EUR 4695

COLLOQUIUM
ON
THE IDENTIFICATION
OF IRRADIATED FOODSTUFFS

Luxembourg, October 27, 1970

Commission of the European Communities

COMMISSION
OF THE
EUROPEAN COMMUNITIES

Directorate General
for Social Affairs

Directorate
for Health and Safety

THE IDENTIFICATION OF IRRADIATED FOODSTUFFS

PROCEEDINGS of a COLLOQUIUM

Luxembourg, October 27, 1970

Editor:
J. SMEETS
Commission of the European Communities

EUR 4695d-f-e

CONTENTS

FOREWORD

Dr. P. RECHT 1

I. INTRODUCTIONS

J. SMEETS 3
J. SPAANDER 9

II. REPORTS ON RESEARCH PERFORMED UNDER CONTRACT 11
A) GERMANY 11

1. H. SCHERZ

Conductivity measurement as a method for differentiation between irradiated and non-irradiated potatoes 13
Discussion 22

2. H. SCHERZ

Evaluation of the structure of the deoxycompounds found in irradiated starch and determination of these products in irradiated wheat and wheat flour 27
Discussion 34

3. B.J. RADOLA

Identification of irradiated meat by thin-layer gel chromatography and solubility studies 37
Discussion 56

4. F. DRAWERT, R. TRESSEL und B. BECK

Gaschromatographisch-massenspektrometrische Untersuchung von bestrahlten tierischen Fetten 59
Discussion 65

5. H. PENNER

Identification of irradiated potatoes by lack of wound periderm formation 67
Discussion 71

6. B.J. RADOLA

Thin-layer isoelectric focusing of sarcoplasmic proteins from irradiated meat 73
Discussion 83

B) THE NETHERLANDS AND BELGIUM 85

7. D. ONDERDELINDEN and L. STRACKEE

Detection of irradiated foodstuffs by means of electron spin resonance 87

Discussion 94

8. L. BUGYAKI and M. VAN DER STICHELEN-ROGIER

Vertical starch gel electrophoresis and immunoelectrophoresis of irradiated and non-irradiated animal proteins 97

Discussion 101

9. A. DESCHREIDER

Identification of irradiated wheat flour, irradiated potatoes and irradiated eggs 105

Discussion 126

10. E. MAES

Changes in irradiated food products measurable by differential thermal analysis 119

Discussion 126

III. REPORTS ON RESEARCH PERFORMED BY OTHER LABORATORIES 129

11. L. SAINT-LEBE, G. BERGER, with the technical collaboration of J.P. AGNEL

Assainissement de l'amidon industriel par irradiation: modifications biochimiques et test d'irradiation 131

Discussion 136

12. C. MAGAUDDA

Evolution de la diminution en poids durant la conservation: Approche pour l'identification des fruits irradiés 141

Discussion 148

13. J. MORRE

Mise en évidence d'aldéhyde malonique dans les solutions de glucides irradiés aux rayons gamma 149

IV. CONCLUSIONS 159

J.F. DIEHL 161

ANNEXE: Participants 163

FOREWORD

A Colloquium organised by the Directorate for Health and Safety of the Commission of the European Communities was held at Luxemburg, 27th October 1970, between experts from the six member countries. The object of the discussions was an exchange of informations on research, completed or in progress, concerning the *measurable* physical, chemical and biological changes which foodstuffs undergo when irradiated for the purpose of preservation. Once these measurable changes have been established, they should enable *practical* methods to be worked out for identifying irradiated foodstuffs.

The proceedings of this Colloquium are to some extent a survey of the present state of research in this field in the six Community countries. It includes reports of studies completed, reports of research still in progress and the discussions during the Colloquium. These discussions were very interesting and fruitful; they are therefore bound to have an inspiring effect on future work. Owing to their great importance, they are included in this publication in extenso.

The Commission is pleased to note that since it began on this research programme only a few years ago, important progress has been made: it has been found possible to identify certain irradiated foodstuffs by certain methods. Several other studies which also may be regarded as completed did not result in such techniques. These latter results are only apparently negative; actually, from the health point of view, they may be considered as positive. In any case, any changes occuring in such cases are not measurable.

The Commission considers itself fortunate with this programme of international cooperation in this field. The collaboration of a number of highly skilled experts in various fields has enabled it to work out an international programme which could hardly have been carried out in any single Community country, since the programme includes specialist studies covering a wide range of disciplines.

I feel sure that this publication, in addition to the monographs in this field already published by the Commission, will be an important contribution both to the continuation of the research work and to the practical application.

Dr. P. Recht,
Director of the Directorate for Health and Safety.

INTRODUCTIONS

J. SMEETS

J. SPAANDER

INTRODUCTION

J. SMEETS

It is a great pleasure for me to welcome you to this colloquium on "The Identification of Irradiated Foodstuffs" on behalf of the Commission of the European Communities. I should like to address a special word of welcome to those experts present here today who have not previously attented meetings of this kind. Previous exchanges of views have hitherto only involved the research scientists who, since 1967, have been working together with the Commission under a number of research contracts. We felt that the research pursued under these contracts has now reached a stage at which a wider exchange of views on the knowledge acquired is worthwhile. This will enable us at the same time to learn of the results of research pursued by colleagues working in the same field.

* * *

Before proceeding to the actual exchange of views, it may be useful to recall the objectives pursued by the Commission in the research and the development of this work in the recent years. At the request of the European Atomic Energy Commission (Euratom), Lafontaine and Bugyaki of the "Institut d'Hygiène et d'Epidémiologie" at Brussels made a litterature survey concerning eventual methods or possibilities of identifying irradiated foodstuffs. This study has been published in 1965 (1).

As a result of this survey a research programme was drafted to be executed in international collaboration in the countries of the European Community. Five laboratories (Belgium, Germany, The Netherlands) expressed their wish to participate in this programme which got financial support of the Commission (see Annexe II). This joint programme which started April 1967, covers measurement techniques to be studied, different types of foodstuffs, radiation sources and doses, storage times for irradiated products, other preservation techniques for the purpose of comparison, etc. The Directorate for Health and Safety of the Commission assures the coordination of the research. The aim of the work is to determine physical, chemical or biologico-anatomical changes in irradiated foodstuffs. Should it prove possible to determine such changes, research should be done of ways in which this knowledge can be used to develop—for practical purposes—suitable analytical control techniques which would make it possible to identify if foodstuffs have actually been irradiated. The discovery and development of such identification methods would create a new tool and a possibility of ensuring the observance of the present legal positions of the Community countries, which generally contain prohibitive clauses (2).

The second aim of this research concerns the protection of public health. Indeed, if certain changes in irradiated foodstuffs are identified, they may provide a basis for studying the toxicological aspect of food irradiation.

Annex I * contains a survey of the programme and the system used in the research and the results obtained up to date, i.e. 27 October 1970. In addition to the various detection and measurement methods to be investigated (column 1) it lists the foodstuffs under investigation and two radiation doses, namely the actual dose, i.e. the normal amount of irradiation required for preservation purposes, and the maximum dose, i.e. the dose, although not applied in practice, for which a maximum radiation effect may be expected.

This table shows that in the present state of the study a number of changes in irradiated foodstuffs may be observed, which could provide the bases for control techniques.

Some investigations were abandoned in 1969, after consultation with the concerned research scientist, Scherz, because the results did not offer prospects. These were: redox potential, polarography, polarimetry.

In redox potential studies of irradiated potatoes and meat it was observed that the method was of little practical use, in view of the fact that the results were too dependent of the presence of traces of air. Polarography proved to be insensitive and insufficiently specific in studies of meat, wheat and flour.

Polarimetry was also considered to show little promise.

The chromatography studies carried out by Drawert on irradiated fruit were also abandoned in 1969 through lack of funds; more emphasis was laid on studies of fatty foods.

Investigations using ultracentrifuges were, on closer study, found to be of little use. Any variations in the molecular weight of proteins may also be determined indirectly with the aid of gel chromatography. It is very doubtful whether any information would be obtained using ultracentrifugation techniques.

---

* The methods marked A were studied by the German laboratories, those marked B were studied in the Netherlands and Belgium.

In contrast to this, isoelectric focusing and turbidimetry were added to the original programme.

Some studies have already yielded positive results for a possible identification method and have been published (3, 4, 5).

Other studies are being prepared for publication.

In conclusion, I am very pleased to note the results which this international programme of research has produced and the team spirit with which scientists of different nationalities and with different specialities pursue a common goal. This international research programme can be considered from many points of view, as very interesting and this programme of international collaboration as a great success.

After these introductory comments, I am very pleased to invite J. Spaander to take the chair for the discussions we shall have this morning. I should be obliged, if J.F. Diehl would guide our discussions during the afternoon session.

REFERENCES

(1) A. LAFONTAINE et L. BUGYAKI: Etude sur les méthodes d'identification des denrées alimentaires irradiées — EUR 2402.f (1965).

(2) J.CH. CORNELIS: Analysis of the international legal and administrative regulations on food irradiation with regard to the public healt aspects — EUR 4466.e (1970).

(3) A.R. DESCHREIDER: Modifications des constituants de la farine irradiée mises en évidence par: spectrophotométrie, spectropolarimétrie et analyse thermodifférentielle — EUR 4417.f (1970).

(4) Fr. DRAWERT et. al.: Meßbare Veränderungen in bestrahlten Lebensmitteln — Anwendung chromatographischer, elektrophoretischer und spektralphotometrischer Methoden zur Untersuchung von Aromastoffen, Amino-Säuren, Fettsäuren, Naturfarbstoffen und Enzymen — EUR 4617.d (1971).

(5) B.J. RADOLA: Identification of irradiated meat by thin-layer gel chromatography and solubility studies — EUR 4618.e (1971).

Annex I. — Summary of results (27 October 1970)

	Potato *Mrad*		Apple Banana *Mrad*		Wheat *Mrad*		Egg *Mrad*		Fish *Mrad*		Meat *Mrad*		Fats *Mrad*			Packing Material *Mrad*	
	0,01	0,2	0,2	0,2	0,025 0,1	1	0,5	1	0,5	1	0,5	5	0,5	1	5	0,1	1
A_1 Electrical conductivity	+	+									—	—					
A_2 Redox potential *	—	—									—	—					
A_3 Polarimetry *																	
A_4 Polarography *					—	—					—	—					
A_5 Chromatography																	
a) gel											+	+					
b) paper and thin layer					+	+											
A_6 Colorimetry			+	+			+	+			+	+					
A_7 Ultracentrifugation **																	
A_8 Gaschromatography			+	+	+	+	+	+			+	+	+	+	+		
A_9 Microstructure	+	+	+														
A_{10} Isoelectric focusing											±	+					
B_1 Electron spin res.						±	—	—	—	—	—	—				±	±
B_2 Electrophorese																	
a) immuno							—	—	—	—	—	—					
b) starch gel						+	+	+	—	—	±	+					
c) disc			+	+													
B_3 Spectrophotometry	—	—			±	+											
B_4 Spectropolarimetry	+	+			±	+	+	+									
B_5 Thermal differ. anal.						±											
B_6 Turbidimetry	—	—			+	+	—	—									

* Work will not be continued.
** Not useful at this stage.

\+ = possibly useful for identification. no sign = experiment not required in the contract or not yet done.
— = not useful.

Annex II. — Collaborating laboratories and institutes

Germany

Bundesforschungsanstalt für Lebensmittelfrischhaltung,
75 Karlsruhe
Engesserstraße 20

Institut für chemisch-techn. Analyse und
chemische Lebensmitteltechnologie
Technische Universität München
8050 München-Freising
Weihenstephan

Belgium

Institut d'Hygiène et d'Epidémiologie
14, rue Wytsman
Bruxelles

Laboratoire Central
Ministère des Affaires Economiques,
17a, rue de la Senne
Bruxelles.

and

CENATRA
Lange Leemstraat, 137
Antwerpen

Netherlands

Rijksinstituut voor de Volksgezondheid
Sterrenbos 1
Utrecht

INTRODUCTION

J. SPAANDER

Since our last meeting in October 1969, some interesting developments have taken place:

1. In the pursuance of the meetings in Brussels (1961) and Rome (1964), a *joint FAO/IAEA/WHO Expert Commitee* (technical report W.H.O. No. 451) has met on the wholesomeness of irradiated food with special reference to wheat, potatoes and onions (Geneva 1970). The decision that a temporary acceptance of irradiated *wheat* and *potatoes* was recommended and more wholesomeness studies were encouraged, has positively influenced the further introduction of irradiated foodstuffs.

2. On October 14, 1970, an agreement on a new *International Food Irradiation Project* was signed in Paris by the countries of the O.E.C.D. This project at the scientific level is a joint undertaking in which participate I.A.E.A., F.A.O., E.N.E.A. and W.H.O.

The structure of combined contract-research ressembles very much that of this Euratom-project. Project leader will be Dr. R. Hickman from Canada and the scientific host will be Prof. Diehl in Karlsruhe. Congratulations to both of them.

3. This year, the Ministry of Health in *the Netherlands* has given a license to food industries for the *free distribution of irradiated mushrooms* (prolongation of shelf-life); there have been no adverse public reactions.

On the contrary, the housewifes have accepted the new and better product without notable hesitation. In the shops, a choice of both products (irradiated and non-irradiated) was offered at the same time and at the same price. The irradiated product was characterised by a distinct emblem. This was demonstrated and explained regularly by television-spots. Care was taken that the public was fully informed of what happened (press conference).

4. On Thursday, November 26, 1970, in Wageningen (ITAL) a short symposium will be held for the information of interested foreign experts. Special attention will be asked for the necessity to draw up a practical system for mutual information of health authorities with regard to the import and export of irradiated foods, now licensed for

free circulation at the national level. Here the recent EURATOM publication EUR 4466 e on the public health aspects of international legal and administrative regulations, by Mr. Cornelis, must be mentioned as a valuable source of up to date information.

As long as no detection method for practical use is available, the administration will have to rely on administrative control systems.

The discussions in the colloque are meant to contribute to the development of the still necessary laboratory-control methods. They will demonstrate the valuable contribution of this Euratom project to international scientific collaboration.

REPORTS ON RESEARCH PERFORMED UNDER CONTRACT

A) Research performed in GERMANY

by

H. SCHERZ, B. RADOLA, H. PENNER
Bundesforschungsanstalt für Lebensmittelfrischhaltung
Karlsruhe

F. DRAWERT
Institut für chemisch-technische Analyse und
chemische Lebensmitteltechnologie der Technischen Universität München
München-Freising

Chairman: J. SPAANDER

1. CONDUCTIVITY MEASUREMENT AS A METHOD FOR DIFFERENTIATION BETWEEN IRRADIATED AND NON-IRRADIATED POTATOES

Progress report 1.4.1967 - 1.10.1970

H. SCHERZ

ABSTRACT

Irradiation causes a decrease in the electrical conductivity of potato tubers. The difference in the conductivity value measured immediately after puncturing the tuber with the electrodes and that measured up to 180 sec later ($\Delta_t - \Delta_{t(180)}$) decreases with increasing irradiation dose, regardless of potato variety. It appears that this difference value can be used to differentiate between potatoes irradiated with low doses (0-20 krad) and those irradiated with high doses (20-100 krad). Storage does not influence conductivity as long as the tubers remain unsprouted. On sprouting, both the absolute conductivity and the conductivity difference decreases.

* * *

INTRODUCTION

The alternating current conductivity of the cells is a measure for ion permeability of the cell membranes. The fundamental studies of Ousterhout (1) have shown that the conductivity of the cell membranes increase on cell death. The semipermeable membrane becomes completely permeable for all electrolytes. In detailed studies on some varieties of fruits and vegetables Paech (2) has found that the conductivity strongly depends on the physiological condition of the material. A number of external factors (cold, heat, mechanical injuries) increase the conductivity; only in a few cases, e.g. on treatment with narcotics a decrease is observed. Hansen (3) studied the influence of irradiation on the conductivity of Nitella-cells and he found also an increase of the conductivity.

In the present investigation an attempt was made to use measurements of alternating current conductivity for differentiation between irradiated and non irradiated food. This approach can be successful only for a material consisting of living cells, which are not much altered by chemical or physical influences during the trade. Potatoes appeared to be a very suitable material since irradiation of potatoes is of great practical interest as an effective means for inhibition of sprouting.

MATERIAL AND METHOD

The following potato varieties were used: Bintje, Maritta, Grata, Sasquia, Sirtima, Erstling, Agora, Ostara, Sieglinde.

The potatoes were irradiated in a linear accelerator with 10 MeV electrons. After irradiation the potatoes were transferred to thermostated storage rooms. For the measurements the potatoes were adjusted to a room temperature of 20° C. The electric conductivity Δ was measured at 50 cycles with an alternating current bridge * equipped with a puncture electrode consisting of steel tips (Fig. 1). The electrodes were calibrated using solutions of 0.1 N and 0.01 N KCl. The electrode-constant was 0.65 at 20° C. The conductivity was measured immediately after the puncture of the electrodes into the potato and for a period of several minutes thereafter. It was found that the values

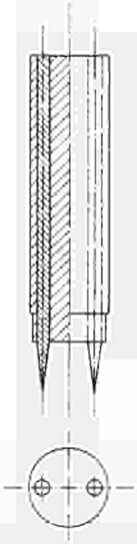


Fig. 1. Puncture electrode.

---

* Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany.

decreased during the first three minutes in the unirradiated material. The effect was followed by using a stop-watch.

The temperature dependence of conductivity was measured with potatoes immediately after their removal from thermostats adjusted to various temperatures.

RESULTS

Irradiation causes a decrease of the conductivity which is measured immediately after puncturing the tuber. Fig. 2 shows the effect for the variety "Bintje". This decrease was found for all varieties which we have studied. The results for eight varieties are presented in Table I. The mean values and the standard deviations were determined with 15 different potatoes each.

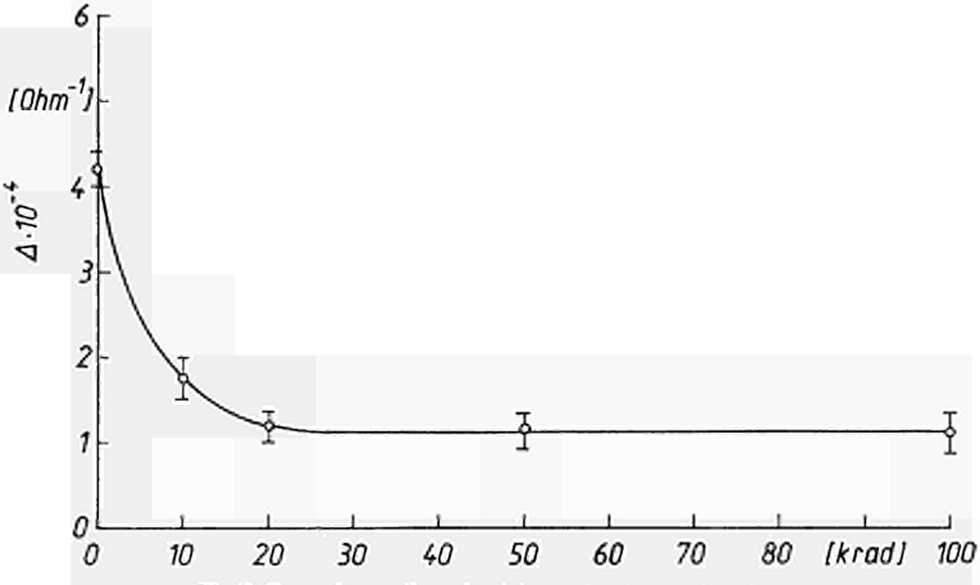


Fig. 2. Dependence of conductivity on irradiation dose for variety "Bintje" measured at 20 °C.

Table II shows the dependence of conductivity on dose for a storage period up to 30 days after irradiation.

In Table III the conductivity of potatoes irradiated with 10 krad is compared with chemically (CIPC **) treated potatoes for storage periods up to 8 months.

** Chlorisopropylphenylcarbamat.

Table I. — Dependence of conductivity on irradiation dose for eight varieties. The values were obtained immediately after puncture; measured at 20 °C.

	Δ . 10^{-4} Ohm^{-1}				
Variety	0 krad	10 krad	20 krad	50 krad	100 krad
Sasquia	2.60±0.16	2.24±0.17	2.19±0.07	1.11±0.03	1.00±0.10
Agora	3.46±0.41	3.24±0.29	2.95±0.23	2.90±0.23	2.72±0.30
Ostara	4.28±0.28	4.03±0.32	3.78±0.42	2.70±0.52	2.22±0.37
Erstling	3.70±0.22	3.38±0.29	3.24±0.20	2.14±0.57	2.04±0.54
Sirtima	4.35±0.33	3.20±0.47	3.12±0.25	2.04±0.38	2.04±0.38
Bintje	4.20±0.19	1.75±0.19	1.18±0.15	1.15±0.14	1.07±0.28
Maritta *	3.78±0.20	—	2.63±0.15	—	—
Grata *	3.20±0.10	—	1.60±0.15	—	—

* Determination 2 months after irradiation.

Table II. — Dependence of conductivity on irradiation dose, up to a storage period of 30 days; measured at 20 °C; variety: Sasquia; storage temperature: 15 °C.

	Δ . 10^{-4} Ohm^{-1}				
Time	0 krad	10 krad	20 krad	50 krad	100 krad
6 hr	2.68±0.16	1.75±0.16	1.75±0.16	1.30±0.17	1.32±0.15
1 day	2.71±0.10	1.92±0.13	1.90±0.16	1.30±0.19	1.00±0.30
2 days	2.80±0.13	1.99±0.03	1.77±0.18	1.38±0.10	1.30±0.12
3 days	2.60±0.16	2.24±0.17	2.19±0.07	1.11±0.03	1.00±0.10
8 days	2.30±0.16	2.20±0.11	2.00±0.10	1.60±0.23	1.35±0.18
14 days	2.68±0.10	2.36±0.10	2.13±0.15	1.75±0.19	1.30±0.17
30 days	2.83±0.10	2.20±0.50	1.75±0.50	1.50±0.15	1.68±0.40

Table III. — Dependence of conductivity on storage time for potatoes irradiated with 10 krad or treated with CIPC; measured at 20 °C; storage temperature 10 °C.

	Δ . 10^{-4} Ohm^{-1}				
	1st month	2nd month	4th month	6th month	8th month
Bintje					
unirrad.	4.85±0.26	4.32±0.28	4.81±0.35	3.78±0.69	— *
10 krad	4.10±0.20	3.64±0.40	4.40±0.32	3.70±0.37	2.76±0.41
CIPC treated	5.10±0.24	4.40±0.29	5.40±0.31	4.70±0.52	3.30±0.58
Maritta					
unirrad.	3.78±0.27	4.90±0.36	4.65±0.28	3.52±0.67	— *
10 krad	3.54±0.44	—	4.34±0.50	3.50±0.55	2.91±0.22
CIPC treated	4.91±0.25	4.96±0.18	4.95±0.24	4.10±0.35	3.61±0.32
Sieglinde					
unirrad.	4.55±0.26	4.55±0.24	3.94±0.42	5.82±0.54	— *
10 krad	4.30±0.37	3.88±0.47	4.44±0.36	3.44±0.13	3.12±0.34
CIPC treated	5.05±0.45	4.43±0.12	4.44±0.30	4.14±0.4	3.06±0.55

* potatoes totally sprouted.

The results demonstrate that the conductivity of irradiated potatoes decreases with increasing dose in a nonlinear manner. The absolute values and also the course of the curves depend both strongly on the variety and on other external conditions e.g. the temperature during the measurement. At higher temperature an increase of conductivity is observed. The storage periods have no significant influence on the conductivity up to four months. After that period the unirradiated samples begin to sprout and a decrease of the conductivity is observed (Table IV).

Table IV. — Dependence of conductivity on temperature during measurements; potato variety: Erstling and Sirtima.

	$\Delta \,.\, 10^{-4}$ Ohm^{-1}			
Sample	0 °C	20 °C	35 °C	45 °C
Erstling				
unirradiated	2.94±0.46	3.70±0.22	4.27±0.39	4.12±0.38
20 krad	2.48±0.22	3.24±0.20	3.78±0.43	4.02±0.46
Sirtima				
unirradiated	2.86±0.13	4.35±0.33	4.30±0.58	4.60±0.57
20 krad	2.60±0.09	3.12±0.25	3.58±0.30	3.70±0.26

It was consistently found that immediately after sticking the electrodes into the potatoes, the conductivity decreased, reaching a constant value after approximately 3 min (Fig. 3). With irradiated potatoes essentially the same effect was noted. However, the conductivity decrease is reduced with increased irradiation dose. The effect was observed for all varieties which were studied. Table V shows the effect for four varieties, namely Sirtima, Ostara, Agora and Erstling. The conductivity was determined immediately after puncture, followed by measurements after 30, 60, 120 and 180 sec. The storage time was eight days, the mean value and the standard deviations were calculated from 15 measurements each.

Table VI shows the dependence of the conductivity decrease on the storage time and Table VII its change with the temperature during the measurement.

The results show that the conductivity decrease $\Delta_t - \Delta_{t(180)}$ depends less on the varieties of the potatoes than does the absolute value of the conductivity. Untreated potatoes or potatoes irradiated with low doses

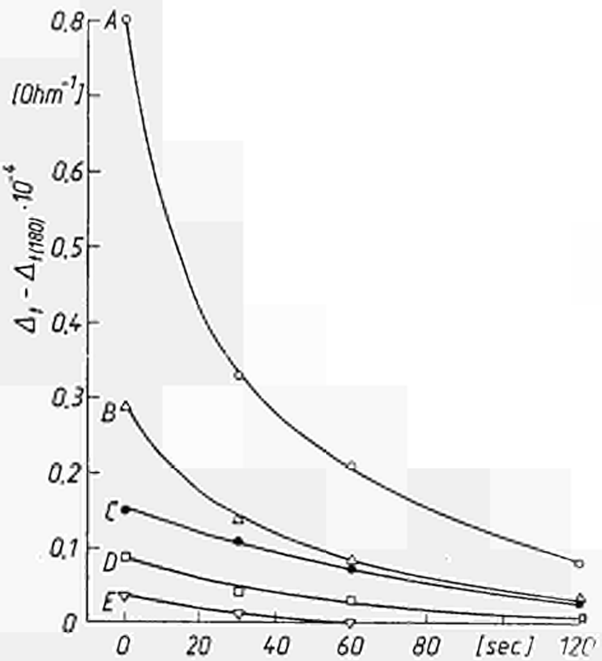


Fig. 3. Dependence of conductivity decrease on irradiation dose for the variety "Sirtima" measured at 20 °C.

A (○): unirradiated, B (△): 10 krad, C (●): 20 krad
D (□): 50 krad, E (▽): 100 krad.
$_{t}$ = Conductivity at indicated time after puncturing the electrodes into the tuber.
$_{t(180)}$ = Conductivity after the first measurement.

could be best distinguished from potatoes irradiated with high doses by determining the differences $\Delta_{t(0)} - \Delta_{t(180)}$ and $\Delta_{t(30)} - \Delta_{t(180)}$.

In Table VIII the ranges of these values for different doses are presented.

For testing the usefulness of the method for practical purposes the conductivity decrease was measured and the dose range was determined according to Table VIII using 15 samples of potatoes of unknown variety and irradiation dose. The results are given in Table IX.

SUMMARY

Irradiation causes a decrease in the electrical conductivity of potato tubers. Although this effect is very pronounced if potatoes of identical variety and provenience are used, it can probably not be used to identify

with certainty irradiated potatoes of unknown variety, stored under unknown conditions.

The difference in the conductivity value measured immediately after puncturing the tuber with the electrodes and that measured up to 180 sec later (Δ_t — $\Delta_{t(180)}$) decreases with increasing irradiation dose, regardless of potato variety. It appears that this difference value can be used to differentiate between potatoes irradiated with low doses (0-20 krad) and those irradiated with high doses (20-100 krad). If law permits irradiation of potatoes with a specific dose (e.g. 15 krad), this

Table V. — Dependence of the differences Δ_t — $\Delta_{t(180)}$ on the radiation dose for four potato varieties; measured at 20 °C; potato variety: SIRTIMA, OSTARA, AGORA, ERSTLING; storage temperature: 10 °C.

	$\Delta_t - \Delta_{t(180)} \cdot 10^{-4}$ Ohm^{-1}			
	0 sec	30 sec	60 sec	120 sec
SIRTIMA				
0 krad	0.80 ± 0.13	0.33 ± 0.08	0.21 ± 0.07	0.08 ± 0.05
10 krad	0.29 ± 0.10	0.14 ± 0.08	0.08 ± 0.08	0.03 ± 0.03
20 krad	0.15 ± 0.08	0.11 ± 0.06	0.07 ± 0.05	0.05 ± 0.03
50 krad	0.09 ± 0.08	0.04 ± 0.05	0.03 ± 0.03	—
100 krad	0.03 ± 0.10	0.04 ± 0.06	—	—
OSTARA				
0 krad	0.96 ± 0.26	0.47 ± 0.12	0.23 ± 0.08	0.45 ± 0.04
10 krad	0.51 ± 0.18	0.18 ± 0.07	0.15 ± 0.09	0.03 ± 0.03
20 krad	0.43 ± 0.21	0.24 ± 0.13	0.11 ± 0.10	0.06 ± 0.05
50 krad	0.30 ± 0.13	0.23 ± 0.12	0.12 ± 0.12	0.08 ± 0.05
100 krad	0.03 ± 0.13	0.04 ± 0.09	0.04 ± 0.03	—
	$\Delta_t - \Delta_{t(180)} \cdot 10^{-4}$			
	0 sec	30 sec	60 sec	120 sec
AGORA				
0	0.71 ± 0.20	0.44 ± 10	0.29 ± 0.08	0.15 ± 0.08
10	0.45 ± 0.09	0.23 ± 0.05	0.15 ± 0.06	0.05 ± 0.04
20	0.36 ± 0.14	0.14 ± 0.08	0.1 ± 0.06	0.07 ± 0.06
50	0.11 ± 0.09	0.09 ± 0.06	0.08 ± 0.05	0.04 ± 0.03
100	0.07 ± 0.05	0.02 ± 0.07	0.03 ± 0.06	—
ERSTLING				
0	0.72 ± 0.21	0.33 ± 0.12	0.22 ± 0.09	0.11 ± 0.07
10	0.41 ± 0.10	0.23 ± 0.15	0.15 ± 0.07	0.06 ± 0.04
20	0.33 ± 0.14	0.16 ± 0.08	0.11 ± 0.06	0.07 ± 0.05
50	0.11 ± 0.052	0.052 ± 0.09	0.02 ± 0.08	—
100	0.08 ± 0.14	0.02 ± 0.08	0.03 ± 0.06	—

Table VI. — Dependence of the conductivity decrease on storage time; measured at 20 °C; storage temperature: 10 °C; variety: Bintje.

	$\Delta_t - \Delta_{t(180)} \cdot 10^{-4}$ Ohm^{-1}			
Time	0 sec	30 sec	60 sec	120 sec
I. month				
unirradiated	0.78±0.09	0.39±0.40	0.17±0.09	0.05±0.07
10 krad	0.45±0.07	0.31±0.05	0.19±0.04	0.08±0.03
CIPC	0.91±0.22	0.58±0.22	0.29±0.15	0.13±0.66
II. month				
unirradiated	1.10±0.16	0.59±0.20	0.34±0.15	0.09±0.10
10 krad	0.45±0.06	0.28±0.06	0.13±0.06	0.06±0.06
CIPC	0.95±0.10	0.45±0.11	0.20±0.12	0.09±0.05
IV. month				
unirradiated	0.64±0.13	0.32±0.11	0.20±0.06	0.03±0.03
10 krad	0.82±0.13	0.62±0.13	0.30±0.09	0.11±0.06
CIPC	0.95±0.13	0.53±0.10	0.32±0.09	0.18±0.07
VI. month				
unirradiated	0.42±0.18	0.32±0.19	0.14±0.09	—
10 krad	0.89±0.26	0.37±0.23	0.09±0.12	—
CIPC	1.4 ±0.02	0.65±0.20	0.20±0.10	—
VIII. month				
unirradiated	—	—	—	—
10 krad	0.53±0.13	0.29±0.12	0.11±0.04	0.03±0.01
CIPC	0.90±0.30	0.43±0.11	0.23±0.13	0.05±0.02

Table VII. — Dependence of the difference $\Delta_t - \Delta_{t(180)}$ on the temperature during measurement; potato variety: Erstling.

	$\Delta_t - \Delta_{t(180)} \cdot 10^{-4}$ Ohm^{-1}			
	0 sec	30 sec	60 sec	120 sec
Erstling				
unirradiated				
0 °C	0.43±0.13	0.33±0.13	0.28±0.09	0.10±0.06
20 °C	0.72±0.21	0.33±0.12	0.22±0.09	0.11±0.03
35 °C	1.0 ±0.26	0.34±0.07	0.16±0.06	0.05±0.04
45 °C	0.56±0.13	0.20±0.05	0.16±0.06	—
Erstling				
20 krad				
0 °C	0.17±0.05	0.13±0.04	0.10±0.05	0.06±0.04
20 °C	0.33±0.14	0.6 ±0.08	0.11±0.06	0.07±0.05
35 °C	0.70±0.24	0.25±0.14	0.13±0.07	0.03±0.04
45 °C	0.39±0.11	0.17±0.03	0.16±0.03	—

Table VIII

krad $\Delta_{t(0)}$	$\Delta_{t(30)} - \Delta_{t(180)}$	$- \Delta_{t(180)}$
0 - 10	0.7 - 1.2	0.3 - 0.6
10 - 20	0.3 - 0.6	0.2 - 0.4
20 - 50	0.2 - 0.4	0.1 - 0.3
50 - 100	0.1 - 0.2	0 - 0.2
100	0 - 0.1	0 - 0.1

Table IX. — Comparison of theoretical values with those which were determined by conductivity (4 potatoes used for each sample)

Sample	Identified dose range	Actual dose applied
1.	20 - 50	50
2.	0 - 10	0
3.	20 - 50	100
4.	0 - 10	0
5.	0 - 10	0
6.	10 - 20	20
7.	0 - 10	0
8.	0 - 10	0
9.	50 - 100	100
10.	10 - 20	0
11.	50 - 100	100
12.	0 - 10	0
13.	10 - 20	50
14.	50 - 100	100
15.	0 - 10	10

method should be useful to make sure that the allowed maximal dose has not been greatly exceeded. In potatoes of known variety this method should be capable of differentiating between irradiated and unirradiated tubers.

Storage time does not influence conductivity as long as the tubers remain unsprouted. On sprouting both the absolute conductivity and the $\Delta_t - \Delta_{t(180)}$ values decrease.

LITERATURE

1) W.J.V. OSTERHOUT, Bot. Gaz. **61**, 148 (1916).
2) K. PAECH, Planta **31**, 265 (1940).
3) U.P. HANSEN, Atomenergie **12**, 447 (1967).

DISCUSSION

CHAIRMAN

Dr Scherz has indeed found a method here by which irradiation can be detected. The extent to which it will be possible to apply this technique in practice will, of course, also have to be determined. I am thinking in particular here of the need for comparing irradiated potatoes with potatoes that have been treated with a chemical sprout-inhibiting agent. Have you made such a comparison?

H. SCHERZ

Yes, with the variety Bintje we have investigated unirradiated potatoes, CIPC-treated potatoes, and potatoes irradiated with a dose of 10 krad. Up to the time when sprouting began in the untreated potatoes the conductivity was the same in untreated and in CIPC-treated potatoes, while irradiation caused a pronounced lowering of both the conductivity itself and of the conductivity differential $(\Delta_t - \Delta_{t(180)})$. We have not studied other chemical sprout inhibitors but I expect that non of them will influence conductivity because they stay on the surface of the tuber while conductivity is measured in the interior of the tuber.

J. PAUWELS

With reference to table III, I believe that the main problems of this method are its degree of sensitivity and precision. I should like to know whether the indicated precision is due to the reproductibility of the method itself or whether different varieties of potatoes were used.

H. SCHERZ

In Table III we have used the varieties Maritta, Sieglinde and Bintje. The values given, e.g. 4.85±0.26 indicate the mean of 15 measurements obtained on 15 different tubers, and the standard deviation.

J. PAUWELS

Does reference-line A for untreated potatoes (Fig. 3) concern no-at-all treated potatoes, or are they treated chemically with CIPC?

H. SCHERZ

No, those were untreated potatoes.

J. PAUWELS

I think CIPC-treated potatoes should be used as reference. From an industrial point of view, untreated potatoes are practically never used and furthermore CIPC seems to have a counter-effect on electrical conductivity, and improves the possibility of detecting low irradiation doses.

H. SCHERZ

In all instances our controls were untreated potatoes so that we could study the influence of various parameters such as storage time and storage temperature independent of radiation treatment or CICP-treatment. We have not carried out any conductivity measurements on potatoes that were both irradiated *and* CIPC-treated. Perhaps such experiments could be carried out later to study the effect of combined treatments.

J. MORRE

I should like to know whether, as well as conductivity, you investigated the coefficient that is normally represented by the Greek letter ε, i.e. the dielectric constant?

H. SCHERZ

No, we have not determined the dielectric coefficient. We have studied the conductivity also at 3,000 Hz. The values were more variable than those obtained at 50 Hz. Therefore we have, in later experiments, used 50 Hz only.

A.R. DESCHREIDER

Figure 2 shows the dependence of conductivity on dose, but after what time, how many seconds, was the measurement made?

H. SCHERZ

As indicated in Table I, "the values were obtained immediately after puncture".

P. KAYSER

I should like to ask a question regarding Table IX. If you had used chemically treated potatoes on the one hand, and irradiated potatoes on the other, would you not have obtained better results for 10 krad? As it is, it is not easy to distinguish between 10 krad and 0. I think that you would then have been more precise. In practice, potatoes that have undergone no chemical treatment are rarely encountered. It would have been useful to compile a table on this basis.

H. SCHERZ

Yes, this is a good idea. We should also include chemically treated potatoes. We hope, however, that radiation treatment will replace chemical treatment of potatoes in the future. At that time the controlling laboraties will have the task of differentiating between irradiated and non-irradiated potatoes only.

CHAIRMAN

In Holland, irradiated potatoes are already permitted for specified purposes. They have not yet been released for general sale. The question of whether irradiation should be used instead of chemical preservatives is still a matter under discussion. In the Netherlands Administration it is quite clear that, in order to prevent sprouting, a choice will be made: either irradiation, or chemical preservatives, but in general not both.

J.F. DIEHL

I would like to add some remarks concerning the investigations of Dr. Scherz. From the questions that were asked it is obvious that the method is not yet considered to be perfect. We readily agree with this. Although much work has been done, these experiments are not finished. Different varieties have been considered, different storage times, different treatment parameters (unirradiated, irradiated and CIPC treated); each variable was tested not on just one potato but on several samples. Thus a very great number of measurements had to be done. The main weakness of the report is the small number of samples in Table IX. This is the basis on which we must decide whether the method is useful or not, but we cannot make this decision on the basis of only fifteen measurements. Probably, a thousand measurements are necessary before we can say on statistically sufficient grounds: The method works or it does not work.

CHAIRMAN

It is certainly extremely valuable to concentrate on its practical implementation. The main thing is that it can be applied in practice, and then under what circumstances and with what degree of certainty and uncertainty. All our testing methods carry a certain percentage of uncertainty.

