization reactions take place and radical reactions such as polymerization, abstraction and disproportionation lead to more stable end products (Güsten, 1972). When the radiation energy is directly absorbed by the individual molecule and produces a change in it, this is referred to as a "direct effect". But if the radiation is absorbed by the surrounding matter and transferred to another molecule by intermolecular energy transfer or the diffusion of radicals which have been formed, this is an "indirect effect". In foodstuffs, most of which contain a relatively large amount of water, the radiation energy is to a great extent absorbed by the water and most of the changes which occur in the other components of the food are indirect effects.

3.1.1 Water

When water absorbs radiation the reactive $OH^{\bullet}$- and $H^{\bullet}$-radicals and the solvated electron e^-_{aq} are formed.

$$H_2O \xrightarrow{h\cdot\nu} H^{\bullet},\ OH^{\bullet},\ e^-_{aq}$$

H_3O^+ and OH^- are also formed and H_2, H_2O and H_2O_2 are formed by recombination of the radicals. The $OH^{\bullet}$-radicals have oxidizing properties, whereas the solvated electrons and the H atoms have a reduction effect. Typical primary reactions of water radicals with the other substances involve the removal of hydrogen and addition of radicals, e.g.

$$RH + OH^{\bullet} \longrightarrow R^{\bullet} + H_2O$$
$$RH + H^{\bullet} \longrightarrow R^{\bullet} + H_2$$
$$RH + OH^{\bullet} \longrightarrow {}^{\bullet}RHOH$$
$$RH + e^-_{aq} \longrightarrow {}^{\bullet}RH^-$$
$$RH + H^{\bullet} \longrightarrow {}^{\bullet}RH_2$$

RH = component substance

In media as complex as most foodstuffs, the radicals $R^{\bullet}$, $^{\bullet}RHOH$, $^{\bullet}RH^-$ and $^{\bullet}RH_2$ formed in these reactions can cause many different reactions.

The primary radiolysis products of water disappear in fractions of a second as they react with each other and with other food components (where these are present). In pure water, only H_2O_2 can still be detected some time after irradiation. The presence of this substance should be taken into account when assessing the cytotoxic effects of irradiated foods on bacteria (Molin and Ehrenberg, 1964; Pollard et al., 1965). Fig. 1 shows the dependence of H_2O_2 formation in pure water on the presence of atmospheric oxygen.

In the absence of oxygen the H_2O_2 formation is so low that the amount detected is still within the margin of error of the measurement method.

The shape of the curve for water containing air is typical of the majority of radiochemical reactions: there is a linear increase in the concentration of the product in the low dose range, followed by a less marked increase

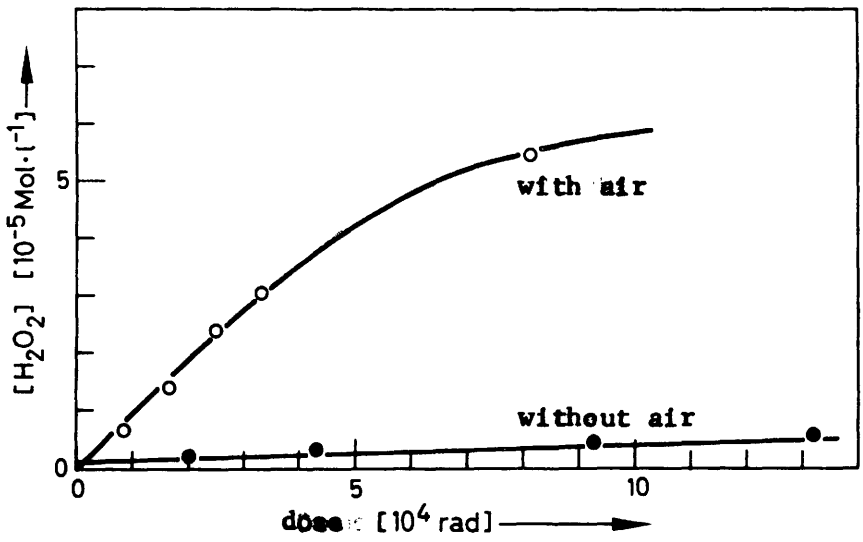


Fig. 1 Hydrogen peroxide formation vs gamma-dose in water containing air and water not containing air.
(from A. Henglein, W. Schnabel, J. Wendenburg: Einführung in die Strahlenchemie, Verlag Chemie, Weinheim 1969, p. 378).

and, finally, by flattening-out at a higher dose level.

We will use this example to introduce a concept which will recur frequently in the following - the G-value, which is used to express the number of molecules formed or destroyed per 100 eV of absorbed energy.

$$G = \frac{c \cdot N_L \cdot 100}{D_a \cdot \rho \cdot 1000 \cdot 6.24 \cdot 10^{13}} = \frac{c}{D_a \cdot \rho} \cdot 9.66 \cdot 10^8$$

where c is the concentration of the substance formed or consumed in moles $\cdot\ l^{-1}$,
N_L = Loschmidt constant (Avogadro constant),
D_a = absorbed radiation dose in rad,
ρ = density.
The figure $6.24 \cdot 10^{13}$ is derived from the conversion of eV to rad (1 rad = 100 erg $\cdot\ g^{-1}$ = $6.24 \cdot 10^{13}$ eV $\cdot\ g^{-1}$).

Once the G-value of a reaction is known the concentration of the substance formed or consumed, in mg/100 g, can be calculated by the following formula:

$$c = 1.04 \cdot G \cdot M \cdot D_a \cdot 10^{-4}$$

where c = concentration in mg/100 g
G = G-value
M = molecular weight
D = radiation dose in krad

From the initial gradient of the curve in Fig. 1 of $1.4 \cdot 10^{-9}$ moles $\cdot$ l^{-1} $\cdot$ rad^{-1} we obtain the value of G = 1.35 for hydrogen peroxide formation in water containing air.

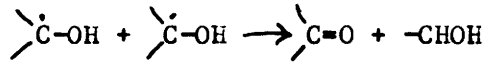
Further data on the radiation chemistry of water are to be found in Hart (1972).

3.1.2 Carbohydrates

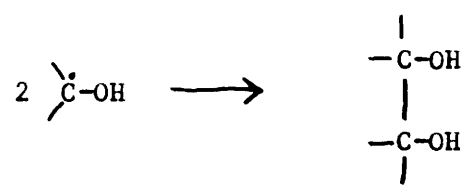
3.1.2.1 Qualitative analysis

Many different reactions can occur in carbohydrates treated by irradiation. 19 radiolysis products were detected by thin-layer chromatography in irradiated aqueous glucose solutions, including arabinose, xylose, erythrose, gluconic acid and glucuronic acid (Scherz et al., 1968). The reactions are mainly caused by $OH^{\cdot}$ radicals and, to a lesser extent, H atoms and solvated electrons.

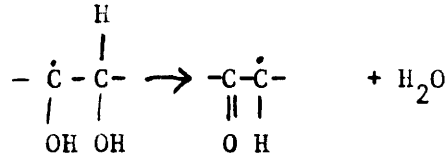
The $OH^{\cdot}$-radicals react primarily with C-H compounds, removing the hydrogen to form water (Phillips, 1972). The carbohydrate radicals which are formed react further with disproportionation



combination reactions



and splitting-off of water



forming a deoxycarbonyl radical which can take part in further reactions involving dimerization, disproportionation or saturation. Also, C-C and C-O compounds can be split by beta-splitting (Normann and Pritchet, 1967; Scherz, 1970; Hartmann et al., 1970; Dizdaroglu et al., 1972).

When polysaccharides such as starch, cellulose, pectin and glycogen are irradiated, the main reaction is decomposition to lower fractions such as dextrin, maltose and glucose (Kertesz et al., 1959; Glegg and Kertesz, 1957; Deschreider, 1970). Parts of these are further degraded forming formic acid (Dauphin et al., 1974), malonic aldehyde (Berger and Saint-Lèbe, 1969), hydroxymethyl furfural, arabinose, xylose (Reuschl and Guilbot, 1964), formaldehyde, dioxyacetone (Adamič et al., 1967) and other substances. In starch irradiated with 5 Mrad 16 low-molecular substances were detected by thin-layer chromatography, and in some cases were identified by gas chromatography and/or mass spectrometry (Scherz, 1971).

3.1.2.2 Quantitative analysis

Phillips was the first to conduct systematic tests of the quantities of reaction products produced by radiolysis of carbohydrates and the various factors which play a part in this (Summary in Phillips, 1963). By way of an example, Table 1 shows products of radiolysis of an aqueous glucose solution with the G-values for glucose decomposition and the main radiolysis products produced in the presence and absence of oxygen. Other factors which can affect G-values, especially the pH and the concentration of the solution, have been researched in great detail but cannot be described individually here. Remarkably high G-values were observed in irradiation of dry crystalline monosaccharides (Phillips, 1972). The crystalline state plays an important part in this; measurements have shown G = 4.8 in amorphous lactose and G = 40 in crystalline lactose (Löfroth, 1967). The high G-values are obviously attributable to chain reactions (von Sonntag and

Dizdaroglu, 1974). These observations are not of immediate interest for foodstuffs irradiation, as irradiation of crystalline monosaccharides would hardly be of practical interest. But the problem would be of importance for radiation sterilization of medicaments, for example, which often have lactose as the carrier substance.

