A C T A A L I M E N T A R I A P O L O N I C A

Vol. XIII (XXXVII), No. 4 1987

JÓZEF FORNAL
MARIA JOUSSELIN-HOSAJA
HALINA KOZŁOWSKA

# EFFECT OF STEAMING, MICROWAVE TREATMENT AND GAMMA-IRRADIATION ON RAPESEED MICROSTRUCTURE

Institute of Food Engineering and Biotechnology, Agricultural and Technical University in Olsztyn

Key words: electron microscopy, rapeseed, steaming, gamma-irradiation, microwaves

Structural changes in rapeseed following hydrothermal treatment are reported. SEM and TEM micrographs revealed significant changes in the seed microstructure. It was found that the intensity of these changes depends on the kind of treatment.

## INTRODUCTION

An important role in technological extraction of oil from rapeseed is played by hydrothermal processing which is meant, among others, to inactivate enzymes having an adverse effect on the course of the process, on the quality, stability and nutritive value of the products. The enzymes most frequently mentioned in this context are lipase, lipoxygenase and thioglucosidase. In most hydrothermal processes employed in technologies the last-mentioned enzyme has favourable conditions for hydrolytic decomposition of glucosinolates, and is totally inactivated only during toasting of ground rapeseed.

To prevent the formation of glucosinolanes decomposition products and to counteract their passing to the oil or ground seed methods are sought to inactivate thioglucosidase in whole seeds immediately before flaking. This is particularly important in view of this enzyme's localization in the cells. Matile [10] and Luthy and Matile [8] found that in *Cruciferae* cells thioglucosidase occurs in inactive form as a so called "mustard oil bomb". The substrate together with the activator are separated from the enzyme by a specific membrane, and the destruction of this system, mechanical or otherwise, brings the substrate into contact with the enzyme

and leads to the formation of harmful derivatives such as isothiocyanates, thiocyanates, oxazolidinethiones and nitriles.

Our previous studies concentrated mainly on determining the effectiveness of selected methods of thioglucosidase inactivation (steaming in fluidal layer, microwave treatment and gamma-irradiation) [6, 7]. We demonstrated that when rapeseed is steamed in a fluidal layer, the enzyme is completely inactivated after 4.5-5.0 min of steam treatment. A similar effect was observed in the case of microwave treatment but here a significant factor was humidity of the initial material. On the other hand, gamma-irradiation turned out to be rather ineffective in inactivating thioglucosidase in whole rape seeds. High radiation doses caused only partial inactivation of the enzyme and did not inhibit glucosinolates decomposition.

One is thus prompted to suspect that differences in the effectiveness of the employed inactivating factors may be due to various degrees of rapeseed cell system destruction. In this research we investigated structural changes in seeds during the above-mentioned thioglucosidase--inactivating treatments with the use of scanning and transmission electron microscopy.

## MATERIAL AND METHODS

The Skrzeszowicki variety of rape (1982 crop) was used in the study. The humidity of seeds was adjusted to 11.7% prior to irradiation, and to 10% before microwave treatment [7].

Irradiation was performed in a radiation chamber with a $^{60}Co$ gamma radiation source with total activity of 20 kCi ($74 \times 10^4$ GBq). Rapeseed was irradiated at room temperature in the presence of air oxygen; irradiation dose was $10 \times 10^3$ Krad.

Microwave heating was carried out in a KM-06 apparatus. Seeds were deposited in a 1-cm layer in glass cuvettes and exposed to 2450 Mhz microwaves for 3.5 min.

Hydrothermal processing of rapeseed in a fluidal layer was performed in an apparatus constructed according to Dahlen [1] at 110°C for 7 min.