H. SCHERZ

I agree, Mr. Chairman, that the method must still be refind. As indicated in Table VIII the method can prove with certainty an irradiation with a dose of 50-100 krad; but in the range of 0-10 krad the situation is very difficult. Perhaps we can still improve our experimental conditions, e.g. by using a different arrangement of the electrodes. We have worked only with commercially available puncture electrodes. Perhaps one should also study different electrode materials.

CHAIRMAN

Are we all of the opinion that we should advance farther along these lines?

J. PAUWELS

If table IX is extended from 15 to 1,000 measurements, one should use chemically treated potatoes instead of untreated ones. In this way, the detection of irradiation would be facilitated. Furthermore, I do not agree completely with Dr. Scherz. I do not believe that in practice comparisons will ever be made with untreated potatoes. Industrially, potatoes are always treated, either chemically or by irradiation. Chemical treatment in combination with irradiation is, I think, excluded for legal and economic reasons.

P. KAYSER

Mr. Chairman, I should like to emphasize once again what the value of this method could be to the inspection authorities. They need to know whether a potato has been irradiated or chemically treated. In my opinion, this is really the main value that this method for potatoes would hold for the inspection authorities. I think that the importance of this fact should be borne in mind in conducting further studies. The method seems extremely promising for making this differentiation.

CHAIRMAN

I think that on this point the conclusion is clear. The investigations should follow along the lines dictated by practice. And the refinement of the method should be investigated for irradiated, non-irradiated and chemically treated potatoes. I agree with Mr. Pauwels and Mr. Kayser that in practice we shall have to use just either one method or the other, even if only for economic reasons.

H. PROST

I should like to back up what you have just said by stating that the trend will very probably be towards prohibiting chemical treatment if irradiation is used, because this is already *res judicata* in all cases as far as French regulations are concerned. In the decree on the irradiation of potatoes that is to be issued very shortly, only potatoes that have not been chemically treated may be irradiated, and irradiated potatoes may no longer be chemically treated. Consequently, as you have said, I think that in the Community, not only from the economic point of view but also from the legal point of view, the trend will be towards a choice between one method or the other, and I think that it is important to recognise this when pursuing experiments and studies.

CHAIRMAN

Have you investigated other methods or means of detection in France?

A. GUILBOT

Mr. President, we have indeed tested several methods to detect irradiated potatoes. One of them consists in making sure that potatoes cannot sprout: the results are positive, but unfortunately very slow to obtain. Consequently, it is not a very good method when a swift control is needed.

Then again we are working just now on the revealing of altered anhydroglucose residues in the starch chains of irradiated potatoes. Even at these very low doses, we have been able to reveal altered anhydroglucose units after hydrolysis. But up till now, it has not been possible to master the use of this method enough to make it quantitative, and moreover we have noticed fluctuations during the storage of irradiated potatoes. However, it is a course we shall go on investigating; no doubt it is longer than the very elegant conductivity method Mr. Scherz has devised.

CHAIRMAN

For practical sprout prevention, we need immediate results, that is, when large batches of potatoes are being inspected it must be possible to give an immediate verdict: irradiated or non-irradiated. This is the aim of our investigations.

I am particularly grateful to Dr. Scherz for his very positive contribution through his investigations in the field of electrical conductivity.

I am now glad to invite him to tell us of his admittedly negative experience in his investigations in the fields of redox potential, polarimetry and polarography.

H. SCHERZ

I will briefly summarize our results concerning a redox potential, polarimetry, and polarography. The measurements of *redox potential* were carried out on meat: We found that in unirradiated meat the values declined after several days of storage due to the growth of bacteria while in the irradiated samples the values remained high for a long time because of the suppression of bacterial growth. However, the measurements are extremely sensitive to the presence of traces of oxygen. Great precautions are necessary to avoid artifacts. We came to the conclusion that the method is not useful for a practical identification of irradiated meats. Our conclusions concerning *polarography* were similar. Any reducing or oxidizing substances present in the foods will influence the redox system and will thus influence the polarographic steps. We have made measurements not only on meat but also on wheat and on starch extracts. In all cases we found polarography to be unsuitable to differentiate between irradiated and unirradiated samples. As to *polarimetry:* We think this method is not sufficiently sensitive to detect small changes in the concentration of the optically active substances present in foods. We have studied the radiation inactivation of the rather radiation-sensitive enzyme invertase. We irradiated invertase in aqueous solutions and studied the effect of radiation on the enzymatic activity by measuring the invertase-catalyzed hydrolysis of sucrose to glucose and fructose polarimetrically. This can certainly not be considered as a general method for identifying irradiated foods.

L. SAINT-LÈBE

I am completely in agreement with Dr. Scherz when he states that polarography is not a sufficiently sensitive method for detecting the irradiation of starch, for example.

2. EVALUATION OF THE STRUCTURE OF THE DEOXYCOMPOUNDS FOUND IN IRRADIATED STARCH AND DETERMINATION OF THESE PRODUCTS IN IRRADIATED WHEAT AND WHEAT FLOUR

Progress report 1.2.1968 - 1.10.1970

H. SCHERZ

ABSTRACT

Chromatography

Malondialdehyde and deoxycompounds are formed on irradiation of carbohydrates. Irradiated potato starch, wheat flour and wheat grain were extracted with a mixture of ethylacetate-acetone-water after lipid removal. The deoxycompounds present in the extract were separated by paper chromatography and detected with the periodic acid-thiobarbituric acid reaction. By visual comparison of spot size and spot intensity, a semi-quantitative determination of the applied dose range was possible. The lower limit at detection was between 100 and 200 krad. For a better quantitative estimation, gas-chromatography following silylation was employed. By reduction with potassium boronhydride, a distinct difference in the pattern of irradiated and untreated material was observed. The combination gas-chromatography and mass spectroscopy was used to identify some of the radiolytic products.

Colorimetry

Direct colorimetric estimation at deoxycompounds in extracts of irradiated wheat flour was unsuccessful. Direct colorimetry of malondialdehyde was possible, but due to the instability of this compound, especially in presence of proteins, unsuitable for identification.

COLORIMETRY AND CHROMATOGRAPHY

Malondialdehyde and deoxycompounds have been found in irradiated carbohydrates (1-4). Both substances can be detected by sensitive color reactions which could provide a basis for identification of irradiated carbohydrate containing food.

The determination of free malondialdehyde appears to be less suitable since it is unstable and disappears within a few days. Determination of the deoxycompounds seemed to be an attractive approach both in view of the stability of this class of compounds and the specificity of their analytical detection. Paper chromatography was necessary for separation and isolation of the deoxycompounds. Gas chromatography was used as an alternative method for the separation and quantitative determination of the deoxycompounds and other radiolytic products.

MATERIALS AND METHODS

Potato starch, wheat flour (Brand "Goldpuder") and wheat grains (Variety: Hanno and Jubilar) were irradiated in sealed polyethylene bags with 10 MeV electrons in a linear accelerator. The irradiated grains were milled to flour. For detection of deoxycompounds 150 g of wheat flour were treated three times with 200 ml of petrolether each for lipid removal. The deoxycompounds were extracted three times with 150 ml each of a mixture of ethylacetate-acetone-water 4 : 5 : 1 v/v. The combined extracts were evaporated to dryness in vacuo at 40° C. The dry residue was treated repeatedly with 0.5 ml of petrolether to remove the small amounts of lipids. The residue was dissolved in 0.2 ml of water.

Descending paper chromatography was carried out on acid washed paper (Schleicher Schüll 2043b). After developing with the solvent *n*-butanol-ethanol-water 4 : 5 : 1 v/v the deoxycompounds were detected by following treatment of the paper with an acetone solution of periodic acid. This causes a splitting of the deoxycompounds with formation of free malondialdehyde. This was subsequently detected with thiobarbituric acid (TBA) according to the procedure of Anderson (5).

For gaschromatographic separation the radiolytic compounds were converted into their trimethylsilylethers according to the procedure of Sweeley *et al.* (6). The separation was performed on temperature programmed equipment (Hewlett-Packard), using steel columns 6 and 20 feet long, filled with diatoport S which was coated with phenylsiliconrubber. The temperature interval was 150-270° C, the temperature gradient 10 and 1.25° C/min.

For getting information about the chemical structure of the radiolytic products we used the combination gaschromatography-masspectrometry (Hitachi-Perkin Elmer).

RESULTS

In a number of experiments it was tried to determine deoxycompounds in the wheat flour extracts by direct colorimetry. For this purpose the extract was treated with 2 ml of 2 % periodic acid in 10 % phosphoric acid. After destroying the excess of periodic acid by adding sodiummetaarsenite the free malondialdehyde was removed by steam distillation. The determination of the malondialdehyde in the distillate by the 2-methylindole reaction (7) showed only traces. Presumably the malondialdehyde reacted on heating with small amounts of amino acids and peptides which were present in the extracts.

Therefore it was decided to separate the deoxycompound by paperchromatography. Fig. 1 presents the chromatogram of the deoxycompounds extracted from wheat flour irradiated with increasing doses. The deoxycompounds were detected by the periodic acid-TBA reaction and gave red spots.

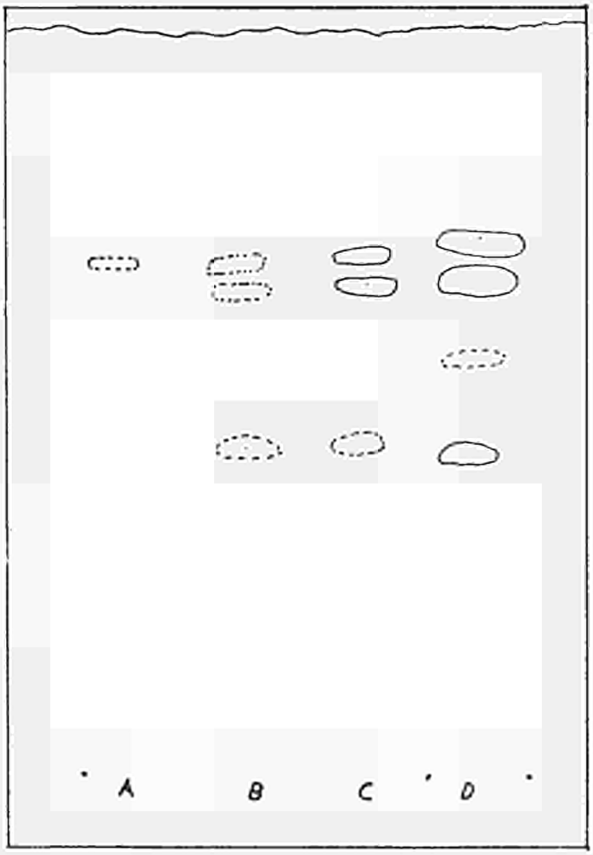


Fig. 1. Paperchromatogram of deoxycompounds extracted from wheat flour prepared of wheat grains (Variety Hanno).
Solvent: n-butanol-ethanol-water: 4 : 5 : 1 v/v.
A: unirradiated, B: 150 krad, C: 500 krad, D: 1000 krad

The red reaction products were extracted from the chromatographic strips with dimethylformamide and subsequently evaluated spectrophotometrically at 532 nm. The results deviated too much and only a semiquantitative determination of the applied irradiation dose was possible. The reaction was very sensitive to external influence (e.g. temperature and humidity during the TBA reaction, purity of paper, etc.).

It was more convenient to carry out visual comparison of color intensity and size of the spots under exactly standardized conditions. The applied dose range could be estimated and the lower limit of detection was between 100 and 200 krad.

In view of the disadvantages of direct colorimetry and paperchromatography an attempt was made to apply gaschromatography for separation and determination of deoxycompounds and other radiolytic products. Since a combination of gaschromatography-masspectrometry was available it was tried to elucidate the chemical structure of a few radiolytic products.

The separation of the trimethylsilylether of the polyhydroxycarbonylcompounds, which were expected to be main products in the extract of irradiated starch, was not satisfactory. Some of the compounds were probably destroyed at the hot metal walls. Different conformations of a single compound could give several peaks on gaschromatographic separation (e.g. monosaccharides). To avoid this disadvantage the aqueous solution of the extract was treated with potassium boronhydride (8). The polyhydroxycarbonylcompounds were reducred to polyhydroxycompounds and their trimethylsilylethers could be separated without difficulty on these columns. Fig. 2 and 3 show the gaschromatograms of starch extracts (Fig. 2: unirradiated sample, Fig. 3: irradiated with 4 Mrad).

Preliminary experiments were performed for the elucidation of the chemical structure of the radiolytic products, especially those of the deoxycompounds, using the combination gaschromatography-massspectrometry. By obtaining the masses (m/e) of the fragmentation ions of the separated trimethylsilylether it is possible to get information on its chemical structure. The localisation of the carbonylgroup can be performed by reducing the extract with sodiumborondeuteride and comparing the mass shifts of some of the fragment ions with those of the nondeuterated sample.

For example Fig. 4 shows the mass-spectra of peak B at Fig. 3.

A: extract reduced with potassiumboronhydride

B: extract reduced with sodiumborondeuteride.

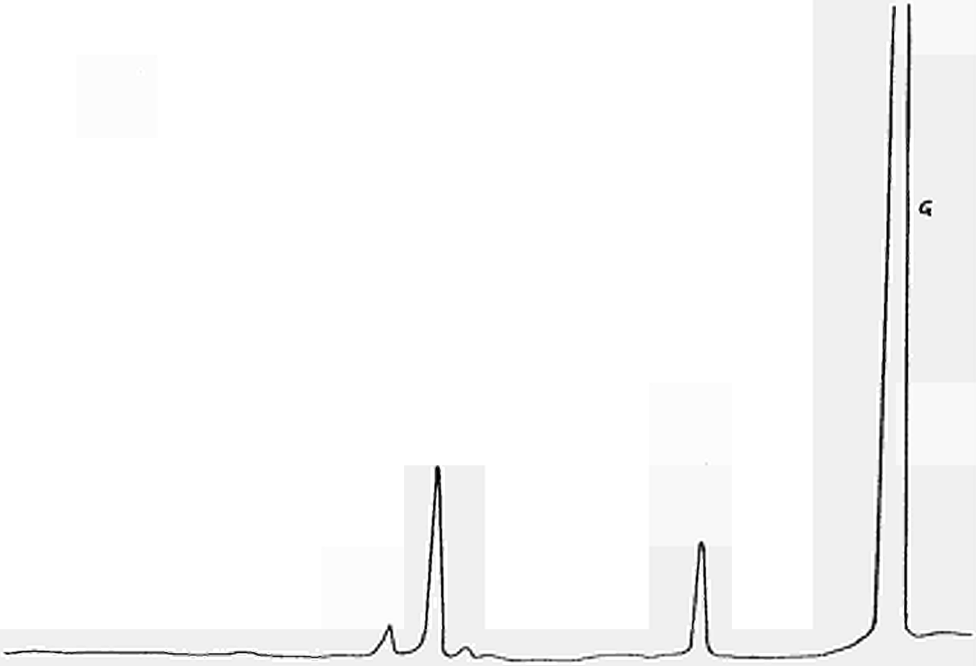


Fig. 2. Gaschromatogram of the silylated extract of unirradiated starch reduced with potassiumboronhydride. Temperature intervall: 150 - 270 °C; Gradient: 1.25 °C/min; Peak G: Mesoerythrol as internal standard.

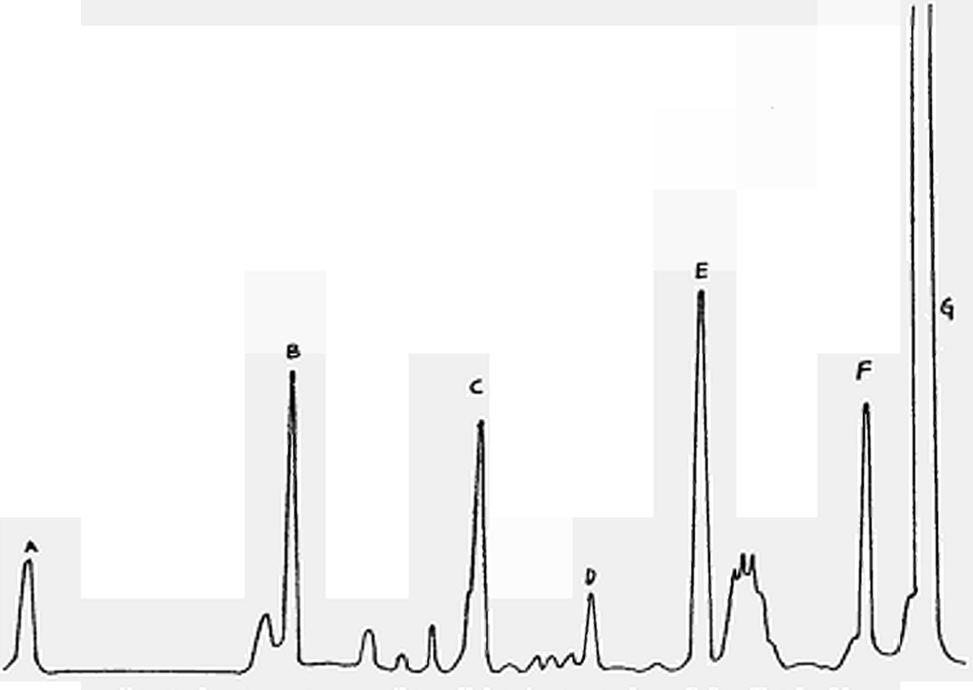


Fig. 3. Gaschromatogram of the silylated extract of starch irradiated with 5 Mrad treated under the same conditions

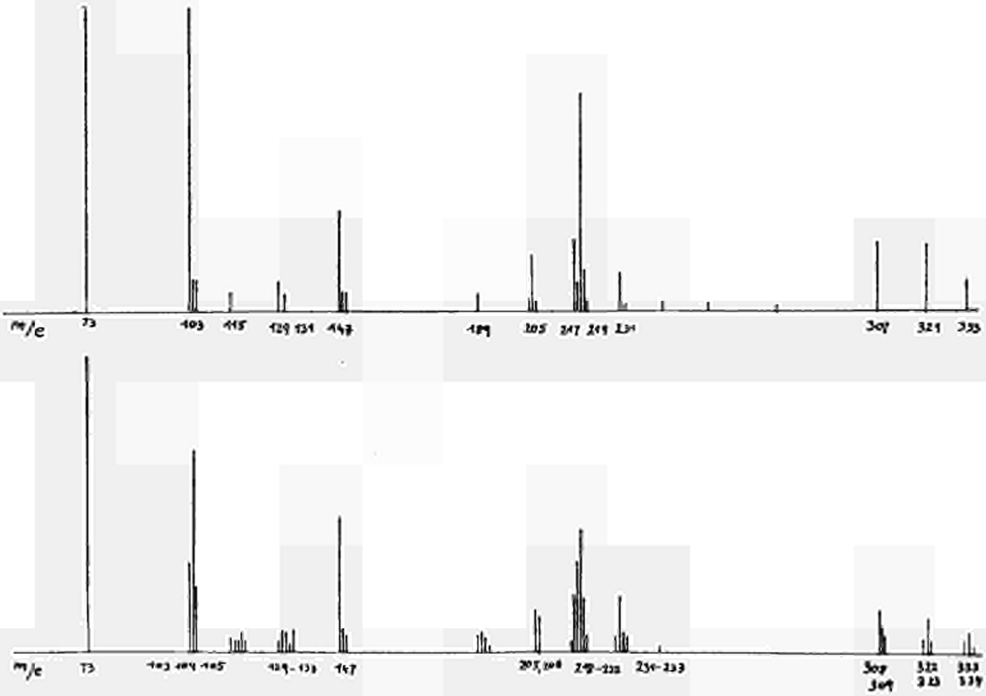


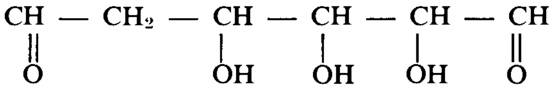
Fig. 4. Masspectra of Peak B in fig. 3.
a) reduced by potassiumboronhydride,
b) reduced by sodiumborondeuteride.

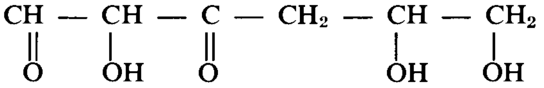
Table I gives the structures of the single fragmentation ions.

Table I.

m/e	Structure (TMSi = $Si(CH_3)_3$)
73	TMSi^{+} —
103	$\mathrm{TMSi}-\overset{+}{O}=CH_2$
147	$\mathrm{TMSi}-\overset{+}{O}=Si(CH_3)_2$
205	$\mathrm{TMSi}-O-CH_2-CH=\overset{+}{O}-\mathrm{TMSi}$
217	$\mathrm{TMSi}-\overset{+}{O}=CH-CH=CH-O-\mathrm{TMSi}$
219	$\mathrm{TMSi}-O-CH_2-CH_2-CH=\overset{+}{O}-\mathrm{TMSi}$
307	$\mathrm{TMSi}-O-CH_2-\underset{\substack{\mid\\ \mathrm{OTMSi}}}{CH}-CH=\overset{+}{O}-\mathrm{TMSi}$
321	$\mathrm{TMSi}-O-CH_2-CH_2-CH_2-\underset{\substack{\mid\\ \mathrm{OTMSi}}}{CH}-CH=\overset{+}{O}-\mathrm{TMSi}$
333	$\mathrm{TMSi}-O-CH_2-CH=\underset{\substack{\mid\\ \mathrm{OTMSi}}}{C}-\underset{\substack{\diagdown\\ \mathrm{OTMSi}}}{CH}-CH=\overset{+}{O}-\mathrm{TMSi}$

This fragmentation pattern was identical with that of 2-deoxy mannitol. It could be assumed that the original structure was a 2-deoxycarbonylcompound. The masspectra of the deuterated sample led to the assumption that by irradiation of starch two deoxyhexoses with the following structure are probably formed.

1.

$$\underset{\underset{O}{\|}}{CH} - CH_2 - \underset{\underset{OH}{|}}{CH} - \underset{\underset{OH}{|}}{CH} - \underset{\underset{OH}{|}}{CH} - \underset{\underset{O}{\|}}{CH}$$

2.

$$\underset{\underset{O}{\|}}{CH} - \underset{\underset{OH}{|}}{CH} - \underset{\underset{O}{\|}}{C} - CH_2 - \underset{\underset{OH}{|}}{CH} - \underset{\underset{OH}{|}}{CH_2}$$

The preliminary results show that gaschromatography in connection with masspectrometry could be a useful way for detection of irradiated grain and flour.

LITERATURE

1) J. MORRE and S. MORAZZANI-PELLETIER C.R., **262** C, 1729 (1966).
2) H. SCHERZ, Nature (London), **219**, 611 (1968).
3) G. BERGER and L. SAINT-LEBE, Stärke, **21**, 205 (1969).
4) N.K. KOCHETKOV, L.I. KUDRYASHOV and M.A. CHLENOV, Dokl. Akad. Nauk SSSR, **179**, 1385 (1968).
5) P.J. ANDERSON, J. Chromatogr., **21**, 163 (1966).
6) C. SWEELEY, R. BENTLEY, M. MAKITA and W. WELLS, J. Amer. Chem. Soc., **85**, 2497 (1963).
7) H. SCHERZ, G. STEHLIK, E. BANCHER and K. KAINDL, Mikrochim. Acta, **1967**, 916.
8) Ju. OVODOV and E.V. EVTUSHENKO, J. Chromatogr., **31**, 527 (1967).

DISCUSSION

L. SAINT-LÈBE

The subject we are working on at Cadarache is very close to the one mentioned by Dr. Scherz, and we also use gas chromatography to identify certain substances which appear when starch is irradiated.

I agree with Dr. Scherz that the malonaldehyde test is very much upset if the protein content is high. However, I am not sure that in the case of carbohydrates gas chromatography is a suitable technique for an irradiation test, as it is complex in application.

With starch the small quantity of lipids and proteins has no effect on the malonaldehyde test. It would be easy to extrapolate this test to grain products, provided that starch, gluten and proteins could be separated—by a dry process.

H. SCHERZ

I thank Mr. Saint-Lèbe very much for his important and stimulating suggestion. Malondialdehyde is quite stable in pure starch; not so when proteins are present. It is your suggestion to investigate the condensation products of malondialdehyde with proteins. Perhaps it will be possible to show bonds between malondialdehyde and protein by some colorimetric method.

B. RADOLA

Have you tried to develop a method for identification based on the interaction of proteins with malondialdehyde?

L. SAINT-LÈBE

We have dropped this approach for the present. With the test devised, the irradiation dose received by the starch can be established regardless of the period of storage, as long as the date of irradiation, the moisture content of the starch and the storage temperature are known. But to answer your question more precisely, we have no technique which might be able to solve your problem.

A. GUILBOT

I should also like to point out that we have been and are just now working to the same effect on starch extracted from irradiated potatoes. We had formerly carried on a study on the alteration of potato starch submitted to very high doses of gamma radiations. Our work differs from Mr. Scherz's in the fact that we are not interested in the soluble fraction of starch, but in the anhydroglucose units which are altered and remain in the macromolecular products. Thus after hydrolysing these products, we can effectively reveal the desoxysugars existing in these chains, and as far as know for the moment, it seems they are not 2-desoxy- but 3-desoxyglucoses.

H. SCHERZ

In my publication in "Nature" I have given only the amounts of deoxysugar. I did not say that it was 2-deoxyglucose. The gaschromatographic studies which we have carried out since then have indicated that these are probably deoxycarbonyl (C—CO—CH_2—) compounds which are generally formed upon irradiation of carbohydrates as a result of an internal splitting off of water. I fully agree with you that different reactions are possible and that possibly 3-deoxyglucose is also formed; but this can happen only if a carbonyl group is present in a 2-position. On the basis of mass spectrometry data I think it is likely that the compounds named on page of my report are also formed. We have not yet investigated whether such groups also occur within the polymer chain of starch after irradiation. This might very well be the case. After enzymatic hydrolysis it should be possible to find such compounds.

A. DESCHREIDER

I should like to ask Dr. Scherz whether he has also turned his attention to the formation of glyoxal under the effect of ionizing radiations, glyoxal which is formed in fairly large quantities.

H. SCHERZ

Yes, we have done such studies because Phillips has reported that glyoxal is present in irradiated glucose solution, but we have not found glyoxal, although we have done many colorimetric tests. Also in our latest investigations using chromatography on thin layer plates and various color reactions we have found that no glyoxal is present in irradiated glucose solution—or if so, it must be present in extremely small quantities.

L. SAINT-LÈBE

I am also replying to Mr. Deschreider's question. I am pleased to note that we have reached the same conclusion as Dr. Scherz. Indeed, a number of authors point out the presence of glyoxal in irradiated glucides or amylose. Well, after 6 month of research using various techniques, especially thin-layer chromatography, we could not detect glyoxal in maize starch irradiated with a dose of 2 Mrad. The work now in progress shows that it is possible to confuse glyoxal, methylglyoxal and erythrose.

A. DESCHREIDER

I should like to put the following question to Mr. Saint-Lèbe and Dr. Scherz. How do they explain the fact that when one examines the ultraviolet spectrum of the coloured substance which forms with thiobarbituric acid, a very pronounced peak is observed at 456 nanometres?

H. SCHERZ

Glyoxal forms another peak in the TBA-reaction—I believe at 480 nm. One should study this color reaction by thin layer chromatography to make sure that it is really glyoxal which gives rise to this color reaction.

J. MORRE

In fact this is a false problem, because although the glyoxal peak is at 455 nm, so are the peaks of a lot of neighbouring compounds, and in my thesis I showed how to distinguish between glyoxal and malonaldehyde: if you perform the TBA reaction below 60° C, glyoxal and the nearby compounds give a non-specific yellow colouration at 455 nm, but malonaldehyde gives a red colouration at 535 nm which *is* specific to this compound. But if you heat to boiling, at 100° C, everything is mixed: glyoxal at 525 and 550 nm cannot be distinguished from malonaldehyde at 535 nm. I think that in a way everyone is right.

CHAIRMAN

To conclude, therefore, the peak is there. To interpret it, recourse has to be had to various methods, not only methods of light measurement but also differentiation by means of chromatography, gas chromatography and mass spectrometry when this is necessary. I think that the most promising method may be mass spectrometry.

3. IDENTIFICATION OF IRRADIATED MEAT BY THIN-LAYER GEL CHROMATOGRAPHY AND SOLUBILITY STUDIES *

Progress Report 1.4.1967 - 1.10.1970

B.J. RADOLA

ABSTRACT

In beef and pork irradiated with a dose of 1 and 5 Mrad, a new radiation-induced, sarcoplasmic protein fraction was observed on thin-layer gel chromatography on Sephadex G-200. Due to its high R_M value, the radiation-induced fraction migrating ahead of all other sarcoplasmic protein fractions of untreated meat could be easily identified in the chromatographic pattern. Irradiation experiments with isolated sarcoplasmic proteins strongly suggest that the radiation-induced fraction results from aggregation of these proteins. The amount of the radiation-induced fraction increased with increasing dose. Irradiation temperature and dose rate were found to affect the formation of the radiation-induced fraction. Storage of meat irradiated with 5 Mrad at room temperature for periods up to 10 weeks has shown that the radation-induced fraction is stable. Solubility studies based on stepwise extraction of a protein precipitate absorbed on an inert carrier with a series of ammonium sulphate solutions of decreasing molarity have shown that the fractions extracted with 1, 1,5 ans 2 M ammonium sulphate are reduced in irradiated meat. The demonstration of the radiation-induced, sacroplasmic protein fraction can serve as a basis for identification of meat irradiated with 5 Mrad. The method is relatively simple and suitable for routine analysis.

INTRODUCTION

Radiation-induced aggregation has been observed for a number of homogeneous proteins (1-8) and for model systems composed of two proteins differing in physicochemical properties (9). The dose dependence and specificity of radiation-induced aggregation and the stability of the aggregates appeared promising as a base for identification of irradiated protein-rich food.

By use of a variety of methods radiation-induced aggregates can be detected (2, 4, 5, 6, 8). Gel chromatography, a method separating substances according to differences in molecular size, affords the

---

* Also published as report EUR 4618.e.

advantage of high resolution and relative simplicity. A thin-layer technique of gel chromatography has been described (10-13) and it appeared to be of potential utility for routine analysis. Meat has been chosen as a model food for our studies because of its technological importance and its high protein content which was considered to be a methodological advantage. A number of factors which may be anticipated to affect the radiation response were studied. These included: the quality of radiation, the dose rate and the irradiation temperature. Our results indicate that all these factors have an influence on the effects observed and are therefore of potential importance in any consideration of identification methods of irradiated food.

Evidence for changes in solubility of proteins in irradiated food has been obtained, rendering extraction of the soluble proteins a critical step for further analysis. An attempt was made, therefore, to study in a more direct way the effect of irradiation on the solubility of proteins. A method based on stepwise extraction of the proteins precipitated by saturated ammonium sulphate solutions by a series of ammonium sulphate solutions of decreasing molarity was used for this purpose (14, 15).

MATERIALS AND METHODS

Fresh beef and pork (longissimus dorsi) was irradiated with a dose of 1 and 5 Mrad either in closed glass vessels or in sealed polyethylene bags. Two irradiation sources were used: 1) a cobalt source with a dose rate of 1.0-1.5.10^4 rad/min, and 2) an electron accelerator (Varian); the energy of the electrons was 10 MeV and the mean pulse dose rate $\sim 10^{11}$ rad/sec. The irradiation temperature was 0 or 30° C. The irradiated meat was analyzed within a few days after irradiation or after storage for several weeks at room temperature.

For preparation of sarcoplasmic proteins 10 g of meat were homogenized for 2-3 min with 30 ml of ice-chilled 0.1 M phosphate buffer (KH_2PO_4-Na_2HPO_4)pH 7.2-7.4 in a Waring blendor with cooling. The homogenates were centrifuged at 4° C at 35,000 g for 15 min. The supernatant was either concentrated directly or dialyzed first against 1 % glycin followed by ultrafiltration on UM-10 or PM-10 Diaflo membranes (Amicon, N.V. Oosterhout, Holland) to an optical density of $E_{280}^{1\,\text{cm}} = 50$, corresponding to a protein concentration of 4-5 %. The concentrated solutions were analyzed within a few days. If kept longer they were preserved with merthiolate 1 : 5000 or 1 : 10000 and kept a 4° C.

Thin-layer gel chromatography was performed essentially as described elsewhere (12, 13). Briefly, 20 × 20 cm glass plates were coated with a 0.5 mm layer of a suspension of Sephadex G-75, G-100 or G-200, all "superfine" (Pharmacia, Uppsala, Sweden), in a 0.02 M phosphate buffer pH 7.2-7.4 containing 0.5 M NaCl. After equilibration overnight 10-20 μl of the concentrated solutions of sarcplasmic proteins were applied. Sperm whale myoglobin and ferritin were run as reference proteins. The flow rate was adjusted to 1 cm/hr for myoglobin. After 4-6 hours the separation was stopped and a print was taken with chromatographic paper Whatman No. 3 or occasionally Whatman No. 1 with the G-75 gel. After contact with the gel for 20-30 sec, the print was removed, dried at 110° C for 15 min and stained in a 9 : 1 v/v methanol-acetic acid solution of amido black 10 B (E. Merck, Darmstadt) or Coomassie Blue R 250 (Serva, Entwicklungslabor, Heidelberg). The prints were destained with a mixture of methanol-acetic acid-water 50 : 10 : 40 v/v. Densitometry (in reflectance) was accomplished either with the Chromoscan densitometer equipped with the thin-layer attachment (Joyce-Loeble & Comp. Ltd., Gateshead, England) or with the Zeiss Chromatogram Spectrophotometer (C. Zeiss, Oberkochen, Germany).

For molecular weight determinations the distance from the starting line to the middle of each zone was measured with an accuracy of 0.5 mm either directly on the print or on the densitogram. The results were expressed by the R_M value defined as the ratio of the migration distance of the sarcoplasmic protein fraction (d_P) to that of myoglobin (d_M) which was used as the reference protein.

$$R_M = \frac{d_P}{d_M}$$

Solubility experiments: 3 g of meat (untreated or irradiated) were homogenized for 3 min at 0° C in a MSE homogenizer with 40 ml of a 4 M ammonium sulphate solution, pH 7.0-7.5. Six grams of cellulose powder (MN Cellulose Pulver, Macherey-Nagel & Co., Düren, Germany) were added as an inert carrier. Twenty ml of the cellulose suspension containing the protein precipitate were placed into a 20 × 1 cm column and allowed to settle. Proteins were extracted from the column with a series of ammonium sulphate solutions of decreasing molarity, 50 ml of each solution being used. Fractions of ~ 5 ml/10 min were collected by means of a fraction collector at a constant flow established by using a peristaltic pump. Absorbancy readings were performed at 280 nm. For some of the fractions absorbancies at two wave lengths were measured.

RESULTS

In preliminary experiments optimal conditions for the separation of sarcoplasmic proteins by thin-layer gel chromatography were determined. With the G-75 gel only two zones were detected—one corresponded to myoglobin (R_M value = 1.0), while the other had an R_M very close to the exclusion limit of this particular gel (Fig. 1). There was no difference in the pattern between the untreated material and the sample irradiated with 5 Mrad. With the G-100 gel besides myoglobin three other components with higher R_M values were observed (Fig. 2). Densitometrically only two peaks could be obtained, one of myoglobin and the other, a strongly asymmetric one, corresponding to the components with higher molecular weights. Meat irradiated with 5 Mrad showed consistently an increased content of fraction F3 which formed the leading part of the asymmetric peak (Fig. 2B).

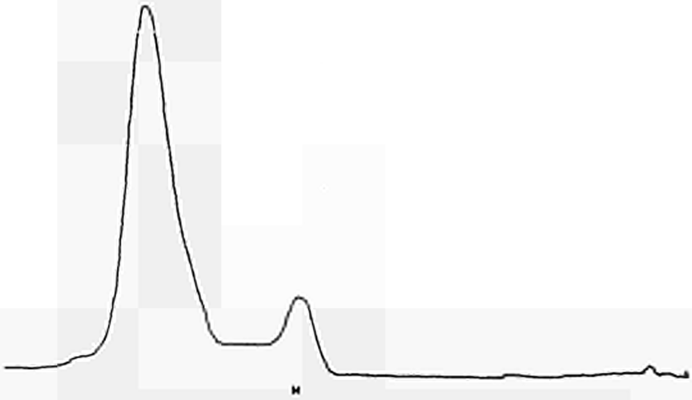


Fig. 1. Thin-layer gel chromatography of beef sarcoplasmic proteins on Sephadex G-75. M-myoglobin.

The best resolution was achieved with the G-200 gel. Sarcoplasmic proteins of beef and pork could be resolved into five fractions termed F1-F4 according to increasing R_M value (Fig. 3). In irradiated meat an additional fraction F5 appeared which migrated ahead of the most rapid fraction F4 of the untreated meat (Fig. 3B and 3C). This radiation-induced fraction with an R_M value of ~ 2.4 could be detected both in samples irradiated with 1 and 5 Mrad the percentage content of the fraction increasing with the dose. In Tables I and II the R_M values for the sarcoplasmic protein fractions of beef and pork are presented for the untreated meat and for meat irradiated with 1 and 5 Mrad. The

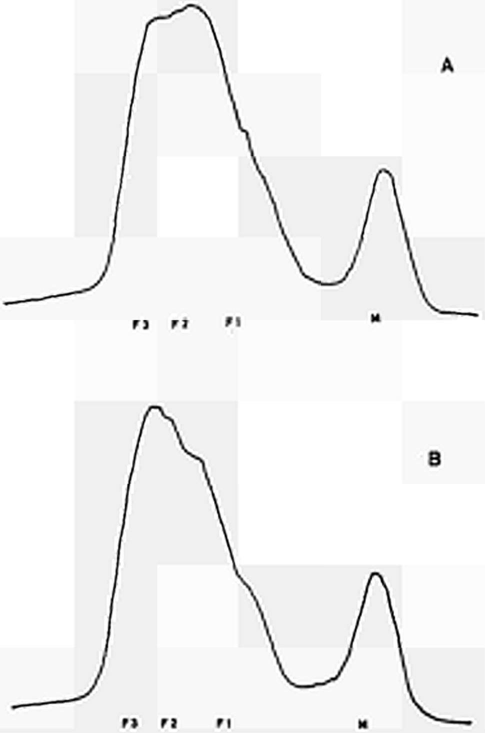


Fig. 2. Thin-layer gel chromatography of beef sarcoplasmic proteins on Sephadex G-100. M-myoglobin, F1 - F3-fractions with increasing R_M value:
A — untreated meat.
B — meat irradiated with 5 Mrad.

molecular weights have been calculated for the fractions of unirradiated meat according to the following equation (12):

$$\log M = 1.172\, R_M + 3.015$$

Only minor differences were noted in the R_M values of the fractions in irradiated meat indicating that there are no changes in the size properties of fractions F1-F4. The R_M value of the radiation-induced fraction corresponds to the exclusion limit of the G-200 gel. Due to this high R_M value the radiation-induced fraction could be easily identified in the pattern of sarcoplasmic proteins. Beef and pork sarcoplasmic proteins showed very similar patterns with only quantitative variations in the content of some fractions. The results presented in Tables I and II demonstrate that there are also only small differences in the R_M values.

