# Influence of Microwave and Steam Heating on Lipase Activity and Microstructure of Rapeseed (*Brassica napus*)

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The level of heat inactivation for rapeseed lipase depended largely on the physical environment of the enzyme (aqueous extract versus seed matrix). After heating, the fair amount of remaining activity observed was possibly due to the activity of a second, heat-stable lipase form. This more heat-stable enzyme started to denature at temperatures of >100 °C. At temperatures of <100 °C, lipase inactivation could be described with a single exponential decay model with a constant level of remaining activity. A correction for the biological age of the rapeseed was included in the analysis. Inactivation of lipase by steam and microwave heating appeared to be significantly different, probably because of different moisture contents during heating and not because of the type of heating. Microwave heating resulted in a higher free fatty acid content in rapeseed oil. Transmission electron microscopy showed that microwave heating may lead to an increased breakdown of oil droplets, leading to a higher availability of oil for reaction with lipase in the early stage of heating.

**Keywords:** *Electromagnetic energy; microwave; lipase inactivation; inactivation kinetics; seed microstructure; oil quality*

## INTRODUCTION

With the introduction of new varieties of rapeseed, which are low in content of glucosinolate and erucic acid, the use of rapeseed (*Brassica napus*) oil in food industry is increasing. The oil of these so-called double-zero varieties is very suitable for human consumption. In addition, there is a tendency to further improve the processing of rapeseed and the products obtained from rapeseed (Fornal *et al.*, 1992). The processing of rapeseed for production of oil involves pretreatment, extraction, and refining. Heating inactivates undesired enzymes (mainly lipase, lipoxygenase, phospholipase, and myrosinase) and improves oil yield. To retain good quality oil products from rapeseed, it is essential to inactivate lipolytic enzymes that may produce rancidity (Vetrimani *et al.*, 1992). Usually, heating is a steam treatment at 77−100 °C for 15−20 min, depending on the rapeseed variety (Ohlson, 1992). Recently, electromagnetic energy has been applied as an alternative heat treatment of rapeseed and soybeans (Maheshwari *et al.*, 1980; List *et al.*, 1990; Kovács *et al.*, 1991). Previous studies have shown that application of microwave energy is suitable for rapid heating of rapeseed and for adequate enzyme inactivation (Ponne *et al.*, 1991; Pelkmans, 1992). It was reported that microwave and steam heating had different effects on oil extraction efficiency and oil quality, especially on the free fatty acid content. In this study, the effects of microwave energy on lipase inactivation and on the oil quality are evaluated in more detail. Heat inactivation parameters of rapeseed lipase were estimated on the basis of inactivation experiments at static conditions (i.e., constant temperatures). The obtained kinetic parameters were used to simulate the dynamic inactivation to compare the effects of conventional (steam) and microwave heating on lipase inactivation. These heat treatments were carried out at dynamic conditions (i.e., temperature increased with time). In addition, the effects of the two heating methods on free fatty acid contents of the oil and on seed microstructure were studied.

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## MATERIALS AND METHODS

**Materials.** Double-zero variety rapeseed (Ronk 00; 42−46% oil) was purchased from Groenbroek Zaden, Scheemda, The Netherlands. The last set of experiments, comparing microwave and steam heating, was carried out with a seed batch from trade mark Unimills, Germany (43% oil). Seeds were stored at room temperature.

**Lipase Extraction.** The rapeseed was conditioned to a moisture content of 13% (w/w) by adding a calculated amount of water to a batch with a known moisture content. The seeds were mixed gently at regular intervals during a total period of 2 h.

Lipase was extracted from 5 g of rapeseed by adding 50 mL of Tris-HCl buffer (5 mM, pH 7.2) and incubating the samples at 4 °C overnight. The seeds were separated from the buffer by filtration at 4 °C. The seeds were crushed in a cool mortar and transferred quantitatively to a blender mixer (Braun). The original extraction buffer was re-added, and the seeds were mixed for 30 s at full speed. The mixture was filtered through cheesecloth, and the crude extract was centrifuged at 4 °C for 30 min at 13 000 rpm. The water phase of the supernatant was filtered again through filter paper. The final extract was kept at 4 °C and used within 24 h.

**Heating Methods.** The decrease of lipase activity was measured during heating of lipase in an aqueous extract of rapeseed (free lipase) and in whole rapeseeds. The experiments were carried out at static conditions (i.e., at various but constant temperatures). A second set of experiments were conducted at dynamic conditions (i.e., at varying temperatures).