Rapeseed was prepared for microscopic analyses by steeping in distilled water for 12 h, and fixation in 3% glutaraldehyde in phosphate buffer at 4°C. Following fixation, the samples were thrice rinsed with buffer and contrasted with 2% $KMnO_4$ for 4 h. Next the seeds were dehydrated in an ethanol series and cut into halves, one half to be examined with a scanning microscope and the other with a transmission microscope [14]. The seed halves dried at critical point were coated with carbon and gold in a vacuum sputterer and viewed through a JSM-1 scanning microscope with working

voltage of 10 KeV. Ultra-thin sections obtained in an LKB-III ultratome were transferred to copper grids treated with uranyl acetate and lead citrate and analysed in a BS 500 microscope.

## RESULTS AND DISCUSSION

### MICROSTRUCTURE OF UNTREATED RAPESEED

In microscopic studies of the Skrzeszowicki rapeseed particular attention was devoted to the size and distribution of protein bodies and fat. It was found that this variety is characterized by a large number of fine-grained structures less than 1 μm in diameter (Photos 1A, 1B). However, most cells were found to contain single spherical aleuronic grains with diameters ranging from 5.5 to 7.5 μm. There were two forms of these grains. One, occupying a central position in the cell (Photo 1A), has a very

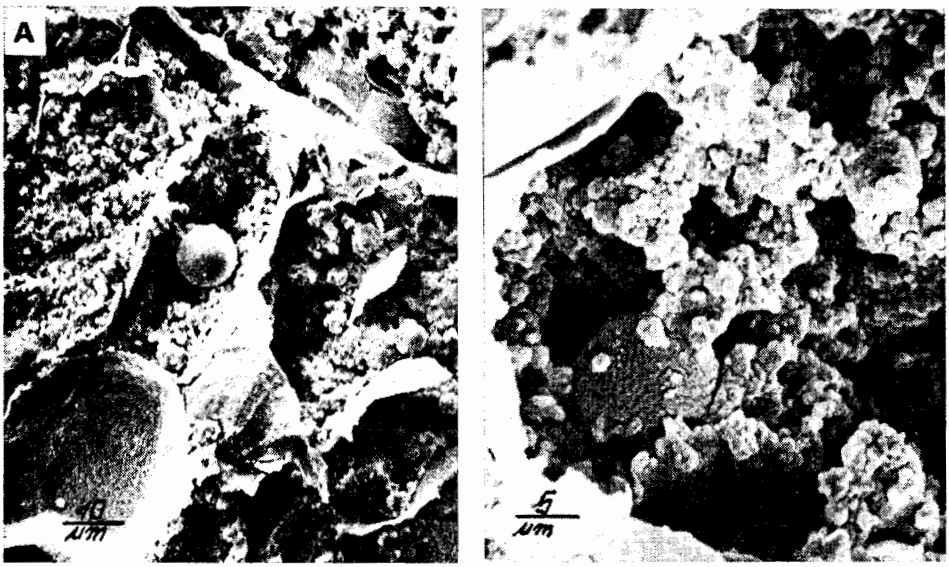


Photo 1. Cells of untreated rapeseed with different protein bodies

smooth surface with remnants of matrix protein (Photo 2A). The other, although similar to the former one as regards shape and size (Photo 1B), has a folded and grooved surface; its bottom part features a distinct projection which may be an element linking this protein structure with the remaining cell elements (Photo 2B). The differences in microscopic appearance of the two protein bodies may be due to different levels of development, different roles performed in the cell or different chemical structure.