Table 1

G-values of radiolysis products of 0.05 M (1 %) aqueous glucose solution and product concentrations calculated for 500 krad dose (in presence and absence of oxygen)

Compound	G-values		Product concentration at 500 krad (mg/100 g)		Reference
	$+O_2$	$-O_2$	$+O_2$	$-O_2$	
glucose (decomp.)	-3.5	-3.5	-32.5	-32.5	Phillips, 1972
gluconic acid	0.35	0.4	3.5	4.1	Phillips, 1972
glucuronic acid	0.9	-	9.0	-	Phillips, 1972
glucosone	-	0.4	-	4.1	Phillips, 1972
erythrose	0.25	-	1.6	-	Phillips, 1972
deoxycarbonyl and other compounds	-	0.3	-	2.7	Scherz, 1970
2-deoxygluconic acid	-	1.0	-	9.4	Schubert, 1973
C_2-fractions	0.85	0.8	2.6	2.4	Phillips, 1972
C_3-fractions	0.80	0.8	3.7	3.7	Phillips, 1972

3.1.2.3 Extrapolation to cover foodstuffs

As stated in the preliminary remarks to this section, an attempt will now be made to predict the amount of reaction products likely to be found in the carbohydrate constituent of complex foodstuffs, basing this prediction on observations on pure carbohydrate solutions. A model food containing 80 % water and 6.6 % each of protein, carbohydrates and lipids, and a radiation dose of 500 krad will be considered for this. It is assumed that the lipid portion is suspended in droplets in the aqueous phase and that the radiation-chemical reactions in the fat droplets are the same as in a homogeneous lipid phase, i.e. that boundary layer phenomena do not have

any effect, and that the reactions in the carbohydrate plus protein solution are the same as in a pure water phase.

As mentioned in 3.1.2.1, it is mainly the OH• radicals which are responsible for radiolysis of aqueous carbohydrate solutions. The velocity constant k is approx. 10^9 M^{-1} s^{-1}, whereas k 10^6 - 10^7 M^{-1} s^{-1} for reactions with H atoms and solvated electrons (Anbar and Neta, 1967). In the model food considered here, there will be equal quantities of carbohydrate and protein, and the OH• radicals formed will, therefore, only partially be available for reactions with the carbohydrate constituent. The velocity constant for the reaction of OH• radicals with proteins is in fact k 10^{10} M^{-1} s^{-1} (Anbar and Neta, 1967), i.e. an order of magnitude greater than for the reaction with carbohydrates. It can, therefore, be assumed that only approximately 1/10 of the OH• radicals formed can react with carbohydrate molecules.

Table 1 showed the concentrations of reaction products, calculated from the G-values, to be expected in a pure glucose solution irradiated with 500 krad. Under aerobic conditions, glucuronic acid is the main product, at 9 mg/100 g, and under anaerobic conditions it is 2-deoxygluconic acid at 9.4 mg/100 g. With a protein concentration the same as the glucose concentration, the maximum level of product formation from glucose should only be 1/10, i.e. approx. 0.9 mg/100 g. Considering that the carbohydrate component of food is never in the form of pure glucose, and that part of the radiation energy is taken up in splitting starch molecules to form dextrin and simpler units down to glucose - and, moreover, that the model food contains fat and thus absorbs yet another part of the radiation energy in reactions with lipids - it can be seen that the maximum concentration of radiolysis products from the carbohydrate component would be 0.5 mg/100 g.

However, it should be borne in mind that we have made several assumptions in arriving at this conclusion and that the assumptions need to be confirmed experimentally. It was assumed that:

a) the radiolysis products formed by irradiation in the carbohydrate component of the model food are the same as in a pure glucose solution;

b) the same G-values apply for the 6.6 % carbohydrate solution as for a 1 % glucose solution (Table 1);

c) the G-values for glucose in the starch polymer molecule are lower than the values for the monosaccharide form;

d) the G-values for carbohydrate radiolysis are lowered rather than raised in the presence of a lipid phase.

Of these assumptions, only (b) has a low probability, as it is quite conceivable that the G-values are higher for the stronger concentration. For the other three assumptions, experimental findings suggest that the G-values would be lower, and possibly much lower.

The assumptions we have made have already been experimentally confirmed to a certain extent in the project by Tajima et al. (1969), which studied the reciprocal action of cysteine and glucose on irradiation of aqueous solutions. While the presence of glucose did not affect the G-values of the radiolysis products from cysteine, the presence of 10^{-3} M cysteine completely suppressed the formation of radiolysis products from glucose. This study is a good example of the type of radiation-chemical research which will be necessary to assess changes in irradiated foodstuffs and eventually to predict them, working up gradually and systematically from the simple system of a pure solution of a substance, to a multi-component system, and then to complex foodstuffs.

3.1.3 Amino acids, proteins

3.1.3.1 Qualitative analysis

The main reaction that occurs when amino acids are irradiated is deamination, with splitting off of ammonia. Also, a series of products such as ketocarbonic acids, fatty acids, aldehydes, carbon dioxide and amines are produced in smaller quantities. Reactions with the radicals formed by radiolysis of the water are probably as follows (Garrison, 1969; Garrison, 1972)

$$e^-_{aq.} + NH_3^+CHRCOO^- \longrightarrow NH_3 + {}^\cdot CHRCOO^-$$

$$OH^\cdot + NH_3^+CHRCOO^- \longrightarrow NH_3^{+\cdot} CRCOO^- + H_2O$$

$$H^\cdot + NH_3^+CHRCOO^- \longrightarrow NH_3^{+\cdot} CRCOO^- + H_2$$

and further reactions are:

$$\cdot CHRCOO^- + NH_3^+CHRCOO^- \longrightarrow CH_2RCOO^- + NH_3^+\cdot CRCOO^-$$

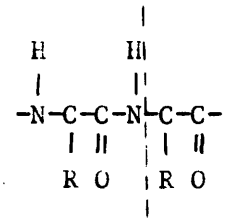
$$\cdot CHRCOO^- + NH_3^+\cdot CRCOO^- \longrightarrow NH_2^+{=}CRCOO^- + RCH_2COO^-$$

$$2\ NH_3^+\cdot CRCOO^- \longrightarrow NH_2^+{=}CRCOO^- + NH_3^+CHRCOO^-$$

$$H_2O + NH_2^+{=}CRCOO^- \longrightarrow NH_4^+ + RCOCOO^-$$

Decarboxylation and dimerization reactions also occur. Amino acids containing sulphur are oxidized; cysteine, for example, forms a disulphide bridge and cystine is produced, and in other products volatile sulphur compounds are produced. In amino acids with a ring structure, there are reactions with the side chains and the rings may be split. Aromatic amino acids are hydroxylated at the benzene rings. The reactions of individual amino acids can vary, however, depending on whether they are free or fixed in a protein structure.

Deamination and the production of carbonyl compounds and substances containing sulphur are also found in proteins. It is likely that further carbonyl compounds, fatty acids and amide products are formed by splitting of peptide chains at the carbon atom, which is in the alpha-position to the NH group in a peptide bond (Garrison, 1972):



3.1.3.2 Quantitative analysis

For reactions of OH· radicals with the various aliphatic amino acids, reaction constants of between $k \sim 10^7\ M^{-1}\ s^{-1}$ and $k \sim 10^9\ M^{-1}\ s^{-1}$ were observed; for the aromatic amino acids, which are more sensitive to radiation, this was between $k \sim 10^9\ M^{-1}\ s^{-1}$ and $k \sim 7 \times 10^9\ M^{-1}\ s^{-1}$. Solvated electrons react especially readily with amino acids containing sulphur and in the reaction with cysteine, for example, the constant is $k \sim 10^{10}\ M^{-1}\ s^{-1}$ (Anbar and Neta, 1967).

The G-values for radiolytic decomposition of amino acids in pure aqueous solution are between 1 and 10 (Table 2). The main radiolysis products of

Table 2

G-values for radiolysis of amino acids in aqueous solution and calculated loss on irradiation with 500 krad

Amino acids	Concentration mole/l	Atmosphere	G-values	Loss at 500 krad (mg/100 g)	Reference
glycine	1.0	N_2	-4.4	-17.2	Weeks and Garrison, 1959
alanine	1.0	N_2	-5.0	-23.1	Sharpless et al., 1955
serine	0.1	O_2	-5.5	-30.0	Pageau and Mehran, 1966
	0.1	N_2	-2.4	-13.1	Pageau and Mehran, 1966
threonine	0.1	O_2	-9.0	-55.6	Pageau and Mehran, 1966
	0.1	N_2	-6.8	-42.0	Pageau and Mehran, 1966
methionine	0.01	O_2	-6.5	-50.1	Kopoldova et al., 1967
	0.01	N_2	-3.8	-29.5	Kopoldova et al., 1967
cysteine	0.01	vacuum	-9.3	-58.5	Wilkening et al., 1968
phenylalanine	0.015	O_2	-2.9	-24.8	Wheeler and Montalvo, 1969
tyrosine	0.003	O_2	-0.62	- 5.8	Wheeler and Montalvo, 1969
tryptophan	0.02	argon	-0.7	- 7.4	Armstrong and Swallow, 1969

all amino acids are known. These will not be listed in full here, but can be found in the summary by Liebster and Kopoldova (1964) and in Garrison (1972). By way of an example, Table 3 shows the main radiolysis products of alanine, cysteine and methionine and their G-values.