In storage experiments meat irradiated with a dose of 5 Mrad was kept at room temperature for periods up to 10 weeks. On thin-layer gel

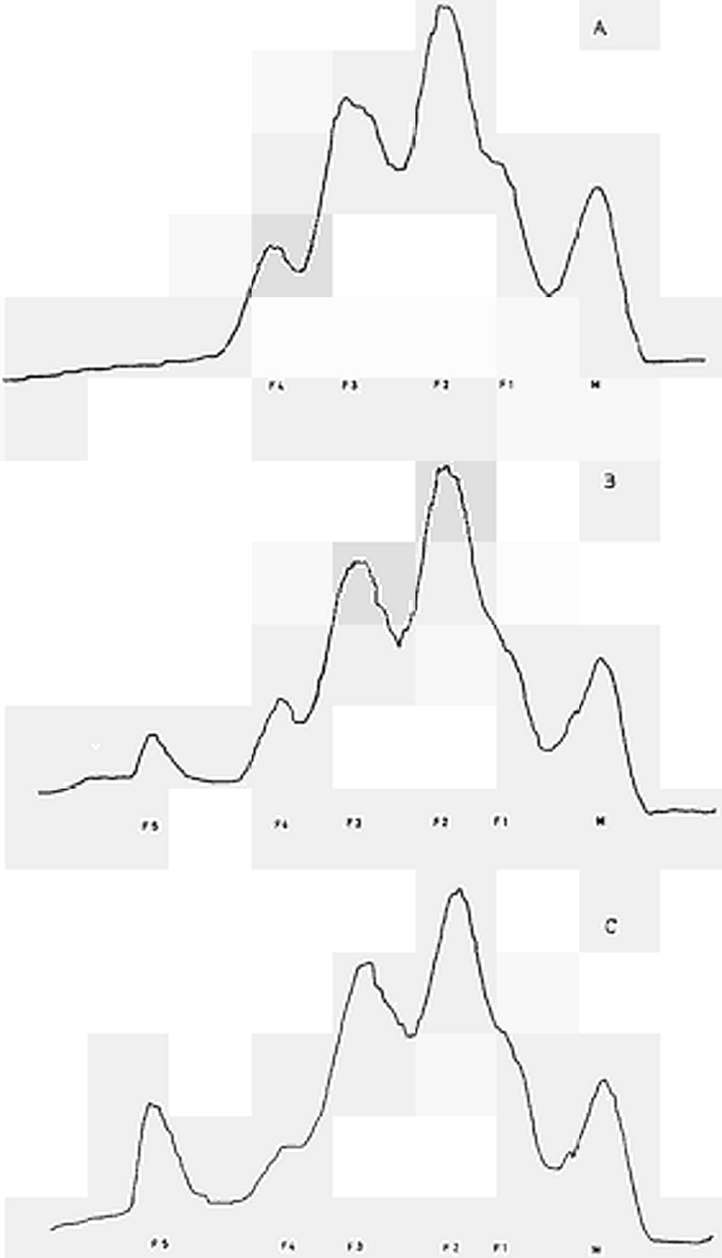


Fig. 3. Thin-layer gel chromatography of beef sarcoplasmic proteins on Sephadex G-200. M — myoglobin, F1 - F5 — fractions with increasing R_M values:

A — untreated meat.
B — meat irradiated at O°C with 1 Mrad.
C — meat irradiated at O°C with 5 Mrad.

Table I. — R_M-Values and molecular weights of beef sarcoplasmic proteins determined by thin-layer gel chromatography on Sephadex G-200.

			Irradiation at 0 °C		Irradiation at — 30 °C	
Fraction	Unirradiated	Molecular weight *	1 Mrad	5 Mrad	1 Mrad	5 Mrad
F 1	1.36	41000	1.36	1.37	1.36	1.39
F 2	1.55	68000	1.53	1.55	1.53	1.55
F 3	1.87	157000	1.86	1.87	1.86	1.84
F 4	2.15	340000	2.09	2.12	2.09	2.09
F 5	—		2.45	2.40	2.45	2.30

* Calculated for the R_M values of unirradiated meat. The R_M values were obtained from 6-12 determinations.

Table II. — R_M-Values and molecular weights of pork sarcoplasmic proteins determined by thin-layer gel chromatography on G-200.

			Irradiation at 0 °C		Irradiation at — 30 °C	
Fraction	Unirradiated	Molecular weight *	1 Mrad	5 Mrad	1 Mrad	5 Mrad
F 1	1.34	39000	1.35	1.36	1.36	1.39
F 2	1.52	63000	1.53	1.54	1.55	1.57
F 3	1.82	143000	1.81	1.84	1.85	1.87
F 4	2.09	300000	2.07	2.10	2.09	2.11
F 5	—		2.45	2.39	—	2.41

* Calculated for the R_M values of unirradiated meat. The R_M values were obtained from 6-12 determinations.

chromatography sarcoplasmic proteins isolated from the stored meat still contained the radiation-induced fraction thus proving the stability of this fraction. In these experiments meat samples preserved at —30 °C for comparable periods were used as a control. No fraction with the R_M ~2.4 value was observed in the frozen meat after prolonged storage. In additional experiments meat was subjected to repeated freezing (—30 °C) and thawing cycles, a treatment considered to be more effective for the induction of aggregates than simple storage for prolonged periods at low temperatures. Following this treating only traces if any of the R_M ~2.4 fraction could be detected in the sarcoplasmic extract.

These results indicate that aggregation of sarcoplasmic proteins appears to be specific for irradiated meat, an important result for distinguishing irradiated meat from that preserved in frozen state.

Distinct differences in the content of this radiation-induced fraction were observed in the 1 and 5 Mrad samples. These differences could provide a basis for dosimetry. However, results obtained on irradiation at low temperature as well as dose rate experiments indicate that this dosimetry should be interpreted with caution. It is well established that irradiation of protein-rich and even of some protein-poor food products at room temperature will induce undesirable organoleptic changes. These changes may be suppressed by irradiation at a sufficiently low temperature. Thus meat irradiated at —70 to —30 °C yielded an organoleptically acceptable product. Irradiation at —30 °C is now considered as adequate. When beef and pork were irradiated at —30 °C and subsequently the soluble proteins analyzed by thin-layer gel chromatography on Sephadex G-200 a fraction with R_M ~2.4 was observed in both samples. The amount of this fraction was consistently found to be reduced when irradiation was carried out at —30 °C (Fig. 4). These results probably reflect differences in the physico-chemical state of the proteins in meat, some of the soluble proteins being not available for radiation-induced aggregation in the frozen state. The temperature of irradiation thus appears to be an important factor in identification: at lower doses (1 Mrad) and at lower temperatures (—30 °C) the radiation induced aggregation was strongly reduced and the irradiated samples could not be easily recognized from the untreated sample.

Dose rate was found to be another important factor. Irradiation of beef and pork at two mean pulse dose rates 10^{10} and 10^{11} rad/sec both yielded the R_M ~ 2.4 fraction. The amount of this fraction was reduced at the lower dose rate. This is presented in Fig. 5. Dose rates of this order are rather high and exceed those of conventional cobalt sources by a factor of 10^4-10^5. These results demonstrate that by the dose alone the irradiated material is not sufficiently characterized. Additional work is needed to compare the results obtained on irradiation in conventional cobalt sources with those of other irradiation sources.

The experiments described above have consistently shown that on thin-layer gel chromatography of sarcoplasmic proteins from irradiated meat a fraction with an increased R_M value appears, which has been tentatively described as radiation-induced fraction. A number of experiments were performed in order to prove the hypothesis that this

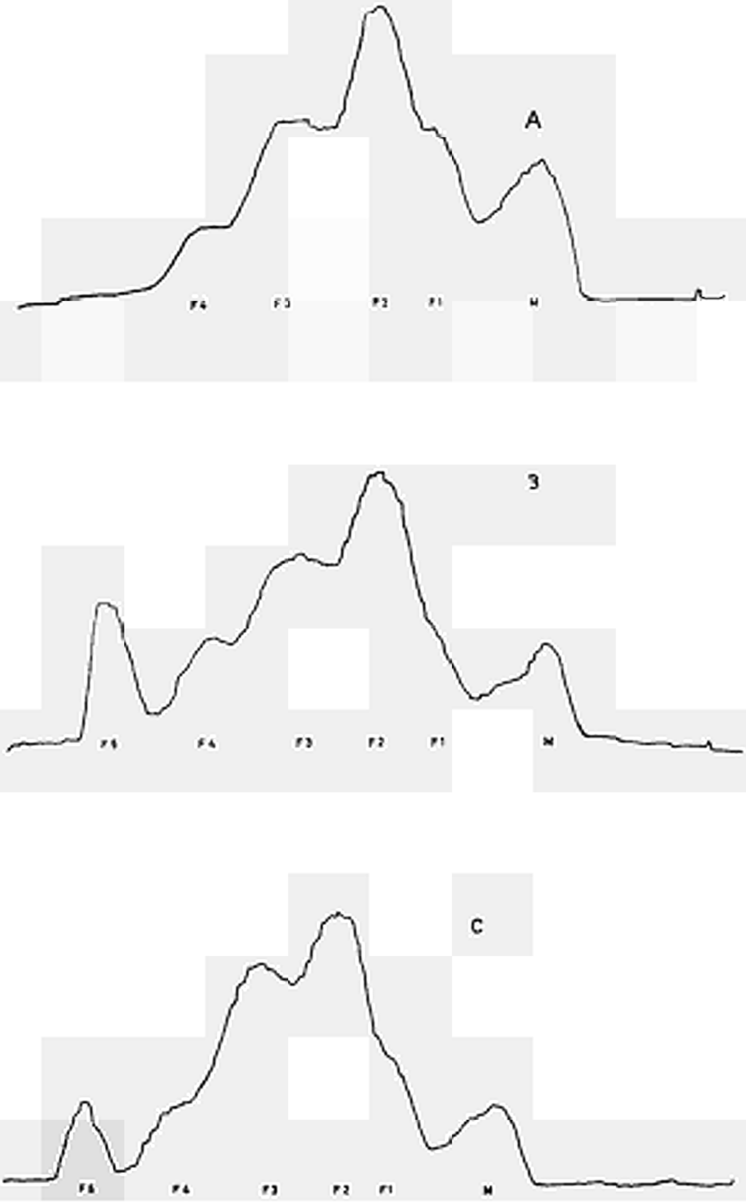


Fig. 4. Effect of irradiation temperature.
Thin-layer gel chromatography of pork sarcoplasmic proteins on Sephadex G-200.
M — myoglobin, F1 - F4 — fractions with increasing R_M values:
A — untreated meat.
B — meat irradiated with 5 Mrad at 0°C.
C — meat irradiated with 5 Mrad at —30°C.

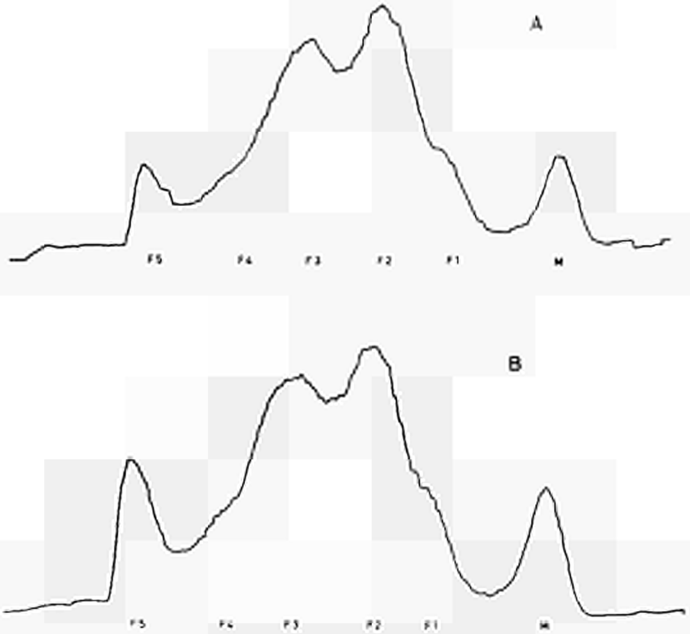
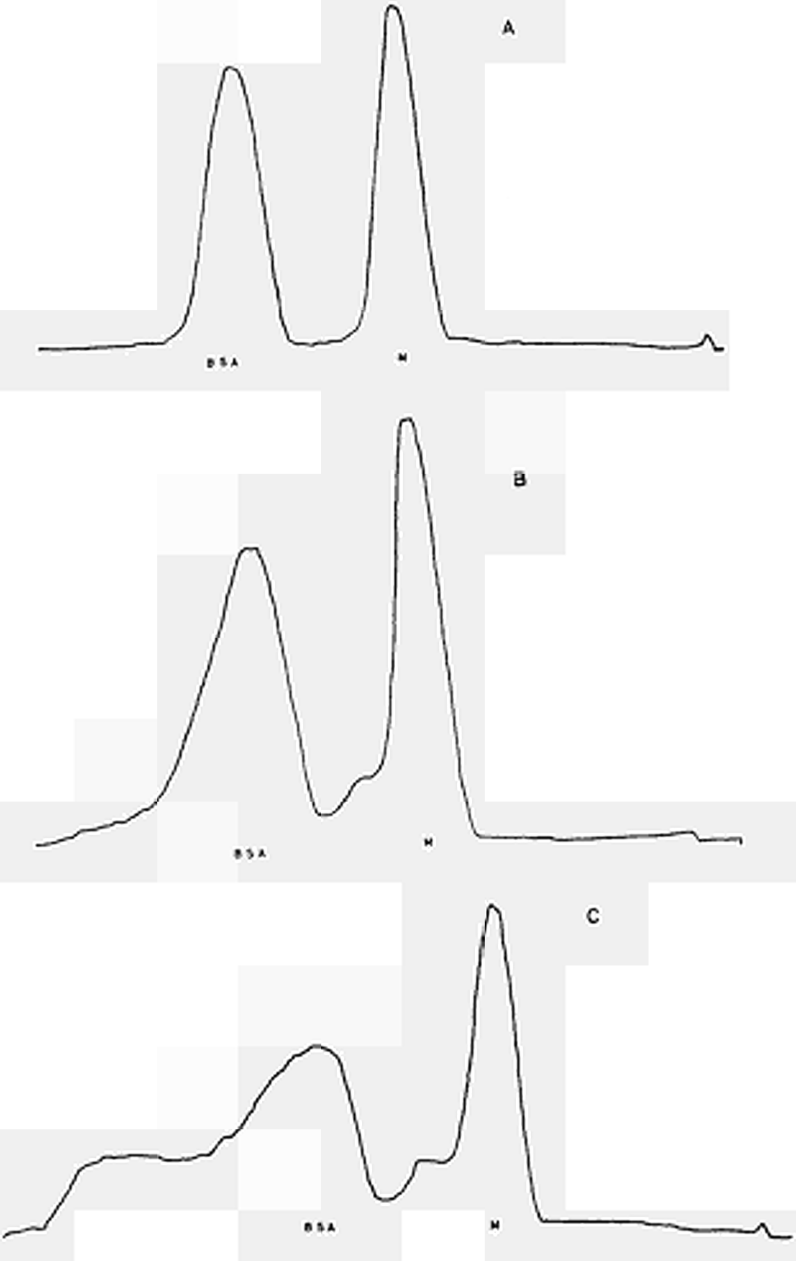


Fig. 5. Effect of dose rate.

Thin-layer gel chromatography of beef sarcoplasmic proteins on Sephadex G-200. Irradiation at 0 °C with 5 Mrad in a linear accelerator with a mean pulse dose rate:
A — 10^{10} rad/sec.
B — 10^{11} rad/sec.

fraction results from aggregation of sarcoplasmic proteins. In Fig. 6 thin-layer gel chromatography patterns are presented for a mixture of 1 % myoglobin and 1 % serum albumin irradiated in phosphate buffer with increasing doses in a cobalt source. The results indicate that with increasing dose the amount of aggregates increases. For the lowest dose (0.5 Mrad) a small amount of dimerized myoglobin and a strong asymmetry of the albumin peak were observed (Fig. 6B). For the highest dose (2 Mrad) almost all the proteins appear as a fraction with an R_M value of $\sim$ 2.4 with only small amounts of residual myoglobin and traces of albumin (Fig. 6D).

The radiation induced aggregation depended on the concentration of the protein mixture. In Fig. 7 patterns are shown of myoglobin and albumin mixtures containing 2, 1, and 0.5 % of each protein irradiated with a constant dose of 1 Mrad. The patterns clearly demonstrate that while at the highest protein concentration only a small fraction of the proteins is aggregated (Fig. 7A), with the lowest protein concentration almost all the protein has aggregated (Fig. 7C). The aggregated



material migrated with an R_M value of $\sim$ 2.4. These findings are important for the interpretation of results obtained on irradiation of food products in which often relatively high protein concentrations will be present.

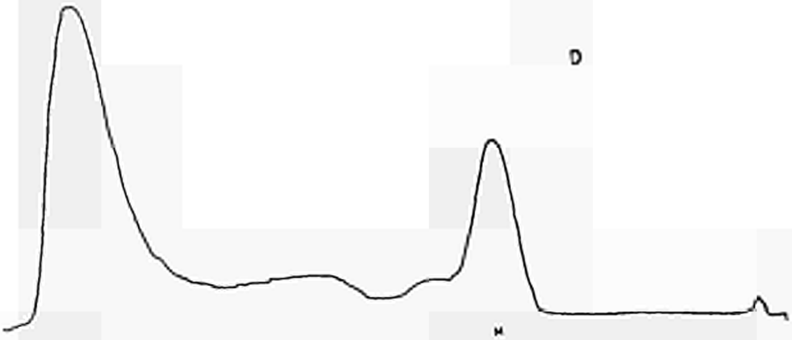


Fig. 6. Radiation-induced aggregation in a model system of two proteins. Thin-layer gel chromatography on Sephadex G-200 of a mixture of 1 % bovine serum albumin (BSA) and sperm whale myoglobin (M) irradiated in a cobalt source in 0.02 M phosphate buffer.

A — unirradiated.
B — irradiated with O.5 Mrad.
C — irradiated with 1 Mrad.
D — irradiated with 2 Mrad.

In addition to these experiments with a model system of two proteins, further experiments were performed with isolated beef sarcoplasmic proteins. The sarcoplasmic proteins were obtained by extraction with 0.1 M phosphate buffer and dialyzed 48 hr against a 0.01 M phosphate buffer of the same pH. The dialyzed solution was concentrated by ultrafiltration on UM-10 Diaflo membranes to an $E_{280}^{1\ \mathrm{cm}} = 50$ value. The protein solution was irradiated in sealed ampoules at 0 °C in a cobalt source (Fig. 8). Already at the lowest dose, namely 0.5 Mrad, a new fraction appears with an R_M value identical with that of the radiation — induced fraction in irradiated meat (Fig. 8B). At the highest dose —2 Mrad the radiation-induced fraction was the dominating fraction of the pattern and only small residual amounts of fractions F2 and M were observed (Fig. 8D). These results obtained on irradiation of sarcoplasmic proteins provide strong support for the assumption that the radiation-induced fraction observed in extracts of irradiated meat results from aggregation of sarcoplasmic proteins.

In a series of experiments optimal conditions for the extraction of the radiation-induced fraction have been investigated. Two phosphate buffers pH 7.2-7.4 differing in molarity — 0.02 and 0.1 M were compared. In addition, a 0.1 M phosphate buffer pH 7.2-7.4 containing 2 % glycerol was also tried. Only extracts prepared with 0.1 M phosphate buffer contained the radiation-induced fraction, thus indicating that solubility may be a critical factor for extraction. An attempt was made therefore to study in a more direct way the effect of irradiation

Total protein concentration:

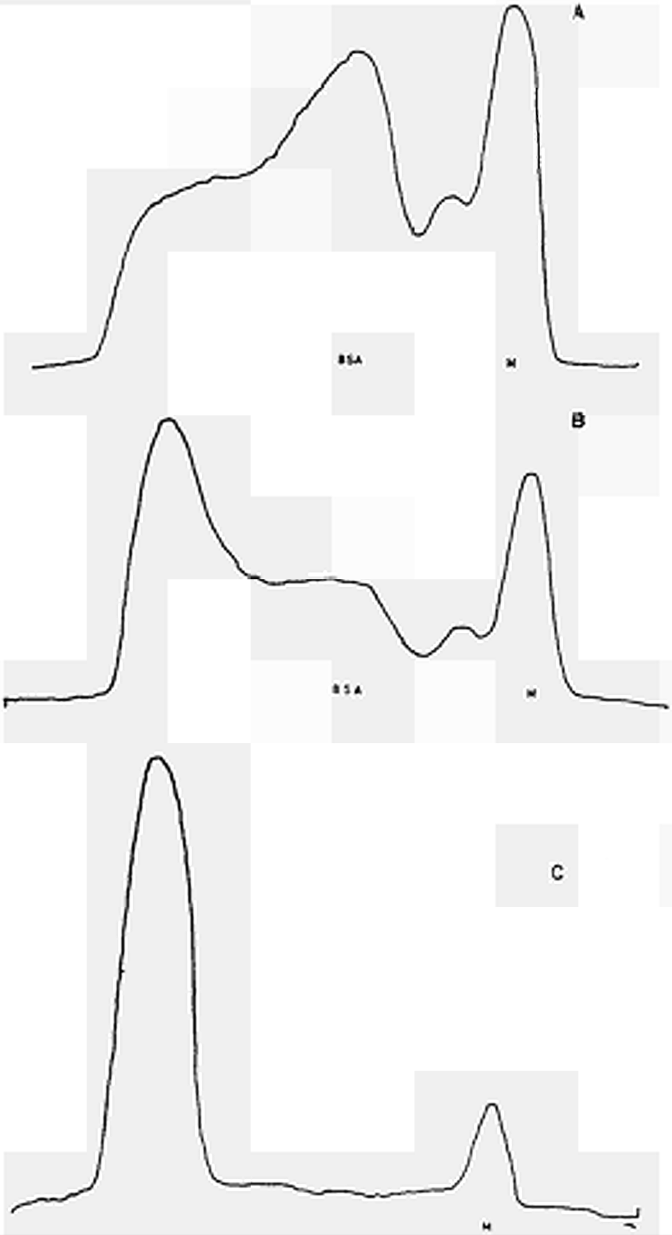


Fig. 7. Effect of protein concentration on radiation-induced aggregation in a model system of two proteins.
Thin-layer gel chromatography on Sephadex G-200 of mixture of bovine serum albumin (BSA) and sperm whale myoglobin (M) irradiated with 1 Mrad in 0.02 M phosphate buffer.
A — 2 % B — 1 %. C — 0.5 %.

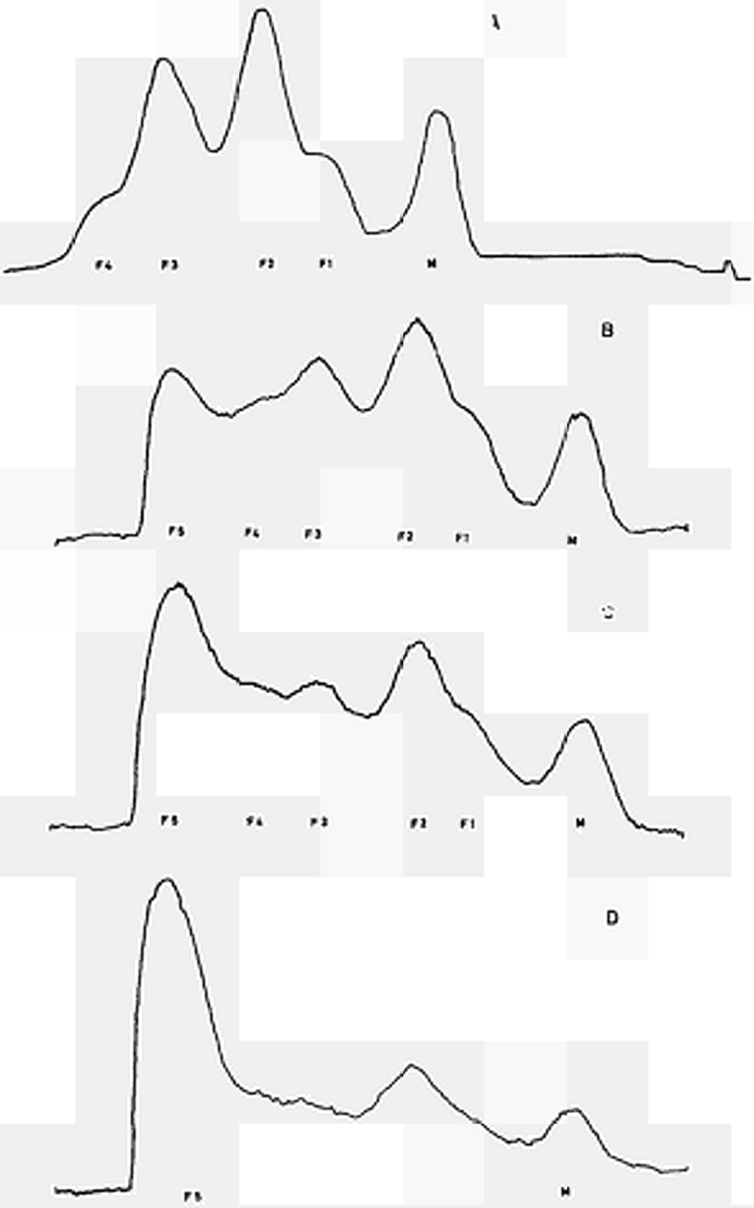


Fig. 8. Radiation-induced aggregation of beef sarcoplasmic proteins irradiated in 0.02 M phosphate buffer, pH 7.2.

Thin-layer gel chromatography on Sephadex G-200.

M-myoglobin, F1 - F4-fractions with increasing R_M value, F5 - radiation-induced fraction.

A — unirradiated sacroplasmic proteins.
B — irradiated with 0.5 Mrad.
C — irradiated with 1 Mrad.
D — irradiated with 2 Mrad.

on the solubility of proteins. A method based on stepwise extraction of the proteins precipitated by saturated ammonium sulphate by a series of ammonium sulphate solutions of decreasing molarity was used for this purpose.

In experiments with four extraction steps most of the proteins were extracted with the 1 and 2 M ammonium sulphate solution, small amounts of material appeared in the 3 M and in the water extract. The 4 M extract contained no proteins, its high absorbancy being probably due to low molecular substances which on the basis of the high A_{260}/A_{280} ratio appear to be nucleic acid derivatives. In the pattern of extracted proteins striking differences were noted between the untreated and the irradiated samples (Figs. 9 and 10). Whereas qualitatively the patterns were very similar for all samples, there were marked quantitative differences in the content of all protein fractions. The greatest difference was found with the 5 Mrad sample (Fig. 9). The percentage content of the 2 M peak was reduced to 40-50 % of the corresponding peak of the untreated sample. Similarly the 1 M peak is reduced to about 50 % of the unirradiated control. Also for samples irradiated with the lower dose (1 Mrad) a distinct decrease of the 1 and 2 M peaks was observed. A small amount of material could always be extracted with the 3 M solution. Determination of the A_{410}/A_{280} ratio indicated that this fraction contains myoglobin. There was a great variation in the A_{280}/A_{260} ratio for different peaks and for different fractions within a single peak indicating differences in the protein composition of these fractions. With the 5 Mrad sample the A_{280}/A_{260} ratio was consistently lower for all the fractions of the 1 M and 2 M peak than for the corresponding fractions of the untreated meat.

In an improved extraction procedure using seven instead of only four extraction steps basically the same results were obtained as described previously (Fig. 10). A strong decrease in solubility was observed on irradiation. The decrease of solubility was most evident with solutions of 2, 1.5 and 1 M molarity. In addition to changes of the absorbancy ratio we have analyzed the fractions obtained on gradient salt extraction by thin-layer gel chromatography. For this purpose the proteins were precipitated from the extracts by raising the concentration of ammonium sulphate. The protein precipitate was centrifuged off, dissolved in buffer, dialyzed and concentrated to the desired protein concentration. Thin-layer gel chromatography of the 2 M extract revealed little difference between the untreated sample and the 5 Mrad sample. Marked differences were however noted in the 1 and 1.5 M extracts.

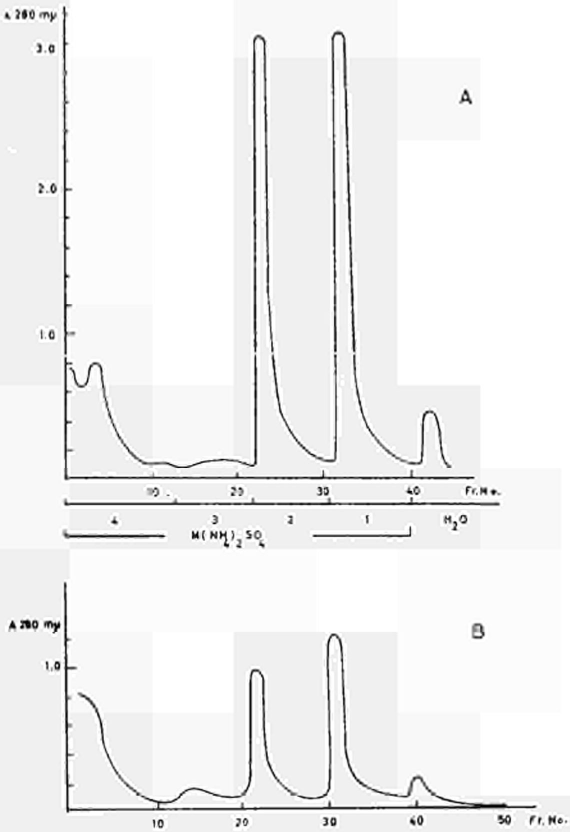


Fig. 9. Gradient salt extraction with ammonium sulphate of beef (4 extraction steps).
A — unirradiated.
B — irradiated with 5 Mrad.

There was a distinct shift to components with higher molecular weights in the irradiated samples. These results could be explained by assuming radiation-induced aggregation.

Most of the techniques used for identification of irradiated food on the basis of changes in proteins require that the protein be obtained in soluble form (thin-layer gel chromatography, electrophoresis and immunoelectrophoresis, ion exchange chromatography). With meat and fish only 20-40 % of the total protein, the sarcoplasmic proteins can be extracted with solutions of low ionic strength (16). The myofibrillar proteins, which amount up to 40-75 % of whole muscle proteins, can be extracted only with high ionic strength salt solutions. Radiation-induced changes of these proteins could not be studied by the above mentioned methods.

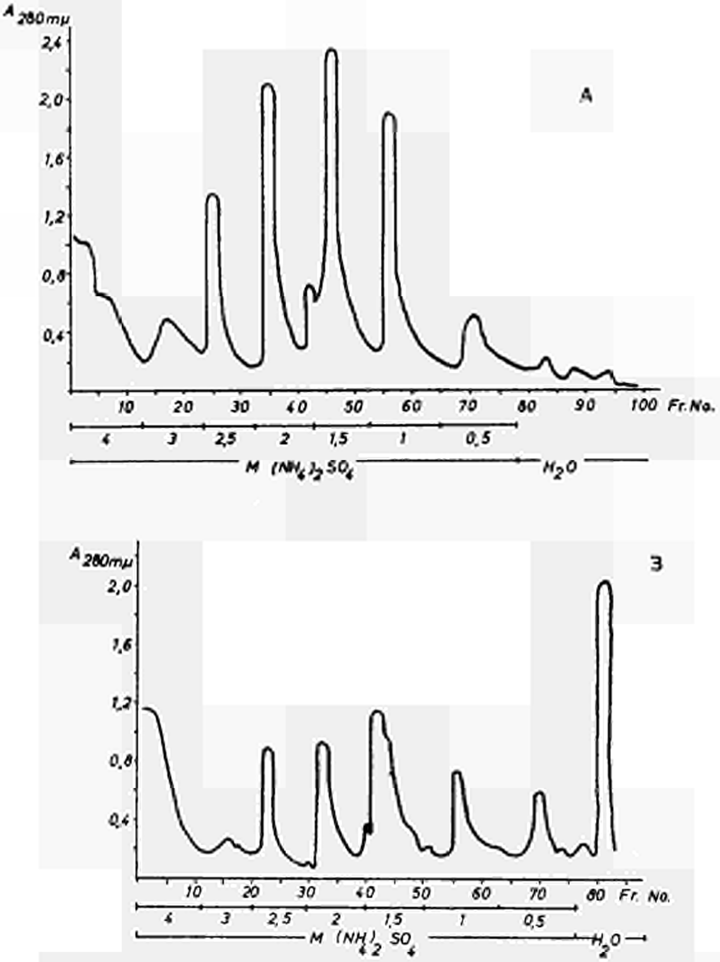


Fig. 10. Gradient salt extraction with ammonium sulphate of beef (7 extraction steps).
A — unirradiated.
B — irradiated with 5 Mrad.

Salt extraction of an ammonium sulphate precipitate of all proteins may be expected to disclose differences both in the sarcoplasmic (myogen) and myofibrillar proteins (myosin). The results obtaines indicate that on irradiation a considerable fraction of the muscle proteins is insolubilized. This insolubilization may be caused either by interaction of these proteins with the insoluble stroma proteins. Radiation-induced changes in proteins in food products may differ considerably from effects observed on irradiation of protein solutions due to different physico-chemical conditions.

While changes of solubility of proteins could provide a promising approach to identification of irradiated meat the column extraction technique appears to be not enough attractive for this purpose since it is too laborious and not suitable for routine analysis. Therefore, most of our work was carried out by thin-layer gel chromatography, which in addition to the relative simplicity affords the advantage of simultaneous analysis on a single plate of the irradiated material and the control.

SUMMARY

In beef and pork irradiated with a dose of 1 and 5 Mrad a new radiation-induced, sarcoplasmic protein fraction was observed on thin-layer gel chromatography on Sephadex G-200. Due to its high R_M value ($\sim$ 2.4) the radiation-induced fraction migrating ahead of all other sarcoplasmic protein fractions (R_M 1 — 2.1) of untreated meat could be readily identified in the chromatographic pattern. Irradiation experiments with isolated sarcoplasmic proteins strongly suggest that the radiation-induced fraction results from aggregation of these proteins.

The amount of the radation-induced fraction increased with increasing dose. At —30°C the amount of this fraction was smaller than at 0°C. Dose rate was found to be another factor affecting the formation of the radiation-induced fraction.

Storage of meat irradiated with 5 Mrad at room temperature for periods up to 10 weeks has shown that the radiation-induced fraction is stable and is still present in the sarcoplasmic extract. Meat preserved at —30°C for comparable periods or subjected repeatedly to freezing and thawing cycles did not contain the R_M $\sim$ 2.4 fraction. These results prove that the radiation-induced fraction is specific for irradiation.

Solubility studies based on stepwise extraction of a protein precipitate absorbed on an inert carrier with a series of ammonium sulphate solutions of decreasing molarity have shown that the fractions extracted with 1, 1.5 and 2 M ammonium sulphate are strongly reduced in irradiated meat. Solubility studies appear to be less suitable for routine analysis than thin-layer gel chromatography.

The demonstration of a radiation-induced, sarcoplasmic protein fraction can serve as a basis for identification of meat irradiated with a dose of 5 Mrad. At the lower dose of 1 Mrad identification depended

on irradiation conditions. The detection of the radiation-induced fraction by thin-layer gel chromatography is relatively simple and suitable for routine analysis. Several samples of irradiated meat can be run simultaneously on a single plate and compared with the untreated control. The radiation-induced fraction appears to be specific for irradiation.

REFERENCES

1) W.R. CARRO, E.R. MITCHELL and M.J. CALLANEN, Arch. Biochem. Biophys., **39** (1952) 232.
2) D. ROSEN, P. ALEXANDER and S. BROHULT, Arch. Biochem. Biophys., **70** (1957) 266.
3) R. SHAPIRA, Int. J. Radiat. Biol., **7** (1963) 537.
4) H. JUNG and H. SCHÜSSLER, Z. Naturforsch., **21b** (1966) 224.
5) J.S. HASKILL and J.W. HUNT, Radiat. Res., **31** (1967) 327.
6) C.O. STEVENS, J.L. LONG and D. UPJOHN, Proc. Soc. Exptl. Biol. Med., **132** (1969) 951.
7) F. FRIEDBERG, Radiat. Res., **38** (1969) 34.
8) J.A. WINSTEAD and T.C. REECE, Radiat. Res., **41** (1970) 125.
9) B.J. RADOLA, Biochim. Biophys. Acta, **160** (1968) 469.
10) B. JOHANSSON and L. RYMO, Acta Chem. Scand., **16** (1962) 2067.
11) C.J.O.R. MORRIS, J. Chromatog., **16** (1964) 167.
12) B.J. RADOLA, J. Chromatog., **38** (1968) 61.
13) B.J. RADOLA, J. Chromatog., **38** (1968) 78.
14) R.K. ZAHN and L. STAHL, Hoppe-Seylers Z. physiol. Chem., **293** (1955) 1.
15) C.A. ZITTLE and E.S. DELLA MONICA, Arch. Biochem. Biophys., **58** (1955) 31.
16) R. CZOK and Th. BÜCHER, Adv. Protein Chem., **15** (1960) 315.

DISCUSSION

A. GUILBOT

I should be interested to know to what extent this method may be applied when meat has undergone a thermic treatment, for example when it has been heated or cooked.

B. RADOLA

On heat treatment the results will be quite different because most of the proteins will be denatured. Inactivation of enzymes can be achieved by a combined heat and radiation treatment of meat. This technology should be considered in future work but we have not yet done the pertinent experiments.

A. DESCHREIDER

Dr. Radola has observed that frozen meat that has not been irradiated does not display the F5 peak, but in certain cases frozen meat could be presented to the consumer in the form of a non-frozen meat. There is a thawing technique which applies microwaves to items that have been frozen. Could not the microwave thawing treatment cause the F5 component to appear?

B. RADOLA

We have kept meat for prolonged periods at low temperatures. We have also performed experiments in which meat has been subjected up to 20 freezing and thawing cycles—without production of noticeable amounts of aggregates. Thus low temperature alone does not induce aggregates of the sarcoplasmic proteins. We don't know whether treatment with microwaves employed for defreezing of meat produces aggregates. This should be checked.

CHAIRMAN

In the discussion two or three topics are coming clearly to the fore. In technology, there is an ever-increasing tendency towards irradiation in combination with other processes such as freezing, drying, etc. It seems inevitable that, in order to continue our investigations, we shall be forced, to begin with, to follow in the direction in which technology is leading. We shall have to familiarize ourselves with this development; just as in the case of potatoes the choice is between irradiation and chemical treatment, so this will also have to be settled in the case of meat.

J. MORRE

Some years ago I showed how to isolate the thiobarbituric acid/ malonaldehyde colourant from irradiated meat. Meat left at the ordinary temperature of 20° C gives you a marvellous positive reaction after ten days or so. What Dr. Scherz succeeds in doing is to isolate the part that will react with malonaldehyde and then another part that will again give malonaldehyde later on.

H. SCHERZ

This is possible only with carbohydrates. With irradiated meat the situation is much more difficult because of the presence of proteins. Although malondialdehyde is formed when amino acids are irradiated, it is formed in very small quantities. And another difficulty is this: In meat we also have some fat and you know that malondialdehyde is formed from fat as a result of oxidative processes, regardless of whether the fat is irradiated or not. So one finds malondialdehyde in meat that has been stored for some time. The appearance of malondialdehyde in meat can therefore not be a proof of irradiation. For this reason I think the formation of aggregates reported by Dr. Radola is a much more specific reaction.