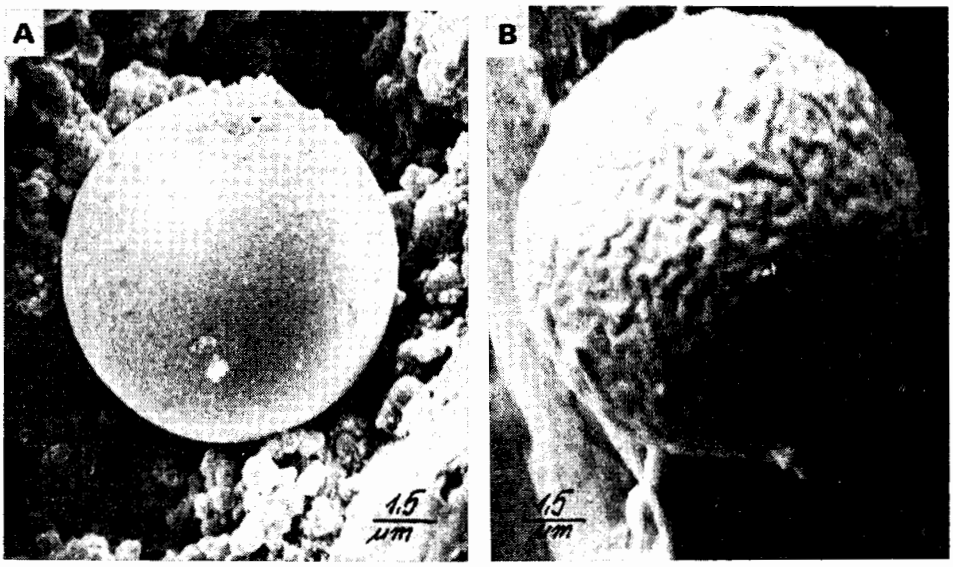


Photo 2. Higher magnification showing protein bodies with different surfaces

Norton et al. [11] report that two types of protein bodies may occur in rapeseed cells. Bodies of one kind which were found to contain globoid inclusions are termed aleuronic grains; the other bodies are referred to as myrosinic grains, they contain much more thioglucosidase and are stained more intensely by Millon's reagent because of their higher content of tryptophane and thyrosine. Our TEM micrographs appear to confirm this division. Photo 3A shows protein bodies, one of which features globoid inclusions; analogous micrographs were obtained by Stanley et al. [12] and Norton et al. [11]. According to the latter researchers, the observed globoid inclusions contain large amounts of Ca, Mg, P and S as well as acid phosphatase. Recent studies by Yiu et al. [13] also confirmed that most aleuronic grains in rapessed contain so called spherical inclusions, i.e. phytin globoid inclusions in the form of myoinositol salt.

A fragment of another cell in the same cotyledon Photo 3B features granules ca. 0.1 μm in diameter inside the protein bodies. This may be 12 S glycoprotein containing arabinose, glucose, inositol and mannose [5, 12].

The protein bodies shown in Photos 2A and 2B may be of significance in determining the localization of the thioglucosidase system. Matile [10] and Luthy and Matile [8] claim that the "oil mustard bomb" is an enzymatic system concentrated mainly in the vacuole in which the enzyme is separated from the substrate and the activator by a membrane. We believe that the presence of both protein forms (on the surface of which, similarly as near the cell walls, this enzyme's activity concentrates) may be an important factor. The presence of sulphur in the protein bodies [11] may be an indication that when the cell system (including also protein structures)