A great deal has been written about the sensitivity of proteins, especially enzymes, to radiation. But apart from some measurements of NH_3 formation, radiolysis products have generally not been identified, let alone measured - as research projects have been more concerned with radiation-induced changes in typical macromolecular properties such as viscosity, solubility, enzyme activity, immune specificity etc. In most cases no direct conclusions about the type and extent of product formation can be drawn from studies of this kind. As regards the activity of certain enzymes, for example, it is known that a whole set of amino acids in certain enzyme molecules can be destroyed without loss of enzyme activity. In other cases the destruction of a single amino acid at the active centre of the enzyme molecule can result in complete loss of activity. The G-values for inactivation of different enzymes can therefore differ widely (Table 4).

Table 3

Radiolysis products of alanine (1.0 M aqueous solution, absence of oxygen) (Sharpless et al., 1955), cysteine (0.01 M aqueous solution, absence of oxygen) (Wilkening et al., 1968), and methionine (0.01 M aqueous solution, absence of oxygen) (Tajima et al., 1972).

Alanine	
Compound	G-value
alanine (decomp.)	-5.0
ammonia	4.48
acetaldehyde	0.59
pyruvic acid	1.92
propionic acid	1.04
ethyl amine	0.17

Cysteine	
Compound	G-value
cysteine (decomp.)	-9.3
alanine	2.6
cystine	3.4
hydrogen sulphide	2.5
hydrogen	1.1

Methionine	
Compound	G-value
methionine (decomp.)	-3.0
methionine sulphoxide	0.30
α-amino-butyric acid	0.46
3-methylthiopropyl amine	0.59
methional	0.08
carboxylic acid	0.24
mercaptan + disulphides	0.61
ammonia	1.48
carbon dioxide	1.45

Table 4

G-values for enzyme inactivation by X-rays (Barron, 1954)

Enzyme	G-value
alcohol dehydrogenase (yeast)	3.4
phosphoglyceraldehyde dehydrogenase	2.9
carboxypeptidase	0.55
D-amino acid oxidase	0.31
ribonuclease	0.09
trypsin	0.077
lysozyme	0.03
catalase	0.009

Nonetheless research on enzymes has provided a fund of information of general interest for assessment of radiation effects on proteins:

a) The extent of radiation-induced damage of protein molecules is greater in dilute solutions than in concentrated solutions.

b) When dry enzyme preparations are irradiated, inactivation requires doses in the Mrad range, while enzymes in aqueous solution are at least partly inactivated by much lower doses (krad range).

c) Pure protein solutions are usually much more sensitive to radiation damage than proteins in raw unpurified products or in situ.

Background to a):

The dose required for a 63 % inactivation of pepsin is

42 krad at a concentration of 0.1 mg/ml
340 krad at a concentration of 1.0 mg/ml
2600 krad at a concentration of 10 mg/ml

(Bellamy and Lawton, 1954; Northrop, 1934). Similar observations were made with trypsin 50 years ago (Hussey and Thompson, 1923). This 'dilution effect' has been described in detail by Dale (1943).

Background to b):

A dose of 30 Mrad is needed for 63 % inactivation of dry pepsin (Bellamy and Lawton, 1953). For complete inactivation, dry pepsin requires a dose 170 times higher than that required for dissolved trypsin (Bier and Nord,

1952). Nevertheless, G-values of dry enzyme preparations are usually higher than those of enzymes in solution (for a detailed discussion see Sanner et al., 1974).

Background to c):

A dose of 25 krad is required for inactivation of pure catalase solution, but inactivation of catalase in chopped potatoes takes 5 Mrad (Bellamy and Lawton, 1954). A dose capable of causing 90 % inactivation in purified hyaluronidase has no inactivating effect on the raw substance from which hyaluronidase is obtained (Byers et al., 1949).

A certain degree of protection from radiation effects can be achieved by adding certain substances to pure enzyme solutions; for example, D-amino acid oxidase can be protected from damage by addition of various carbohydrates, leucyl-glycine and sodium nitrite (Dale, 1942), and catalase can be similarly protected by addition of fumaric acid, cysteine, cystine and glutathione (Dale and Russell, 1956; Forssberg, 1947). In research on the protective effects of D-isoascorbic acid on pepsin, Procter and Goldblith (1952) found that a dose of 500 krad caused 80 % inactivation in a pure pepsin solution, but that only 63 % of pepsin activity was lost if 0.1 mg ascorbic acid/ml was added, 42 % with 0.25 mg/ml and just 18 % with 1 mg/ml. A protective effect can also be obtained by absorption on insoluble carrier material. For example, Fletcher and Okada (1955) found that a radiation dose causing complete inactivation in a pure solution of deoxyribonuclease had no effect at all when the enzyme was adsorbed onto a sufficient quantity of cellulose.

A great deal has also been written on the ways the pH value, the gas atmosphere, the temperature during irradiation and other factors can affect the radiation resistance of enzymes, but these works cannot be mentioned individually here.

3.1.3.3 Extrapolation to cover foodstuffs

It would obviously be rather pointless to attempt to use the G-values for pure undiluted amino acid solutions to calculate the levels of radiation-induced reaction products from proteins in foodstuffs. As the previous section has shown, although some amino acids in undiluted solutions are

extremely sensitive to radiation damage, proteins in complex plant or animal tissues are very resistant.

What conclusions, then, can be drawn about the quantity (or to be more precise, the concentration) of radiolysis products on the basis of analyses of amino acids in food proteins (or their hydrolysates)?

The first study of the effects of radiation on the amino acid composition of a food was carried out by Proctor and Bhatia (1950) who found a 7 % drop in tryptophan and a 6 % drop in phenylalanine and threonine in haddock fillet irradiated with 5.3 Mrad. In other amino acids losses were less than 5 %, and in some cases an increase was observed (for example, + 8 % in the case of histidine). At that time techniques for analysis of amino acids were still fairly unreliable and we should not attach too much importance to the results of this early research. The same applies to the papers published by E.C. Johnson's team in the fifties, reporting considerable amino acid losses in the proteins of irradiated beef and milk powder (especially in serine, glycine, threonine, glutamine and aspartic acid) (Tsien and Johnson, 1959a). It was stated that 2.8 Mrad irradiation of pea proteins resulted in a 14 % loss of arginine and a 17 % loss of lysine, while other amino acids remained unchanged (Tsien and Johnson, 1959b).

In more recent research reported from the same laboratory these results have, at least for beef, been extensively revised. The later results indicate that losses of serine, glycine, threonine, glutamine and aspartic acid (which had previously been stated to be the most sensitive amino acids) were less than 5 % (i.e. within the margin of error of the method) even at a gamma radiation dose of 10 Mrad. Irradiation of beef with electron beams up to a dose of 50 Mrad produced losses of less than 10 % in these amino acids. Cystine and cysteine were now considered to be most sensitive to radiation (44 % loss with gamma radiation at 4.5 Mrad; 15 - 42 % loss with electron radiation at 4.5 Mrad). Next in order of sensitivity was tryptophan (15 % loss at 4.5 Mrad gamma radiation and 6 - 13 % at 4.5 Mrad electron radiation), followed by proline (9 % at 4.5 Mrad gamma radiation, 3 - 12 % at 4.5 Mrad electron radiation). (Johnson and Moser, 1967)

Kennedy (1965) reported a 6 % methionine loss in wheat gluten irradiated with 5 Mrad. Cod irradiated with 4.5 Mrad showed a 29 % loss of cysteine (Underdal et al., 1973). Other authors found no significant change in the

amino acid composition of protein hydrolysates from the following foodstuffs and feedstuffs:

Irradiated item	Radiation dose	Reference
Beef	20 Mrad	Rhodes, 1966
Cod fillet	10 Mrad	Maslennikova, 1969
Clams	4.5 Mrad	Brooke et al., 1964
Haddock fillet	2.5 Mrad	Brooke et al., 1966
Wheat gliadin (in a nitrogen atmosphere)	20 Mrad	Booth, 1970
Wheat bran	5 Mrad	Moran et al., 1968
Feed mix	3.5 Mrad	Sickel et al., 1969
Feed mix	7.0 Mrad	Eggum, 1969
Feed mix	10.0 Mrad	Udes et al., 1971

We will now attempt to estimate the maximum concentration of radiolysis products likely to be formed from protein when a food containing 6.6 % protein is irradiated with 500 krad, as we did in section 3.1.2.3 for the radiolysis products of carbohydrates. The level of the radiation-sensitive amino acids such as cysteine, tryptophan and methionine in food protein is not more than 10 % each - that is, at most 660 mg/100 g in the hypothetical model.

If it is assumed that 10 % of these amino acids are destroyed on irradiation with 5 Mrad (the maximum level which would be used for irradiation of foodstuffs) this gives a maximum of 66 mg amino acids destroyed per 100 g of food. As each amino acid is split into a whole range of products during radiolysis it is unlikely that the concentration of any of these products exceeds 20 mg/100 g. The maximum concentration is more likely to be 5 mg/100 g on irradiation with 500 krad, and most of the radiolysis products will be substances such as ammonia, carbon dioxide, hydrogen, pyruvic acid and propionic acid which are harmless at these concentrations (Table 3). It is most improbable that irradiation with a dose of 500 krad could form toxicologically significant compounds from the protein component of the model food in concentrations higher than 1 mg/100 g.