L. BUGYAKI

The technology of meat irradiation performed an important evolution and the period is far away when meat was irradiated simply at room temperature. As joined treatment, heat (71° C) was used to inhibit undesirable organoleptic changes resulting from the enzyme activity after the irradiation (Modification in texture and water holding capacity of the meat). Unfortunately this heat-treatment joined to irradiation is not able to avoid all the undesirable effects of the irradiation, e.g. taste and odour changes of the irradiated meat. Further researches have proved that these undesirable changes can be avoided if the irradiation is realized on the frozen meat. Therefore, the industry introduces the pretreatment with heat followed by irradiation under 0° C.

B. RADOLA

At the present moment the combined heat and radiation treatment appears to provide the best technological approach. Therefore, the effect of temperature alone and of the combined treatment should be studied in future experiments.

CHAIRMAN

That is correct. It could possibly be that the changes caused by heat treatment are so much greater that proteins disintegrate, so that the sensitive technique that you have developed is in fact no longer usable.

B. RADOLA

In experiments with model protein systems (we have studied the mixture of bovine serum albumin and sperm whale myoglobin in some detail) we could not detect on heat treatment soluble aggregates but only insoluble coagulates. We aim to develop a specific method and it is therefore important to prove that the soluble aggregates observed in irradiated meat are not produced by any other treatment.

L. BUGYAKI

On the basis of our researches with electrophoresis, we are able to affirm that the distinction between the fresh and irradiated or heat treated meat is possible. We have observed in our experiments that the majority of the proteins, coagulated by the heat, lacks the electrophoresis patterns. Unfortunately the identification is more difficult if we intend to distinguish the meat irradiated at low temperature or that preserved by freezing, because the electrophoretic patterns of these meats are very similar to that of fresh meat.

A. DESCHREIDER

I should like to ask Dr. Radola, who according to this report used beef for his studies, whether other meats display the same phenomenon to as great an extent or to a lesser extent than the observed for beef.

B. RADOLA

We have studied both beef and pork. The results are basically the same. In our report in Fig. 4 the effect of temperature is shown for pork. The chromatographic pattern is very similar for pork and beef (Fig. 3 and Fig. 4 of the report).

CHAIRMAN

We can see that a highly definite method has been discovered, but that we need to bring technology into the matter again in order to see where we are to go from here. And perhaps one should also consider whether to irradiate first and then carry out temperature treatment (perhaps the aggregates have already been formed by then and can be detected thereafter), or whether temperature treatment is to be carried out first, with coagulation of the proteins, and irradiation afterwards, so that we can no longer obtain aggregate formation. Have you already studied this, or is it still a question to be answered?

B. RADOLA

That is still an unsolved question which should be studied.

CHAIRMAN

I can well imagine that, if a coagulation is obtained, aggregation no longer takes place.

B.J. RADOLA

I agree with you. If irradiation is applied after a heat treatment aggregates can be expected only for the residual soluble proteins which have not coagulated.

CHAIRMAN

I should then like to ask Dr. Bugyaki: in your opinion, does heat treatment come before irradiation or after?

L. BUGYAKI

At present, heating is carried out before irradiation; if it were done after irradiation, the result would perhaps be the same, but to my knowledge no studies have been carried out in this field. The character of the denaturation of the proteins during heating is certainly such that it would be difficult to render them soluble again, while it is precisely this that offers us the possibility of drawing a clear distinction.

4. GASCHROMATOGRAPHISCH-MASSENSPEKTROMETRISCHE UNTERSUCHUNG VON BESTRAHLTEN TIERISCHEN FETTEN

Progress report 1.7.1969 - 1.10.1970

F. DRAWERT, R. TRESSEL UND B. BECK

ABSTRACT

In animal lipids (pork, beef) irradiated with a dose of 6 Mrad about 60 hydrocarbons, ketons and alcohols as well as about 30 free fatty acids have been detected by gas chromatography and subsequently identified by mass-spectrometry. Most of these components do not occur at all or only in trace amounts in the unirradiated material. Isolation and identification of some of the compounds by the combination gas-chromatography and mass-spectrometry will provide a basis for identification of irradiated lipid-containing material by a simplified procedure.

Irradiated fruits can be identified by gas-chromatography of aroma substances.

1. EINLEITUNG

Im Rahmen des Forschungsprogramms werden mögliche Veränderungen der physikalisch-chemischen oder biologischen Beschaffenheit bestrahlter Lebensmittel untersucht.

Der Schwerpunkt der Untersuchungen liegt auf einem *gesicherten Nachweis der Bestrahlung.*

Absprachegemäß sind im Versuchszeitraum 1. Januar 1968 bis 31. März 1969 als *Orientierungsnahrungsmittel* Obst (Äpfel, Bananen, Erdbeeren) und als *Hauptnahrungsmittel* Fleisch und Trockenerpulver bestrahlt und unbestrahlt vergleichend untersucht worden.

Diese Ergebnisse sind in einem 1. und in einem 2. zusammenfassenden Bericht ausführlich dargestellt und erläutert worden.

Gemäß Protokoll der Sitzung vom 26./27. Juni 1969 in Luxemburg (A-8 Seite 4) sollten die gaschromatographischen Untersuchungen bei bestrahlten Früchten nicht weitergeführt werden. Statt dessen wurde geplant, die gaschromatographischen Arbeiten auf die schon begonnenen Untersuchungen von *fettreichen Lebensmitteln* zu konzentrieren.

Demzufolge wurden im Untersuchungszeitraum zunächst verschiedene tierische Fette mit hohen Dosen *bestrahlt* und vergleichsweise *unbestrahlt* untersucht, um vorab mit leistungsfähigen gaschromatographischen Trennsystemen und durch Kopplung mit der nachweisempfindlichen und strukturspezifischen Massenspektrometrie eine Bestandsaufnahme der Stoffe vorzunehmen, die vorwiegend nach Bestrahlung auftreten. Wie die Tabellen zeigen, entstehen durch Bestrahlung sehr zahlreiche Verbindungen. Die experimentellen Bemühungen konzentrierten sich zunächst auf deren Trennung und Identifizierung, so daß beim gegenwärtigen Stand der Arbeiten vorwiegend über diese Ergebnisse zu berichten ist. Dementsprechend wurde der 3. Bericht als *Zwischenbericht* ohne Literaturangaben abgefaßt.

2. MATERIAL UND METHODEN

Fette

Fettgewebe vom Schwein, Hammel und Rind wurde im Fleischwolf zerkleinert, durchgemischt, in 500-g-Portionen abgeteilt und in Kunststtofffolien versiegelt. Die Proben wurden anschließend im Elektronenbeschleuniger (Varian-Linearbeschleuniger) am Institut für Strahlentechnologie der Bundesforschungsanstalt für Lebensmittelfrischhaltung, Karlsruhe, mit 6 Mrad bestrahlt. Diese hohe Dosis ist gewählt worden, um entstehende Bestrahlungsprodukte in bestimm- und nachweisbaren Mengen zu erhalten. Später wird auf geringere Dosen übergegangen.

Jeweils 500 g (Probe) wurden nach der Bestrahlung in einem 2-1-Kolben mit 750 ml Wasser versetzt und der Wasserdampfdestillation unterworfen. Als Vorlagen dienten 4 Kühlfallen. Die ersten beiden Kühlfallen hatten ein Aufnahmevolumen von 100 ml. Sie wurden mit Eis gekühlt. Nachgeschaltet waren zwei Kühlfallen von etwa 8 ml Fassungsvermögen. Sie wurden mit Pentan gefüllt und waren so angeordnet, daß sie als Waschflaschen für die leicht flüchtigen Bestandteile dienten. Die Kühlung dieser Fallen erfolgte mit Trockeneis.

Das Wasserdampfdestillat wurde mit NaCl gesättigt und 8 Stdn. mit Pentan:Methylenchlorid 2 : 1 extrahiert. Der Extrakt ist mit dem Inhalt der Kühlfallen 3 und 4 vereinigt und in einem Spitzkölbchen auf 0,25 ml eingeengt worden.

Gaschromatographische Untersuchung von jeweils 1,5 μl. Für die weitere gaschromatographisch-massenspektrometrische Untersuchung sind 0,25 ml des Extraktes weiter auf etwa 0,05 ml eingeengt und hiervon 0,1 μl über einen mit einem Massenspektrometer gekoppelten Gaschromatographen analysiert worden. Als Blindprobe dienten 500 g unbestrahltes Fett, das unter den gleichen Bedingungen untersucht wurde.

Gesamtfettsäuren

Die nach Verseifen der Fette mit KOH erhaltenen Fettsäuren sind mit Diazomethan verestert und als Methylester gaschromatographisch untersucht worden.

Freie Fettsäuren

Bindung an einen stark basischen Ionenaustauscher, dadurch Trennung von anderen Substanzen und Elution mit ätherischer Ameisensäure. Gaschromatographische Bestimmung der Methylester.

Gaschromatographie

Geräte der Firmen Siemens und Varian. Die genauen Bedingungen werden im Abschlußbericht angegeben.

Massenspektrometrie

Gerät CH 7 der Fa. Varian MAT (Bremen) mit Helium-Separator nach Biemann-Watson.

3. ERGEBNISSE

Bei der gaschromatographisch-massenspektrometrischen Untersuchung der bestrahlten Proben (Schweine-, Hammel-, Rinderfett) wurden bislang ca. 60 *Kohlenwasserstoffe, Ketone* und *Alkohole* gefunden und über die Massenspektren weitgehend identifiziert. Tab. I enthält die bei bestrahltem Schweinefett erzielten Ergebnisse. Die Resultate bei Rinder- und Hammelfett sind vergleichbar. Die bei den bestrahlten Proben gefundenen Verbindungen sind in den *unbestrahlten Vergleichsproben nicht oder nur in kaum nachweisbaren Mengen vorhanden.*

Im bestrahlten Schweine-, Hammel- und Rinderfett konnten etwa 30 freie Fettsäuren weitgehend identifiziert werden. Tab. II zeigt die Ergebnisse für Hammelfett, Tab. III für Rinderfett.

4. ZUSAMMENFASSUNG UND SCHLUSSFOLGERUNGEN

In bestrahltem tierischen Fett wurden ca 60 *Kohlenwasserstoffe,* Ketone und Alkohole sowie etwa 30 *freie Fettsäuren* gaschromatographisch nachgewiesen und größtenteils massenspektrometrisch identifiziert. Ein großer Teil dieser Verbindungen tritt im unbestrahlten Fett *nicht* oder *nur in geringer Menge* auf.

Diese *außerordentlich aufwendigen* Untersuchungen waren erforderlich, um eine Bestandsaufnahme der Stoffe durchzuführen, die nach Bestrahlung von tierischen Fetten grundsätzlich auftreten. Weiterführende Arbeiten können bei entsprechender Finanzierung quantitative Beziehungen herstellen, um dann bei abgestuften Bestrahlungsdosen zu entscheiden, welche der gefundenen Verbindungen bevorzugt für den Nachweis der erfolgten Bestrahlung geeignet sind.

Tabelle I. — Nach Bestrahlung von Schweinefett gaschromatographisch-massenspektrometrisch gefundene Kohlenwasserstoffe Ketone u. Alkohole (Hauptkomponenten hervorgehoben).

Nr.	Verbindung	Massenzahlen	Nr.	Verbindung	Massenzahlen
1	**n-Heptan**	100	32	*	
2	**Hepten**	98	33	Okten-(1)-ol-(3)	128
3	n-Oktan	114	34	Heptanol-(1)	126
4	**Okten**	112	35	*	
5	**Okten**	112	36	n-Tetradecan	198
6	Okten	112	37	*	81 153 110
7	**n-Nonan**	128	38	Tetradecen	196
8	**Nonen**	126	39	Tetradecen	196
9	Nonen	126	40	*	
10	Nonen	126	41	*	
11	Nonen	126	42	*	
12	Toluol		43	Oktanol-(1)	130
13	*	81	44	**n-Pentadecan**	212
14	**n-Decan**	142	45	Pentadecen	210
15	**Decen**	140	46	*	91
16	Decadien	138	47	Pentadecen	210
17	*n-Undecan*	156	48	*	
18	*Methylamylketon*	114	49	*	
19	**Undecen**	154	50	*	85
20	*	81	51	*	
21	*		52	*	81
22	Pentanol-(1)	88	53	Hexadecen	224
23	*	81	54	*Hexadecadien*	222
24	*n-Dodecan*	170	55	*	
25	*Methylhexylketon*	128	56	*	85
26	**Dodecen**	168	57	n-Heptadecan	240
27	*		58	Heptadecen	238
28	Dodecadien	166	59	*	81
29	Hexanol-(1)	102	60	Heptadecen	238
30	*n-Tridecan*	156	61	Heptadecadien	236
31	Methylheptylketon	142	62	Heptadecadien	236

* noch nicht sicher identifiziert.

Tabelle II. — Gaschromatographisch-massenspektrometrische Untersuchung der freien Fettsäuren in bestrahltem Hammelfett.

Säuren	Massenzahlen					
Buttersäure	74	87				102
Isovaleriansäure	74	87	43	57	85	116
Capronsäure	74	87				130
Önanthsäure	74	87				144
Caprylsäure	74	87				158
Pelargonsäure	74	87				172
Caprinsäure	74	87				186
Benzoesäure	74		77	105		136
Laurinsäure	74	87				214
Tridecansäure	74	87				228
Tetradecansäure	74	87	43			242
*	74	87	41	43		157
Myristinsäure	74	87				242
Tetradecansäure	74	87				
Pentadecansäure	74	87				256
Pentadecansäure	74	87				256
Pentadecansäure	74	87				256
Hexadecansäure	74	87				270
*	74	87				185
Palmitinsäure	74	87				270
Palmitoleinsäure	74	87				268
Heptadecansäure	74	87				
Heptadecansäure	74	87				
Heptadecansäure	74	87				
Heptadecensäure	74	87				
Stearinsäure	74	87				298
Stearinsäure	74	87				298
Ölsäure	74	87				296
*	74	87				312
Linolsäure	74	87				294
Linolsäure	74	87				294

* noch nicht sicher identifizierte Säuren.

Tabelle III. — Gaschromatographisch-massenspektrometrische Untersuchung der freien Fettsäuren in bestrahltem Rinderfett.

Säuren	Massenzahlen								
Buttersäure	74	87						102	
Isovaleriansäure	74	87	43	57	85			116	
Capronsäure	74	87						130	
Önanthsäure	74	87						144	
Caprylsäure	74	87						158	
Pelargonsäure	74	87						172	
Caprinsäure	74	87						186	
Benzoesäure	74		77	105				136	
Laurinsäure	74	87						214	
Tridecansäure	74	87						228	
Tetradecansäure	74	87						242	
*	74	87	28	32	41	43	55	59	69
		83	97	115	125	128		157	
Myristinsäure	74	87						242	
(Tetradecansäure)	74	87						242	
Tetradecensäure	74	87						240	
Pentadecansäure	74	87						256	
Pentadecansäure	74	87						256	
Pentadecansäure	74	87						256	
Hexadecansäure	74	87						270	
*	74	87	59	69	83	98		111	124
		125	144		132	186			
Palmitinsäure	74	87						270	
Palmitoleinsäure	74	87						268	
Heptadecansäure	74	87						284	
Heptadecansäure	74	87						284	
Heptadecansäure	74	87						284	
Heptadecensäure	74	87						282	
Stearinsäure	74	87						298	
Ölsäure	74	87						296	
Linolsäure	74	87						294	
Linolsäure	74	87						294	
Linolensäure	74	87						292	
*	74	87						326	

* noch nicht sicher identifizierte Säuren.

DISCUSSION

CHAIRMAN

With your studies on lipids you have opened up a new method. It could possibly constitute a feasible procedure for the monitoring of meat and fat irradiations. But it is not practicable to equip every meat inspection station with a mass spectrometer, that would be too expensive, so we must look for a further refinement for its practical implementation. You have, however, now demonstrated the fundamental possibility.

F. DRAWERT

These investigations should not be taken to mean that a mass spectrometer has to be used. It is rather that the mass spectrometer serves only for the identification of a compound, and with suitable precautions regarding the quantitative basis it is possible to settle on a small number of compounds directly with the aid of gas chromatography alone, and to analyse them quantitatively via the start of retention.

J.F. DIEHL

I think it is remarkable that Prof. Drawert and his collaborators have been able to find distinct effects or irradiation in the gaschromatograms of fats. On the basis of several American publications I would have expected that the changes resulting from irradiation of fats are quite unspecific and that gaschromatography is probably not suited for the identification of irradiated fats. This shows again that we must not be satisfied with the often heard opinion that all experiments in the field of food irradiation have already been carried out in the United States. Prof. Drawert's studies are still in an early phase. Particularly the effects of heating should be considered in future experiments; it may be that heating causes the appearance of the same compounds which have now been found in irradiated fats. But I do believe that a promising basis for another method of identifying irradiated foods has been laid.

CHAIRMAN

I am in complete agreement with you that the investigations should be directed towards practical implementation. Dr. Drawert worked with a rather high dose, viz. 6 Mrad. The Americans have possibly not taken their doses as high as this. It is therefore absolutely essential to be able to establish these effects again at low doses too. Have you not yet got as far as this, Dr. Drawert?

H. DRAWERT

We have already made preliminary studies at lower irradiation doses also, and found that essentially the same compounds occur, but in a different gradation. We are better able to find them now that we have seen them once.

L. SAINT-LÈBE

I am not well acquainted with the subject, in this field at least, but the results of the promising work carried out by Dr. Drawert provide me with proof that is supplementary to the line of investigation that we have decided to follow within the scope of our study on the irradiation of industrial starch. Everything that has been done, whether in the United States or elsewhere, should not be accepted word for word, and a certain number of approaches should be pursued, particularly in this field of the identification of products formed during the irradiation of starch. I very deeply regret not having known about this work in time for the meeting that we organized at Cadarache in June, which was attended, by Dr. Diehl. It would have been very useful in the discussions to have such strong evidence of the value, in the toxicological evaluation of irradiated food, of not confining oneself to experiments on animals.

J. SMEETS

Have you also made any experiments with irradiated mushrooms?

H. DRAWERT

No; I do not even know the composition of fungi. What fungi do have, at any rate, are a whole series of considerable activities from what are called oxido-reductase, polyphenoloxidase, catalase, and peroxidase. I am in fact almost certain that the use of irradiation can be detected in fungi, simply because the mushroom has such a high content. We ourselves previously isolated these enzymes from mushrooms, as working enzymes.

5. IDENTIFICATION OF IRRADIATED POTATOES BY LACK OF WOUND PERIDERM FORMATION

Progress report 1.10.1969 - 1.10.1970

H. PENNER

ABSTRACT

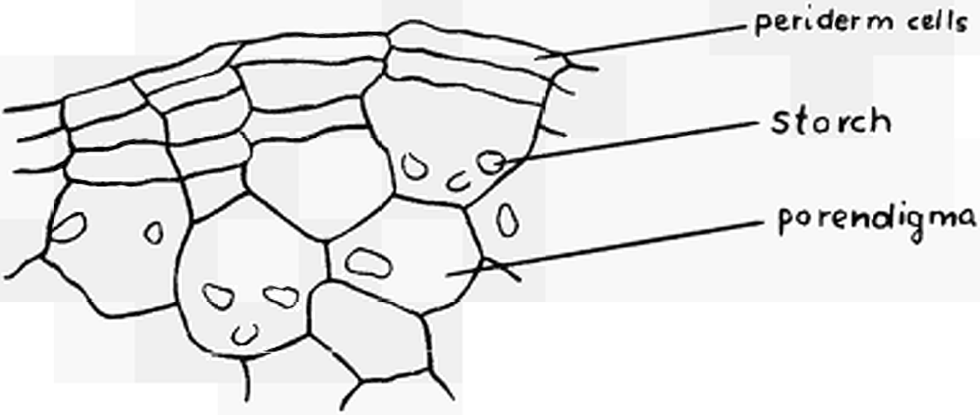
Irradiated potatoes can be identified histologically. After irradiation, the potato tissue loses its ability for wound periderm formation. Chemical treatment (CIPC-Chlorisopropylphenylcarbaminate) does not inhibit wound healing so that chemically-treated potatoes can be discerned from irradiated ones. The described wound-healing method being a negative one, it is necessary to exclude all wound-periderm inhibitivity factors other than irradiation. Above 10 krad, no estimation of the applied dose is possible.

INTRODUCTION

Due to the very small and unspecific chemical changes in potatoes after irradiation with sprout inhibiting doses it is difficult to identify those tubers by chemical methods. On the other hand doses of the order of 10 krad not only inhibit sprouting but also other mechanisms of cell division. It is possible to use the lack of wound periderm (WP) formation in irradiated potatoes as a histological method for identification of irradiation. In a damaged potato tuber the parenchyma cell layer beyond the destroyed cells is redifferentiated. This cell layer regains the ability of cell division and forms several layers of characteristic brick-shaped WP cells. If other parameters can be excluded, the absence of those cells under microscopic observation is an indication of irradiation.

METHODS

Potato tubers are cut into halves and stored for 5-8 days in an atmosphere of high air humidity and room temperature. Afterwards tissue samples are taken from the cut surface, embedded in paraffine, cut by microtome, and stained by gentiana-violet according to Jensen (Botanical Histochemistry, San Francisco 1962). Microscopic slides are prepared and viewed under the microscope.



RESULTS

1. Lack of WP formation in irradiated potatoes

Irradiated potatoes of the Datura and Bintje varieties (harvest October 1969) gave no wound periderm. In several cases very few periderm-like cells were found but no sample showed a continuous cell layer. Results are give in table I.

2. WP formation in untreated potatoes

The untreated varieties of Datura and Bintje showed 3-4 periderm cell layers. The potatoes were harvested in October 1969. It seems that after 10 months of storage the ability of WP formation is reduced. Results in table II.

3. WP formation in CIPC-treated potatoes

The treatment with CIPC (Chlorisopropylphenylcarbaminate) is commonly used for sprout inhibition in potatoes. It was found that CIPC did not inhibit WP formation in potatoes harvested and treated in October/November 1969. However, in September 1970 the WP-forming ability was greatly reduced. Results in table III.

DISCUSSION

In agreement with reports by other authors it was found that in irradiated potato tubers no WP is formed. CIPC-treatment does not inhibit the WP formation. By this way the sprout inhibition by CIPC-treatment can be discerned from that by irradiation. The described wound-healing-method being a negative one it is necessary to exclude all WP inhibiting factors other than irradiation. Very long storage can be such a factor. The influence of chemicals other than CIPC needs further investigation.

Table I. — Lack of WP formation in irradiated potato tubers.

Variety	Date of irradiation	Date of evaluation	Time allowed for wound healing	Number of samples	Number of periderm all layers
Datura	Nov. 69	March 70	8 days	20	0
Datura	March 70	March 70	8 days	20	0
Datura	Nov. 69	April 70	8 days	10	0
Datura	March 70	April 70	8 days	10	0
Bintje	Nov. 69	March 70	8 days	10	0
Bintje	Nov. 69	April 70	8 days	10	0

Table II. — WP formation in untreated potato tubers.

Variety	Date of evaluation	Time allowed for wound healing	Average number of periderm layers (10 samples)	Remarks
Datura	March 70	8 days	3.5	distinct
Datura	April 70	8 days	3.1	distinct
Bintje	March 70	8 days	3.5	distinct
Bintje	April 70	8 days	3.2	distinct
Bintje	August 70	8 days	3.9	distinct
Bintje	September 70	10 days	2.0	weak

Table III. — WP formation in CIPC-treated potato tubers.

Variety	Date of evaluation	Time allowed for wound healing	Average number of periderm layers (10 samples)	Remarks
Bintje	March 70	8 days	3.5	distinct
Bintje	April 70	8 days	3.2	distinct
Bintje	August 70	8 days	1.8	weak, but dist.
Bintje	September 70	10 days	0	not distinct
Bintje	September 70	10 days	0	not distinct
Maritta	September 70	10 days	0	not distinct
Sieglinde	September 70	10 days	0	not distinct

FURTHER INVESTIGATION

1. The method should be improved in order to simplify and standardize it.

2. It is necessary to determine the length of storage during which the method can be applied.

3. It should be verified that no chemical treatment for sprout inhibition inhibits WP formation.

4. It should be studied whether the method can be applied to other vegetables like onions or carrots.

SUMMARY

A method is described by which irradiated potatoes can be identified histologically. After irradiation the tissue of potatoes loses its ability for wound periderm formation. CIPC-treatment does not inhibit wound healing so that CIPC treated potatoes can be discerned from irradiated ones.

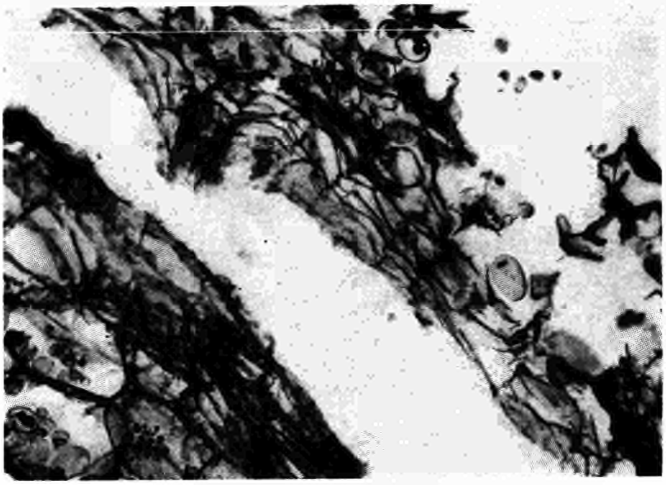


Fig. 1. Wound periderm formation in non-irradiated potatoes after 9 days. 512 X.

Fig. 2. Lack of wound periderm formation in irradiated potatoes after 9 days. 512 X.

DISCUSSION

A. GUILBOT

I do not bear in mind the details of the work made by Pr. Sandret about the detection of irradiated potatoes, by examining whether they are able to sprout or not, but I know he could make the difference between the potato which CIPC stops from sprouting and the irradiated potato. Besides he is just now investigating a method using tissue cultures, which seems to be also full of promise. But unfortunately the experiment is long. Of course 5 days is a short time compared to the storing perior of potatoes; but when it is necessary to know, for having them cross the frontiers, whether potatoes have been irradiated or not, it is a long time.

CHAIRMAN

Do you wish to comment on that, Dr. Penner?

H. PENNER

At present about 5 days are needed for this method, but is seems to be possible to shorten it. Considering the long storage period of potatoes as well as the lack of a reliable alternative method, the application of the wound healing method seems to be useful.

A. GUILBOT

Compared with the storage like of potatoes, a period of five days is not, of course, a long time; but if it is necessary to detect at international frontiers whether or not potatoes have been irradiated, it is a long time.

F. DRAWERT

The mentioned wound healing process is connected with various enzymatic processes. When plants are damaged, enzymes catalyzing oxidation reactions, for instance, may be increased severalfold. These are generally speaking the oxydoreductases. As these enzymes are involved in the plant's response to wounding, it should be possible to find changes in their activity within a short time after the wound is inflicted.

H. PENNER

The mentioned mechanism of suberization, which is connected with the activity of oxydoreductases, is not fully inhibited by irradiation. The activities of those enzymes do not seem to be very much altered by irradiation. But I agree that one should attempt to find enzymatic reactions which preceed the histological changes which I described. Perhaps this would lead to a faster identification of irradiated potatoes.

J. CROIZET

A decree is soon to be issued, authorizing the irradiation of potatoes in France. These potatoes will have to be placed in lead-coated packaging before being irradiated. This lead would have to be retained up to consumer level. It is, perhaps, not a very scientifc mode of control. In most countries it is agriculture which is the source of these procedures.

CHAIRMAN

But it is possible to safeguard the health aspect with administrative measures; it is surely not always necessary to have a detection method, if none exists. For sterilization processes and in vaccine manufacture, at least, control procedures are carried out during production and not during marketing.

6. THIN-LAYER ISOELECTRIC FOCUSING OF SARCOPLASMIC PROTEINS FROM IRRADIATED MEAT

Progress report 1.9.1969 - 1.10.1970

B.J. RADOLA

ABSTRACT

Thin-layer isoelectric focusing was used to study radiation-induced changes of the charge properties of proteins. A strong decrease of the most basic proteins and some components present in the middle part of the focusing pattern was consistently observed in meat irradiated with doses of 1 and 5 Mrad. In addition, similar effects were observed for the peroxidase isoenzymes of meat. Isoelectric focusing offers a further criterion for identification on the basis of radiation-induced changes of the charge properties of proteins and enzymes. The combined use of two identification methods based on independant molecular parameters may be anticipated to yield more reliable results than application of a single method.

INTRODUCTION

The size properties of sarcoplasmic proteins from irradiated meat have already been successfully studied by thinlayer gel chromatography on Sephadex G-200. An attempt was now made to study radiation-induces changes of the charge properties of sarcoplasmic proteins from irradiated meat. Isoelectric focusing appeared to be attractive for these studies for a number of reasons. On isoelectric focusing proteins can not only be separated with a hitherto unprecedented resolution but in addition the separated components can be characterized by an important physico-chemical parameter, namely the isoelectric point. Molecular size does not interfere with isoelectric separations; this is an advantage, when compared with some electrophoretic methods, with which components with very high molecular weights can not be separated due to steric hindrances. Finally, a thin-layer technique of isoelectric focusing has been developed, which could be useful for routine analysis.

MATERIALS AND METHODS

Irradiation was carried out as reported previously. The sarcoplasmic proteins were isolated as described in the previous report with the following modifications. The crude 0.1 M phosphate buffer extract was dialyzed for 24-48 hours against a 1 % glycin solution with a pH adjusted to 7.2-7.4. The dialyzed extracts were concentrated by ultrafiltration on Diaflo membranes (Amicon, Oosterhout, Holland) to an $E_{280\ \mathrm{mm}}^{1\ \mathrm{cm}} = 50$ corresponding roughly to 4-5 % protein concentration.

Thin-layer isoelectric focusing was performed on plates 20 × 20 cm in size, which were coated with a suspension containing 8 g of Sephadex G-75 "superfine" (Pharmacia, Uppsala, Sweden), in 100 ml of a 1 % ampholyte solution (LKB, Bromma, Sweden). The plates were mounted on a metal cooling block through which water thermostated at 4° C was circulated. Contact with the electrode vessels containing 0.2 M sulphuric acid at the anode and 0.4 M ethylene diamine at the cathode was established by strips of cellulose acetate. Usually 20 μl of the sarcoplasmic extract, treated before application with a twofold molar excess of $K_3[Fe(CN)_6]$ were applied on plates with pH 3-10 ampholytes and 40 μl on plates with pH 5-8 ampholytes. Five samples were run on a single plate. Focusing was complete in 5-7 hours with a voltage gradient of 10 V/cm with the pH 3-10 ampholytes and 20 V/cm for the pH 5-8 ampholytes. After focusing a print was taken by rolling a sheet of chromatographic paper Schleicher & Schüll SS 2043 b mgl on the gel layer. The print was dried at 110° C. The carrier ampholytes were washed off in three successive bathes of 20 % sulfosalicylic acid. The washed prints were stained with Coomassie Blue R 250. Densitometry was accomplished with a Zeiss Chromatogram-Spectrophotometer (C. Zeiss, Oberkochen, Germany), operated in reflectance.

RESULTS

In preliminary experiments optimal conditions for thin-layer isoelectric focusing of sarcoplasmic proteins were determined. On the basis of these experiments the pH 3-10 and pH 5-8 ampholytes were chosen for further work. In Fig. 1 densitometric tracings of beef sarcoplasmic proteins in pH 3-10 ampholytes are shown. Sarcoplasmic proteins from untreated meat and from beef irradiated with a dose of 1 and 5 Mrad were separated on a single plate, equal amounts of all proteins being applied. Between 15 and 20 components were detected in the untreated meat. With increasing dose a decrease of the number of protein zones was consistently observed. Very distinct changes were noted especially in the region of the basic proteins and some of the components in the middle of the isoelectric pattern.

An improved resolution was achieved in the pH 5-8 ampholytes. The unirradiated meat contained 20-25 zones, which could be demonstrated with high reproducibility (Fig. 2). With irradiated meat again a

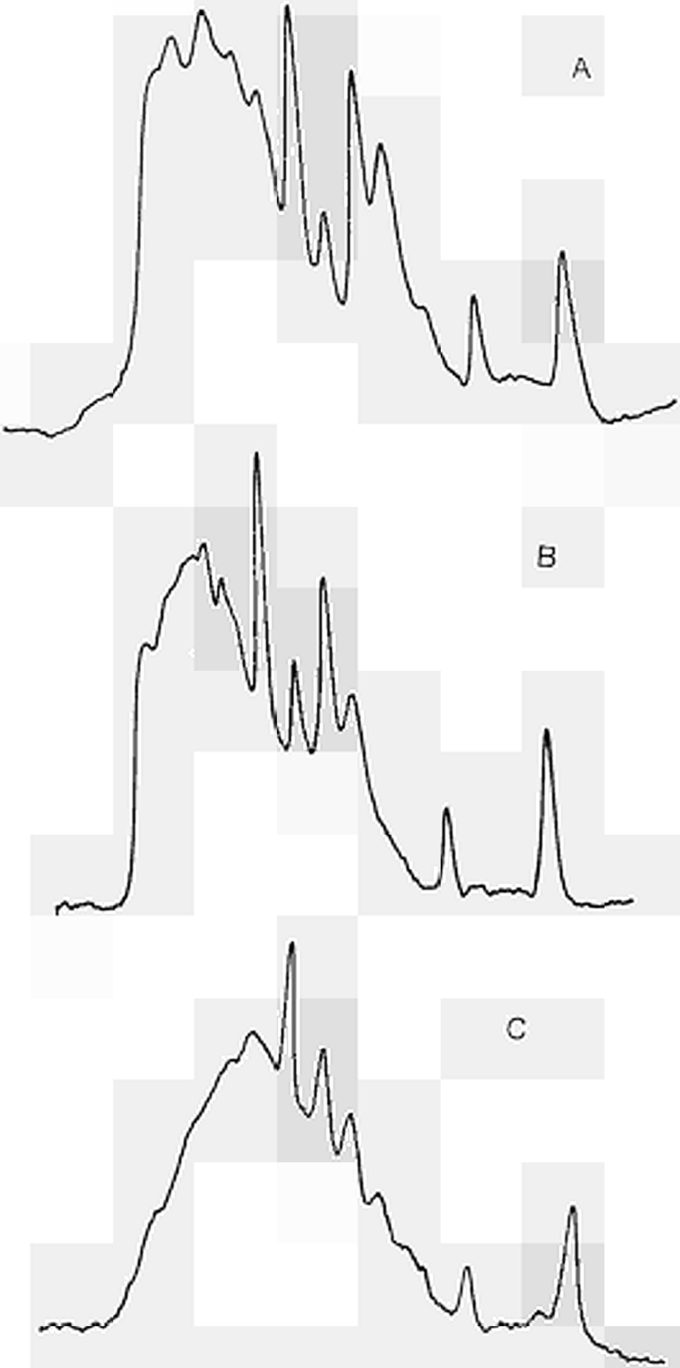


Fig. 1. Thin-layer isoelectric focusing of sarcoplasmic proteins from beef in pH 3 - 10 ampholytes. Cathode on the left.
A - unirradiated meat,
B - irradiated with 1Mrad.
C - irradiated with 5 Mrad.

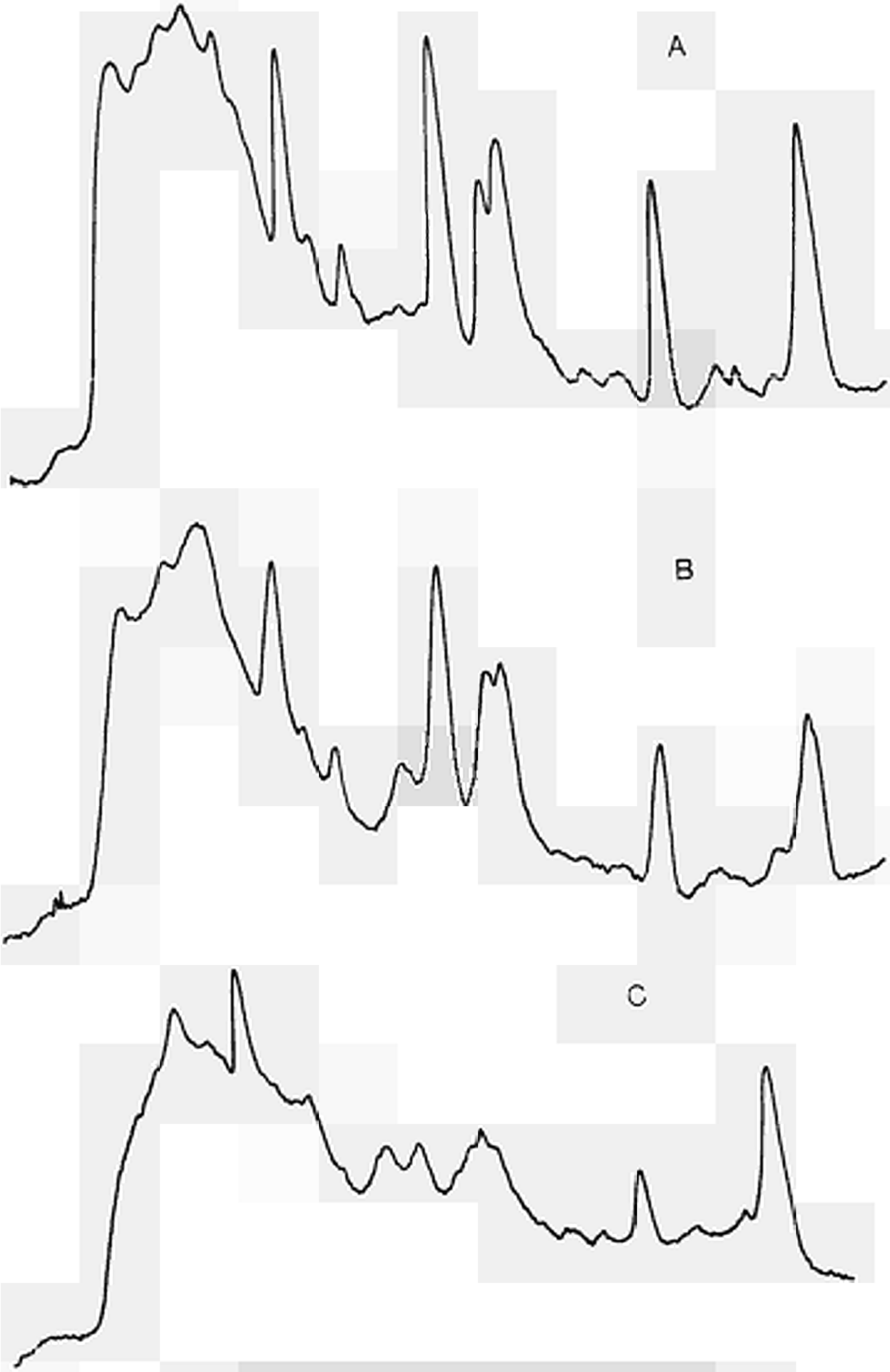
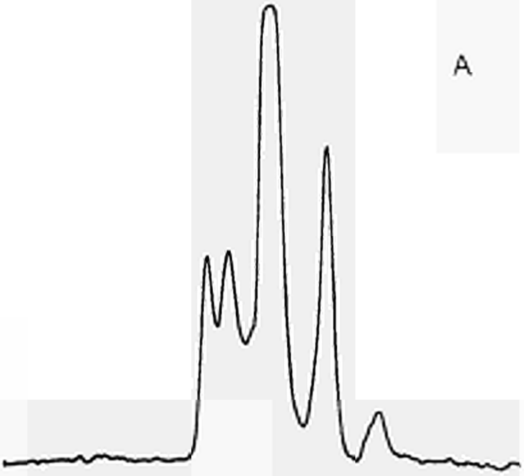


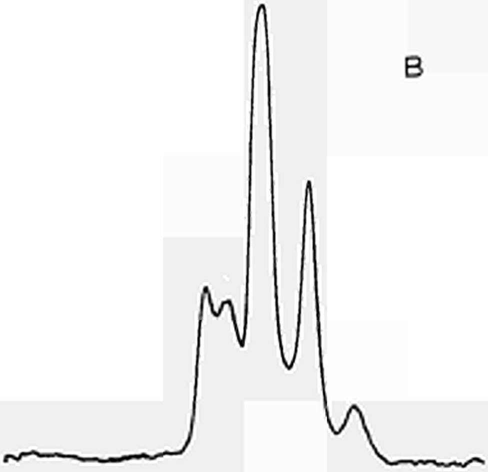
Fig. 2. Thin-layer isoelectric focusing of beef sarcoplasmic proteins in pH 5 - 8 ampholytes. Cathode on the left.