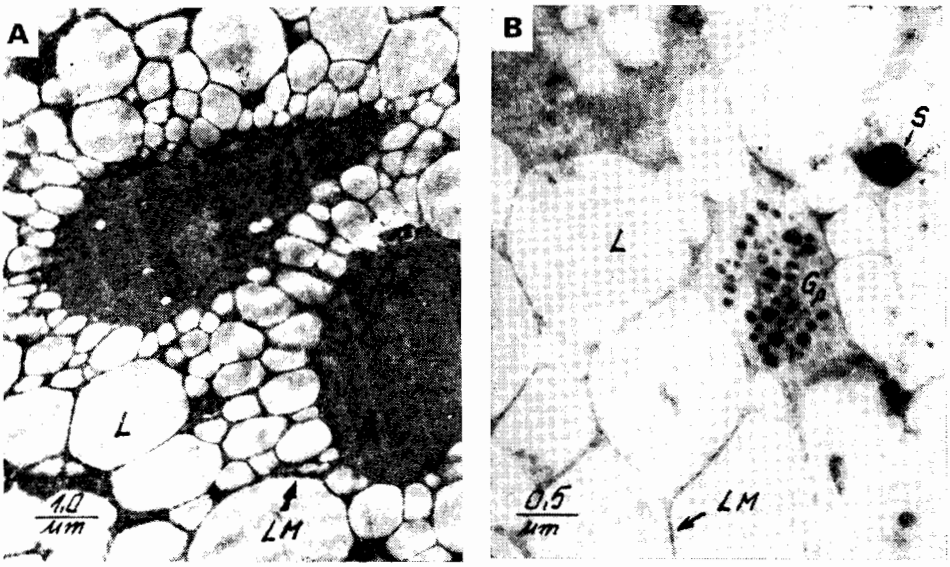


Photo 3. TEM micrographs of untreated rapeseed showing: A — aleuronic grains with globoid inclusions (G), L — lipid droplets, LM — lipid droplet membranes, $P_m$ — protein body membranes, $G_p$ — glycoprotein, S — starch granule

is disrupted, the enzyme has easier access to the substrate and the activator, and sulphur is more readily liberated into the oil.

The distribution of lipid droplets in the investigated seeds conformed to the typical distribution in most rapeseed varieties [2, 3, 5, 9, 11-14]. Their diameter ranged from 0.25 to 3.0 µm. Fine-grained membranes separating the individual droplets can be seen (Photos 3A and 3B).

#### HYDROTHERMAL PROCESSING

This treatment led to drastic changes of rapeseed microstructure. Protein underwent severe denaturation, the aleuronic grain membranes, clearly visible in the untreated material, were destroyed and the grains themselves coalesced to form a compact amorphous mass (Photo 4). In some parts of the preparation the protein mass is concentrated in the central part of the cell, while in others it occupies the entire cell. The surface of cell sections featured numerous smooth, spherical or flattened structures completely covering the cell content. The structures are probably the result of thermal gelation of rapeseed proteins, a phenomenon reported by Gill and Tung [4, 5]. They are characterized by axial fissures which may have been caused by limited expansion surface or may have arisen during cutting the specimens.

TEM micrographs show that the membranes surrounding lipid droplets have vanished and that the droplets coalesced into large concentrations

Photo 4. SEM micrograph of rapeseed steamed in fluidal layer. $P_m$ — protein mass, $P_c$ — protein cover

immediately next to the cell wall surface (Photo 5). The remainder of the cell is filled by protein in which it is no longer possible to distinguish the granular forms characteristic for the untreated material. It is also interesting to note that this treatment also led to structural changes in cell walls.

#### MICROWAVE TREATMENT

The microstructure of the studied rapeseed subjected to microwave treatment for 3.5 min is presented in Photos 6 and 7. Like other authors [9]

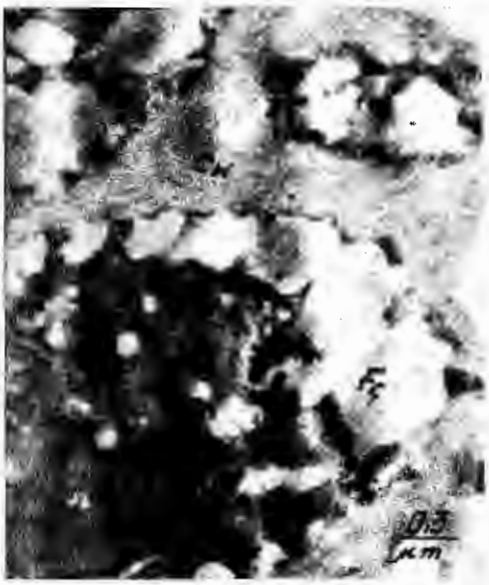

Photo 5. TEM micrograph of rapeseed steames in fluidal layer. $F_c$ — fat complex, $C_w$ — cell wall

Photo 6. SEM micrograph of rapeseed after microwave treatment

we observed a strong denaturation of proteins and their concentration in the cell's center. The aleuronic grains were also strongly denaturated and their smooth surface became quite irregular (Photo 6). Also observed, similarly as in the case of steaming, was the appearance of spherical, probably protein membranes on the cell surface. The behaviour of lipid droplets was also similar to that induced by steaming (Photo 7).