Even assuming 50 % destruction of cystine/cysteine at 5 Mrad, to tally with the observations of Johnson and Moser (1967) mentioned above, the conclusion would be the same. The beef protein analysed by these authors only

contained 1.3 % cystine/cysteine. The cod protein studied by Underdal et al. (1973) contained 1.7 % cystine/cysteine. If the 6.6 g of protein in the prototype food contains 1.7 % cystine/cysteine, this makes 112 mg per 100 g food. With 5 Mrad and 50 % destruction, 56 mg would be destroyed - which again means that a maximum of approx. 20 mg of individual radiolysis products would be formed, and more like 5 mg/100 g with 500 krad.

3.1.4 Lipids

3.1.4.1 Qualitative analysis

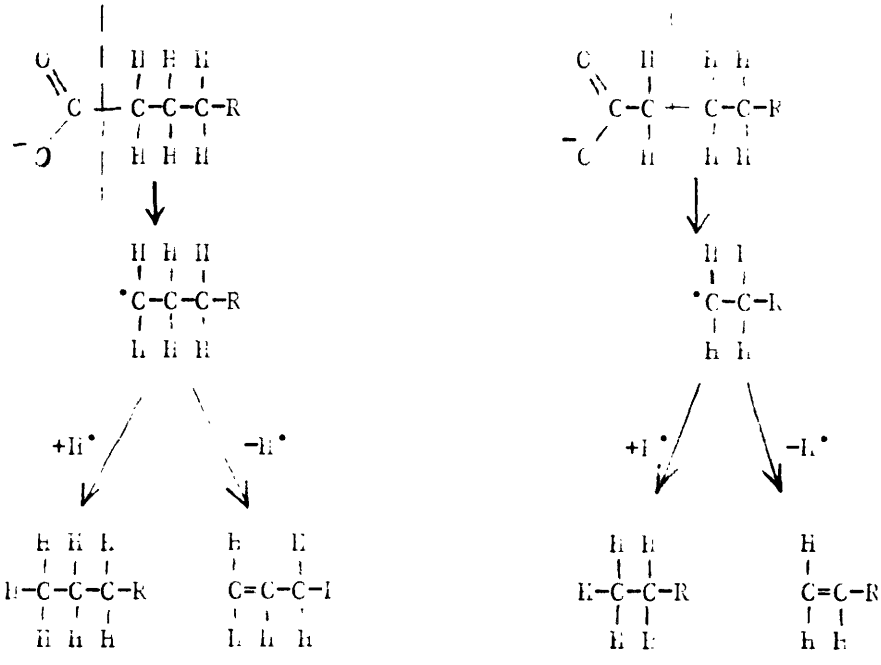
In the dose range which would be used for irradiation of foodstuffs, the range below 5 Mrad, the usual indices for fat quality show only very slight changes in fat. Distinct rises in the acid number, the trans-fatty acid content, the carbonyl content and the peroxide number of various fats were found only with doses between 10 and 100 Mrad (Lück and Kühn, 1959). Also, radiation-induced polymerization (Lück et al., 1964) and changes in physical properties such as melting point, refractometric and dielectric constants, density and viscosity occur only in this high dose range (Partmann, 1962).

Oxidative decomposition of fat during irradiation is heavily dependent on the dose rate. Lück et al. (1966) found a peroxide number of 14.1 in lard after electron irradiation with 10 Mrad at a dose rate of 2000 Mrad/hour, but this level was 62.5 with 50 Mrad/hour and 134 with 5 Mrad/hour (the peroxide number in non-irradiated lard was 13.1). But it is not certain how far the peroxide number can be taken as a real measure of peroxide formed. On the basis of their research using linoleic and linolenic acids, Milch and Klassen (1965) showed that on irradiation - as with thermal decomposition - the formation of hydroperoxides is only an intermediate stage, and they are immediately converted to aldehyde compounds (see also Monty et al., 1961). Presumably only a very small fraction of this is malonic aldehyde. The positive thiobarbituric acid reaction on irradiated fats used to be taken to indicate the degree of malonic aldehyde formation, but should be regarded only as an indication of the presence of unknown carbonyl compounds (Saslaw and Waravdekar, 1965).

Oxidative changes in fats can be reduced but not completely eliminated by irradiation in a vacuum or in a nitrogen atmosphere (Chipault et al., 1957).

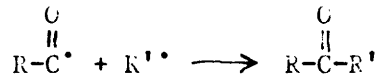
The oxygen needed is apparently taken from the ester groups of the glycerides. Although pure fats irradiated in a vaccum or nitrogen atmosphere have a greater tendency to oxidation on subsequent contact with air than do non-irradiated fats, this is not always the case with the fats in foodstuffs. Tipples and Kerris (1965) report that the fat component of irradiated wheat flour oxidized less, in 6 months of storage, than that of non-irradiated flour. Irradiated beef, pork and poultry meat stored in airtight conditions for several months also showed less tendency to oxidize (as indicated by oxygen absorption and thiobarbituric acid counts) than non-irradiated meat. It was thought that this was due to the radiation-induced formation of an anti-oxidant, possibly an aldehyde-amine complex (Green and Watts, 1966; cf. also Chipault and Mizuno, 1966).

When free fatty acids are irradiated, carbon dioxide, hydrogen, hydrocarbons and lower fatty acids are formed (Burton, 1949). It is mainly thanks to gas chromatography, used together with mass spectrometry, that we can now elucidate the radiochemical reactions which occur when fats are irradiated. Triglycerides of saturated fatty acids have primarily alkanes and 1-alkenes as radiolysis products, whereas unsaturated fatty acids produce alkadienes, alkatrienes and alkatetraenes as well as 1-alkenes (Dubravcic and Nawar, 1968). The reactions occur via radical mechanisms:

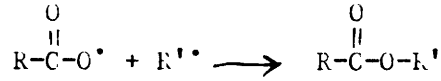


hydrocarbons with longer chains are formed by the combination of two alkyl radicals: $R^{\bullet} + R''^{\bullet} \longrightarrow R\text{-}R'$.

Ketones are formed from the acyl and alkyl radicals:



Esters are formed from acyl oxyradicals and alkyl radicals



(Nawar, 1972).

A comparison of the hydrocarbons formed in irradiated and heated edible fats (Table 5) shows that of 28 hydrocarbons formed by irradiation, 22 are also formed by heating. 6 cyclohexenes were found in heated fats which were not found in irradiated fats. Oxygen compounds were also identified: in pork fat irradiated with 6 Mrad, 4 alcohols, 11 aldehydes, 4 ketones and 9-oxononanoic acid were found (Drawert, 1973).

3.1.4.2 Quantitative analysis

Most of the detailed information on the quantities of volatile (i.e. identifiable by gas chromatography) radiolysis products of fats have been published by Nawar and collaborators, studying triglycerides (Dubravcic and Nawar, 1968), mackerel oil (Dubravcic and Nawar, 1969), edible vegetable oils (Kavalam and Nawar, 1969) and beef and pork fat (Champagne and Nawar, 1969).

In both beef and pork fat, hexadecadiene was the most predominant product (respectively 0.564 and 0.486 mg/100 g on irradiation with 500 krad - Table 6). In the model food with a 6.6 % fat content this would represent 0.037 mg/100 g or 0.032 mg/100 g food.

This should however be qualified by adding that precise quantitative data are at present only available for those radiolysis products of fats which can be detected by gas chromatography, i.e. the volatile products. Little is known about the levels of polymerization products formed in fats. But available data do indicate that the effect of irradiation in the dose range of interest here is primarily one of fragmentation. It may be assumed that the concentration of individual non-volatile radiolysis products in fats is no higher (and probably much lower) than the concentration of the main

Table 5

Hydrocarbons identified in irradiated or heated fats (pork fat, sunflower seed oil, olive oil) and in smoked ham (Drawert, 1973).

Irradiation 0.5 - 6 Mrad	Heating 24 hours, 170° C	Outer layers of smoked ham
1. Octane	1. Octane	1. Nonane
2. Octene	2. Nonane	2. Nonene
3. Nonane	3. Nonene	3. Decane
4. Nonene	4. Decane	4. Decene
5. Decane	5. Decene	5. Undecane
6. Decene	6. Undecane	6. Undecene
7. Undecane (4 isomers)	7. Undecene	7. Dodecane
8. Undecene	8. Dodecane	8. Dodecene
9. Dodecane	9. Dodecene (2 isomers)	9. Tridecane
10. Dodecene (2 isomers)	10. Tridecane	10. Tridecene
11. Tridecane	11. Tridecene (2 isomers)	11. Tetradecane
12. Tridecene (2 isomers)	12. Tetradecane	12. Tetradecene
13. Tetradecane	13. Tetradecene (2 isomers)	13. Pentadecane
14. Tetradecene (2 isomers)	14. Pentadecane	14. Pentadecene
15. Tetradecadiene	15. Pentadecene (2 isomers)	15. Hexadecane
16. Pentadecane	16. Hexadecane	16. Hexadecene
17. Pentadecene	17. Hexadecene (2 isomers)	17. Heptadecane
18. Pentadecadiene	18. Heptadecane	18. Heptadecene
19. Hexadecane	19. Heptadecene	19. Octadecane
20. Hexadecene	20. Heptadecadiene	20. Octadecene
21. Hexadecadiene	21. Octadecane	
22. Hexadecatriene	22. Octadecene	
23. Heptadecane	23. Ethylcyclohexene	
24. Heptadecene (2 isomers)	24. Propylcyclohexene	
25. Heptadecadiene	25. Butylcyclohexene	
26. Heptadecatriene	26. Pentylcyclohexene	
27. Octadecane	27. Hexylcyclohexene	
28. Octadecene	28. Heptylcyclohexene	

volatile products. It is therefore unlikely that any compound could be formed from the fat component of the model food, irradiated with 500 krad, at a concentration exceeding 0.05 mg/100 g.