**Heating−Static Conditions.** *Heating of Lipase in Aqueous Extract.* The lipase extracts were heated in thin-walled

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glass tubes in a water bath at 45, 50, 65, and 75 °C. Samples were taken at selected times up to 2000 s, transferred into Eppendorf tubes, immediately frozen in liquid nitrogen, and stored at −20 °C until analysis of lipase activity.

*Heating of Lipase in Whole Seeds.* The moisture content of the rapeseed was adjusted to 13% (w/w) prior to every heating session. Heating experiments were carried out at 75, 90, 100, and 130 °C with an oil bath (silicon oil). Plastic bags containing single layers of seeds were vacuum sealed. At selected time intervals, up to 120 s, one sealed bag was removed and immediately frozen in liquid nitrogen. Seeds were stored at −20 °C until further analysis.

**Heating−Dynamic Conditions.** *Steam Treatment.* Batches of 55 g of rapeseed with standardized moisture content (13% w/w) were exposed to steam at atmospheric pressure. Temperatures were recorded with two thermocouples in the layer of seeds. The batches were heated during time periods up to 120 s in steps of 10 s. After heating, the seeds were immediately frozen in liquid nitrogen and kept at −20 °C until further analysis. For each point of time, the heat treatment was carried out in triplicate, each time exposing a new rapeseed sample.

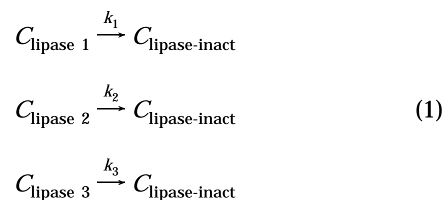
*Microwave Treatment.* Batches of rapeseeds with standardized moisture content (13% w/w) were heated in closed petri dishes in a 600 W household microwave oven (Moulinex, Germany) with maximum power. Temperatures were measured at four locations within the sample layer, using fiber optic temperature sensors (Luxtron, U.S.A.). Layers of 1 cm of rapeseed (50 g) heated up homogeneously throughout the whole sample. A linear increase of temperature in time with the same heating rate on all four locations was found. A load of 150 mL of tap water was placed in a separate beaker at a fixed location in the oven. This was carried out to increase the total product mass in the oven to reach the desired heating rate of the seeds. For every time period (up to 120 s in steps of 10 s), new rapeseed samples were heated and immediately frozen for lipase activity analysis. All heating experiments were carried out in triplicate.

**Measurement of Lipase Activity.** Whole seeds were defrosted, and lipase was extracted as described before. Heated lipase extracts were defrosted and used as such. Lipase activity was measured according to a colorimetric method described by Mosmuller *et al.* (1992), with 2,4-dinitrophenyl butyrate as a substrate.

**Free Fatty Acid Analysis.** Samples of rapeseed from the dynamic steam and microwave heating experiments were freeze dried. Oil was extracted from the dried seed samples with petroleum ether in a Soxtec extraction unit (Perstorp Analytical, Herndon, VA). After extraction and cooling, oil samples were immediately analyzed for free fatty acid (FFA) content. Samples of 1 g of oil were weighed and dissolved in a mixture of 20 mL of hexane and 20 mL of ethanol. Titration with 0.01 N KOH was carried out with phenolphthalein as indicator. The FFA content was measured in triplicate and expressed in % FFA (g of oleic acid per 100 g of oil).

**Transmission Electron Microscopy of Seed Microstructure.** For transmission electron microscopy (TEM), a new series of samples was prepared according to the dynamic steam and microwave heating procedures. To prevent damage to the seed structure, the seeds were not frozen after heating but cut in half and immediately imbedded in a fixation medium of 2% formaldehyde and 3% glutaraldehyde in phosphate−citrate buffer (pH 7.2) and incubated at 4 °C for 6 days. After fixation, the seeds were rinsed five times in buffer for 5 min. Samples were colored for lipids by a second fixation in 1% osmium oxide in a dark room for 1 h at room temperature. The seeds were rinsed in purified water and dehydrated in a graded ethanol series. Seeds were embedded in Spurr (an epoxy resin). Samples were cut in ultra thin coupes of 60−90 nm with a diamond knife (Microtome LKB, Bromma, Sweden). The coupes were collected on 100 mesh copper grids and washed in 2% uranyl acetate. Seed microstructure was studied with a Philips EM 400 transmission electron microscope.