A - unirradiated meat,
B - irradiated with 1 Mrad,
C - irradiated with 5 Mrad.

decrease of the number of focused zones was observed. Essentially the same change of the pattern was observed as already described above for the pH 3-10 ampholytes.

In addition to studies on radiation-induced changes of proteins, attempts were made to characterize some enzymes in the sarcoplasmic extract. Peroxidase activity was studied first, because the detection of the activity of this enzyme can be readily accomplished with a substrate-impregnated paper. Guaiacol was used as a substrate due to its relative high sensitivity and relative stability of the coloured reaction products.





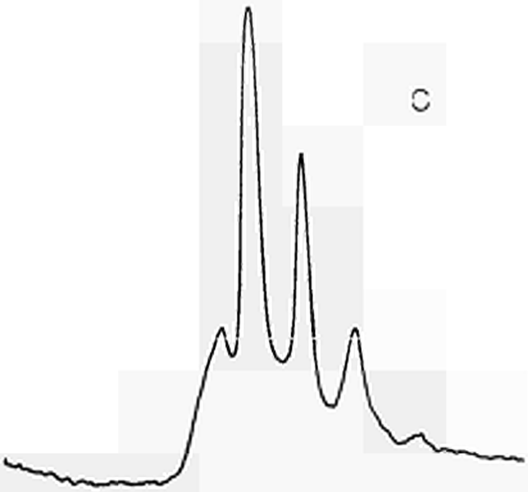
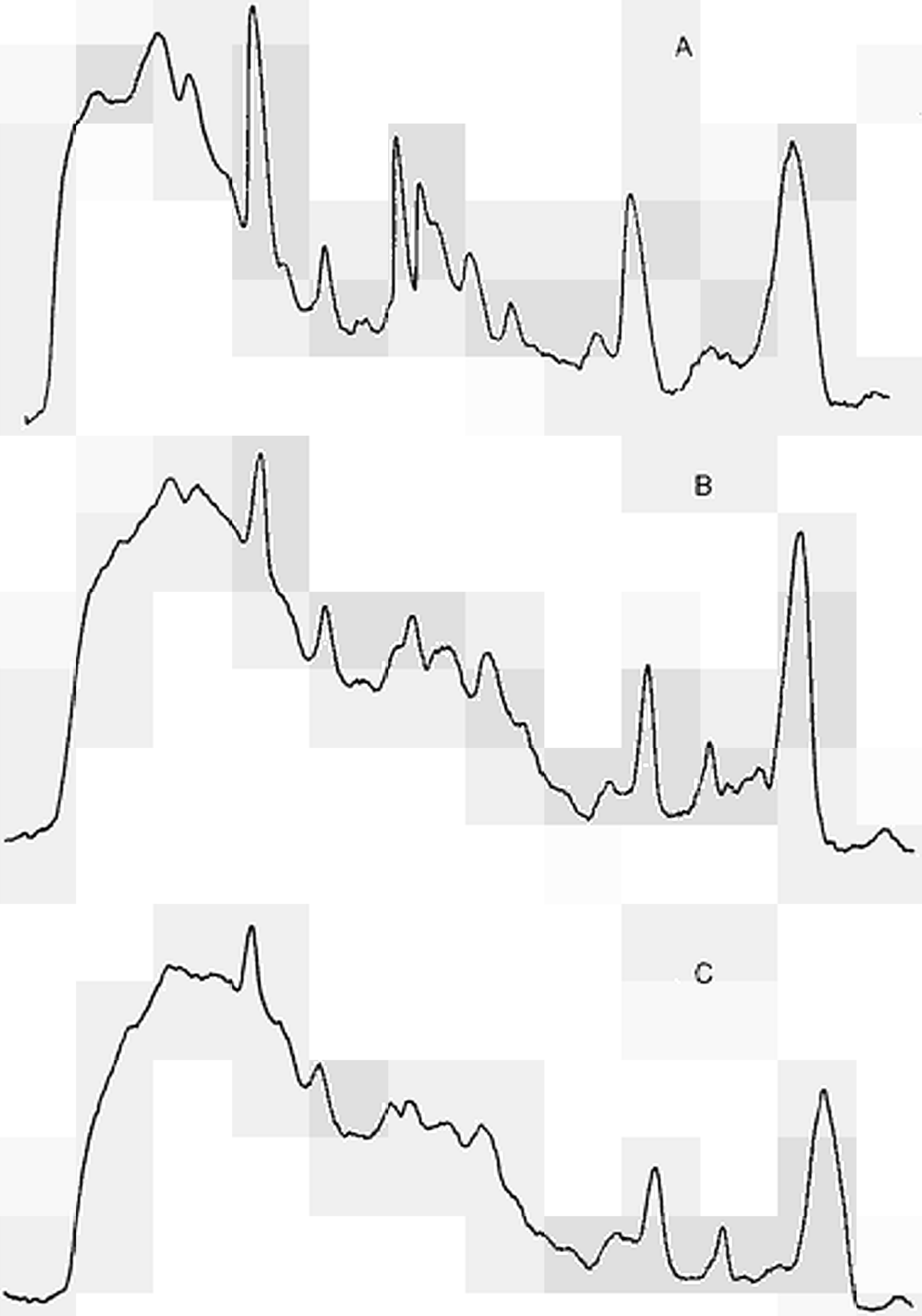


Fig. 3. Thin-layer isoelectric focusing of peroxidase isoenzymes from beef in pH 3 - 10 ampholytes. Cathode on the left. Detection of enzyme activity with a buffered (pH 5), substrate-impregnated paper (guaiacol and urea peroxide).
A - unirradiated meat,
B - irradiated with 1 Mrad,
C - irradiated with 5 Mrad.

In Fig. 3 the peroxidase isoenzyme pattern is presented for the pH 3-10 ampholytes. Five isoenzymes can be clearly seen. With sarcoplasmic proteins from irradiated meat the pattern changed. Beef irradiated with a dose of 1 Mrad contained a distinctly smaller amount of the two most basic isoenzymes. In beef irradiated with a dose of 5 Mrad only one basic isoenzyme was detected while simultaneously the amount of the most acidic isoenzyme increased distinctly.

A series of irradiation experiments were performed with isolated sarcoplasmic proteins. Beef sarcoplasmic proteins were irradiated in 0.02 M phosphate buffer pH 7.2-7.4 in a cobalt source with doses of 0.5-2.0 Mrad. Densitometric patterns following thin-layer isoelectric focusing of the irradiated sarcoplasmic proteins in pH 5-8 ampholytes are presented in Fig. 4. The results were in complete agreement with those obtained with sarcoplasmic proteins isolated from irradiated meat. With increasing irradiation dose the number of focused zones decreases, especially by a strong reduction of the most basic components. The pattern of the irradiated sarcoplasmic proteins contained only poorly defined peaks when irradiated at the highest dose (Fig. 4D). In Fig. 5 the patterns of peroxidase activity following focusing in pH 5-8 ampholytes are shown. The improved resolution obtained in this ampho-


A
B
C

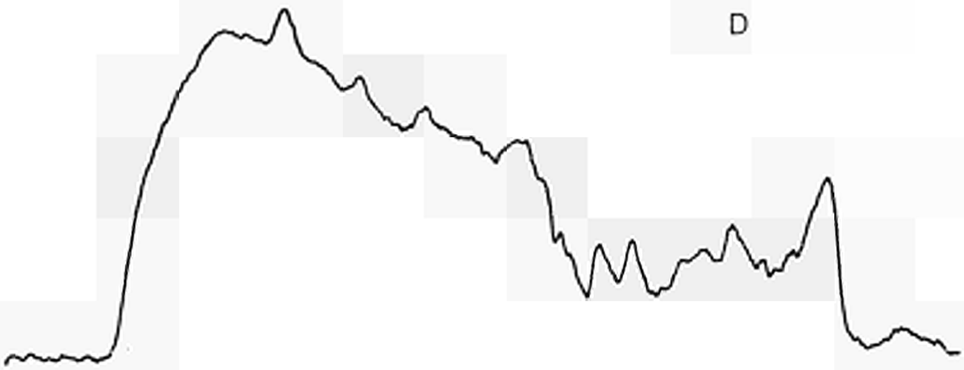
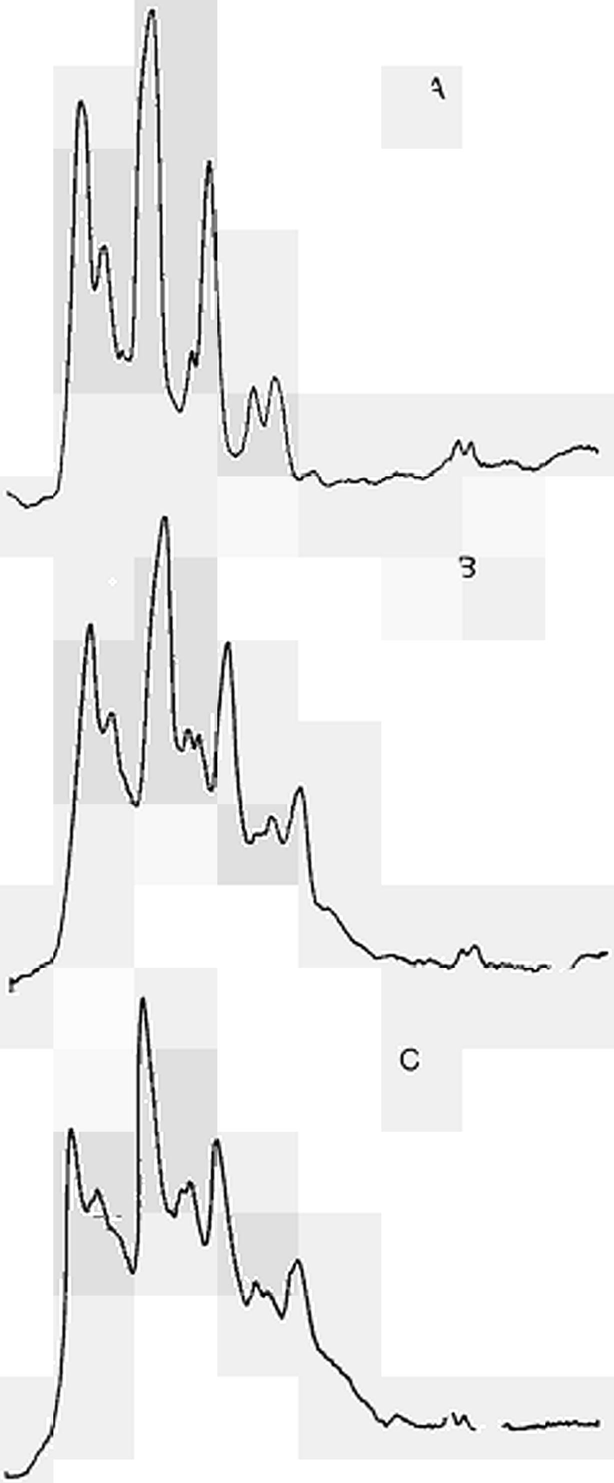


Fig. 4. Thin-layer isoelectric focusing of sarcoplasmic proteins irradiated in phosphate buffer.
Focusing in pH 5 - 8 ampholytes. Cathode on the left.
A - unirradiated proteins,
B, C, D — irradiated with 0.5 — 1 and 2 Mrad at 0°.

lyte system becomes clearly evident in the increased number of isoenzyme peaks. Instead of 5 isoenzymes of the pH 3-10 pattern (Fig. 3A) 9 isoenzymes were observed in the pH 5-8 ampholytes (Fig. 5A). With increasing radiation dose there was a shift of the isoenzymes towards components with lower isoelectric points (Fig. 5C and 5D). These results obtained with peroxidases of sarcoplasmic proteins irradiated in phosphate buffer are again in agreement with those obtained with the proteins extracted from irradiated meat. Comparative studies thus prove that the radiation-induced changes of the charge properties both of the proteins and the enzymes are basically of the same type, irrespectively of whether the proteins are irradiated in meat or in a simplified system, namely in phosphate buffer.

Previously we have reported that in meat irradiated with a dose of 1 and 5 Mrad a new radiation-induced sarcoplasmic protein fraction was observed on thin-layer gel chromatography. Evidence was presented that this fraction results from aggregation of the sarcoplasmic proteins. In addition to radiation-induced changes of the size properties of sarcoplasmic proteins detected by thin-layer gel chromatography, isoelectric focusing offers further a criterion for identification on the basis of radiation-induced changes of the charge properties of the proteins. The combined use of two identification methods based on independent molecular parameters may be anticipated to yield more reliable results than application of a single method.


A
B
C

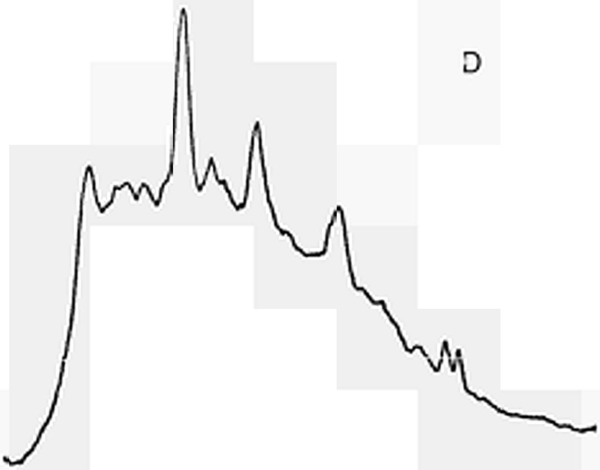


Fig. 5. Thin-layer isoelectric focusing of peroxidase isoenzymes from beef sacroplasmic proteins irradiated in phosphate buffer.
Focusing in pH 5 - 8 ampholytes. Detection of peroxidase activity with a mixture of guaiacol and o-tolidin. Cathode on the left.
A - unirradiated sarcoplasmic proteins,
B, C, D - irradiated with 0.5 - 1 and 2 Mrad.

DISCUSSION

CHAIRMAN

Dr. Radola has discovered a new method which again appears to be an exacting one.

L. BUGYAKI

I would like to draw your attention to the difficulty of identifying irradiated meat which has been previously heat treated. The quantity of its soluble and extractable proteins being very low, I am wondering if the isoelectric-focusing could be useful.

B.J. RADOLA

We have not unintentionally included peroxidase in our studies. It is well established that peroxidase is a rather heat stable enzyme. It thus could survive a heat treatment at e.g. 70° and changes of the charge properties of peroxidase could perhaps provide a basis for identification following a combined heat and radiation treatment.

CHAIRMAN

Although our colleagues in the milk and cheese industries carry out peroxidase detection, the pasteurization of milk is still a very practical procedure. If an enzyme can perhaps be detected in this way, it offers a very important method for inspection procedures.

B.J. RADOLA

We have studied the effect of heat and irradiation on horse-radish peroxidase. The effects were quite different and we hope that the isoelectric properties could provide a useful test for identification of irradiated food.

B) Research performed in the NETHERLANDS and in BELGIUM

by

D. ONDERDELINDEN and L. STRACKEE
Rijksinstituut voor de Volksgezondheid, Utrecht/Bilthoven

L. BUGYAKI and M. VAN DER STICHELEN-ROGIER
Institut d'Hygiene et d'Epidémiologie, Bruxelles

A.R. DESCHREIDER and E. MAES
Ministère des Affaires Economiques, Laboratoire Central, Bruxelles
and CENATRA, Antwerpen

Chairman: J.F. DIEHL

7. DETECTION OF IRRADIATED FOODSTUFFS BY MEANS OF ELECTRON SPIN RESONANCE

Progress report April - October 1970

D. ONDERDELINDEN and L. STRACKEE

ABSTRACT

In this progress report some preliminary results of measurements on the irradiation yield of polyvinylchloride, polyethene, polystyrene and cellophane are communicated. Irradiations were performed both at 77° k and at room temperature, while irradiation dose rates were 0.66, 0.22 and 0.11 Mrad/hr. The irradiation yield of polyethene, polystyrene and cellophane was found to be smaller than 0.02 spins 100 eV, assuming a hypothetical line width of 10 gauss. Measurements on polyethene at 77° k revealed a broad spectrum with several lines and a free radical yield of the order 0.2 spins 100 eV. For PVC irradiation yields up to 8 spins/100 eV were found. Furthermore a strong dose rate effect showed up in these measurements. It may be remarked here that in later measurements this dose rate effect was appreciably smaller. Decay measurements on PVC showed two decay times about 2 days and about 60 days respectively. Thusfar no influence of the ambient atmosphere on the decay time was found.

Measurements at intermediate temperatures and with an improved sensitivity of the spectrometer are in preparation.

1. INTRODUCTION

In April 1970 the electron spin resonance study on irradiated materials was taken up again. The results obtained thusfar were not very promising. Dried foodstuffs were found to contain a large amount of stable radicals up to concentrations of 5×10^{17} spins/gr at least partly as result of the drying process. No significant change in the number of free radicals was observed after irradiation of these samples up to a dose of 1 Mrad. Measurements on irradiated grains were more promising. Irradiation of wheat for example gives an approximately 19 gauss broad signal and a radical yield of approximately 3 spins/100 eV. The stability of the resonance signal however was low. In a nitrogen atmosphere the half value time observed was about 40 days. Samples kept in open vessels showed a 4-fold decrease in about 3 days. The electron spin resonance as a detection method for irradiated foodstuffs seems therefore not the most qualified method thus far.

Because it was thought that radicals created by irradiation in packaging materials are more stable and therefore useful as identification of irradiated foodstuffs and investigation on this subject was started as agreed on in the June-meeting in Luxemburg in 1969. In the present report the results of measurements on polyethene, polyvinylchloride and cellophane are communicated. Irradiation and measurements were performed both at room temperature and at liquid nitrogen temperature. Some of the earlier measurements on grains were repeated at low temperature.

2. EXPERIMENTAL

The electron spin resonance apparatus as described earlier (1) has been equiped with a Varian temperature controller to allow measurements both at low (77°K) and high (up to 300°C) temperatures. The cavity used in the earlier studies was replaced by an AEG rectangular cavity with external modulation coil and a fixed coupling to the wave guide system. The resonance frequency of the cavity with temperature tubes installed is approximately 8950 MHz. The modulation amplitude was chosen as 2 gauss, small enough to prevent modulation broadening of the spectral lines. Furthermore the input power was such that no saturation effects were observed. The maximum sensitivity at a time constant of 0.5 sec and a line width of 1 gauss is estimated to be 10^{13} free spins.

Irradiations were performed with a 850 Ci Co^{60} source of the standard dosimetry group at the Institute. The samples were kept in open or closed quartz tubes or in thin walled aluminium vessels. Since the dimensions of the samples were of the same order of magnitude as the range of the secondary electrons no complete electron equilibrium was obtained. This means that the calculated dose is overestimated systematically. No attempt was made to correct for this effect. The dose rate was changed by varying the distance of the samples to the source. For a square law dependence of the exposure rate the dose rate at distances of 4.1 cm, 7.1 cm and 10.0 cm is estimated as 0.66 ± 0.07, 0.22 ± 0.02 and 0.11 ± 0.01 Mrad/hr respectively. The finite size of the source introduces an additional uncertainty in the calculated dose rate. For the shortest distance this incertainty is estimated as 10 %.

The irradiation of the samples in quartz tubes induces a strong signal in the material of the tubes. This makes it necessary to transfer the samples to another tube or to anneal the irradiated tube without heating the sample. This manipulation still offers a problem especially for irradiation at low temperatures.

Experiments have been performed on polyethene-, polyvinylchloride- and polystyrene samples in the form of cylinders with a length of 5.3 mm and ∅ = 2 mm. The cellophane sample consisted of thin layers packed to a cylinder of about the same dimensions. These materials were of industrial quality and no attempts were made to arrive at better defined samples at present.

3. RESULTS

Room temperature measurements

Irradiation of polyethene, polystyrene and cellophane both in a nitrogen atmosphere and in air gave no measurable ESR signal up to a dose of 2 Mrad.

Polyvinylchloride gave a single gaussian type absorption peak with some small hyperfine structure. The width between the maxima of the derivative spectrum was found to be 35 gauss. The Landée g factor is estimated as 2.0023 ± 0.0004. The number of spins is found by double integration of the derivative curve and comparison with a standard charcoal powder sample which contains 3.6×10^{16} spins. The absolute accuracy of this number is estimated to be better than a factor 2. The results of PVC, irradiated in air, are given in Fig. 1, where the number of free spins is given as a function of the dose at different dose rates. As can be seen the radical yield strongly depends on the dose rate. The number of free radicals produced per 100 eV absorbed energy from the incident beam of radiation, the G value, as deduced from the initial slope is given in the inset of Fig. 1. The experimental points for fixed dose rate are fitted with an expression of the form

$$N(x) = A\ (1 - \exp - Bx) \tag{1}$$

where A and B are constants, $N(x)$ the number of free radicals and x represents the dose. A values of 3.0×10^{17}, 4.7×10^{17} and 13.3×10^{17} radicals were found for the dose rates 0.66, 0.22 and 0.11 Mrad/hr respectively. B was taken to be 0.2 Mrad^{-1}. This follows from a measurement close to saturation. For a dose of 12.6 Mrad at a dose rate of 0.11 Mrad/hr a yield of 11.7×10^{17} radicals was found. With the assumed values of the parameters A and B equation (1) gives the result 12.3×10^{17} spins. A measurement in a nitrogen atmosphere yields a result that is compatible with the dose rate dependence of G for irradiation in air as can be seen in the inset of Fig. 1. The stability of the free radicals

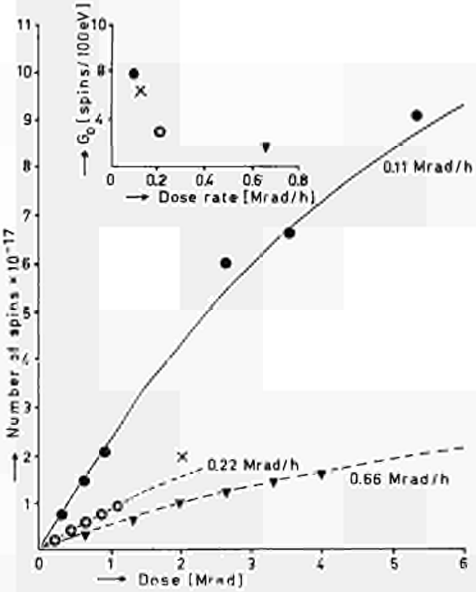


Fig. 1. The number of free radicals in PVC samples (0.05 gr) as a function of irradiation dose at different dose rates. Measurements were performed within 15 min after the end of the irradiation.

The cross refers to a single experiment in a nitrogen atmosphere both during the ESR measurement and the irradiation. The other experiments were performed in air. The solid and broken curves are fitted functions as given in equation (1). The inset shows the initial free radical yield as a function of dose rate.

in PVC is shown in Fig. 2, where the decay of the ESR signal at room temperature is given. It seems justified to conclude from the results that two decay times may be distinguished. The long time decay is approximately exponential as demonstrated by the straight line fit in Fig. 2. Half value times for the short and the long time decay are of the order of 1 and 60 days respectively. The phenomenon of two decay mechanisms has also been observed in irradiated wheat. For this case the half value times are estimated as 40 hr and 40 days for decay in a nitrogen atmosphere. From the slope of the straight lines in Fig. 2 it follows that the decay of the free radicals in irradiated PVC depends only weakly on the surrounding atmosphere. The decay in a nitrogen atmosphere is slightly slower than in air.

In order to speed up the decay process an annealing experiment has been performed at 80° C in a tube evacuated to about 10^{-3} Torr. The PVC sample was irradiated up to a dose of 3.6 Mrad at a dose rate of 0.11 Mrad/hr. For this case two decay times were also found: 6 and 180 min.

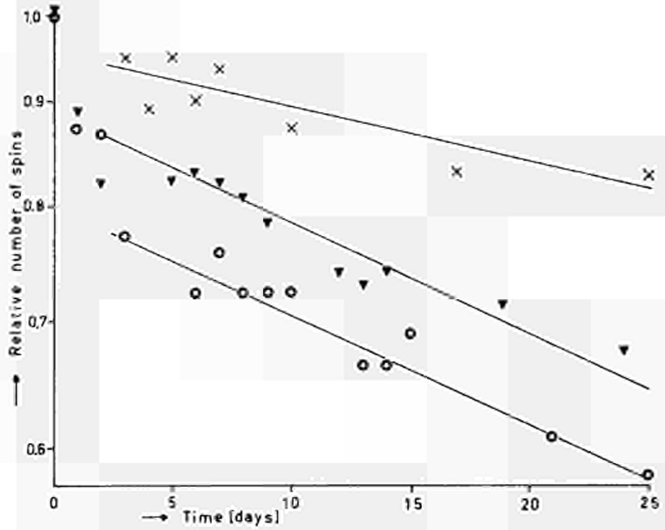


Fig. 2. Relative number of free radicals in PVC on a logarithmic scale versus time. All values are reduced to the initial value, directly after the irradiation. The straight line shows an exponential decrease for a fraction of the radicals.

☆ dose rate 0.22 Mrad/hr; dose 1.1 Mrad; in air,
● dose rate 0.11 Mrad/hr; dose 2.6 Mrad; in air,
× dose rate 0.13 Mrad/hr; dose 2.0 Mrad; in nitrogen.

Low temperature measurements

Irradiation of polyethene at liquid nitrogen temperature revealed a broad spectrum with several lines. For polystyrene no ESR signal was found. At a dose rate of 0.3 Mrad/hr and a dose of 1.8 Mrad the free radical yield of polyethene was found to be 0.25 spins/100 eV. On heating the sample to 0° C the ESR signal disappears completely within 15 min. The signal of PVC at these temperatures shows a more detailed fine structure, while the free radical yield was about the same as at room temperature.

Previous measurements on grains were repeated at liquid nitrogen temperature. The form of the ESR signal was similar to the form observed in samples irradiated at room temperature; a single gaussian type line with a width of about 20 gauss. The free radical yield at a dose rate of 0.3 Mrad/hr and a dose of 1.8 Mrad was found to be 0.7 spins/100 eV. This may be compared with the earlier result of 3 spins/100 eV at a dose rate of 0.025 Mrad/hr and a dose of 1.2 Mrad.

In order to check the experimental procedures, notably at low temperatures, some measurements on polycrystalline glycine and L-α alanine were performed. The 5 line spectrum of glycine changed in a 3 line spectrum when the sample was heated to room temperature. The free radical yield at a dose rate of 0.3 Mrad/hr and a dose of 1.8 Mrad/hr was found to be in the order of 3 spins/100 eV. These results are not in contradiction with measurements found in the literature (2, 3).

4. DISCUSSION

In order to estimate the upper limit of the free radical yield in the case of polyethene, cellophane and polystyrene, where no signal was observed elsewhere after irradiation, we will assume a single line spectrum with a hypothetical line width of 10 gauss. It then follows from the sensitivity of the spectrometer that the yield in these materials should be smaller than 3×10^{16} radicals/gr. This means for the used doses a G value smaller than 0.02 spins/100 eV, not in contradiction with measurements of Schneider (4). From the fact that a higher yield occurs at low temperatures in polyethene it may be concluded that the low yield at room temperature is due to annihilation of diffusing radicals. The disappearence of the ESR signal on warming up to 0° C shows that in this picture a fraction of the radicals is highly mobile above 0° C. The low response of polystyrene may be due to the high radiation stability of the benzene ring (5). It seems that the benzene ring is sometimes capable of absorbing the energy of the incident radiation and converting it to thermal energy. The high response of PVC as compared with polyethene must be due to the halogene atom. The shape of the spectrum is in agreement with older measurements on polyvinylbromide of Abraham *et al.* (6). They also found a relatively high radical yield. The large width of the line can either be due to a number of partially overlapping spectra or to electron-dipole — nuclear-dipole interaction. This interaction gives according to Ingram (7) broadening effects of the order of 25 gauss. As can be seen from Fig. 1 the number of spins vs dose curves show a strong saturation effect. This has been found by many authors (see for instance ten Bosch (8)). The exponential behaviour is also often found in literature. However, the strong dose rate dependence of both the saturation value and the initial slope seams to be uncommon. It may be remarked that from the few results on wheat it follows that the same type of dose rate effects occur possibly also in grains. Ten Bosch (8) gives a series of models to explain the saturation effect without however considering the dose rate dependence. Therefore none of these models was capable of describing our results. The mechanism of radical formation in solids is closely related to diffusion and interaction of these highly reactive molecules. Since these processes, underlying the interaction of ionizing radiation with matter are not yet fully understood we feel that a more detailed investigation along the lines suggested in this report is desirable. At present a model is in study with the ultimate aim of explaining both the dose rate dependence and the saturation effect in terms of diffusing free radicals. To test this model the experiments has to be extended to intermediate temperature ranges and to better defined sample material. Furthermore the sensitivity of the ESR spectrometer and the dosimetry has to be improved.

5. CONCLUSION

From the previsiously reported work it was concluded that the ESR measurements do not form a very successful method to identify irradiated foodstuffs directly. This conclusion holds also when the method is applied indirectly via the packaging materials. However the type of study reported here seems to be very promising to gain useful information on the mechanisms of radical formation and radical diffusion both in polymers and in foodstuffs. Since chemical changes in irradiated foodstuffs occur very probably via a free radical mechanism better insight in the basic process is desirable.

REFERENCES

1) Progress report on activities in the period October 1967-February 1968.
2) S. DESCOURS, J. ASSAYRENC, F. BERMANN, B. CONDERC, H. DE CHOUDENS, R. DELARD, A. RASSAT AND P. SERVOZ-GAVIN, Centre d'Etudes Nucleaires de Grenoble, Rapport CEA-R-3913, 1970.
3) G. SCHOFFA, 1964. Electronenspinresonanz in der Biologie, Verlag G. Braun, Karlsruhe.
4) E.E. SCHNEIDER, Disc. Faraday Soc., **19** (1955) 158.
5) A. CHARLESBY, Radiation Research 1966, (ed. G. Silini), North. Holl. Publ. Comp. Amsterdam, Page. 265.
6) R.J. ABRAHAM and D.H. WHIFFEN, Trans. Farady Soc. **54** (1958).
7) D.J.E. INGRAM, 1958, Free radicals as studied by electron spin resonance, Butterworths Scientific Publications, London.
8) J.J. TEN BOSCH, 1967, Thesis, Utrecht.

DISCUSSION

J. SPAANDER

I should like to draw your attention to Document EUR 4466 e, the report by Cornelis. In Appendix B (page 37), 14 types of packaging material are listed, of which only a few have been investigated. A great many foodstuffs are packaged. The packaging remains intact up until the time of consumption, and this means that the packaging material is a component of the foodstuff unit. By measuring the packaging material, with the aid of electron spin resonance, for example, it is therefore possible to detect the irradiation of foodstuffs.

H. SCHERZ

You have studied packaging materials irradiated in the presence of air. I am surprised that you found only one ESR-signal with polyvinylchloride under these conditions. As a result of irradiation, chloride is probably split off; oxygen will react with the radical thus formed, producing a peroxide radical. I wonder what kind of signal you would find after irradiation in an oxygen-free atmosphere, when peroxide radicals cannot be formed.

CHAIRMAN

There seems to be a misunderstanding. If we say that a signal has been found in electron spin resonance measurements we say nothing about the shape of the spectrum. This signal may be a doublet or a triplet or it may have any other characteristic. Isn't that so?

D. ONDERDELINDEN

We did find a single Gaussian type line both for measurements in air and in a nitrogen atmosphere. In experiments on radicals trapped in solids it is possible that the anisotropic electron-dipole-nuclear-dipole interaction causes the lines of the randomly oriented radicals to be broadened which makes identification of the species difficult.

From the inset of figure 1 it can be seen that initial G value does not depend strongly on the kind of atmosphere in which the radiation has taken place. This means that we were not able to demonstrate the formation of peroxy radicals in our experiment.

CHAIRMAN

In Fig. 2 you have indicated that irradiation has been carried out once under nitrogen and twice in air, but the radiation dose was different in each case. Now one does not know how much of the difference is due to different dose and how much is due to different atmosphere. I believe this point should be clarified. In general, I think that the ESR-method is of interest for the identification of irradiated foods even if an ESR signal is found in the packaging material only, and not in the food itself.

F. DRAWERT

In Figure 1, the dose is plotted against the number of spins × 10^{-17}. It thus seems that at a dose of 0.11 Mrad/hr more radicals are formed than at a dose of 0.66 Mrad/hr. Is that correct?

D. ONDERDELINDEN

The highest signal occurs when the lowest radiation intensity is used. This, if we irradiate with, for example, 1 Mrad in 9 hr, at in intensity of 0.11 Mrad/hr, we obtain a higher signal than if we irradiate up to 1 Mrad in a smaller number of hours. The higher the dose rate, the lower the signal.

CHAIRMAN

We must differentiate dose and dose rate. The fact that a lower number of spins was observed when a higher dose rate was applied is in agreement with many other studies. The explanation is, that the use of a high dose rate will lead to the simultaneous formation of many radicals which will react with each other and thus disappear. With a low dose rate more free radicals will not find a partner to react with, and therefore a higher number of spins is observed.

J. SPAANDER

What are the dose rates most commonly used in practice for the irradiation of mushrooms? Are they comparable with fairly low or with fairly high dose rates?

CHAIRMAN

This depends on whether an accelerator is used or a cobalt source. With an accelerator we may have dose rates of 10^{10} rad/sec, with a cobalt source the dose rate may be 10^{4} rad/sec or even less.

L. SAINT-LÈBE

I think that the problem of identifying an irradiated product is a dual one and that we see only one side of it. Why are products checked for irradiation? Firstly, to make sure that the firm which performed the irradiation has not exceeded the dose laid down by the law. Another reason, however, is to see that there is no irradiation fraud, i.e. to check that no one profits from marketing a product labelled "irradiated" if it is in fact not irradiated. Now if irradiation is detected on the packaging, it is quite possible that the contents have not been irradiated.

H. PROST

There is a third reason for identifying irradiated food: to prevent it from being irradiated twice. Without identification that it has been irradiated, there is a chance of its inadvertently being irradiated again, and thus receiving a double dose.

CHAIRMAN

I believe that with all these results we may have to admit that a definite conclusion "irradiated or not irradiated" cannot be made by using one method alone. By combining informations obtained with several methods, some studying changes in the foods, others studying changes in the packaging material, we should come to a saver judgement. For this reason I believe that we should continue to proceed with many different analytical techniques.

8. VERTICAL STARCH GEL ELECTROPHORESIS AND IMMUNOELECTROPHORESIS OF IRRADIATED AND NON-IRRADIATED ANIMAL PROTEINS

Progress Report: April 1969-April 1970

L. BUGYAKI and M. VAN DER STICHELEN ROGIER

ABSTRACT

The first method does not enable fresh codfish to be distinguished from irradiated cod. Conversely, the differences between the electrophoretic pattern from the non-irradiated meat and egg or that irradiated at room temperature are important enough to enable the treated and non treated samples to be differentiated.

The finding to the effect that the difference does not seem to be as important if the irradiation is carried out at low temperature indicates that investigations must be undertaken in this field.

In spite of the improvement of the separating power of the electrophoresis and of the precipitating ability of the immune serums, the results of our research do not enable us to differentiate the irradiated and non irradiated meat, fish and eggs. We therefore see no point in pursuing our research with immunoelectrophoresis.

1. INTRODUCTION

We have evaluated the usefulness of starch gel electrophoresis and immunoelectrophoresis as laboratory methods for the identification of irradiated meat, fish and chicken egg. In the previous reports it was shown that it is not possible to differentiate between irradiated and non-irradiated samples by immunoelectrophoresis in agar gel. Conversely, the vertical starch gel electrophoresis patterns of liquid hen egg and meat showed differences between the irradiated and the untreated samples.

In view of the discouraging results with immunoelectrophoresis, experiments were planned with more potent (complete) immune serums used in agarose gel. The research was backed up by starch gel electrophoresis to improve the technique.

The present report relates to the work carried out between April 1969 and April 1970.

2. MATERIAL AND METHODS

Irradiation

The preparation, the irradiation and the preservation of samples were performed as described in the previous reports.

Immunoelectrophoresis in agarose gel

We hoped to obtain better results with the agarose for the following reasons: The agarose is free of polar groups and its gel does not produce electroendosmose during electrophoresis.

The migration path is longer, because the sample is deposited nearer the cathode, giving better separation.

The slides are prepared in a similar way as with agar; the migration period is 60 min.

Meat immune serum

Plain muscle, taken immediately after slaughtering, is extracted in a similar manner as described in our previous reports. Merthiolate to a concentration of 1/10,000 is added, after which the extract is concentrated by dialysis at 4° C against Carbowax M20 to a final concentration of 10 % of protein. This concentrated extract is distributed in flasks, with 1 ml of it in each.

The closed flasks are kept at —20° C. Before intramuscular injection in rabbits, 1 ml of Carbowax M20 30 % solution is added to the rapidly defrosted extract; 2 cc of antigen obtained by this method are injected twice a week into rabbits intramuscularly. A total of 10 injections are given, totalling 1 gr of protein per animal.

Fish immune serum

This is prepared in the same way as the meat with the difference that the "fresh" codfish was bought in a fish-shop.

3. RESULTS AND DISCUSSION

Meat

Agarose gel immunoelectrophoresis

We obtained better results with this new technique; the number of lines is greater and they are clearcut. Important differences can be detected in the patterns of irradiated and fresh meat (disappearance of some lines and weakening of others). Unfortunately this difference is not significant enough, especially in the absence of a control sample, to state categorically whether the sample has been irradiated or not.

Vertical starch gel electrophoresis

The new experiments confirm the results of our previous work.

In the patterns of 5 Mrad irradiated meat, the whole lenght of the trace of migrated proteins bands is diffusely coloured. The most typical difference compared with fresh meat is the disappearance of a band corresponding to a chromoprotein (myoglobin). Several other bands are weakened.

The patterns of samples irradiated with 0.5 Mrad give practically the same picture as the fresh meat during the first few days after irradiation. But an evolution takes place progressively in the samples stored at +4° C and they show a typical pattern at the end of three weeks, which remains stable after that period. The difference is important for distinguishing between the irradiated and non-irradiated samples.

Fish

Immunoelectrophoresis in agarose gel

Many precipitation lines are seen on the gels but the difference between the irradiated and non-irradiated samples is not marked.

Vertical starch gel electrophoresis

Only slight differences are observed on the slides between irradiated and non-irradiated samples. We are not able to differentiate them on this basis.