Table 6

Main products of irradiation of beef and pork fat in mg/100 g as detected by gas chromatography. Dose 500 krad. (Champagne and Nawar, 1969).

	Beef fat	Pork fat
1-tetradecene	0.423	0.188
n-pentadecane	0.285	0.196
1-hexadecene	0.418	0.101
hexadecadiene	0.564	0.486
n-heptadecane	0.418	0.077
heptadecene	0.378	0.317
all other products	< 0.1 mg/100 g	

3.1.5 Other components of foodstuffs

So far only the main components of foodstuffs - water, carbohydrates, proteins and fats - have been mentioned. However it is quite conceivable that a number of secondary ingredients are considerably more sensitive to radiation than the main components and that radiolysis products from them are present in higher concentrations in irradiated foodstuffs than the radiolysis products of the main components.

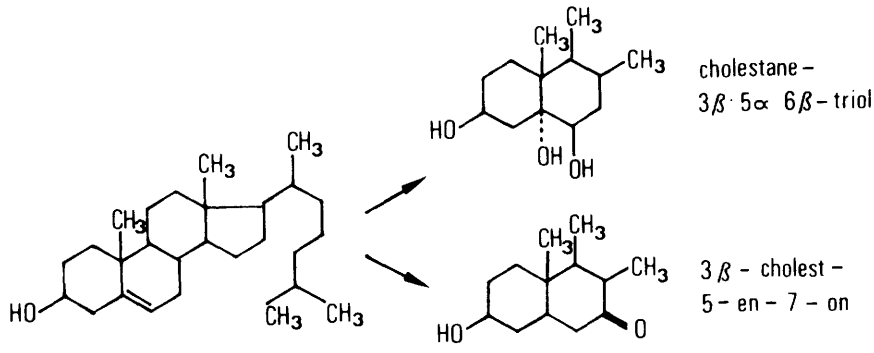
Here the nucleic acids come to mind, as they are known to be especially sensitive to radiation. Even relatively low radiation doses can cause splitting of the phosphate diester bonds in the polynucleotide chains. Chains can also be broken in this way as a result of heating and the action of enzymes in the digestive tract. The fragments formed in this process are therefore not likely to be of toxicological significance.

Irradiation can also cause deamination and oxidation of pyrimidines and splitting of purine rings. But changes of this kind can only be detected by analysis when the radiation dose is relatively high. When calf thymus-DNA (5 mg/ml) in 0.01 M phosphate buffer was irradiated with 1 Mrad only 10 % of the purine and pyrimidine bases were chemically changed (Hems, 1960; Scholes et al., 1960). With the exception of a few organs - such as the thymus - the nucleic acid content of foodstuffs is not more than 10 mg/100 g, and purines and pyrimidines make up less than 1/3 of this. As they form a whole series of different radiolysis products on irradiation, and as

the level of radiation damage is likely to be lower in an actual food than in a dilute solution in phosphate buffer, the maximum concentration of any one radiolysis product after irradiation of a food with 500 krad should not exceed 0.1 mg/100 g.

Some vitamins are also known to be sensitive to radiation. Among the fat-soluble vitamins, this is true particularly of vitamin E (tocopherols), and, among the water-soluble vitamins, primarily vitamin B_1 (thiamine). The concentration of the latter substance in foodstuffs is less than 1 mg/100 g. The radiolysis products formed from it can therefore only be formed in concentrations considerably lower than 1 mg/100 g. The situation with vitamin E is of more interest, as this vitamin is present in many foodstuffs in concentrations of up to 10 mg/100 g and in some cases (especially in nuts) up to 50 mg/100 g. A radiation dose of 500 krad can destroy 50 % of this substance - more in some foodstuffs. The nature of the radiolysis products is unknown, but provisional results of chromatographical research indicate that at least five compounds are formed (Diehl, 1974). In this case food irradiated with 500 krad could easily contain 1 mg/100 g of individual radiolysis products from vitamin E, and foods especially rich in vitamin E, such as nuts, could even contain 5 mg/100 g. Nothing is known about the toxicological properties of these compounds.

Steroids are also of interest in this connection. The Argentinian cancer researcher Roffo (1938) advanced the theory that cancer could be caused by the effect of sunlight on cholesterol which could form carcinogenic substances in the human body. Because of this there was a great deal of research in the thirties on the compounds formed by ultraviolet irradiation of cholesterol and other steroids and the possible carcinogenic effect of these compounds. Roffo's theory was disproved experimentally (Bergmann et al., 1940). The effects of ionizing radiation on cholesterol were also studied in depth. Keller and Weiss (1950) state that cholestane-3β:5α:6β-triol is formed with 27.5 % yield in a 0.6 % acetic acid cholesterol solution irradiated with X-rays at 3.6 Mr in the presence of air, and 3β-cholest-5-ene-7-on with 17.5 % yield. Both compounds are present in animal tissue, but little is known about their biological function or their physiological significance. Cholestane-3β:5α:6β-triol is found in aortas affected by arteriosclerosis (Hardegger et al., 1943), in pigs' testicles (Ruzicka and Prelog, 1943) and in ox liver (Haslewood, 1941). 3β-Cholest-5-ene-7-on has



been isolated from pigs' spleen (Prelog et al., 1943) and pigs' testicles (Prelog et al., 1947).

The volatile alkanes and isoalkanes formed by splitting of the side chain of cholesterol have been identified by gas chromatography (Merritt et al., 1967). No research appears to have been done on the extent of radiation-induced cholesterol decomposition in foods. Even if we assume that only approximately 1/10 of the cholesterol decomposition observed in an acetic acid solution takes place in a complex food, a product such as hen's egg, which contains 1.8 g total cholesterol/100 g (Kritchevesky and Tepper, 1961), can be expected to contain as much as 50 mg cholestane-3β:5α:6β-triol/100 g after irradiation with 3.6 Mrad.

3.1.6 Food additives

Ishizeki et al. (1972) have published work on the radiolysis of the preservative sorbic acid. The behaviour of the food colours amaranth and tartrazine in irradiated sausages has been studied by Kim et al. (1973). Lane (1973) described the radiation resistance of the organo-chlorine pesticide Mirex in duck eggs. The stability of the antibiotics tylosin, chlortetracycline and furyl furamide on irradiation in buffer solutions and in foodstuffs was tested by Kawabata et al. (1968).

Substances of this kind can only be present in foodstuffs in very low concentrations (the addition of the antibiotics mentioned is prohibited in most countries), so the concentration of radiolysis products from these

substances would be even lower. However almost nothing has been written as yet about the toxicological aspects of radiolysis products from such additives and impurities. Only the irradiation products from DDT in the fat component of foods have been studied in depth; they were found to be no more toxic than DDT itself (Kimbrough and Gaines, 1971).

3.2 Radiochemical changes in complex foodstuffs

In the discussion of the radiochemical changes in individual components of foodstuffs in Chapter 3.1 it was not possible to make sufficient allowance for the fact that, in a complex food, reactions can also take place between the radiolysis products formed from each component, and that different components can form the same radiolysis products. Particular interest is therefore attached to research carried out on complex foodstuffs such as meat or fish rather than on a pure substance.

Underdal et al. (1973) gave G-values for the formation of hydrogen (G = 0.2), carbon dioxide (G = 0.2) and methane (G = 0.04) in cod. From these G-values we can calculate that the products would be present in the following quantities after irradiation with 500 krad:

H_2 : 0.02 mg/100 g
CO_2 : 0.46 mg/100 g
CH_4 : 0.03 mg/100 g.

These values are considerably lower than the values calculated in section 3.1.3.3 for the formation of carbon dioxide, hydrogen and similar compounds from the protein component of the model food after irradiation with 500 krad, which was max 5 mg/100 g.

The formation of amines in irradiated beef was studied by Burks et al. (1959) who found that the total volatile bases, calculated as ammonia, increased by 25 ppm or 2.5 mg/100 g with 3.75 Mrad. Under these circumstances individual amines are unlikely to be produced in quantities exceeding 0.5 mg/100 g.

Nawar and Balboni (1970) found a linear increase in heptadecene and hexadecadiene with an increase in dose in irradiated pork. These data indicate that approx. 10 µg of these compounds per gram of fat are formed in pork

irradiated with 500 krad. The authors state that the meat used had a fat content of 30 %, which seems unusually high. The concentration of the product formed was therefore 0.3 mg/100 g of meat.