Photo 7. TEM micrograph of rapeseed after microwave treatment

GAMMA-IRRADIATION

Unlike in the previous two methods, in this case microscopic analysis revealed no significant morphological changes. SEM studies showed only slight structural changes in aleuronic grains in cotyledon cells (Photo 8), namely visible subsidence of their suface and deformation of their spherical shape. This was especially evident in large bodies in which these deformations were multiplanar. Numerous small protein bodies (0.3-0.5 μm in diameter) additionally formed sizeable conglomerates.

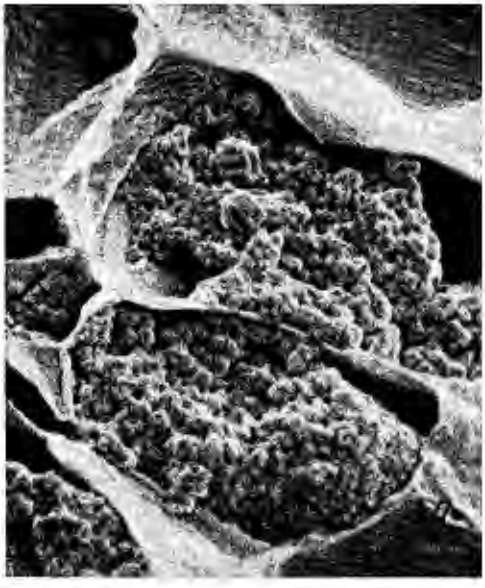

Photo 8. SEM micrograph of rapeseed after gamma-irradiation

TEM micrographs (Photo 9) confirmed the information collected with the scanning microscope. The membranes surrounding the protein bodies disappeared in part only, although they were strongly deformed. However, the internal structure of protein bodies remained comparable with that of protein in untreated rapeseed. The vast majority of lipid droplets were separated from one another, but in some places of the sample the membranes surrounding them apparently disappeared and the droplets there coalesced to form larger structures. However, this process was not as evident as in rapeseed treated with microwaves or steamed in a fluidal layer.

CONCLUSIONS

1. Of the three methods of inactivating thioglucosidase in whole rapeseed only steaming and microwave treatment significantly altered the structure of cell components, mainly proteins and fat. The considerable

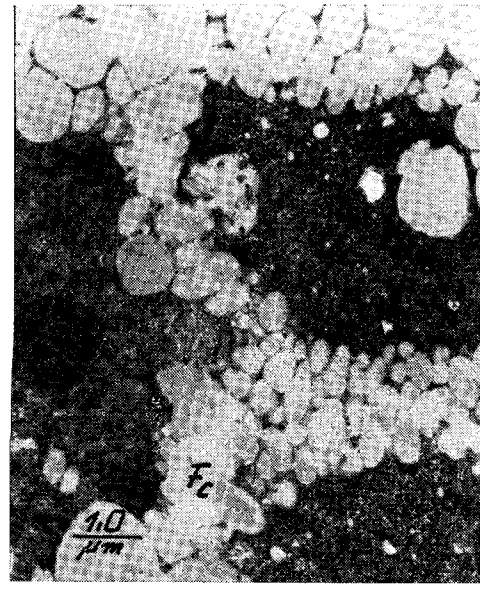


Photo 9. TEM micrograph of rapeseed after gamma-irradiation

destruction of these components corresponds well with the previously determined complete inactivation of the enzyme.

2. Following microwave treatment and steaming in a fluidal layer, the fat, occurring in untreated samples in the form of distinctly isolated droplets, loses the membranes encasing each droplet and accumulates next to the cell wall.