Egg

Immunoelectrophoresis in agarose gel

Both for eggs and for meat and fish, treatment with agarose does not permit differentiation of the irradiated and non-irradiated samples.

Vertical starch gel electrophoresis

The results of our previous experiments with whole liquid egg, with white or brown shell, irradiated at room temperature, are confirmed. Between the two samples a significant difference is observed, consisting in the absence of bands in the irradiated sample and the presence of diffusely coloured bases between the bands.

Conversely, preliminary experiments performed on eggs irradiated at low temperature show a slight difference between the irradiated and non-irradiated samples.

4. CONCLUSIONS

Immunoelectrophoresis in agarose gel

In spite of the improvement of the separating power of the electrophoresis and of the precipitating ability of the immune serums, the results of our research do not enable us to differentiate the irradiated and non-irradiated meat, fish and egg. We therefore see no point in pursuing this method for use with these foodstuffs.

Starch gel electrophoresis

This method does not enable fresh codfish to be distinguished from irradiated cod. Conversely, the differences between the electrophoretic pattern from the non-irradiated meat and egg or that irradiated at room temperature are important enough to enable the treated and non treated samples to be differentiated.

The finding to the effect that the difference does not seem to be as important if the irradiation is carried out at low temperature indicates that investigations must be undertaken in this field.

Proposal for future research

General problem:

1) reproducibility of the electrophoretic separation,

2) quantification of the result by densitometry with Chromoscan, meat: irradiation at low temperature,

Electrophoresis in acrylamide gel

This method has the following advantage in comparison with the starch gel technique:

— ease of preparation,

— reproducibility,

— better discoloration and greater transparency of the zones between the rings,

— fairly good quantitative evaluation of coloured zones (by transmitted light).

DISCUSSION

CHAIRMAN

It is interesting that you have found differences between irradiated and unirradiated eggs and meat using starch gel electrophoresis, even with a relatively low dose of 0.5 Mrad. This is the dose range required for destruction of salmonellae, which is of particular interest for egg products.

B. RADOLA

It is gratifying that on application of electrophoretic methods basically the same results were obtained as those presented in our report on isoelectric focusing. There is a decrease of the most basic proteins and at the higher dose of 5 Mrad the zones become diffuse. We have also observed both effects on isoelectric focusing.

It is known that irradiation of meat is accompanied by a conversion of oxymyoglobin to metmyoglobin. Have you treated your samples with potassium ferricyanide? I would like also to ask whether you have standardized the protein concentration of your preparations. In some of your figures the irradiated samples appear to have less total staining than the untreated samples.

M. VAN DER STICHELEN ROGIER

The quantity of proteins contained in the sarcoplasmic protein extracts is evaluated by the biuret method. This process is not highly sensitive but however it gives a fairly good appreciation. The protein content is about 2 % in the extract from the fresh meat, and it is less than 1 % in the extract from 5 Mrad irradiated meat. In order to have at least 2 % of proteins in all the extracts, those from the irradiated samples are concentrated by means of filtering membranes under vacuum. Two red coloured bands appear in the pattern of starch gel electrophoresis from the fresh meat extract. The faster migrating represents the oxymyoglobin and the slower is metmyoglobin. The same two bands are present in the pattern of the extract of 0.5 Mrad irradiated meat, if the extraction has been realised immediately after the irradiation. Later on, the oxymyoglobin disappears progressively, which takes 3 weeks keeping the samples in a refrigerator. The samples do not seem to show more evolution after these 3 weeks. The fresh meat sample shows the same evolution, but it is much slower than that of the irradiated sample.

The effect is immediate with 5 Mrad irradiation. As you can see on the plate this type of electrophoretic separation appears immediately after irradiation. In addition, the coloured background between the bands represents, in my opinion, a series of proteins-aggregates of different molecular weight, as you mentionned this morning.

B. RADOLA

The conversion of oxymyoglobin to metmyoglobin seems not to be suitable for identification of irradiated meat because this conversion can also be observed on a variety of other treatments. We have therefore added potassium ferricyanide to our samples thus converting the myoglobin to metmyoglobin. In our chromatographic and focusing experiments we have tried to obtain comparable results by application of equal amounts of protein. We have determined protein concentration by measurements at two wavelengths, 280 and 255 nm. The ratio 280/255 is for irradiated meat usually slightly lower than for the untreated sample.

The background staining is probably only partly due to aggregates because most of the aggregates with a molecular weight of more than 1,000,000 don't enter the gel at all. The background staining could be explained by assuming charge changes of the very heterogenous sarcoplasmic protein system leading to a population of molecules with almost continuously modified molecules.

M. VAN DER STICHELEN ROGIER

I have the intention to try the electrofocusing in acrylamide gels. The data of the scientific publications mainly concerns this method. You have used the electrofocusing on Sephadex. Does it represent some advantages on the acrylamide?

B. RADOLA

On disc-electrophoresis and disc-focusing part of the proteins, especially most of the aggregates will not enter the gel. This difficulty can be avoided when working with Sephadex. Soluble proteins from irradiated meat have been already studied by disc-electrophoresis, but no distinct differences have been noted between the untreated and the irradiated samples.

A. DESCHREIDER

I should like to ask M. van der Stichelen whether he has tried porosity gradients.

J. MORRE

I should like to ask whether you have carried out any tests with eggs preserved in lime, frozen eggs, and stabilized eggs, because I enjoyed myself carrying out this experiment. I then obtained electrophoresis bands on starch, which differed widely according to the method of preservation. Be careful not to confuse a lime egg with an irradiated egg. But I did not use irradiated eggs.

M. VAN DER STICHELEN ROGIER

The electrophoretic pattern of the whole fowl egg, homogeneised by mixing, presents some bands, which are not present on the patterns taken separately either from the white or from the yellow. These are perhaps aggregates or protein combinations resulting from the mixing. In the aim to elucidate this phenomenon, independent from the irradiation, we have started investigations with fresh eggs.

F. DRAWERT

May I ask a question relating to experimental procedure? On page 2 you describe the use of "Carbowax 20M"; may I ask what the purpose of using Carbowax 20M was in this case?

L. BUGYAKI

The Carbowax is an immunity-adjuvant.

B. RADOLA

I would like to add a methodological hint. Concentration of dilute protein solutions can now be easily achieved by ultrafiltration on Diaflo membranes distributed by Amicon (Oosterhout, Holland). The method is more rapid and simple than concentration with Carbowax.

J. SPAANDER

I should like to draw attention to the conclusions that M. Morre has drawn in this publications, despite his modesty. He has sait that: (1) the sale of irradiated products is surrounded by a wealth of precautions such as have never before been taken for any method of preservation; (2) the body of regulations governing these products should be international, to avoid costly and lengthy inspection procedures in each country; and (3) the identification of these fraudulently irradiated foodstuffs is a problem that has not yet been resolved.

I think, M. Morre, that in these three sentences you have summed up the entire problem, and I congratulate you on having so clearly stated what is happening in our field.

9. IDENTIFICATION OF IRRADIATED WHEAT FLOUR, IRRADIATED POTATOES AND IRRADIATED EGGS

Progress report 1.10.1967 - 1.10.1970

A. DESCHREIDER

ABSTRACT

Spectrophotometry

Spectrophotometry in U.V. cannot be helpful for the detection of the irradiation of wheat, wheat flour, eggs and potatoes, out perhaps in the case of meat and fish. It seems that spectrophotometry in IR gives encouraging results in the field of irradiated eggs.

Spectropolarimetry

This method can be used for the detection of the irradiation of wheat and wheat flour. The technique is also useful in the case of irradiated potatoes and eggs. We study now a modification of the method in order to improve its sensitivity.

Turbidimetry

This technique is useful for the detection of irradiated wheat and wheat flour, but we try to improve its sensitivity. In the case of irradiated eggs and potatoes, no positive results have been obtained.

In this investigation, covering a period of 3 years, we have studied the application of spectropolarimetry, spectrophotometry, turbidimetry and thermodifferential analysis to the analysis of irradiated foodstuffs.

Because the modifications observed in irradiated wheat flour already have been published in the report EUR 4417 f, these results are here summarized and we will develop those obtained for irradiated potatoes and eggs.

1. IDENTIFICATION OF IRRADIATED WHEAT FLOUR

Gamma rays induce physical and chemical changes in wheat flour, some of which are detected by one of the above mentioned techniques. The detection depends on the nature and intensity of the modification in question.

The results obtained can be summarized in the following way:

1.1. *Spectrophotometry*

a) in visible light: gives a measure of the degree of destruction of the carotenoids. The complete disappearance of their typical absorption curve takes place only at the dose level of 1 Mrad.

b) in U V light: cannot be used for detection of low irradiation doses.

c) in I R light: detection in this way is only possible at the level of 1 Mrad.

1.2. *Spectropolarimetry*

By this technique it is possible to detect an irradiation at the dose of 0,1 Mrad, sometimes 0,025 Mrad and one may hope that some modification of this method might succeed to improve the sensibility still more.

1.3. *Turbidimetry*

This method is more sensitive than spectropolarimetry and in a certain manner completed it. In some cases the turbidimetric curve of an irradiated sample at the dose of 0,025 Mrad is quite different from that obtained for the blanks.

It is possible that the sensitivity of this technique can be increased by the modification of the conditions of its application.

1.4. *Differential thermoanalysis*

This technique did not allow to detect the irradiation of wheat flour.

In conclusion, the work done in the field of irradiated wheat flour has demonstrated that only spectropolarimetry and turbidimetry can be used in order to detect a dose between 0,025 and 0,050 Mrad, but it is still necessary to increase the sensitivity of both techniques.

2. IDENTIFICATION OF IRRADIATED POTATOES

A batch of potatoes, variety "Bintje" was irradiated at the dose of 10,000 rad, at the CEN of Mol and the samples were stored during 9 months at +5° C.

The peeled potatoes were treated with absolute alcohol, and the solution used for spectrophotometric examinations. The residue, dried and crushed in a mortar, gave a powder used for turbidimetric and spectropolarimetric analysis.

Neither spectrophotometry in UV and IR nor in visible light gave a differentiation useful for the detection.

In the field of turbidimetry, soluble substances from starch in the extract from the potatoes powder give a turbidimetric curve. But unfortunately, as can be seen in figure 1, contrary to the results obtained for wheat flour, the non irradiated samples show a very pronounced turbidimetric curve which during storage more and more approches to the curve of the irradiated samples. For these reasons turbidimetry cannot help in differentiating between irradiated and non irradiated potatoes.

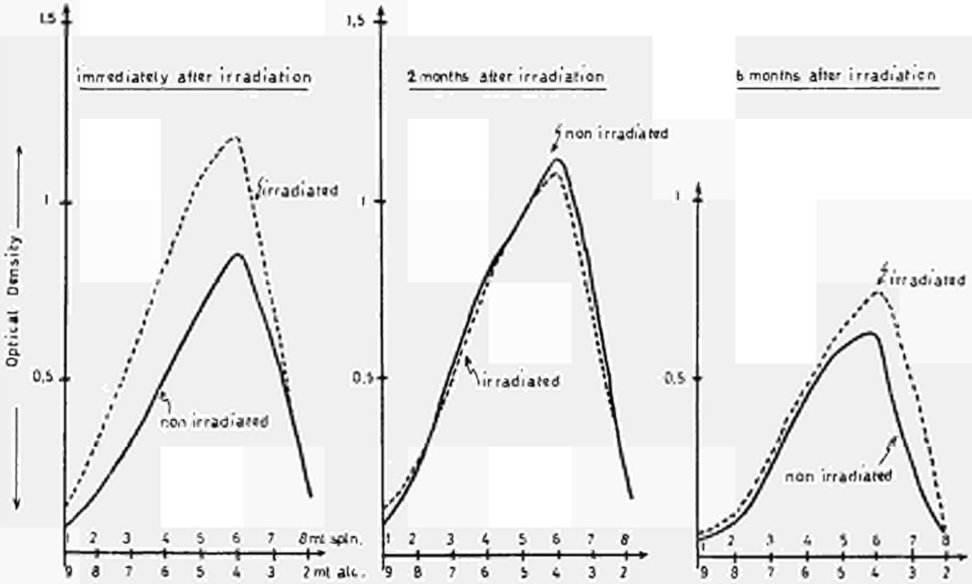


Fig. 1. Turbidimetric curves.

As one can see from figure 2, the spectropolarimetric curves obtained from the extract used for turbidimetry, show that after irradiation the optical rotation decreases with increasing wavelenghts and tends to zero for the irradiated samples.

The sharp difference between blank and irradiated samples shows that spectropolarimetry is a potential detection method at the dose used for blocking germination.

That means that further systematic research must be carried out

1) in order to increase sensitivity
2) on different potato varieties
3) during storage periods up to 12 or 15 months.

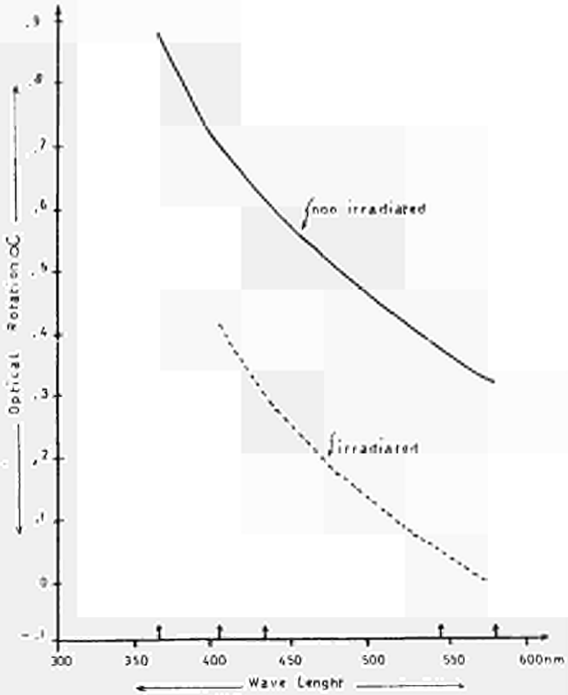


Fig. 2. Spectropolarimetric curves of potato six months after irradiation.

3. IDENTIFICATION OF IRRADIATED EGGS

3.1. *Experimental*

3.1.1. Conservation of the eggs in the frozen state and irradiation

The trial samples were batches of fresh eggs coming from the same poultry battery.

We worked on whole eggs without mixing yolk and white, after mixing them and on separated yolk and white of eggs.

Equal quantities of product were put in polyethylene containers and deepfrozen at —35° C, then stored at —20° C. The samples were kept in the frozen state during the dispatch to and return from the CEN of Mol.

At the CEN, they were irradiated in a gamma cell with a cobalt 60 source of 3,100 curies.

They received a dose of 0,5 and 1 Mrad and in each case, the non irradiated sample was joined to the shipment.

Before taking analytical samples, the product was thawed during 24 hours at 2-3° C, then a few hours at 15° C. Immediately after sampling, the material was again deepfrozen at —35° C and kept at —20 C.

3.1.2. Separation of the egg components

The chalazion was eliminated by filtration of the white of egg through cotton gauze.

3.1.2.1. Treatment of the egg white

3.1.2.1.1. Lyophylisation

5 ml of the white of an egg of each sample were lyophilised in order to have a powder sample for analysis.

3.1.2.1.2. Dialysis

A quantity of 10 gr of egg white was put in a dialyser tubing and dialysed against acetic acid 0,05 N during 8 hours at ambiant temperature and 12 hr in the refrigerator; then 4 hr against pure water. The solution after dialysis was put in a volumetric flash of 50 ml and completed to volume with water.

3.1.2.2. Treatment of egg yolk

On 10 gr of egg yolk one pours, under vigorous stirring, 100 ml of a chloroform-ethanol mixture (1/1). After centrifugation, the residue is washed twice with the same solvents mixture and dried. On the other hand, the yellow solution obtained is evaporated in a Rotavapor, and then dried under vacuum.

In this manner one has isolated the carotenoïds and lipids of the yolk.

3.1.2.3. Treatment of whole egg

After irradiation of the white plus yolk, without mixing them, the yolk was separated from the white of the egg and the treatment of both was the same as described above.

3.1.2.4. Treatment of the mixed white and yolk

When white and yolk were mixed before irradiation, the separation of the components was made in the following manner:

On 20 gr of the mixture, 100 ml of chloroform-ethanol (vol/vol) were poured under stirring.

The insoluble matter was separated by centrifugation and after two washings with the same solvents, was dried under vacuum.

The yellow extract was evaporated in the Rotavapor and the residue dried under vacuum.

The dry insoluble matter was treated with 30 ml of water, centrifugated after 20 min of agitation. The supernatant solution was decanted in a 50 ml volumetric flask. The residue was washed twice with a little of water and the washing water added to the flask.

The volume was completed with water.

The residue obtained after the water extraction was dried under vacuum on phosphorus pentoxide.

A part of the water extract was dialysed against acetic acid 0.05 N and then against water, then brought to a volume of 50 ml; the other part of the extract was lyophilised.

An other extraction process was also applied, giving three components in one operation. To 30 ml water 10 gr of product is added under magnetic stirring. After a few minutes 30 ml of chloroform are added and after 15 minutes of stirring the mixing is centrifugated. One becomes an aqueous phase containing the proteins of the white egg, a solid phase with the proteins of the yolk and a chloroformic phase with the carotenoïds and lipids. The liquid phases are separated with the aid of a decantation funnel. After careful washing, they are brought to a volume of 50 ml and the solid matter is dried under vacuum.

3.1.3. Analytical investigations on the isolated components

Carotenoïds and lipids were extracted by treatment with chloroform-ethanol (1/1), the egg white proteins were obtained by lyophilisation or dialysis of the white as well as by water extraction. The proteins of the egg yolk are contained in the insoluble residue remaining after solvent extraction and/or water extraction of the samples.

Spectropolarimetry was used on an aqueous solution of the egg white or on an aqueous extract of the mixed white and yolk, on the carotenoïds-lipids dissolved in hexane and on yolk proteins dissolved in NaOH 0.1 N.

Turbidimetry was applied on the aqueous extract containing egg white proteins.

Spectrophotometry was performed in visible light on the carotenoïds-lipids dissolved in chloroform, and UV photometry on the solutions used for spectropolarimetry.

IR spectrophotometry was applied to the lyophilised egg white, to the proteins of the yolk using KBr pellets and to the carotenoïds-lipids dissolved in chloroform.

3.2. *Results and discussion*

3.2.1. Spectrophotometry

3.2.1.1. In visible light

The solution in chloroform of carotenoïds has a spectrum with the following absorption bands: 435-455 and 485 nm, the strong pronounced being at 455 nm. The irradiation has no influence on the nature of the

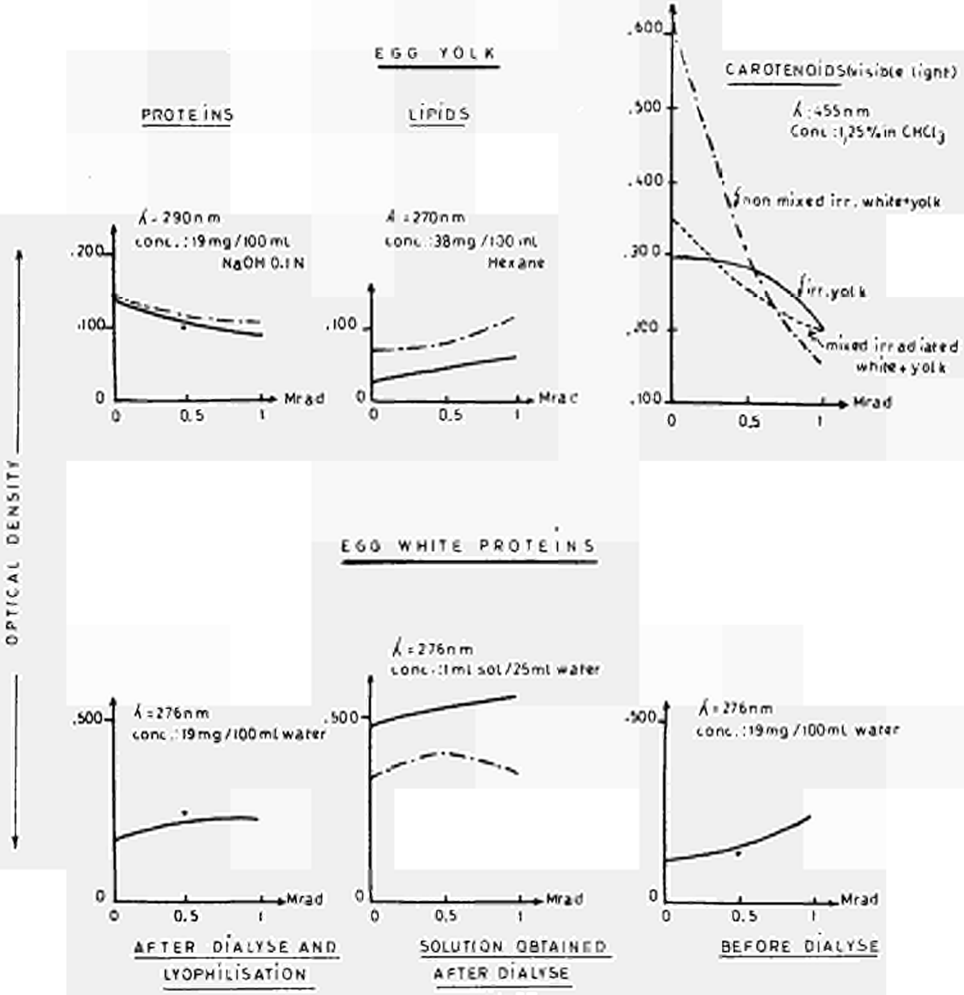


Fig. 3. Spectrophotometry in U.V. light, variation of the max. absorption.

absorption curves, but as can be seen on fig. 3, the general absorption decreases with the dose of gamma rays.

This is due to the activation of the autooxydation, which is more important in the case of irradiated white and yolk together.

3.2.1.2. In UV

3.2.1.2.1. Lipids

Lipids of egg yolk dissolved in hexane have an absorption spectrum with two peaks at 270 and 280 nm. In figure 3 we observe that the maximum of the curve increases with the irradiation dose. This is in agreement with the above mentioned point, the autooxydation has an action on the lipids producing more derivates of them, having an absorption at 270 nm.

3.2.1.2.2. Proteins

Aqueous solutions of proteins from egg white have an absorption peak at 276 nm and those of yolk dissolved in NaOH 0,1 N, at 282 and 290 nm.

It can be seen from figure 3, that whatever technique of extraction is used, the maximum of the yolk proteins decreases whereas for the proteins of the white it increases with the irradiation dose.

This increase is clearly visible when one reports on a graph the area of the UV curve as a function of the irradiation dose from the maximum till the point where it reaches the zero optical density.

This is illustrated in figure 4, where it can be observed that the increase is much more important where the egg white was irradiated alone.

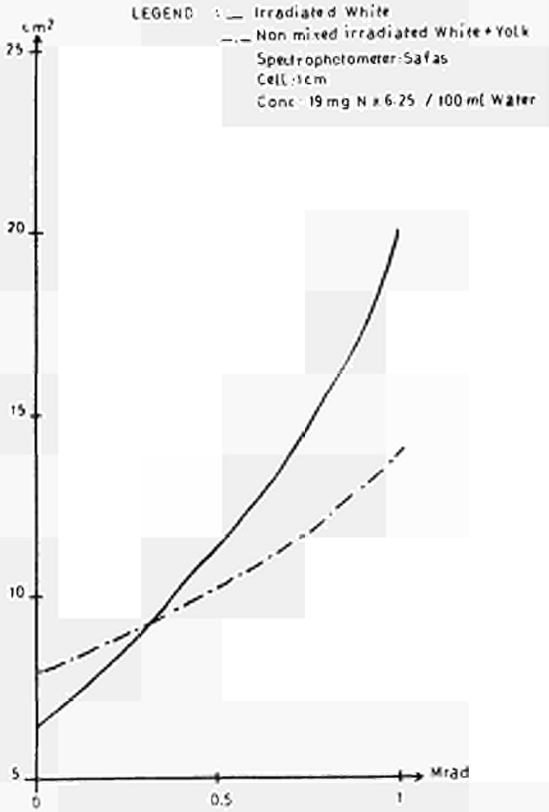


Fig. 4. Areas of the U.V. curves — Proteins.

We can conclude that UV and visible spectrophotometry show no modification of the nature of the absorption curves, only the general absorption value may be affected either in a negative or in a positive way.

This fact can be useful in studies of modifications compared to a blank, but cannot serve as a means of detection.

3.2.1.3. In IR

The assays in this field are still under way and we can present no conclusion at the present time for this technique.

3.2.2. *Turbidimetry*

Turbidimetry occurs in aqueous proteins solutions of egg white after dilution in order to have a concentration in proteins of 0,25 %.

Typical turbidimetric curves are given in figure 5 and the histograms of their maximum show that in each irradiation condition there appears a drop in their value, thus in the areas of the corresponding curves.

Unfortunately if the diminution of the turbidimetric values can be used in comparative studies, it cannot be helpful for the detection of the irradiation of an unknow sample at the dose generaly applied in the case of this product.

3.2.3. *Spectropolarimetry*

The spectropolarimetric curves have been drawn on the base of the $(\alpha)\lambda$ values obtained by the formula:

$$(\alpha)\,\lambda = \frac{100 \times \alpha}{l \times c\,(\mathrm{g}/100\ \mathrm{ml})},$$

in which α is the optical rotation at the wavelenght used, l the length of the polarimeter tube in dcm and c is the concentration in g per 100 ml of the solution.

3.2.3.1. Carotenoïds and lipids

Carotenoïds and lipids extracted from the egg yolk and dissolved in hexane give spectropolarimetric curves in which there are two inflection points, one at 312 nm and the other at 434 nm, the second being more pronounced.

As one can see from figure 6, the $(\alpha)\ \lambda$ values of the non-irradiated eggs are positive and become negative after 0,5 Mrad, the negative rotation being very pronounced after 1 Mrad.

The values at 312 nm and 404 nm reported on the histograms of figure 7 show that the negative values are the most important when the irradiation occurs on the mixed yolk and white.

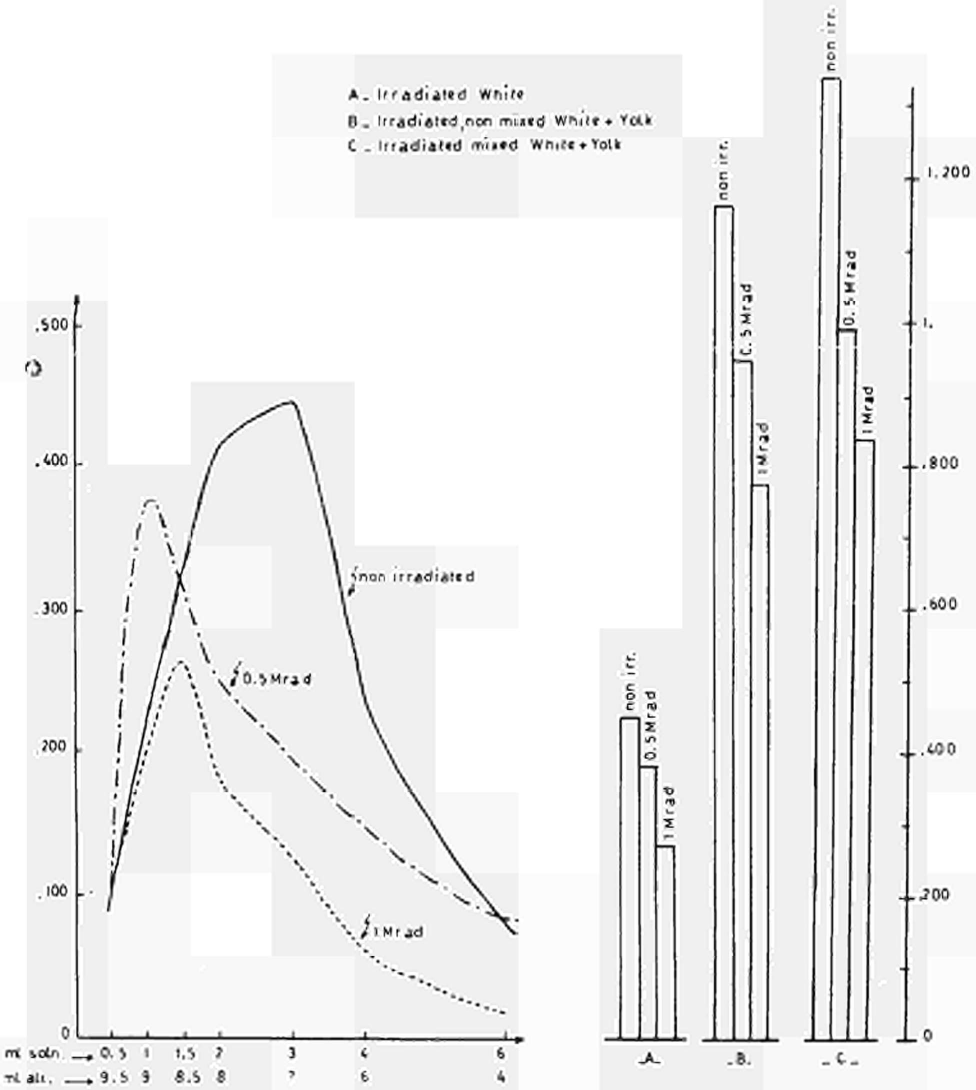


Fig. 5. Turbidimetry of dialysed egg white.

Pending confirmation of the registered results by complementary assays on fresh eggs and irradiated eggs, the spectropolarimetry of the carotenoïds-lipids can be used for the detection of the treatment.

2.3.2. *Proteins*

At the present time of this research we can only rely upon the results obtained for the irradiation of non mixed yolk and white.

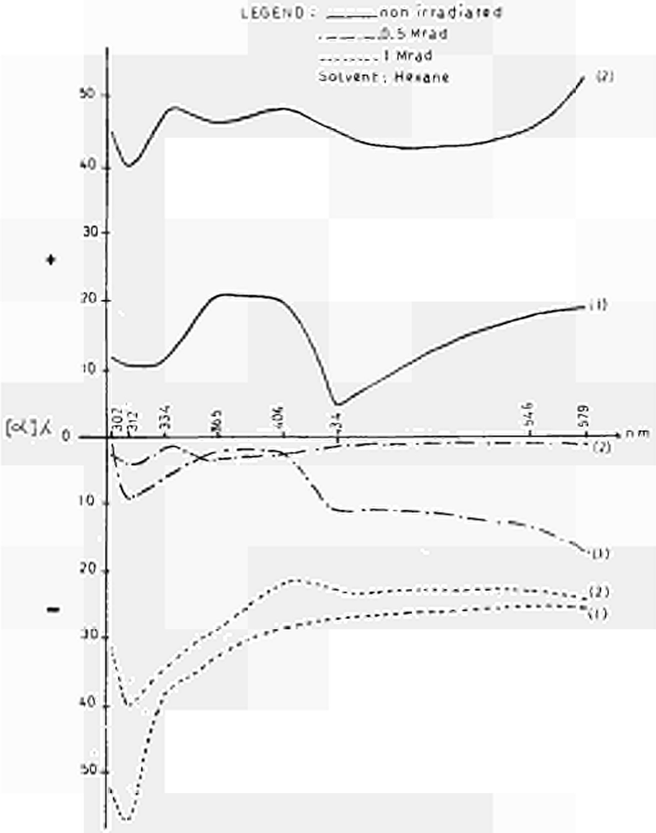


Fig. 6. Carotenoids + lipids.
(1) Curves of irradiated mixed white + yolk.
(2) Curves of irradiated non mixed white + yolk.

Aqueous solution of lyophilised egg white and of yolk proteins dissolved in NaOH 0,1 N, show negative values for the $(\alpha)\lambda$. Only after an irradiation dose of 1 Mrad, does egg white show a very important negative deflection at 324 and 365 nm, the $(\alpha)\lambda$ at the other wavelengths being very near those of the blank, as we can see in figure 8.

3.3. *Conclusion*

We have tried to detect the irradiation of wheat, wheat flour, potatoes and eggs by the techniques mentioned in this contract. In the field of irradiated eggs, IR spectrophotometry and spectropolarimetry are always in way. For irradiated wheat and flour, spectropolarimetry and turbidimetry give promise for the detection. But further research must be done in order to increase the sensitivity.

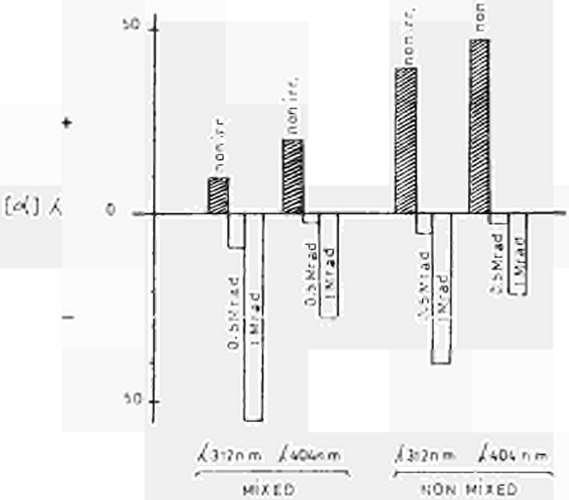


Fig. 7. [α] λ at 312 and 404 nm of irradiated eggs.

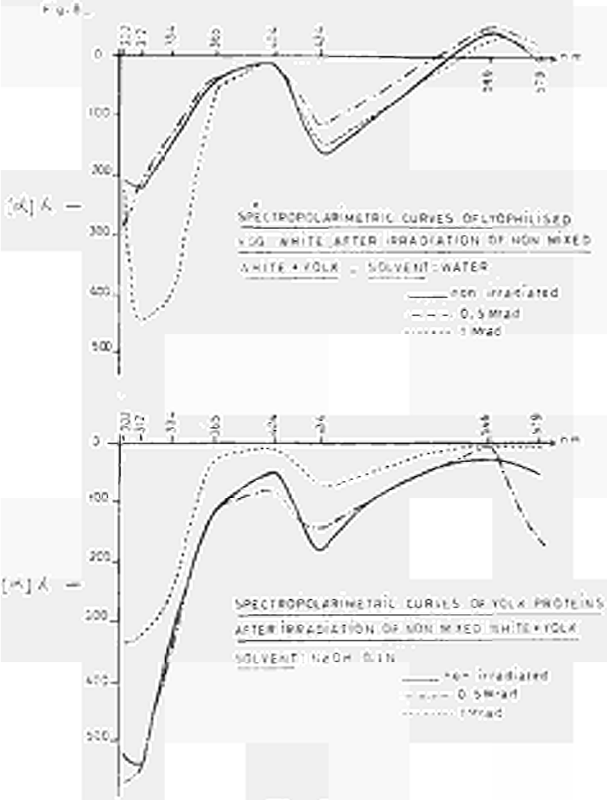


Fig. 8.

Only spectropolarimetry has given encouraging results for irradiated potatoes but they must been confirmed and we must try to increase the sensitivity of the method.

For irradiated eggs, spectropolarimetry seems also to be useful, here also we must have confirmation of the obtained results and the investigations in the field of IR spectrophotometry must been completed.

10. CHANGES IN IRRADIATED FOOD PRODUCTS MEASURABLE BY DIFFERENTIAL THERMAL ANALYSIS

Progress reports 1.4.1969 - 1.4.1970 and 1.4.1970 - 1.10.1970

E. MAES

ABSTRACT

A marked decrease can be observed in the peak area in function of the irradiation dose for eggs.

The work will be carried out on irradiated wheat and wheat products and also on eggs, in order to confirm the preliminary results obtained.

1. *Period 1.4.1969 - 1.4.1970*

Apparatus:

— Perkin-Elmer Differential Scanning Calorimeter DSC-1 B;
— temperature range: 173-825° K;
— maximum load: 20 mgr;
— scan speed: 0,5-64° C/min.

The great advantage of the apparatus lies in the fact that during the operation a given mass (solid or liquid) can be added to the pans through a window in the ovenwall.

The temperature's increase and decrease system can be linearly programmed or operated manually; besides the thermogram, the real oventemperature is continuously recorded in the course of the operation.

As the differential scanning calorimetry is not a real DTA-system the differential temperature between sample and reference is not measured (by thermocouples) but on the contrary the quantity of energy necessary to maintain the temperature of both pans at a constant level is recorded; so e.g. when 10 microliter of aq. dist. are evaporated, at a given temperature, in one pan, the recorded thermogram represents an energy-versus-time graph; the area under the curve is proportional to the sum of millicalories necessary to heat the water up to the given temperature and to evaporate the water at this temperature; this is true for an isothermic as for a programmed temperature increase as well.

Since May 1969 we carried out several preliminary tests with the intention to study the possibility of the D.S.C.

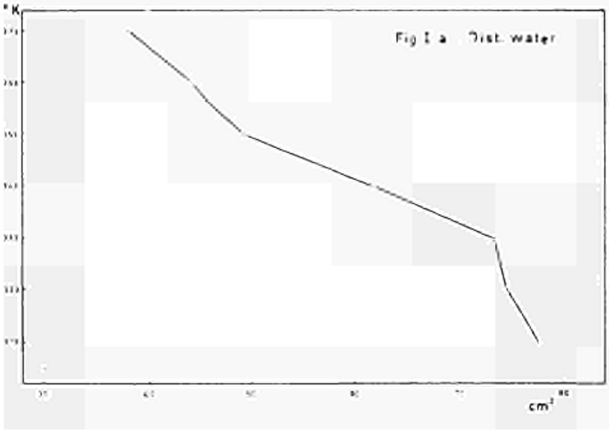
Later on the apparatus was tested with aq. dist. and we detected some anomalies in the energy measurement during evaporation, probably caused by the formation of vapour bubles, which can only escape if the increase of the vapour pressure (with increasing temperature) is high enough.

The differences between the values of heat energy calculated from the peak area and the theoretical values are the greater as the temperature is higher.

On the other side one has to take into account that in the D.S.C. the produced water vapour is immediately removed from the water surface by a dry nitrogen stream; in this way the mass transfer from the liquid surface to the vapour phase will be accelerated, as the rate of the transition from liquid to vapour is controlled by the rate at which vapour is removed from the region of the liquid-vapour interface.

On the attached figure one can see the peak areas (planimetry) for temperatures (isothermic) between 313 and 373° K for aq. dist.; the theoretically calculated area was obtained for 313° K but the peak surface decreases continuously in function of temperature.

If both pans of a given mass (14 mgr, ± 0.05 mgr) are filled with the same mass of dry product, lyophilized shrimp, potato, pea, etc., (7 mgr, ± 0.05 mgr), and if to one of the pans 10 microliter of aq.dist. are added and evaporated at given temperatures (isothermic) the peak area will be smaller at 313° K and greater at 373° K, than for water only (figure).