This agrees with the following maximum values given by Merritt (1972) for the formation of volatile compounds in beef irradiated with 6 Mrad:

17	alkanes, total	1.2 mg/100 g
26	alkenes	1.4
4	aldehydes	0.15
5	S-containing compounds	0.10
5	alcohols	0.10
2	ketones	< 0.05
4	alkylphenyls	< 0.01
1	ester	< 0.01

Scherz (1972) studied the formation of malonic dialdehyde in a large number of foods. After irradiation with 500 krad the highest concentration, 0.1 mg/100 g, was found in milk powder.

Schubert (1973) determined the level of total carbonyls in irradiated strawberries. His data indicate that some 8 mg carbonyls/100 g are formed with 500 krad. The reagent used (2.4-dinitrophenylhydrazine) reacts with many compounds and, this being the case, it may be assumed that the concentration of individual radiation-induced carbonyls is less (and probably much less) than 1 mg/100 g. Batzer et al. (1957) measured carbonyls in irradiated beef and found approx. 0.5 mg carbonyls/100 g after irradiation with 2 Mrad.

In wheat flour irradiated with 500 krad, 0.02 mg deoxy sugars/100 g were found and 0.05 mg hydroxymaltol was detected after irradiation with 100 krad (Scherz, 1974). In section 3.1.2.3 the maximum level for formation of radiolysis products from the carbohydrate component of the model food was calculated as 0.5 mg/100 g.

It is clear from the experimental results of research on irradiated foodstuffs that the extrapolations and calculations in chapter 3.1 were based on cautious assumptions. The levels of products found in irradiated food are actually lower than the levels calculated.

4. Radiobiological changes in the composition of foodstuffs

The previous sections have described radiochemical effects; we will now move on to biological phenomena and the effects of radiation on living vegetable matter. Irradiation with relatively low doses (e.g. 10 krad) causes metabolic changes which are initiated by radiation-chemical effects on genetic substances (nucleic acids), enzymes or cell walls, or by other mechanisms - effects which are virtually impossible to detect by chemical analysis. These changes then affect both the main components (e.g. causing starch to change into glucose) and the secondary components (vitamins, flavouring substances, etc.). Irradiation with a higher dose (e.g. 200 krad) can cause both radiobiological and radiation-chemical changes in the vegetable matter subjected to irradiation.

The resulting changes are partly of nutritional interest (loss of vitamins, etc.) and partly too - at least theoretically - of toxicological interest. These two aspects will be discussed separately in the following.

4.1 Effects on vitamin content

4.1.1 Potatoes

As the potato is an important source of vitamin C, the effects of irradiation on the vitamin C content of potatoes have been thoroughly researched. Different kinds of potatoes vary quite considerably in initial vitamin content and in the way this changes during storage and after irradiation. This is no doubt one of the reasons why some authors have found a drop in vitamin C content after irradiation (Burton and de Jong, 1969; Tajima et al., 1967) while some have noted an increase (Egiazarov, 1960) and still others have reported no change (Panaaks and Pelletier, 1960; Boffi et al., 1969). The other reason is that during storage the vitamin content changes with time. It is important to note how long after irradiation the vitamin C contents of irradiated and non-irradiated specimens are compared. In the first few months after irradiation it is normal to find less vitamin C in irradiated tubers than in non-irradiated ones. With longer storage this difference evens out, or can actually be reversed in favour of the irradiated potatoes. Most authors have only studied

ascorbic acid levels, but Wills (1965) also studied dehydroascorbic acid. The results show that during storage, changes in the levels of dehydroascorbic and ascorbic acid balance each other out, with the result that the differences between irradiated and non-irradiated tubers are less marked if the total vitamin C content is considered than if only the ascorbic acid level is taken.

Because dehydroascorbic acid is converted into ascorbic acid, the ascorbic acid level in cooked potatoes, both irradiated and non-irradiated, is higher than in raw tubers (Lewis and Mathur, 1963). Herrmann and Raths (1958) report that in isolated potato slices, the capacity to synthesize ascorbic acid can be increased or decreased depending on the time of irradiation and the length of storage thereafter. Comparing the total vitamin C content of irradiated potatoes with that of chemically sprout-inhibited ones during 10 months' storage, Scherz (1973) found no significant difference between the varieties Bintje and Maritta. In Sieglinde, the vitamin C content was higher in the chemically treated specimens than in those which had been irradiated (12 krad).

All the authors who have studied this problem seem to agree that the changes in the vitamin C content of potatoes induced by irradiation are insignificant by comparison with the differences which result from the variety of potato selected, and from growth and storage conditions.

As for the thiamine content, Wills (1965) detected no losses in potatoes irradiated with 16 krad and stored for up to six months, when they were compared with non-irradiated potatoes. Derid et al. (1967) confirmed this for potatoes irradiated with 5 krad. However, potatoes irradiated with 10 krad and stored until July had only approximately half as much thiamine as non-irradiated ones.

The same authors also studied the riboflavine content and found that this decreased slightly more in irradiated potatoes stored until July than in non-irradiated ones.

In potatoes irradiated with 10 krad and cooked immediately after irradiation, it was found that the nicotinic acid content was unchanged in comparison with non-irradiated controls. The level only decreased with doses over 50 krad (Washüttl, 1971).

4.1.2 Fruit

The radiation doses used for insect control (e.g. in papayas), mildew prevention (e.g. strawberries and citrus fruit) and to delay ripening after harvesting (e.g. bananas) are in the range of up to 200 krad. In this dose range changes due to physiological factors are observed, as well as direct radiation-chemical decomposition reactions. Here again interest centres mainly on the behaviour of vitamin C.

After 12 days' storage at 5° C strawberries irradiated with 200 krad had 84 - 97 % the ascorbic acid level of non-irradiated fruit (Maxie and Sommer, 1963). Other authors noted a 23 % drop in ascorbic acid in strawberries immediately after irradiation with the very high dose of 500 krad. After eight days' storage the decrease had risen to 35 - 46 %. Non-irradiated controls showed losses of between 32 and 100 % at the same point in time, depending on the degree of deterioration (Tomana et al., 1963, cf also Kim et al., 1969). A dose of 100 krad caused a 36 % decrease in ascorbic acid in grapes. After 15 days' storage the decrease in non-irradiated grapes was 66 %, and 69 % in irradiated fruit (Tomana et al., 1963).

In the course of their extensive research on the effects of radiation on different types of fruit, Maxie and Sommer (1963) found that the ascorbic acid level in lemons irradiated with 100 and 200 krad was unchanged immediately after irradiation; after 40 days' storage the irradiated fruit still had, respectively, 94 % and 29 % of the ascorbic acid content of non-irradiated controls. Oranges irradiated with 200 krad showed no differences in ascorbic acid level from non-irradiated fruit after 95 days' storage. Where doses of more than 200 krad were applied, ascorbic acid losses were observed immediately after irradiation and these were greater as the dose increased, but within 24 hours they had largely balanced out (Romani et al., 1963).

Bananas irradiated with 80 krad showed no ascorbic acid losses (Ali et al., 1968), while those irradiated with 200 krad showed losses of 23 - 31 % after nine days' storage in comparison with non-irradiated ones (Ferguson et al., 1966). Slight ascorbic acid losses were noted in papayas irradiated with up to 300 krad (Wilker and Young, 1966; Jiravatana et al., 1970). In peaches stored for 10 days after irradiation at 5° C, there were ascorbic acid losses of 23 % (150 krad) and 35 % (300 krad) compared with

non-irradiated controls (Maxie et al., 1964). After 10 days' storage at 5°C irradiated cherries showed losses of 3 % (200 krad) and 2 % (400 krad); these levels are within the margin of error of the method (Maxie et al., 1964).

Irradiation of haws with doses of 50 and 500 krad caused an increase in the level of dehydroascorbic acid at the expense of ascorbic acid. The total vitamin C level was unaffected by irradiation (Bancher et al., 1969). This study is of particular interest as it shows that measurement of the ascorbic acid level alone can lead to the wrong conclusions. As far as nutrition is concerned, it is the total vitamin C level which matters, as dehydroascorbic acid can be metabolized by the human body in the same way as ascorbic acid.

The same authors found that there was no decrease in β-carotene immediately after irradiation with 500 krad. No changes in the total carotene content were found in papayas irradiated with doses up to 100 krad (Jiravatana et al., 1970).

4.1.3 Vegetables

Here the purpose of irradiation is usually to improve keeping qualities by destroying some of the spoilage microorganisms or by delaying ripening. The radiation doses used are up to approx. 200 krad.

Effects on the vitamin C content of tomatoes were studied in relation to the degree of ripeness on harvesting, the radiation dose and storage time. Immediately after irradiation the vitamin losses were found to increase with an increasing radiation dose. In tomatoes harvested before they were completely ripe the level balanced out during storage after irradiation. In ripe irradiated tomatoes, however, the ascorbic acid levels were clearly below those of non-irradiated tomatoes at all times (Abdel-Kader et al., 1966).

The total vitamin C content of paprika remained unchanged in the dose range up to 500 krad; the dehydroascorbic acid content increased and the ascorbic acid content decreased (Bancher et al., 1970b). The ascorbic acid level of onions irradiated with 6 krad did not differ from that of non-irradiated onions, whether cooked or raw, not even after up to six months' storage

without refrigeration or 10 months' storage at 11 - 12° C (Lewis and Mathur, 1963).