3. The accumulation of fat next to the cell wall may be due to strong denaturation of protein bodies of rapeseed, their coalescence into large conglomerates, and to the extrusion of fat from intergranular space.

4. The results of microscopic analyses indicate the advisability of thermal processing of whole seeds. Such treatment may facilitate oil extraction, the more so since it creates conditions in which thioglucosidase is completely inactivated.

LITERATURE

1. Dahlen J. A.: Patent No. 23/3224. Szwecja 1975.
2. Fornal J.: Materiały II Krajowego Sympozjum Chemii i Technologii Tłuszczów, Gdańsk 1985, 172.
3. Fornal J., Kozłowska H.: Materiały II Krajowego Sympozium Chemii i Technologii Tłuszczów, Gdańsk 1985, 175.
4. Gill T. A., Tung M. A.: Can. Inst. Sci. Technol. J., 1976, 9, 75.
5. Gill T. A., Tung M. A.: Cereal Chem., 1978, **55**, 180.
6. Kozłowska H., Nowak H., Nowak J.: J. Sci. Food Agric., 1983, **34**, 1171.
7. Kozłowska H., Rotkiewicz D., Fornal J., Bock H. D.: Getreidewirtschaft 1984, **18**, 247.
8. Luthy B., Matile P.: Biochem. Physiol. Pflanzen 1984, **179**, 5.

9. Maheshwari P. N., Stanley D. W., van de Voort F. R., Gray J. I.: Cereal Chem., 1984, **58**, 381.
10. Matile Ph.: Biochem. Physiol. Pflanzen 1980, **175**, 172.
11. Norton G., Harris J. F., Tomlinson A.: Development and deposition of protein in oliseeds. Plant Proteins., ed. G. Norton, Butterworths London-Boston 1978, 59.
12. Stanley D. W., Gill T. A., de Man J. M., Tung A.: Can. Inst. Food Sci. Technol. J. 1976, **9**, 54.
13. Yiu S. H., Poon H., Fulcher R. G., Altosaar I.: Food Microstructure 1982, **1**, 135.
14. Yiu S. H., Altosaar I., Fulcher R. G.: Food Microstructure 1983, **2**, 165.

*Manuscript received: July, 1986*
*Authors address: 10-957 Olsztyn, bl. 43*

*J. Fornal, M. Jousselin-Hosaja, H. Kozłowska*

WPŁYW PAROWANIA, MIKROFAL I PROMIENIOWANIA NA MIKROSTRUKTURĘ NASION RZEPAKU

Instytut Inżynierii i Biotechnologii Żywności, AR-T, Olsztyn

Streszczenie

W prezentowanej pracy przedstawiono wyniki badań zmian mikrostruktury nasion rzepaku poddanych wybranym procesom mającym na celu inaktywację enzymu tioglukozydazy. Za pomocą skaningowego i transmisyjnego mikroskopu elektronowego wykazano zróżnicowany wpływ tych procesów na podstawowe składniki chemiczne nasion rzepaku — białko i tłuszcz.

W kontrolnych nasionach rzepaku odmiany Skrzeszowicki zaobserwowano występowanie dwóch typów ciał białkowych. Różniły się one głównie wielkością i rodzajem powierzchni (fot. 1-3). Wysunięto przypuszczenie, że ciała te mogą mieć znaczenie w lokalizacji tzw. „bomby olejku gorczycznego".

Wykazano, że parowanie nasion w warstwie fluidalnej oraz działanie mikrofal powodują praktycznie identyczne zmiany mikrostruktury. Przejawiają się one w silnej denaturacji ciał białkowych, gromadzeniu się masy białkowej w centralnej części komórki, a tłuszczu w pobliżu ścian komórkowych (fot. 4-7).

Zastosowanie promieniowania γ spowodowało jedynie nieznaczną denaturację ciał białkowych, a także miało minimalny wpływ na zanik membran wokół kropel tłuszczu (fot. 8 i 9).