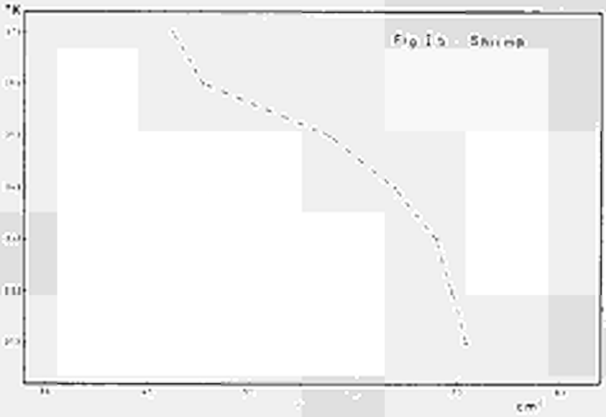


Fig I b : Shrimp

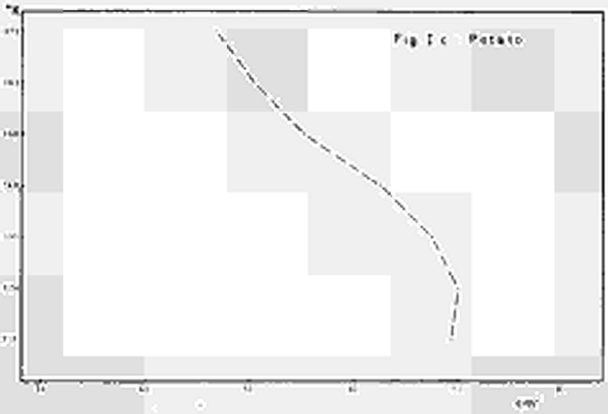


Fig I c : Potato

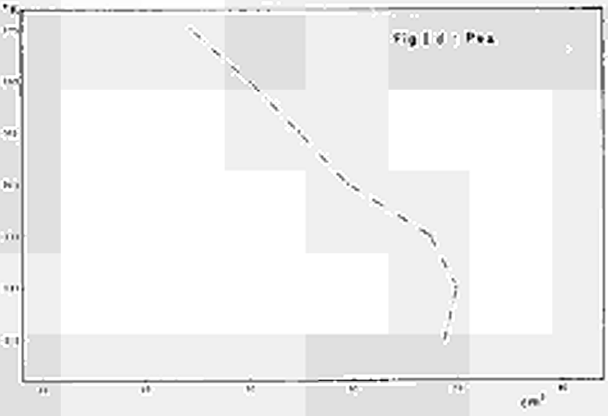


Fig I d : Pea

Imbibition heat and retention of bound water, after rehydration of the dry product, could be at the origin of these changes.

For the preliminary tests we choose shrimp, potato, pea, apple, etc. with very different protein and starch contents, as in cereals and eggs these contents are practically constant. The further intention is to verify if the changes in the structure of the biocolloids are important enough to permit the detection of changes in the quantity of heat needed for the evaporation, at a given temperature, of the imbibition water after rehydration of the dry product.

Possible changes in the composition of the biocolloids will surely have an influence on the rehydration process (water binding) and on the evolution of the evaporation (departure of the imbibition water).

We will also study the DTA characteristics of non-irradiated wheat and egg and of extracted protein fractions of both.

In the last stage irradiated samples and fractionated components of them will be studied.

2. *Period 1.4.1970 - 1.10.1970*

In the first report we discussed the results of tests in which the evaporation temperature was adjusted manually in a few seconds.

From these results one could conclude that the amount of energy required to evaporate an identical mass of water at 373° K is smaller than the amount required at 313° K, with a gradual course between those both temperatures, this is naturally inadmissible from a theoretical viewpoint.

In extensive tests we studied the different operation conditions and the influence of several parameters. The results led to the conclusion that the measurement of the energy after a too rapid temperature increase was not correct, therefore the modus operandi was adapted, in this way that the increase between room temperature and working temperature was progressive and much slower, by using the programmation (8° C/min). From then the measured amount of millicals, proceeding from the peak area determined by planimetry, agreed much better with the theoretically calculated amount and remained more or less constant for the working temperatures between 313 and 373° K.

The new modus operandi is described in annex 1.

The new tests such as the former were carried out with lyophilized products (table I).

The results are given in table II.

DISCUSSION

However the figures of several products show some parallelism, the contents of the components (protein, starch, fat and sugar) seem not to have an influence. More work will be needed before we can try to give some conclusions.

IRRADIATED EGGS

Mr. Deschreider transferred to our laboratory three series of three samples each, treated with 0, 0.5 and 1 Mrad gamma rays:

Nos 1 to 3: separated egg white, irradiated in the frozen state (—35° C), defrozen and lyophilized.

Nos 4 to 6: the same as nos 1 to 3, but dialyzed after irradiation.

Nos 7 to 9: proteins isolated from the yolk, irradiated in the frozen state (—35° C), defrozen, extraction with chloroform/ethanol 1 : 1 vol., centrifugation, dessication under vacuum of the insoluble.

All the eggs originated from the same battery of hens. From each sample from 1 to 6, 3 to 4 mgr were put in one pan (6 to 7 mgr for the Nos 7 to 9), to this pan were added 10 microliter of aqua dist., the sample was allowed to regenerate for 15 min. The pans were heated with a temperature increase of 8° C/min (programmed), till complete evaporation of the water. The results are given in table III.

DISCUSSION

A marked decrease can be observed in the peak area in function of the irradiation dose; this argues a less stronger binding of the water in the irradiated colloids.

The next work will be carried out on irradiated wheat (and wheat products) and eggs as is, because on October 1st a Caesium-137 source will be available in this laboratory. As before the extracted components will be placed at our's disposal by Mr. Deschreider.

Annex I. — Modus operandi

Both pans of the same weight (14 ± 0.05 mg) are filled with 7 ±0.05 mg of the lyophilized product and put in the apparatus.

Starting temperature 273° K, manually increased till room temperature, varying from 305 to 310° K, mostly 305 or 306° K.

Addition of 10 microliter ±0.02 microliter aqua distillata in one pan.

Programmed temperature increase 8° C/min, in seven different tests, respectively to 313, 323, 333, 343, 353, 363 and 373° K.

Isothermic till the complete evaporation of the water.

Control by weighing the pans.

All tests in duplicate; the maximum deviation between peak areas of duplicates is ±4 %.

Annex II

Table I. — Composition of products.

Product	Approximative percentage (on dry basis) of				
	Protein	Starch	Fat	Sugar	Acids
Shrimp	82	—	10	—	—
Leek	20	50	3	—	+
Celery	10	20	1	—	+
Pea	20	55	2	+	—
Tomato	20	60	4	+	++
Plum	3	—	—	65	++
Pear	3	+	—	65	+
Apple	—	+	—	75	++
Potato	8	75	0.5	+	+

Annex III

Table II. — Peak areas at different evaporation temperatures for different products (cm²)

°K	313	323	333	343	353	363	373
Shrimp	68.5	69.1	68.4	67.7	68.3	68.0	70.1
Leek	66.2	65.4	66.2	66.1	66.7	65.5	64,8
Celery	69.2	67.2	68.2	68.6	68.4	69.7	69.5
Pea	69.8	69,8	69.3	68.4	68.2	67.3	68.7
Tomato	65.6	66.5	67.4	67.2	69.0	68.3	66.7
Plum	65.9	66.2	66.7	66.6	66.9	66.6	67.0
Pear	—	—	68.3	68.0	67.2	68.8	69.0
Apple	66.8	68.5	69.2	69.3	69.3	69.7	71.2
Potato	70.3	68.2	68.9	67.5	68.4	69.0	68.5

Annex IV

Table III. — Irradiated eggs. Peak areas for the evaporation of bound water (programmed temperature increase).

Samples No.	Radiation dose Mrad	Peak area cm^2
1	0	37.0
2	0.5	31.9
3	1	26.0
4	0	26.7
5	0.5	25.0
6	1	21.4
9	0	78.3
8	0.5	77.3
7	1	73.7

DISCUSSION

A. DESCHREIDER

I should like to add that if any of the methods are to be rejected for the products examined, this does not mean that they would not be suitable for meat, fish or other products.

CHAIRMAN

This is true for all experiments reported here. Our conclusions are limited to those food stuffs which we have actually examined.

H. SCHERZ

I believe that spectro-polarimetry is a very promising method in the case of starch. I do have one question: The changes in the spectropolarimetric measurements which you have reported are, I believe, due to changes in secondary structure. Did you investigate the possibility that such changes in the spectropolarimetric behaviour also occur after heating?

A.R. DESCHREIDER

At least as far as wheat and its derivatives are concerned, heating the product does not give rise to a spectropolarimetric curve, at any rate below 120° C—and this temperature is not in fact exceeded. No method would heat wheat or flour to such temperatures to improve it. In some cases you heat to 60 or 70° C for improvement, or else the wheat has to be heated with hot air to dry it, but these temperatures have no effect on turbidimetry and spectropolarimetry. As for potatoes, the problem does not arise: they are not heated to preserve them. With eggs, since they may be pasteurized, we still have to study the effect of this action, either of heat or of some other means, such as microwaves, to establish whether the response is similar to irradiation or not.

J. SPAANDER

Could you explain to me what you are attempting to identify by means of the differential thermal analysis technique?

A. DESCHREIDER

I am sorry that Professor Maes is not here. Briefly, a measurement is made of the number of millicalories needed to eliminate the bound water of a product, both irradiated and non-irradiated. To do this, we take a given quantity of a sample (7 mg) add to it 10 mg of water, and leave the sample at rest to fix a certain quantity of water depending upon its capacity. This water is then eliminated with the aid of a programmed temperature, and the disappearance of this water as the temperature rises is expressed in the form of a curve, the area of which is measured. When the product has been irradiated, the area of the curve obtained with the thermal analysis apparatus is smaller than the area that is observed for a product that has not been irradiated. That is, the non-irradiated product fixes water more energetically than the same product does when it has been irradiated. Hence the reduction in the area of the curves.

This is the explanation that I am able to give you, but as regards entering into the details of the technique, it is Professor Maes who is conversant with them. I should, however, like to add that, in my opinion, we need to wait until the work has been pursued further, and then see in what direction we can advance with this technique.

CHAIRMAN

I believe these diagrams reflect not only the loss of water but rather any process that requires heat, e.g. melting processes or changes in crystal structure. When well defined polymers are studied by DTA-analysis, several humps are often found corresponding to changes in structure which occur when the polymer is gradually heated. With foods of very complex chemical composition I would not expect a very characteristic DTA-curve. Perhaps DTA will be useful for studying rather homogeneous products such as starch or pure proteins. The more complex the mixture, the less characteristic will be the DTA-curve. If the unirradiated food shows a rather uncharacteristic curve, it appears that radiation will not cause very characteristic changes in such a curve.

B. RADOLA

I would like to ask a question concerning Table 3 of Annex 4. You have compared three samples: 1) irradiated and untreated proteins, 2) dialyzed proteins and 3) proteins from egg yolk. The greatest difference appeared in the undialyzed samples. Perhaps it would be interesting to do some experiments with the diffusable material.

A. DESCHREIDER

Your question is pertinent, and this is obviously to be considered. In the present state of things, it is a little premature. But you are quite right, it is possible that the products which dialyse and pass through the membrane would give a more significant response than the product which is situated in the membrane, and thus in the dialytic sac. We shall see; this is a good suggestion.

REPORTS ON RESEARCH PERFORMED BY OTHER LABORATORIES

Research performed by

L. SAINT-LÈBE, G. BERGER,
with the technical collaboration of J.P. AGNEL
Service de Radioagronomie, CEN, Cadarache, France

G. MAGAUDDA
Laboratorio applicazioni in Agricoltura del CNEN, Rome, Italie

J. MORRE
Laboratoire de Radiobiologie du Service Vétérinaire, Paris, France

Chairman: J.F. DIEHL

11. ASSAINISSEMENT DE L'AMIDON INDUSTRIEL PAR IRRADIATION: MODIFICATIONS BIOCHIMIQUES ET TEST D'IRRADIATION

L. SAINT-LÈBE, G. BERGER, with the technical collaboration of J.P. AGNEL

ABSTRACT

Radiolysis products are identified and titrated with a view to supplementing and elucidating studies on the toxicological evaluation of irradiated starch, the subjects being microorganisms and higher animals. At the moment the only substances certainly identified are malonic aldehyde, formic aldehyde and hydrogen peroxide. The formation of malonic aldehyde is specific to the irradiation of starch, its titration forming the basis of a test which makes it possible to determine the dose absorbed to ±30 krad, provided the humidity of the sample, the date of irradiation and the storage temperature are known.

L'étude entreprise au Commissariat à l'Energie Atomique par le Service de Radioagronomie (Département de Biologie) s'inscrit dans le cadre général de recherches sur la pasteurisation des produits alimentaires pulvérulents. Pour assainir de tels aliments destinés aux hommes et aux animaux le technologue est aujourd'hui démuni et fait parfois appel à des produits qui risquent d'être toxiques et dont l'utilisation est de plus en plus controversée. L'irradiation, seule ou combinée à d'autres traitements, a une chance de s'imposer car elle conduit à une pasteurisation efficace du produit à l'intérieur de son emballage et que le coût sera peu élevé en raison du caractère continu de la production. Les risques de toxicité en principe mineurs, restent à évaluer.

Le problème pris en compte dans un premier temps est la radiopasteurisation de l'amidon de maïs, composant de base d'un grand nombre de produits alimentaires manufacturés (plats précuisinés, potages en sachet, farines diététiques, baby foods... aliments du bétail), et représentant caractéristique de la classe des amidons et de certains de leurs dérivés. L'évaluation toxicologique de l'amidon irradié constitue la partie la plus importante de ces travaux dans la mesure où elle conditionne l'autorisation par les instances nationales (Conseil National Supérieur d'Hygiène, Académie de Médecine) d'irradier le produit. Cette démarche est abordée par trois voies: essais sur animaux, essais sur microorganismes (1), et enfin recherche des produits de radiolyse dont on évoque ici les premiers résultats.

A l'heure actuelle, trois produits ont été identifiés avec certitude: aldéhyde malonique, aldéhyde formique, peroxyde d'hydrogène; on a étudié leur formation en fonction des conditions d'irradiation, et on propose un test qui permet de déterminer la dose reçue par l'amidon.

a) *Aldéhyde malonique*

L'aldéhyde malonique (2) est mis en évidence par colorimétrie grâce à la formation d'un complexe avec l'acide 2-thiobarbiturique, absorbant à 532 mμ (3-10). La chromatographie sur couche mince, l'examen au microscope des cristaux obtenus, la position du maximum d'absorption, ont confirmé que dans l'amidon irradié le produit responsable de cette coloration est bien l'aldéhyde malonique et non le glyoxal. La densité optique croît proportionnellement à la dose reçue (fig. 1) et décroît avec l'hydratation de l'amidon ainsi qu'avec la température d'irradiation. La nature des liaisons 1-4 ou 1-6 ne semble pas avoir d'influence et il est vraisemblable que cet aldéhyde provient de la coupure oxydative du 2-désoxyglucose présent dans les sucres irradiés (11).

b) Aldéhyde formique

L'aldéhyde formique (12) est identifié dans l'amidon irradié par les méthodes de Schryver (13), de G. Denigès (14) et de T. Nash (15) qui

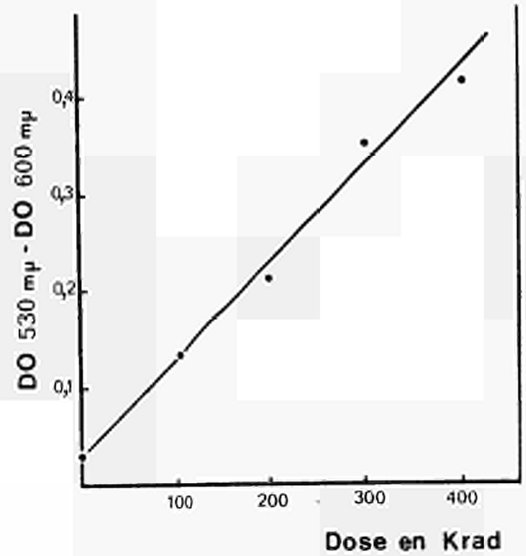


Fig. 1. Densité optique, après réaction avec l'acide thiobarbiturique, des extraits aqueux d'amidon industriel (12,5 % d'humidité) irradié à différentes doses.

donnent des résultats concordant à 15 % près. La spécificité de ces techniques a été vérifiée sur un grand nombre d'aldéhydes, de cétones, de sucres et d'acides susceptibles de se former lors de l'irradiation.

La quantité d'aldéhyde formique croît proportionnellement à la dose (fig. 2) et il y en a de l'ordre de 20 µg/g/Mrad à 25 °C immédiatement après irradiation d'un amidon à 12,5 % d'humidité. A dose constante la quantité d'aldéhyde augmente avec la température et la teneur en eau ce qui permet de supposer que son mode de formation est différent de celui de l'aldéhyde malonique. Stable à 5 °C, elle décroît rapidement au cours du stockage à la température ambiante pendant les 15 premiers jours; après 3 mois elle est encore, selon les échantillons, comprise entre 9 et 13 µg/g/Mrad.

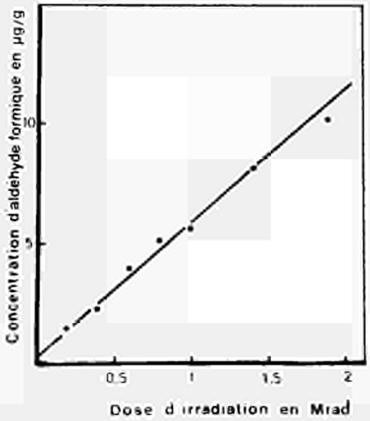


Fig. 2. Quantité de formaldéhyde par gramme d'amidon, obtenue lors de la première extraction aqueuse ($\rho = 60$ %), en fonction de la dose. Ici les mesures ont été effectuées un mois après l'irradiation.

c) *Peroxyde d'hydrogène*

L'amidon industriel contient environ 13 % d'eau. Sous irradiation, il se forme du peroxyde d'hydrogène (16) que l'on dose par le réactif au thiocyanate d'ammonium-sulfate ferreux (17) ou par le sulfate de titane (17). Les deux méthodes donnent des résultats pratiquement identiques. D'autre part, la catalase supprime toute coloration.

Le peroxyde d'hydrogène n'apparaît dans l'amidon irradié (13,75 % d'humidité, température de 25 °C) qu'à partir d'une dose de 100 krad. L'amidon industriel contient en effet de l'anhydride sulfureux (35 µg/g environ) provenant de la technologie de fabrication, qui empêche, tant qu'il est présent, le peroxyde de se former. La concentration de ce dernier est proportionnelle à la dose d'irradiation entre 100 et 400 krad, avec un coefficient de 6,6 μg/g/100 krad, après quoi la courbe s'infléchit (fig. 3). Elle augmente avec la teneur en eau, est négligeable au dessous de 0°, passe par un maximum entre 25 et 60 °C, puis décroît au delà. Un autoclavage de 1 h 30 à 130 °C détruit la totalité du peroxyde formé, mais ce traitement est sans effet lorsqu'il précède l'irradiation. La présence d'oxygène est évidemment nécessaire à la production de peroxyde d'hydrogène. L'influence de la structure du glucide n'a pas encore pu être élucidée.

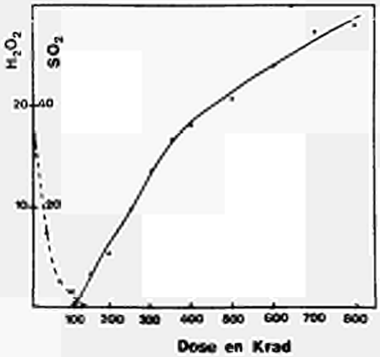


Fig. 3. Formation de peroxyde d'hydrogène et disparition de l'anhydride sulfureux en fonction de la dose. Amidon à 13,75 % d'humidité, irradié en présence d'air, à la température ordinaire.

—— μg de peroxyde d'hydrogène par gramme d'amidon.

- - - - μg d'anhydride sulfureux par gramme d'amidon.

L'évolution au cours du temps dépend encore ici de la température: stable à — 50°C pendant plus d'un mois, la concentration est presque nulle au bout de 2 jours à 25 °C. Il y a vraisemblablement interaction de l'eau oxygénée avec les sucres (18), les cétones et les aldéhydes radioformés.

d) Test d'irradiation

Le test proposé (19) est basé sur le dosage de l'aldéhyde malonique; sa spécificité a été vérifiée sur des amidons autoclavés ou provenant de maïs ayant subi divers traitements (maïs échauffés en cellule ou en tas, conservés en cribs ou sous atmosphère confinée), la coloration obtenue est comparable à celle enregistrée après une irradiation à une dose de 10 à 30 krad.

L'évolution au cours du temps a été étudiée sur 3 échantillons différents (amidons de maïs, fécule de pomme de terre, amidon prégélatinisé) d'humidités voisines des valeurs commerciales et conservés à diverses températures (5-15-25° C). La vitesse de disparition de l'aldéhyde croît avec la température de stockage et avec l'humidité de l'amidon. On a pu montrer que l'eau oxygénée radioformée était responsable de cette décroissance.

Lorsque la nature de l'amidon, la température et la durée de conservation sont connues cette méthode permet de déterminer la dose avec une précision, variable selon les cas et de l'ordre de 15 à 30 krad. Il est encore possible de détecter l'irradiation d'un amidon même après trois mois de stockage à 15° C à condition qu'il ait reçu une dose d'au moins 25 krad. Cependant il faut souligner que le test ne peut s'appliquer ni au maïs irradié dès la récolte par suite d'une teneur en eau trop élevée et de la présence de protéines, ni à de l'amidon extrait par voie humide du maïs irradié.

Les études dans ce domaine, entreprises depuis environ 3 années, sont poursuivies à l'aide de techniques telles que la colorimétrie, la chromatographie sur couche mince, sur colonne, ou en phase gazeuse. Dans un deuxième temps, il est envisagé de coupler la chromatographie en phase gazeuse à la spectrographie de masse, et d'utiliser de l'amidon marqué au carbone 14 afin d'augmenter le pouvoir de résolution de certaines méthodes d'identification.

BIBLIOGRAPHIE

1) A. MUCCHIELLI, D.E.A. Marseille (1970).
2) G. BERGER, L. SAINT-LÈBE, C.R. Acad. Sci. **268** (1969) 2620.
3) G. THIEULIN, J. MORRE, L. RICHOU, Ann. Al. Nut. **17B** (1963) 385.
4) J. MORRE, Ann. Chim. **4**, (1969) 227.
5) J. MORRE, C.R. Acad. Sci. **265**, (1967) 462.
6) N.L. SMITH, J. TINSLEY, E.C. BUBL, Food Technol. **14** (1960) 317.
7) R.O. SINNHUBER, T.C. YU, Food Technol. **12**, (1953) 9.
8) R.O. SINNHUBER, T.C. YU, Food Research **23**, (1958) 626.
9) W.G. JENNINGS, W. DUNKLEY, H.G. REIBER, Food Research **20**, (1955) 13.
10) A.R. DESCHREIDER, R. MEAUX, Ind. Al. Agr. **86** (1969) 519.
11) H. SCHERZ, Nat. **219** (1968) 611.
12) G. BERGER, L. SAINT-LÈBE, C.R. Acad. Sci. **271** (1970) 552.
13) SCHRYVER, Proc. Roy Soc. **B 82** (1910) 226.
14) G. DENIGES, C.R. Acad. Sci. **150** (1910) 529.
15) T. NASH, Biochem J. **55** (1953) 416.
16) G. BERGER, L. SAINT-LÈBE, C.R. Acad. Sci. à paraître (1971).
17) A.C. EGERTON, J.A. EVERETT, G.J. MINKOFF, S. RUDRAKANCHANA, K.C. SALOOJA, Ann. Chim. Acta. **10** (1954) 422.
18) G.J. MOODY, Nat. **198** (1963) 781.
19) G. BERGER, L. SAINT-LÈBE, Die Stärke **21**, n° 8 (1969) 205.

DISCUSSION

CHAIRMAN

Although your main interest is in the area of toxicology, I do believe that your investigations provide many stimulating suggestions for the identification of irradiated carbohydrate-rich foodstuffs.

A. DESCHREIDER

I should like to ask M. Saint-Lèbe what amount of SO_2 is present in a starch.

L. SAINT-LÈBE

In a normal industrial starch there are 35 μg/g.

H. SCHERZ

Did you study the toxicological properties of malondialdehyde in bacterial cultures or tissue culture? Malondialdehyde being a very reactive compound, I could imagine that it is interesting from the toxicological point of view.

L. SAINT-LÈBE

So far we have not tested every product; our work has concentrated on the development of cultures of Escherichia Coli K12 and of Pseudomonas Putida NCMB 128 on irradiated starch-based media. The target is perfect reproducibility. As to whether the increase in the latency periods recorded is due to the formaldehyde present in the irradiated starch, I think it is much too early to tell.

J. MORRE

To reply indirectly to your question, David L. Craword and R.O. Sinnhuber have published an article on the toxicity of malonaldehyde (1). I carried out an experiment on rats, injecting large quantities of malonaldehyde subcutaneously for a period: they did not appear to suffer from it in the least.

J. SPAANDER

I am also extremely interested in toxicological studies, although this is not really the subject of our discussions here today; toxicological investigations are very necessary as a means of obtaining the signal to go ahead with irradiated foodstuffs. I should like to ask whether you are not of the opinion that studies carried out on bacteria or cell strains are somewhat dubious from the toxicological point of view. An organism in its entirety like the human body always possesses a liver for ridding itself of many substances, and so there is a danger of drawing conclusions regarding products that are harmful to the cell

---

(1) "Acute Toxicity of Malonaldehyde". Toxicology and Applied Pharmacology 7, 826-832 (1965).

or bacterium but are not at all so on the scale of the complete human being or animal.

L. SAINT-LÈBE

I agree with you entirely, Dr. Spaander, but I must have failed to make myself clear in my improvised address, as the studies on microorganisms are not intended only for the evaluation of the toxicity of radiolysis products. We in fact hope that these experiments will allow us to specify irradiation conditions for minimum formation of these products. It is certainly very difficult to extrapolate from microorganisms to man, as it is in the case of rats and mice, but in this case, because of the speed of the response, we shall be able to study a multitude of parameters, which is impossible with animals.

J. SPAANDER

I am in complete agreement on the fact that organisms will give you responses, but I object somewhat to the use of the word "toxicology" or "toxic" in this connection. It is quite possible as diagnosis; when you say these are diagnostic procedures or procedures aimed at identifying certain chemical substances, I agree with you, but they must not be regarded as toxicological studies.

L. SAINT-LÈBE

I agree, Dr. Spaander, when you ask me to be careful about using the term "toxicological" where microorganisms are concerned. But our aim is not to evaluate the toxicity of irradiated starch by these experiments alone but to specify the irradiation conditions for this apparent toxicity to be at a minimum. However, we also propose to perform some complementary experiments on ordinary consumer products because you read periodically in the press that particular irradiated products give rise to chromosomic fractures...

CHAIRMAN

I fully agree with your viewpoint. At Karlsruhe we study the effects of irradiated sugar solutions and of other irradiated substances on certain microorganisms — not because we want to extrapolate from effects in microorganisms to effects in the human organism. There are several reports in the literature on cytotoxic effects of irradiated substrates. And the question is asked again and again: What are the substances in these irradiated solutions which cause these cytotoxic effects? Once we can say it is hydrogen peroxide or is malondialdehyde or some other compound, we have made another step forward. Once we know how much malondialdehyde is present in the irradiated solution we can ask: Is this concentration toxic for humans or not? Thus the use of microorganisms may be a useful auxiliary tool in toxicological considerations.

F. DRAWERT

I have a very simple question to ask. When we talk of starch, we are using a collective concept; we mean by this a substance that consists partly of amylose and partly of amylopectin. Amylose has a chain structure, with essentially α-1-4 linkages, while amylopectin contains essentially 1-6 linkages. What I am really waiting for are complete representations, and the answer to what is, in my opinion, a fundamental question, i.e. which of the two structures it is that is primarily attacked as a result of irradiation. Can this question be answered?

H. SCHERZ

I do not believe that there is any difference. The effect of radiation is primarily on the ether bridges. These ether bridges exist both in the 1-4 and in the 1-6 position. The radiation induced reaction occurs between neighbouring OH-groups. I do not believe that the secondary structure plays an important role here.

F. DRAWERT

I am always rather sceptical when belief is mentioned in science. For example, I believe, contrary to what you believe, that the configuration plays an extremely important part. And amylose differs fundamentally from amylopectin as regards configuration. This is also the reason why some are attacked by an enzyme, and others are not. Thus, why should it not be for reasons of differences in configuration that in this case too a totally different reaction occurs as a result of irradiation?

H. SCHERZ

We have not especially studied this with malondialdehyde but we have done it with deoxycompounds. In spite of their different configuration, cellulose and starch yielded approximately the same quantities of deoxycompounds upon irradiation.

A. DESCHREIDER

To reply more directly to the question put by Dr. Drawert, the investigations that we have carried out specifically on starch indicate that the polysaccharides of degradations originating from starch are due principally to amylopectin, and not to amylose.

L. SAINT-LÈBE

Like Dr. Scherz, I too think there is no difference between amylose and amylopectin. We have performed a number of experiments to determine the specificity of the appearance of malonaldehyde during irradiation. We irradiated amylose and amylopectin and the ATB response was virtually the same. The differences noted cannot easily be attributed to α-1-4 or α-1-6 links. However, we shall bear your comment in mind in our future research.

A. GUILBOT

I think everybody is right somehow. When the question in hand is micromolecules, formed during radiolysis, there cannot be much difference between the results obtained with amylose or amylopectin. On the contrary, as regards macromolecular residues, I agree with Prof. Drawert about there being actual differences between starches.

CHAIRMAN

I would like to ask a question to Mr. Saint-Lèbe: This morning Mr. Scherz has stated during the discussion of his colorimetric studies that he can differentiate with certainty, on the basis of the deoxysugars formed, between irradiated and non-irradiated wheat if the dose was 150 krad or higher. Now I would be very interested to know: Can you make a similar statement for your experiments? If you are given a sample of starch, to which lower dose limit can you state by your methods whether the starch has been irradiated or not?

L. SAINT-LÈBE

We can tell if the irradiation of the starch is genuine or not, because a commercial starch has a starch content of 99.2 or 99.5 %

The remainder consists of impurities such as cellulose, traces of lipids and traces of proteins; the small amounts of proteins and lipids present in commercial starch do not present any difficulty. We have carried out tests on delipidated starches, and have found no difference as against commercial starch. If the date of irradiation and the storage temperature are known, it will be possible to say after three months' storage that a starch has received a dose of 25 krad.

F. DRAWERT

Have Maillard products actually been found as products of the irradiation of pure carbohydrates?

H. SCHERZ

The formation of Maillard products is possible only if nitrogen compounds such as amino acids are present.

CHAIRMAN

On the basis of reports in the literature I think that Maillard reactions play a much smaller roler in irradiation than in heating. We know for instance that much of the lysine in feeds is lost upon heating due to Maillard reactions while very little lysine is lost upon irradiation, even in the Mrad-range.

F. DRAWERT

This, you see, was the reason for my asking this question, since this is a case of very substantial differences between processes. Thus, if no Maillard reactions occur, or not to any appreciable extent, this constitutes a quite definite "yes" for irradiation.

12. EVOLUTION DE LA DIMINUTION EN POIDS DURANT LA CONSERVATION: APPROCHE POUR L'IDENTIFICATION DES FRUITS IRRADIES

G. MAGAUDDA

ABSTRACT

A promising method of identification of irradiated fruit (tomatoes and oranges) appears to be provided by the determination of the weight loss during storage. It seems to be a constant and reproducible phenomenon for irradiated fruit to loss more weight than the control. By calculating this time-dependent difference by means of the regression line interpolating the experimental points and comparing the latter with the relative angular coefficient b, it can be shown that this phenomenon is a function of the dose absorbed.

Au cours d'expériences sur l'application de radiations gamma à différents produits d'origine végétale, dans le but d'obtenir une conservation plus longue et dans des meilleures conditions, des observations ont été effectuées sur l'évolution de la diminution en poids pendant la conservation.

En général, nous avons observé que les fruits irradiés accusent une perte en poids, au cours de la conservation, supérieure à celle du témoin. Quelques indications dans ce sens avaient déjà été obtenues l'année passée au cours d'expériences conduites sur des tomates de la variété « Supermarmande ». Dans la première figure, il est possible de constater que cela s'observe surtout pour les doses les plus élevées.

Pour vérifier le bien fondé de cette hypothèse, en analysant également son évolution dans le temps, on a décidé de reprendre l'expérience sur deux autres variétés de tomates: « Pantano », qui est consommée aussi à l'état frais et « San Marzano », qui est surtout utilisée par l'industrie des conserves.

Ces dernières expériences, montrent déjà par les premières analyses une diminution en poids nettement supérieure pour le lot de tomates irradiées par rapport au témoin. La figure 2 se réfère à la variété

Publication N° 287 du Laboratorio Applicazioni in Agricoltura del C.N.E.N., Centro Studi Nucleari della Casaccia, S. Maria di Galeria, Roma, Italie.

% loss of weight compared with the initial one

Dose krad	Days of storage at 25 °C			
	0	6	13	21
0	0	2.9	5.7	9.6
100	0	3.2	6.3	10.5
200	0	3.5	6.6	10.8
250	0	3.2	6.3	10.7
300	0	3.7	6.9	11.1

Fig. 1. Dans ce tableau sont groupées les valeurs en poids de trois caisses (± 15 kg) de tomates de la variété « Supermarmande » exprimées en % du poids initial des caisses immédiatement après irradiation.
Conditions de la conservation: 25 °C; 85 % H.R.

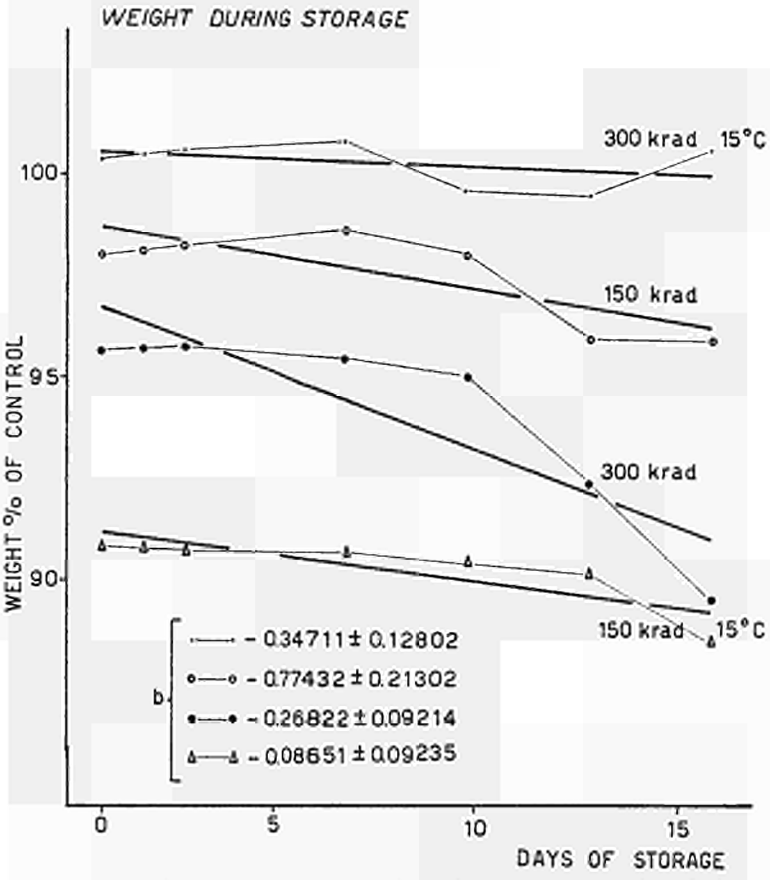


Fig. 2. Dans cette figure, ainsi que dans les suivantes, sont représentés les points expérimentaux et les droites de régression correspondantes, avec leur coefficient angulaire *b*; calculés durant la conservation, point par point en % par rapport au témoin. Tomates de la variété « Pantano », irradiées à maturité au stade vert-rose.
Conditions de la conservation:
1) 15 °C, 85-90 % H.R.
2) température ambiante (20 °C), H.R. 60 %.

« Pantano » irradiée au stade de maturation vert-rose, la figure 3 à la même variété au stade vert et enfin la figure 4 à la variété « San Marzano » au stade de complète maturation. Il est, de plus, possible d'observer combien sont importantes les conditions de conservation (humidité et température); en effet, pour la San Marzano (fig. 4) cette différence disparaît à 5° C, tandis que pour la « Pantano » irradiée au stade vert (fig. 3) elle disparaît à 15° C.

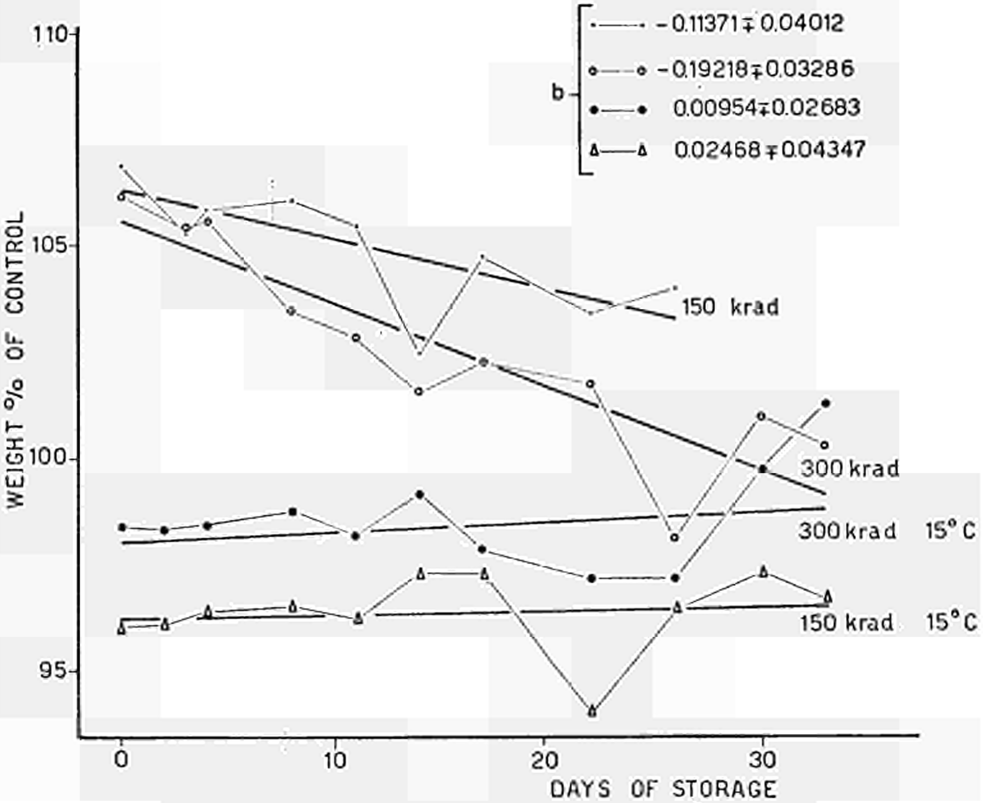


Fig. 3. Tomates de la variété « Pantano », irradiées au stade vert.
Conditions de la conservation:
1) 15 °C, 85-90 % H.R.
2) température ambiante (20 °C), H.R. 60 %.

Les mêmes observations, avec des résultats encore plus clairs, car obtenus lors d'expériences conduites sous conditions de température et d'humidité contrôlées rigoureusement, ont été faites avec des oranges des variétés « Valencia Late » et « Ovale ».

Les résultats reportés sont la moyenne des analyses effectuées sur 10 kg d'oranges pour chaque point expérimental.