According to Lukton and McKinney (1956) the β-carotene content of tomatoes remains unchanged, even with doses in the Mrad range. Irradiated tinned carrots stored for six months showed no significant carotene losses, even at doses of 2.8 and 5.6 Mrad, while tinned peaches lost approx. 50 % of their carotene content under the same conditions (Calloway and Thomas, 1961).

In spinach, provitamin A was not affected by irradiation with 500 krad (Bancher et al., 1970a). In paprika immediately after irradiation a β-carotene loss of 26 % (50 krad) and 31 % (500 krad) was observed (Bancher et al., 1970b).

4.2 Effects on protein value

In industrialized countries most of the protein consumed by human beings is of animal origin and research into the possible effects of irradiation on the biological value of proteins has been concerned primarily with animal foodstuffs (cf. 3.1.3.3). But a few studies have also dealt with radiobiological changes in the amino acid or protein content of irradiated potatoes and irradiated fruit.

4.2.1 Potatoes

Several authors report that changes in the amino acid content of potatoes during storage do not follow the same course in irradiated and non-irradiated tubers (Gantzer and Heilinger, 1964; Jaarma, 1967). In particular, increased levels of γ-amino butyric acid (GABA) and free proline, and decreased levels of free glutamic acid, have been reported in irradiated lots. Similar changes also occur in tubers treated with chemical germicides (Jaarma, 1969). Four months after irradiation with 30 krad Boffi et al. (1969) found levels of amino acids 50 % higher than in non-irradiated potatoes (except for methionine which decreased). The capacity to synthesize proteins from amino acids is not lost as a result of irradiation (with 7, 15 and 30 krad) (Fujimaki et al., 1968).

Rat feeding tests showed that the PER (protein efficiency ratio) value of potatoes was not affected by irradiation with 8 krad (Varela and Moreiras-Varela, 1966).

4.2.2 Fruit

Drawert et al. (1971) report that irradiation of apples with 200 krad leads to an increase in the free amino acids glutamic acid, isoleucine, valine and alanine. Similarly, in bananas irradiated with the same dose an increase was observed in the concentrations of free amino acids, especially histidine, glutamic acid and aspartic acid. Only the valine level dropped after irradiation. Clarke and Fernandez (1961) found that the protein content increased in irradiated pears.

4.3 Effects on non-essential components

Radiobiological changes in the main components of foods, especially the starch and sugar content, can slightly affect the caloric value of irradiated vegetable matter; changes in the level of vitamins and essential amino acids may be of importance in nutritional evaluation, but a large number of other ingredients of irradiated products have also been studied, primarily because of their contribution to the characteristic flavours and aromas of foods. Depending on the types of fruit in question and depending on the radiation dose applied, irradiation can help to delay or speed up the process of ripening. Here we only have room to mention a few of the many research projects on this topic.

Kawakishi et al. (1971) studied the effect of irradiation on the development of characteristic aromatic substances in onions. Chachin and Kato (1965) have described the effect of radiation on the ripening process in bananas. Similar research has been done on lemons (Maxie et al., 1966a), pears (Maxie et al., 1966b), mangoes (Dennison and Ahmed, 1967; Cuevas-Ruiz et al., 1972; Khan et al., 1974) and papayas (Hilker and Young, 1966).

As indicative parameters of the ripening process, these studies measured ethylene and CO_2 production, oxygen consumption, the level of various carbohydrates, etc, or identified characteristic aromatic substances by gas chromatography (Drawert et al., 1971; Khan, 1973; cf. also summary article

by Clarke, 1971). In some cases the effects of radiation on processes of cell metabolism, such as the pentose-phosphate cycle and the Embden-Meyerhof scheme, were traced individually. On the basis of this type of research on irradiated carrots, Massey and Bourke (1967) worked out the following diagram (Fig. 2) to show how the rates of various stages in intermediate metabolism are speeded up or slowed down by irradiation.

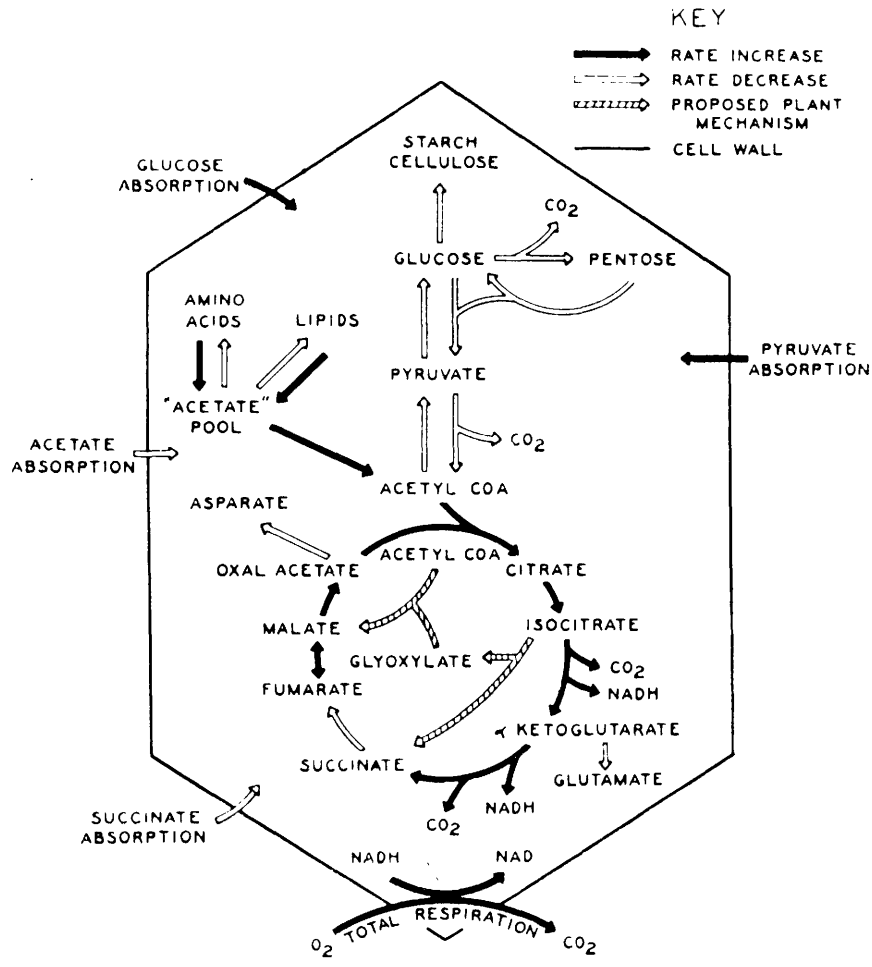


Fig. 2: Influence of gamma radiation on intermediate metabolism in sliced carrot (Massey and Bourke, 1967).

The immediate cause of the biological phenomena observed after irradiation, such as inhibition of growth and sprouting, etc., is probably connected with changes in the activity of certain plant hormones and enzymes. The concentrations of the hormones gibberellic acid and indolyl acetic acid which stimulate plant growth are lower in irradiated seedlings than in non-irradiated ones - not because they are destroyed by irradiation, but because

irradiation inhibits biosynthesis of these hormones (Ananthaswamy et al., 1972).

Many of the physiological reactions of plants to irradiation are identical to their reactions to other stress factors. The increase in ethylene production following irradiation also occurs after mechanical damage and microbial infection. The same applies to the increase in phenylalanine-ammonialyase activity (PAL). Increased PAL activity causes increased conversion of phenylalanine into cinnamic acid and the phenol compounds produced from cinnamic acid, down to the hydroxycoumarin scopoletin. According to Riov et al. (1972) increased phenol biosynthesis is a typical reaction of plant tissue to irradiation and other stress factors. Herrmann (1962) described how the hydroxycoumarin level in plants increases by several hundred percent as a result of microbial attack or mechanical damage. The following scheme indicates the pathway for the synthesis of phenols and coumarins (page 40).

The increase in scopoletin and scopolin levels in the flavedo of irradiated grapefruit is shown in Fig. 3.

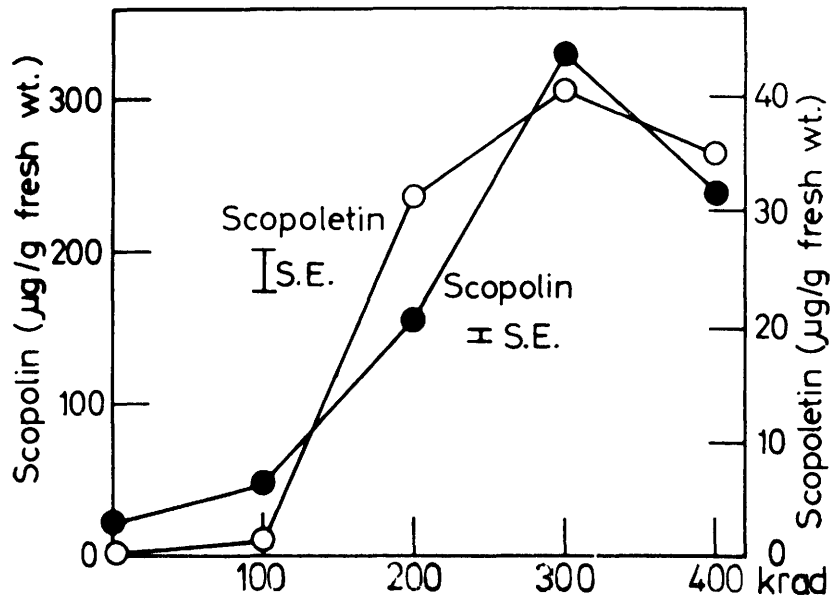
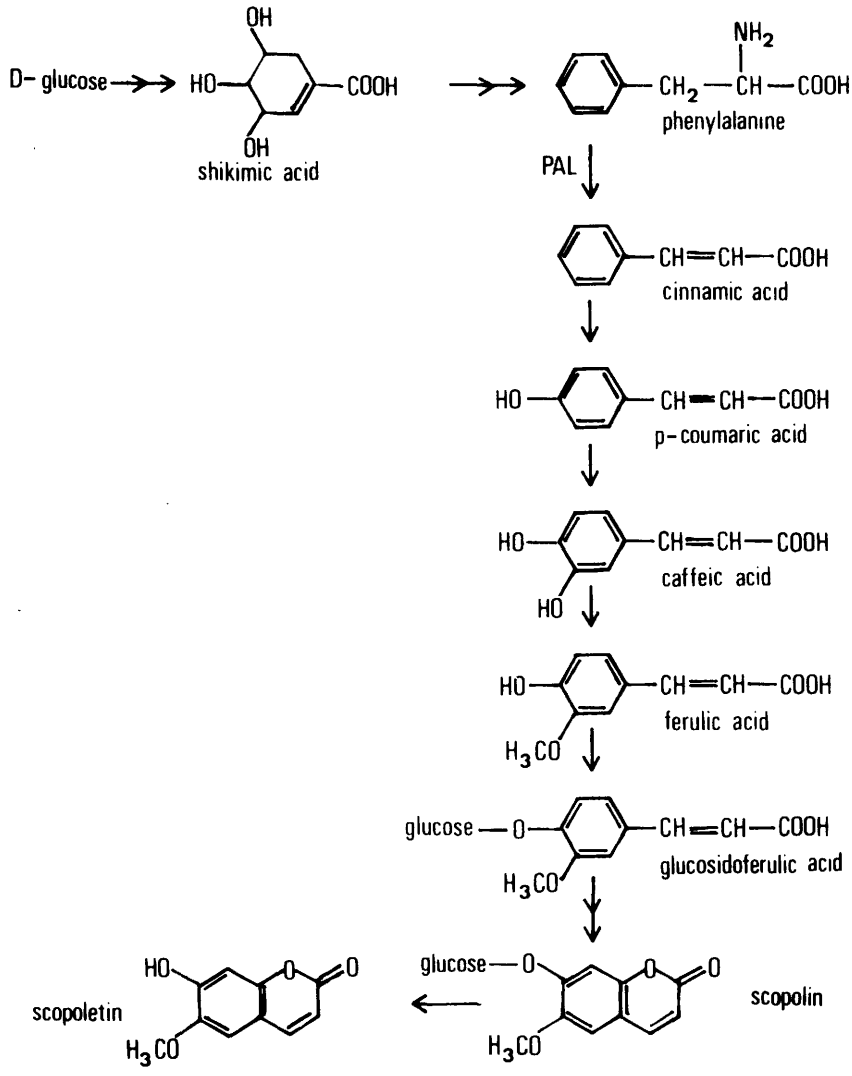


Fig. 3: Scopoletin and scopolin levels in the flavedo of grapefruit seven days after irradiation (Riov et al., 1972).


D- glucose
OH
HO
COOH
OH
shikimic acid
NH2
CH2 — CH — COOH
phenylalanine
PAL
CH=CH—COOH
cinnamic acid
HO
CH=CH—COOH
p-coumaric acid
HO
HO
CH=CH—COOH
caffeic acid
HO
H3CO
CH=CH—COOH
ferulic acid
glucose — O
H3CO
CH=CH—COOH
glucosidoferulic acid
glucose — O
O
O
H3CO
scopolin
HO
O
O
scopoletin
H3CO

This study is particularly interesting in the light of the hypothesis advanced some years ago by Kuzin (1962) on the formation of 'radiotoxins'. This term refers to substances, especially phenols (Kuzin et al., 1965), which have been identified in extracts from irradiated plants and are found to inhibit mitosis and growth in various test systems. As Table 7 shows, the outer layer of irradiated potatoes was found to contain 50 % more chlorogenic acid and 70 % more caffeic acid than in non-irradiated potatoes. It is worth noting that mitosis and growth are also inhibited in the plant test systems by extracts from non-irradiated plants, but to a lesser degree than by extracts from irradiated ones. Certain compounds (especially phenols) which are present in the non-irradiated plant are found in higher concentrations in the irradiated plant. As yet, there is no proof that irradiated plants contain any cytotoxic compounds which are not normally found in non-irradiated plants. In this respect, the use of the term 'radiotoxins' is misleading.

Table 7

Chlorogenic and caffeic acid (mg %) in the outer layer of non-irradiated and irradiated (15 krad) potato tubers, 24 hours after irradiation (Kuzin et al., 1965)

	Chlorogenic acid			Caffeic acid		
Experiment	Control	Experiment	% of level in control	Control	Experiment	% of level in control
1	15.7	30.0	191	1.61	2.75	171
2	16.2	23.2	143	1.88	2.90	155
3	16.0	22.0	138	1.74	2.80	160
4	19.7	24.8	126	171	3.32	195
mean value	16.9±1.1	25.0±2.5	150	1.73±0.7	2.9±0.18	170

The increase in hydroxy-coumarins observed by Riov et al. (1972) in grapefruit flavedo is even more pronounced than the increase in phenols described by Kuzin. Nine days after irradiation with 300 krad, 160 µg scopoletin/g were found in the flavedo and 8.2 µg in non-irradiated controls.

At present there are hardly any other quantitative data on the levels of

phenols and particularly hydroxy-coumarin compounds in irradiated vegetable foods, not have the toxicological aspects of these compounds been properly evaluated. The natural occurrence of scopoletin and other hydroxy-coumarins in carrots, celery, plums and apricots (Hermann, 1957, 1958) and potatoes (Reppel, 1959) is well-known - further studies should investigate whether the concentrations induced by irradiation are high enough to have physiological effects when the food is consumed. In the particular case of grapefruit, the presence of a high coumarin concentration in the flavedo is not really serious as this part of the fruit is not - or not normally - consumed. But the main question remains, how much irradiation increases the level of such compounds in other foods, possibly in parts which are consumed. Coumarins were once used in flavouring essences, and it has recently been claimed that woodruff (Asperula odorata L.) is potentially harmful because of its characteristically high coumarin content.

5. Conclusions

To return to the stated purpose of this study (cf. Chapter 1), it is clear that a great deal of information is available on the effects of ionizing radiation on foodstuffs and their components. It is evident, especially from the research projects aimed at developing methods to identify irradiated foodstuffs, that the chemical changes induced by radiation are far from specific and almost imperceptible in the low dose range (Diehl, 1973b; International Colloquium 1973). However, the information available on radiation-induced changes in the composition of irradiated foodstuffs does not yet constitute a sufficient basis (without animal feeding tests for individual irradiated foods) for an evaluation of the wholesomeness of irradiated foods.

The reasons why it is not possible to answer these questions on the basis of animal feeding tests alone, have been presented in chapter 2. Further research should aim to extend our knowledge of the chemical changes which occur in irradiated foods so that, initially, animal feeding tests can concentrate on specific problems or areas, and eventually sufficient knowledge will have been accumulated to make animal tests superfluous.

As shown on chapter 3, a great deal of research has been carried out on the effects of radiation on pure substances (amino acids, carbohydrates, nucleic acids, vitamins, etc.), either in dilute solutions or as pure solids. Most analysis of foodstuffs has been concerned with the decrease in the concentration of individual compounds (especially vitamins and amino acids) and only in relatively few cases with the radiation-chemical formation of reaction products created in this process. Here the decomposition products of the fat component in irradiated foodstuffs have been studied in the greatest detail, but much of this research has been restricted to gas chromatography, with the result that volatile reaction products have been identified, but little is known about the non-volatile products.

Before we can transfer conclusions about radiation-chemical processes in dilute solutions of pure substances to the processes which take place in complex foodstuffs, it will be necessary to study the transition from simple to complex systems in model tests. For example, when we know the identity and yield of the main reaction products formed by irradiation of pure glucose solutions, we can proceed to systematic tests to see how the simultaneous presence of certain concentrations of proteins, fats, etc., can influence the effects of radiation on the glucose. The same applies for solids - a great deal has already been written about the radiation-chemistry of pure starch. But little is known about the effects of varying amounts of water, proteins, vitamins, etc., on the type and extent of radiation-induced changes in starch.

This study has also shown that, compared with the amount of research on the chemical effects of radiation on the main components of foodstuffs, relatively little is known about the chemical effects on secondary components. As we have pointed out, it is at least theoretically possible for the secondary components in certain foods, such as vitamin E or cholesterol, to form higher concentrations of radiation-induced reaction products than the main components. More research should be done on this topic.

Finally, the points made in chapter 4 have shown how irradiation can stimulate biological processes in living vegetable matter so that they are enriched in certain natural substances. Of these, only phenol compounds have been studied in detail, and only in very few vegetable foods. In this sector too, there is a need for more research, and it should aim to give not

only qualitative descriptions, but where possible also quantitative data on radiation-induced changes.

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