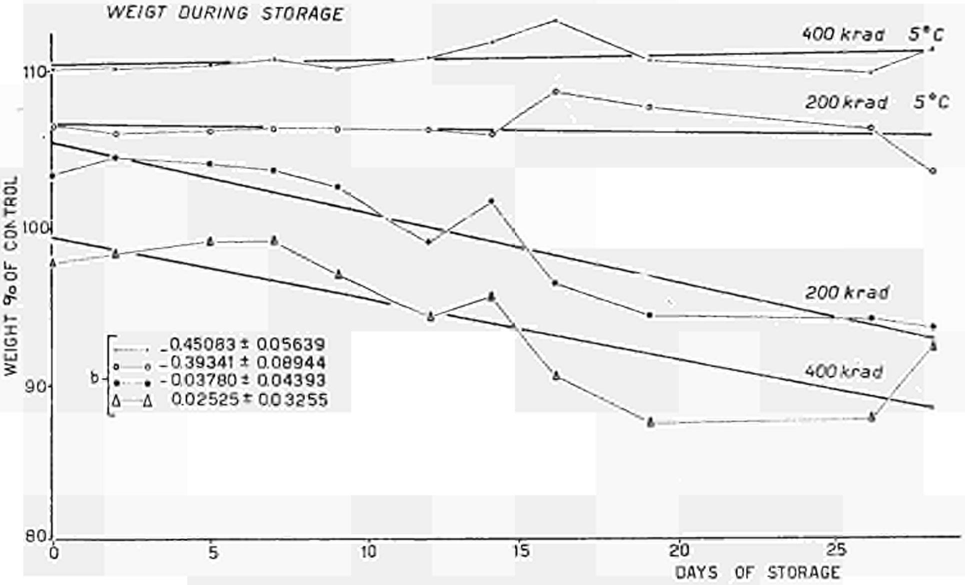


Fig. 4. Tomates de la variété « San Marzano », irradiées à maturité complète (rouge).

Conditions de la conservation:
1) 5 °C, 95-100 % H.R.
2) température ambiante (20 °C), H.R. 60 %.

Dans la figure 5 est représentée l'évolution du poids dans le cas de l'essai sur oranges « Ovales ». Il est possible de constater comment, en suivant pour quelques semaines l'évolution du poids, et en la comparant à celle du témoin porté à la valeur 100, on peut facilement mettre en évidence des inclinaisons des droites de régression qui incluent les points expérimentaux, différents suivant les doses d'irradiation. Les coefficients angulaires *b* des droites de régression, permettent de relever plus facilement ces différences entre doses.

La figure 6, relative aux expériences sur l'orange « Valencia Late », montre clairement combien ce phénomène est uniquement une conséquence directe du traitement ionisant gamma; en effet, tant un lavage avec le Tween qu'un traitement fongicide avec le thiabendazol (TBZ), n'influencent pas les valeurs de *b*.

La figure 7 se réfère à une autre série d'expériences sur « Valencia Late ». L'examen des valeurs de *b* montre une fois de plus combien le

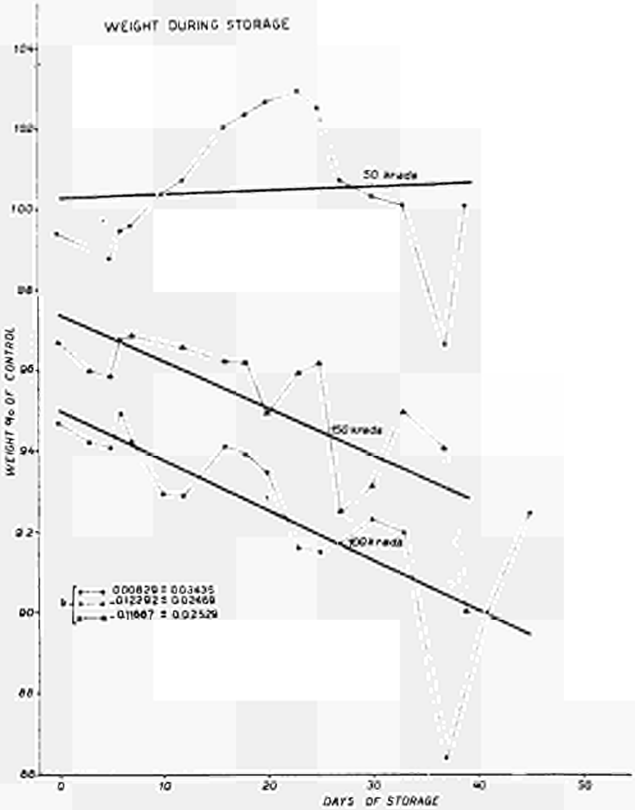


Fig. 5. Oranges de la variété « Ovale ».
Conditions de la conservation: 25 °C, 95-100 % H.R.

phénomène est lié à la dose: une dose de 50 krad n'a pratiquement aucun effet, tandis qu'une dose de 200 krad donne une valeur de *b* environ doublée par rapport à celles obtenues avec des doses de 100 et 150 krad. On observe aussi dans cette figure, comme d'ailleurs dans la précédente, que les traitements fongicides, même lorsqu'ils sont drastiquement associés à une température de 50° C, ne modifient pas sensiblement les valeurs de *b*.

La comparaison des différentes expériences réalisées semble montrer des valeurs constantes de *b* pour les mêmes doses et pour la même variété, tandis que ces valeurs sont différentes avec d'autres variétés et, plus encore, avec d'autres espèces.

Les résultats obtenus jusqu'à présent sont assez prometteurs pour une possible application pratique et facile de l'identification des fruits irradiés.

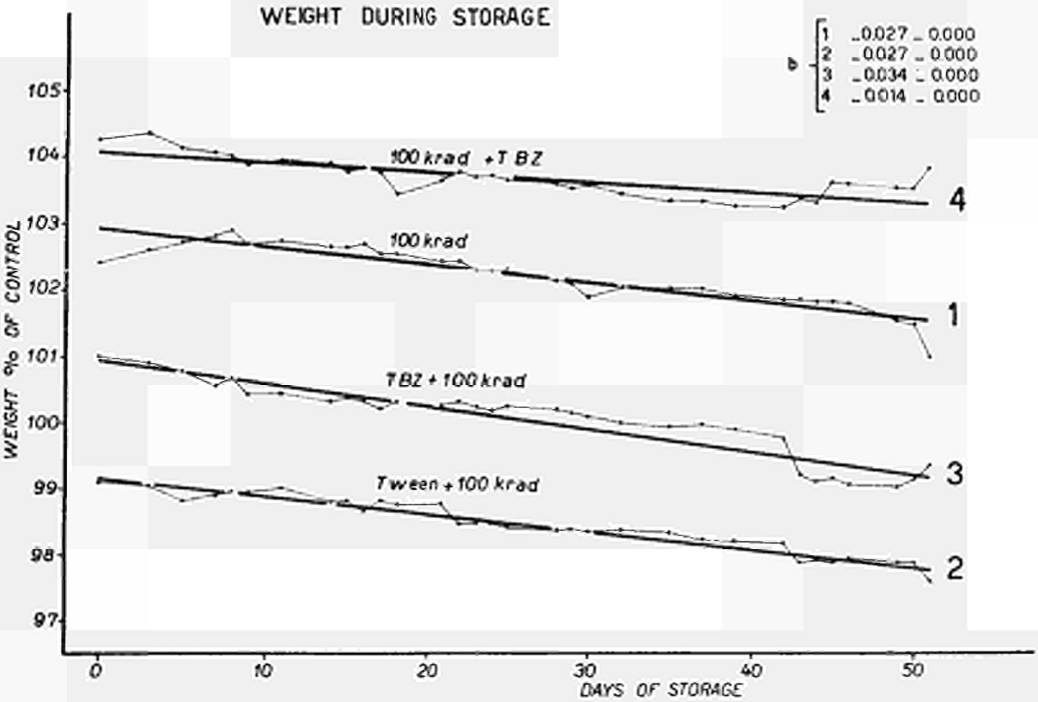


Fig. 6. Oranges de la variété « Valencia Late ».

1) irradiation gamma de 100 krad,
2) lavage avec du Tween avant l'irradiation gamma,
3) traitement avec le fongicide TBZ avant l'irradiation,
4) traitement avec le fongicide TBZ après l'irradiation.

Conditions de la conservation: 25 °C, 95-100 % H.R.

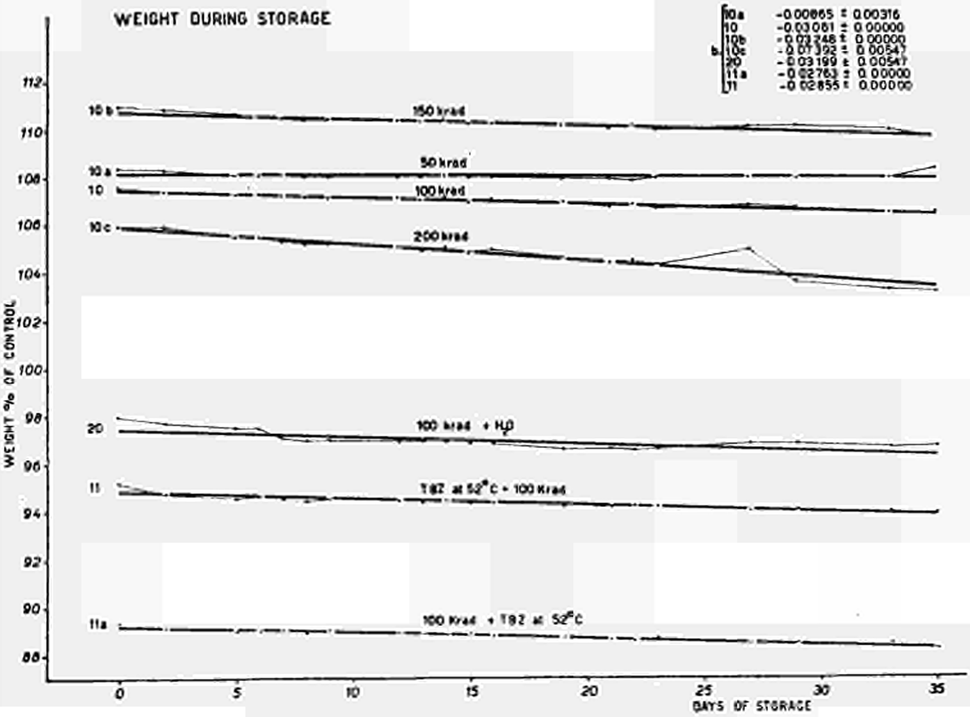


Fig. 7. Oranges de la variété « Valencia Late ».

10a) Irradiation gamma de 50 krad,
10) Irradiation gamma de 100 krad,
10b) Irradiation gamma de 150 krad,
10c) Irradiation gamma de 200 krad,
20) Immersion dans l'eau pendant 30 s après l'irradiation gamma (100 krad)
11) Traitement avec le fongicide TBZ à chaud (52 °C) pendant 5 mn, avant l'irradiation gamma (100 krad),
11a) Traitement avec le fongicide TBZ à chaud (52 °C) pendant 5 mn, après l'irradiation gamma (100 krad).

Conditions de la conservation: 25 °C, 95-100 % H.R.

DISCUSSION

CHAIRMAN

I assume that these weight losses are related to the stimulation of metabolism which is caused by irradiation. I had not previously seen data on weight loss in irradiated oranges, tomatoes and other vegetables but there are many reports on the weight changes in irradiated potatoes. As I recall it, the effect there is in the opposite direction as the one you have reported: irradiated potatoes show somewhat lower weight losses than unirradiated potatoes—at least over long storage periods. I am sure that such investigations are of considerable interest in view of a practical application of irradiation; a weight loss of 10 % such as you reported would be of considerable financial interest. Whether these changes can be utilized for the identification of irradiated products is another question.

P. KAYSER

I should just like to ask whether, instead of measuring the weight loss, it would not be better to measure the gases or water vapour; this would possibly yield more precise information on the loss. If only the weight loss is measured, we still do not know what happens. We need to make specific measurements of what it is that is lost, and to do so rapidly.

G. MAGAUDDA

As such differences, the values obtained never reach the 10 % compared to the control but are much lower. The investigation carried out by following for some days the weight loss allows to ascertain not only that fruit has been irradiated but also the approximate dose rate. The suggestion to measure the water rather than the weight loss does not seem to be the most appropriate to me since there are probably in the water other substances which leave the irradiated fruit more quickly than the control, as for instance CO_2 or other gaseous substances. Storage conditions are very important; in our case, for instance, oranges were stored under almost saturated humidity conditions and high temperature.

13. MISE EN EVIDENCE D'ALDEHYDE MALONIQUE DANS LES SOLUTIONS DE GLUCIDES IRRADIES AUX RAYONS GAMMA

J. MORRE

ABSTRACT

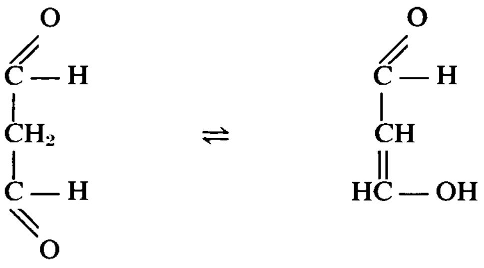
The author shows that the absorption in UV that gamma irradiated in medium buffered at pH 9.5 sugar present and that different authors point out is due to malonaldehyde.

The colouring formed with this dialdehyde is isolated by means of a reaction with thiobarbituric acid; it is identifed by means of a characteristic spectrum (maximum 535 nm with a shoulder between 480 and 520 nm) and by means of a chromatography on silicagel (butanol, methanol, water, sodium hydroxid: 37, 37, 20 cm^3 and 0.50 gr or butanol, methanol, water, acetic acid: 37, 37, 20.5 cm^3.

INTRODUCTION

Ce sont les travaux de Smith, Tinsley et Bubl qui ont attiré l'attention des chercheurs sur la production d'aldéhyde malonique lors de l'irradiation des aliments par les rayonnements ionisants. L'aldéhyde malonique bien que présent dans de nombreux processus biologiques a été peu étudié: il est difficile à isoler et instable d'où une grande difficulté pour fixer ses propriétés.

L'aldéhyde malonique existe sous deux formes: anionique et énolique (N. Bacon, W.O. Georges et B.H. Stringer)



Les solutions acides sont très instables. Les diacétals sont plus faciles à utiliser, de même que les composés d'addition nitrés ou halogénés. Les solutions basiques sont plus stables, car il y a formation d'un sel:

l'aldéhyde malonique est un acide d'une force non négligeable: *p*K = 4,6 (Mashio et Kimura).

ORIGINE DE L'ABSORPTION EN ULTRAVIOLET DES SUCRES IRRADIES

Ce qui nous a frappé dans ces études, c'est que les auteurs: Laurent, Bothner-By, Phillips qui ont étudié les sucres irradiés en solution aqueuse, n'ont effectué que des mesures en ultra-violet, alors que Patton, Schmidt, Smith, Sinnhuber, Taufel, Von Streuli, n'ont employé que la réaction à l'acide thiobarbiturique pour l'étude des aliments irradiés. Ayant trouvé que l'aldéhyde malonique présente une absorption en ultra-violet (268 nm et 245 nm) et dans le visible (535 nm) nous avons voulu comparer ces résultats.

RECHERCHES EFFECTUEES

L'irradiation a eu lieu au Centre d'Energie Nucléaire de Saclay (Service Physico-chimie appliquée). Nous avons utilisé le rayonnement du cobalt 60.

Vingt-quatre heures après l'irradiation, une première mesure a été effectuée en ultra-violet au pH d'irradiation, puis une deuxième à pH 1,5 par addition de H_2SO_4 concentré. Enfin la réaction à l'acide thiobarbiturique a été réalisée sur les solutions irradiées de la façon suivan-

Tableau I. — Irradiation de divers sucres en solution tamponnée à pH 9,5 à la dose de 0,4 Mrad. Lecture à pH 1,5 et 9,5 en UV et à pH 1 après réaction à l'action thiobarbiturique.

Glucides irradiés Con = 0,01 M	pH 1,5 E_{245}	pH 9,5 E_{268}	E_{535} après réaction à l'acide thiobarbiturique à pH 1,00	
Alhédyde glycérique	0,50	0,90	0,36 (465)	1,78 (535)
D. Xylose	1,10	2,40		5,60 (535)
L. Arabinose	1,02	2,70		5,20 (535)
D. Ribose	1,00	1,60	0,18 (455)	4,10 (535)
D. Galactose	1,15	2,20		5,60 (535)
D. Glucose	1,04	2,50		5,10 (535)
D. Mannose	1,00	2,20		5,60 (535)
D. Fructose	1,20	2,20		5,00 (535)
Maltose	0,75	1,60	0,32 (455)	2,64 (535)
Lactose	0,87	1,60	0,36 (455)	2,84 (535)
Saccharose	1,88	5,40		5,90 (535)
Cellobiose	0,82	1,45	0,14 (455)	1,86 (535)
Raffinose	2,90	5,90	1,30 (535)	11,60 (535)

te: à 2 cm^3 de la solution, on ajoute 2 cm^3 d'une solution fraîchement préparée d'acide thiobarbiturique Fuka à 0,72 % dans de l'acide trichloracétique à 15 % dans de l'eau. Après 30 min au bain-marie à 60° C et refroidissement sous courant d'eau, la lecture est effectuée au spectrophotomètre Leres. Si la solution de sucre irradiée est trop concentrée, un trouble apparaît, il y a lieu de la diluer. On mesure à 535 nm la valeur de E_{1cm}.

Tous les sucres irradiés en milieu basique en solution diluée présentent une absorption à 268 nm en UV et à 535 nm dans le visible après réaction avec l'acide thiobarbiturique. Après acidification de la liqueur irradiée, le pic en UV est déplacé à 245 nm. Il s'agit donc d'un fait très général.

Tableau II. — Valeur de 0,01 $E_{1\,cm}$ à divers pH pour les maxima d'absorption, (λ) longueur d'onde en nm.

Composés	Mesure en UV à pH 1,5	Mesure en UV à pH 9,5		Après réaction avec l'acide thiobarbiturique	
Acide ascorbique	92 (245)	4 (220)	22 (268)	0,13 (300)	
Aldéhyde glycérique	54 (245)		127 (268)		0,35 (455)
Déhydroxyacétone					
monomètre	0,92 (245)	2,4 (245)			
dimètre	0,15 (270)		0,34 (285)	0,03 (368)	0,05 (455)
Glyoxal	0,03 (210)	0,015 (235)	0,05 (295)	4,6 (370)	9,5 (455)
Aldéhyde malonique	130 (245)		300 (268)		780 (535)

COMPOSES RESPONSABLES DE CETTE ABSORPTION

Les divers composés que les auteurs citent comme susceptibles de se former lors de l'irradiation des sucres ont été passés en revue et étudiés suivant la même méthode que les sucres irradiés.

En comparant le tableau I (sucres irradiés) et le tableau II (composés de désintégration) la similitude des pics d'absorption de l'aldéhyde malonique et des sucres irradiés apparaît: seuls des composés étudiés, l'aldéhyde malonique a en UV des maximums pour les mêmes longueurs d'onde que les glucides irradiés: 245 nm à pH 1,5; 268 nm à pH 9,5. Le spectre dans le visible après réaction à l'acide thiobarbiturique en milieu acide est tout à fait identique à celui des glucides irradiés: maximum à 535 nm et « épaulement » caractéristique entre 480 nm et 520 nm (fig. 1 et 2).

Notons que l'importance des coefficients d'extinction moléculaire de l'aldéhyde malonique $1{,}30.10^4$ et 3.10^4 en ultraviolet pour les pH de 1,5 et 9,5 explique qu'une concentration très faible en aldéhyde malonique produise une intense absorption en ultra-violet.

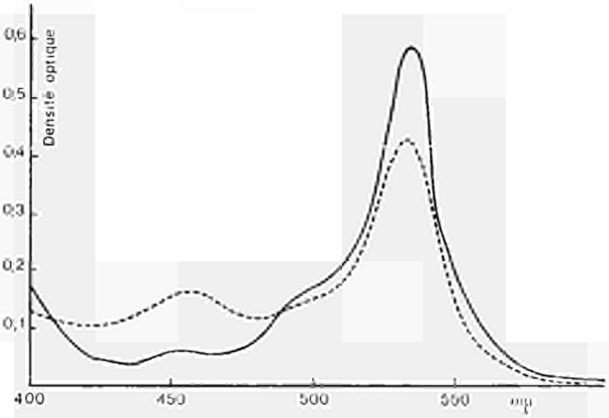


Fig. 1. Spectres du galactose irradié (trait plein) et du D-ribose irradié (trait interrompu) après réaction à l'acide thiobarbiturique. Blanc: acide thiobarbiturique dans l'acide trichloracétique.

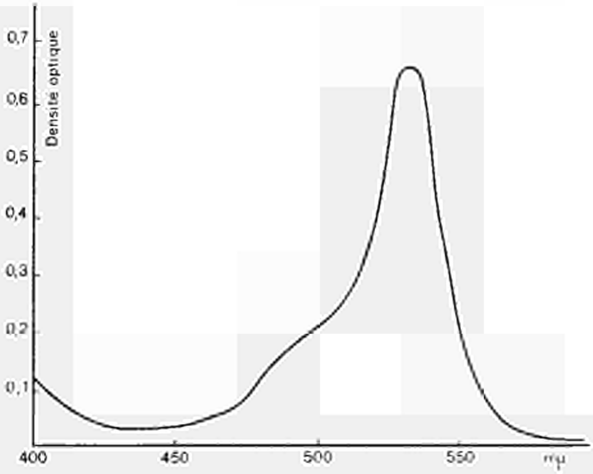


Fig. 2. Spectre de l'aldéhyde malonique après réaction à l'acide thiobarbiturique. Blanc: acide thiobarbiturique dans l'acide trichloracétique.

Nous avons calculé le rendement radiolytique initial G_0 pour l'aldéhyde malonique produit et le galactose détruit pour une dose de 2 Mrad.

Notons que grâce à l'extrême sensibilité de la réaction à l'acide thiobarbiturique lue au spectrophotomètre et à la relation linéaire existant entre la dose absorbée et la concentration en aldéhyde il est possible de reconnaître une solution de sucre irradiée et d'en calculer la dose reçue jusqu'à 2,5 Mrad.

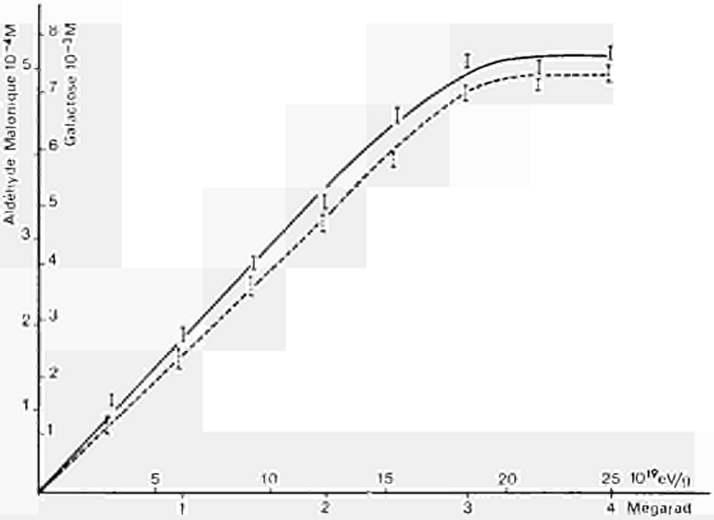


Fig. 3. Aldéhyde produit (trait plein) et galactose détruit (trait interrompu) en fonction de la dose d'irradiation exprimée en électronvolt et en Mégarad.

Les valeurs de G_0 sont: pour l'aldéhyde malonique: 0,18; pour le galactose: 2,30. La différence de valeur des deux rendements montre que d'autres composés sont formés et peuvent intervenir pour provoquer l'absorption en UV des sucres irradiés. Pourtant l'aldéhyde malonique en est le principal responsable: le colorant formé à 60 °C avec l'acide thiobarbiturique présente un maximum d'absorption à 535 nm qui est spécifique de ce dialdéhyde.

INFLUENCE DE LA VARIATION DU PH D'IRRADIATION

L'irradiation au lieu d'être effectuée à pH 9,5 est pratiquée à divers pH étagés de 11,5 à 1,5 dans des milieux tamponnés choisis pour leur résistance aux rayonnements: Na_2CO_3, $NaHCO_3$, NaH_2PO_4, $(NH_4)_2SO_4$, $NaHSO_4$ ajustés au pH désiré par addition de soude, d'acide phosphorique ou sulfurique.

Tableau III. — Rôle du pH d'irradiation.
Irradiation de solution de galactose 0,01 M à divers pH pour une dose de 0,5 Mrad.

Mesures	Réaction avec l'acide thiobarbiturique; pH 1,5	
pH	E_{535}	E_{455}
11,5	21	
9,5	6,3	
7,5	2,1	
5,5	—	—
3,5		i,i
1,5		1,3

L'expérimentation montre que les oses irradiés ne présentent les absorptions caractéristiques de l'aldéhyde malonique que pour les pH alcalins, quelques osides font exception: il y a production d'aldéhyde malonique quel que soit le pH d'irradiation.

EXTRACTION DE L'ALDEHYDE MALONIQUE DES SOLUTIONS IRRADIEES

Nous avons essayé d'isoler de la liqueur irradiée non pas l'aldéhyde malonique ce qui était difficile, mais le colorant formé entre l'aldéhyde malonique et l'acide thiobarbiturique. Ce colorant est en effet peu soluble en milieu acide et possède un poids moléculaire élevé (324) ce qui permet un isolement aisé.

TECHNIQUE

L'expérience a été faite sur le galactose en solution aqueuse, irradié à la dose de 2 Mrad par le cobalt 60 (500 cm^3 de galactose 0,01 M dans $NaHCO_3$ 0,1 M ajusté à pH 9,5). La liqueur est distillée sous vide et recueillie sur 0,10 g d'acide thiobarbiturique dans 50 cm^3 d'acide chlorhydrique. Par prudence on intercale entre le sommet du réfrigérant et la trompe à vide une fiole de barbotage avec la même quantité de réactif. Quand l'appareil est purgé d'air par un courant d'azote, la solution irradiée est acidifiée par addition de 60 cm^3 d'acide chlorhydrique pur. La distillation est poursuivie jusqu'à ce qu'il ne reste plus que 50 cm^3. Un léger courant d'azote régularise l'ébullition. Le distillat est alors chauffé à reflux au bain-marie bouillant pendant 1 h et concentré à basse pression avec arrivée d'azote jusqu'à un volume de 100 cm^3. Après 48 h à +4° C une fine poudre bleu acier précipite;

elle est lavée sur un filtre en verre fritté d'abord avec 100 cm^3 de HCl 0,6 M refroidi à 10° C, puis très rapidement avec quelques centimètres cubes d'eau froide, enfin très abondamment avec de l'éther éthylique: 3 à 4 fois 100 cm^3. Après séchage à l'étuve à 60° C la poudre est pesée. La solution irradiée après distillation ou le distillat lors de la concentration ne doit plus donner la coloration rouge avec l'acide thiobarbiturique.

PREPARATION DU COLORANT SYNTHETIQUE

Il était intéressant de comparer le colorant ainsi isolé avec celui obtenu par hydrolyse acide du diacétal de l'aldéhyde malonique ou tétra-1,1,3,3 éthoxypropane. On obtient de l'aldéhyde malonique et de l'alcool éthylique suivant la réaction:

$$(C_2H_5O)_2CH - CH_2 - CH(C_2H_5O)_2 \rightarrow CHO - CH_2 - CHO + 4\ C_2H_5OH).$$

Nous avons employé la méthode de Sinnhuber, Yu et Chang-Yu. Un flacon de 500 cm^3 contenant:

acide thiobarbiturique	:	0,012 5 M soit 1,802 0 g
tétraéthoxypropane	:	0,006 25 M soit 1,375 0 g
HCl à 12 p. 100	:	250 cm^3

est chauffé 90 min à reflux au bain-marie bouillant. Après 48 h à 4°C, le précipité est lavé sur filtre en verre fritté avec 100 cm^3 d'HCl 0,6 M refroidi à + 10° C, puis avec 4 fois 100 cm^3 d'éther éthylique et finalement séché à l'étuve à 60° C, le rendement est de 97,5 %.

Le poids moléculaire du colorant est de 324 g (Sinnhuber).

Le colorant est peu soluble dans les acides concentrés: ClH à 12 %; acide trichloracétique à 15 %. Il est stable avec tendance à précipiter peu à peu au bout de quelques jours. Il est soluble dans l'eau chaude, dans l'alcool absolu, rigoureusement insoluble dans l'éther éthylique ce qui permet de le séparer aisément de l'acide thiobarbiturique en excès qui y est très soluble. Enfin avec la soude à 3 % (P/V) il donne un nouveau colorant rouge framboise extrêmement soluble qui se décolore à l'air et à la lumière en 24 à 48 h; la pyridine le stabilise. Les spectres d'absorption de ces deux composés ont la même allure; seul le maximum d'absorption est déplacé de 535 nm (milieu acide) à 550 nm (milieu basique). Ils présentent tous les deux le même « épaulement » caractéristique. En milieu acide trichloracétique, le coefficient d'extraction moléculaire à 535 mm est de 1,56.10^5 M^{-1} cm^{-1} (Sinnhuber) en accord avec le $E^{AM}_{1\,cm}$ trouvé ici ($7{,}8.10^4$ M^{-1} cm^{-1}) où l'aldéhyde malonique est dilué de moitié pour effectuer la réaction. En milieu basique (soude à 3 %) nous avons mesuré ce coefficient, il est de $9{,}26.10^4$ M^{-1} cm^{-1} à 550 nm. Le colorant extrait des solutions irradiées présente toutes ces propriétés.

COLORANT: *glyoxal, acide thiobarbiturique*

De nombreux auteurs signalent la présence de glyoxal dans les produits irradiés. Le glyoxal avec l'acide thiobarbiturique en milieu acide donne à 60° C après 30 mn un colorant jaune qui absorbe à 455 nm. Si la réaction est effectuée à 100° C pendant 15 mn, il apparaît un colorant rouge présentant deux maximums à 525 et 550 nm. Ces colorants sont stables. En milieu basique par addition de 3 % de soude le colorant rouge acide se décolore immédiatement et devient jaune. En conséquence dans une solution irradiée basique, le colorant rouge restant est dû uniquement à l'aldéhyde malonique.

CHROMATOGRAPHIE

Afin d'apporter une nouvelle preuve de l'identité du colorant préparé synthétiquement et de celui extrait des solutions irradiées, il a été procédé à la chromatographie comparative des deux composés sur plaque de gel de silice Merck F. 254.

TECHNIQUE

Quelques cristaux de colorant sont dissous dans de la soude à 3 %. Les deux mélanges suivants ont donné satisfaction.

1)	butanol	37 cm³
	méthanol	37 cm³
	eau	20 cm³
	soude en paillettes	0,50 g
2)	butanol	37 cm³
	méthanol	37 cm³
	eau	20 cm³
	acide acétique R.P.	5 cm³

Les taches migrent régulièrement. Il y a identité entre les taches provenant du colorant extrait du galactose et celles du colorant préparé synthétiquement à partir du tétraéthoxy-propane. La couleur rouge parfaitement nette à la fin de l'expérience disparaît peu à peu en un quart d'heure. Il n'y a pas de taches en UV, si le colorant a été débarrassé de l'acide thiobarbiturique en excès (lavage à l'éther). Il peut rester une tache de couleur brique dans certains cas.

pour l'éluant n° 1 basique Rf = 0,78 à 20° C
pour le n° 2 acide Rf = 0,76 à 20° C.

La sensibilité est excellente puisque la chromatographie peut être réalisée avec 0,1 μg de colorant.

CONCLUSION

L'irradiation gamma des glucides en solution diluée tamponnée à pH 9,5 produit de l'aldéhyde malonique. L'aldéhyde malonique présente en UV à pH acide ou basique un spectre semblable à celui des sucres irradiés, mais il est le seul à avoir dans le visible après réaction à l'acide thiobarbiturique un spectre superposable à celui des sucres irradiés.

L'extraction du colorant aldéhyde malonique-acide thiobarbiturique a permis de montrer par spectrophotométrie ou par chromatographie sur gel de silice la parfaite similitude du produit provenant du sucre irradié et de celui préparé par synthèse à partir du diacétal. La présence de l'aldéhyde malonique dans les solutions de glucides irradiés en solution basique ou neutre est ainsi bien établie.

IV. CONCLUSIONS

CONCLUSIONS

J.F. DIEHL

This Colloquium has been named "The Identification of Irradiated Foodstuffs". But the interest in the results presented at this meeting goes beyond the legal problem of identifying irradiated foods. The hesitation of national and international health authorities to grant more permissions for marketing irradiated foodstuffs is hardly caused by the lack of methods for the identification of such foods. It is rather caused by still persisting doubts concerning the wholesomeness of irradiated produce. No matter how many animal feeding studies have been carried out and will be carried out in the future—there are still the questions "What happens when foodstuffs are irradiated? What chemical changes are initiated by the radiation energy? What compounds are formed that could perhaps, under special, unfavorable conditions be toxic?" The more answers we can give to these questions, the more solid will be the scientific basis upon which the health authorities can draw their conclusions.

The experiments carried out with the support of the Commission of the European Communities in laboratories in Belgium, the Netherlands and Germany, and the additional information provided notably by our French colleagues during today's discussions, have shown clearly identifiable chemical change when a radiation dose in the Mrad range was employed: aggregations of soluble proteins, various breakdown reactions in starch and other carbohydrates, formation of hydrocarbons, aldehydes and ketones in fats. Some of these reactions are sufficiently specific to make them the basis for an identification method. In the dose range below 1 Mrad, however, the problem of finding specific proof of irradiation by chemical means becomes almost impossibly difficult. Only minute chemical changes are induced in this range—enough to trigger various physiological responses in plants, such as the inhibition of sprouting of potatoes, or the effect on weight loss described by our Italian colleagues—but not enough to permit proof of irradiation on the basis of radiochemical changes per se. Or perhaps I should say "not yet". We shall continue to improve our methods and perhaps we will come up with reliable chemical identification procedures in the lower dose range in the future.

In the meantime we should continue to work on methods which are based on the physiological changes occuring after low-dose irradiation: changes in electrical conductivity in potatoes, histological observation of wound-healing ability, etc. I assume that the change in spectropolarimetric behavior of extracts of irradiated potatoes described by Prof. Deschreider is also a reflection of such physiological changes.

Should we fail in the long run to find absolutely reliable methods for quick and simple identification of irradiated foodstuffs, our work would still be useful in judging the wholesomeness of these foods. Toxicologists making such judgement will find their decision easier if they know what the main chemical changes are in the Mrad range, and if they know that in the krad range chemical changes are so unspecific and so minute, that they can hardly be detected by the most sophisticated analytical methods.

Annexe I. — Participants

Germany

J.F. DIEHL, chairman afternoon session
Bundesforschungsanstalt für Lebensmittelfrischhaltung
75 Karlsruhe
Engesserstraße 20

F. DRAWERT
Institut für chemisch-technische Analyse und chemische Lebensmitteltechnologie
Technische Universität
8050 München-Freising
Weihenstephan

H. PENNER
Bundesforschungsanstalt für Lebensmittelfrischhaltung
75 Karlsruhe
Engesserstraße 20

J.P. RADOLA
Bundesforschungsanstalt für Lebensmittelfrischhaltung
75 Karlsruhe
Engesserstraße, 20

H. SCHERZ
Bundesforschungsanstalt für Lebensmittelfrischhaltung
75 Karlsruhe
Engesserstraße 20

Belgium

L. BUGYAKI
Institut d'Hygiène et d'Epidémiologie
14, rue Wytsman
Bruxelles

A.R. DESCHREIDER
Ministère des Affaires Economiques
Laboratoire Central
17a, rue de la Senne
Bruxelles

and
et CENATRA
137, Lange Leemstraat
Antwerpen

M. VAN DER STICHELEN-ROGIER
Institut d'Hygiène et d'Epidémiologie
rue Wytsman, 14
Bruxelles

France

J. CROIZET
Service Central de Protection contre les Rayonnements Ionisants
Boîte Postale 35
78 Le Vesinet

A. GUILBOT
Institut National Agronomique
C.E.R.D.I.A.
91 Massy

C. MADELMONT
Commissariat à l'Energie Atomique
Département Protection Sanitaire C.E.N.F.A.R.
Boîte Postale no 2
Fontenay-aux-Roses

J. MORRE
Laboratoire Radio-biologie
39, rue de Dantzig
Paris 15e

H. PROST
Service de la Répression des Fraudes et du Contrôle de la Qualité au Ministère de l'Agriculture
42bis, rue de Bourgogne
Paris 7e

L. SAINT-LEBE
Commissariat à l'Energie Atomique
Service de Radioagronomie, Département de Biologie
C.E.N. Cadarache
Boîte Postale no 1
13 St-Paul-lez-Durance

Italy

Mme BELLI-DONINI, M.L.
Laboratorio Applicazioni in Agricoltura del CNEN Casaccia
Via Anquillarese Km 1,300
Roma

G. MAGAUDDA
Laboratorio Applicazioni in Agricoltura del CNEN Casaccia
Via Anquillarese Km 1,300
Roma

Luxembourg

P. KAYSER
Direction de la Santé Publique
3, rue Auguste Lumière
Luxembourg

Netherlands

J. SPAANDER, Chairman morning session
Rijksinstituut voor de Volksgezondheid
Sterrenbos 1
Utrecht

D. ONDERDELINDEN
Rijksinstituut voor de Volksgezondheid
Sterrenbos 1
Utrecht

J.F. STOUTJESDIJK
I.T.A.L.
Postbus 48
Wageningen

Commission des
Communautés
Européennes

J. SMEETS, Secretary, Editor
Direction Générale des Affaires Sociales
Direction Protection Sanitaire
29, rue Aldringen
Luxembourg

J. PAUWELS
Directorate general of Industries
Bureau Eurisotop
rue de Loi 200,
1040 Bruxelles

SALES OFFICES

This report published by the Commission of the European Communities is on sale at the offices listed below. When ordering, specify clearly the EUR number and the title which are shown on the internal title page.

OFFICE FOR OFFICIAL PUBLICATIONS
OF THE EUROPEAN COMMUNITIES
P.O. Box 1003 - Luxembourg 1
(Compte chèque postal N° 191-90)

BELGIQUE — BELGIË
MONITEUR BELGE
Rue de Louvain, 40-42 - B-1000 Bruxelles
BELGISCH STAATSBLAD
Leuvenseweg 40-42 - B-1000 Brussel

DEUTSCHLAND
VERLAG BUNDESANZEIGER
Postfach 108 006 - D-5 Köln 1

FRANCE
SERVICE DE VENTE EN FRANCE
DES PUBLICATIONS DES
COMMUNAUTÉS EUROPÉENNES
rue Desaix, 26 - F-75 Paris 15e

ITALIA
LIBRERIA DELLO STATO
Piazza G. Verdi, 10 - I-00198 Roma

LUXEMBOURG
OFFICE DES
PUBLICATIONS OFFICIELLES DES
COMMUNAUTÉS EUROPÉENNES
Case Postale 1003 - Luxembourg 1

NEDERLAND
STAATSDRUKKERIJ-
en UITGEVERIJBEDRIJF
Christoffel Plantijnstraat - Den Haag

UNITED KINGDOM
H. M. STATIONERY OFFICE
P.O. Box 569 - London S.E. 1

Price: B.Fr. 210.—

Commission of the
European Communities
D.G. XIII - C.I.D.
29, rue Aldringen
Luxembourg