**Lipase Heat Inactivation Model.** *Heat Inactivation Kinetics−Static Conditions.* These heat inactivation data sets indicate the existence of at least two isoenzymes (see Results and Discussion). In addition to two different inactivation mechanisms found in the lipase extract, a fair amount of lipase activity remained after heating. For this reason, three different inactivation steps were distinguished in the model:



$$C_{\text{lipase 1}} \xrightarrow{k_1} C_{\text{lipase-inact}}$$
$$C_{\text{lipase 2}} \xrightarrow{k_2} C_{\text{lipase-inact}} \qquad (1)$$
$$C_{\text{lipase 3}} \xrightarrow{k_3} C_{\text{lipase-inact}}$$

The method applied in measuring lipase activity does not discriminate between the two lipases: the sum of both activities is measured (eq 2):

$$C_{\text{tot}} = C_{\text{lipase 1}} + C_{\text{lipase 2}} + C_{\text{lipase 3}} \qquad (2)$$

Heat-induced inactivation of enzymes can usually be described by a first-order decay, which results in the analytical solution shown in eq 3:

$$C_i = C_{0,i}\, e^{-k_i t} \qquad (3)$$

The inactivation rate $k_i$ is a function of temperature most probably according to Arrhenius' law (eq 4), where $T_{\text{ref}}$ is the reference temperature chosen within the range of the measurements:

$$k_i = k_{i,\text{ref}}\, e^{\frac{E_i}{R}\left(\frac{1}{T_{\text{ref}}} - \frac{1}{T_{\text{abs}}}\right)} \qquad (4)$$

Analyses were made assuming a constant level of remaining activity, $C_{\min}$ ($k_2$ and/or $k_3$ equal zero). This assumption leads to the following model (eq 5), known as the Mitscherlich function (Genstat 5 Reference Manual, 1993):

$$C = C_{\min} + (C_{\max} - C_{\min})\, e^{-kt} \qquad (5)$$

where $C_{\max}$ represents the initial maximal activity.

For lipase in aqueous extract, separate analysis of the lower and higher temperatures was carried out. In the data set of lipase inactivation in whole seeds, only temperatures up to 100 °C were analyzed, assuming $k_2$ and $k_3$ to be zero. Because different series had different initial activities, it was obvious that the individual substrates (rapeseeds) had a different biological age or at least a different history of decay. Therefore, a correction should be applied in an integral analysis of the data. This correction was developed by rewriting the Mitscherlich function with a series dependent time shift Δt (eq 6) and where $C_{\max}$ represents the maximal concentration on an arbitrarily chosen time scale:

$$C = C_{\min} + (C_{\max} - C_{\min})\, e^{-k(t+\Delta t)} \qquad (6)$$

Solving the equation for $\Delta t$ with $C = C_0$ at $t = 0$ and simplifying the expression leads to eqs 7 and 8, respectively.

$$\Delta t = \frac{\ln\left(\frac{C_{\max} - C_{\min}}{C_0 - C_{\min}}\right)}{k} \qquad (7)$$

$$C = C_{\min} + (C_0 - C_{\min})\, e^{-kt} \qquad (8)$$

In these equations, $C_0$ is the initial activity for each data series separately.

**Data Analysis.** Equation 8 was used together with the Arrhenius equation for $k$ (eq 4) in a nonlinear regression analysis on all data of static heat inactivation experiments, with time $t$ and temperature $T$ simultaneously as explaining

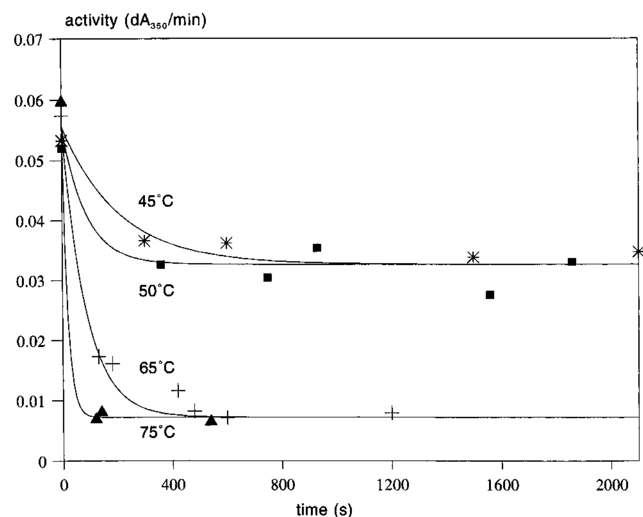



**Figure 1.** Activity of free lipase as a function of time at different temperatures. (*) 45 °C; (■) 50 °C; (+) 65 °C; (▲) 75 °C. Simulation results are indicated with lines with $k_1 = 5.27$ s$^{-1}$, $k_2 = 0.46$ s$^{-1}$, and $E = 150$ kJ·mol$^{-1}$ (Table 1).

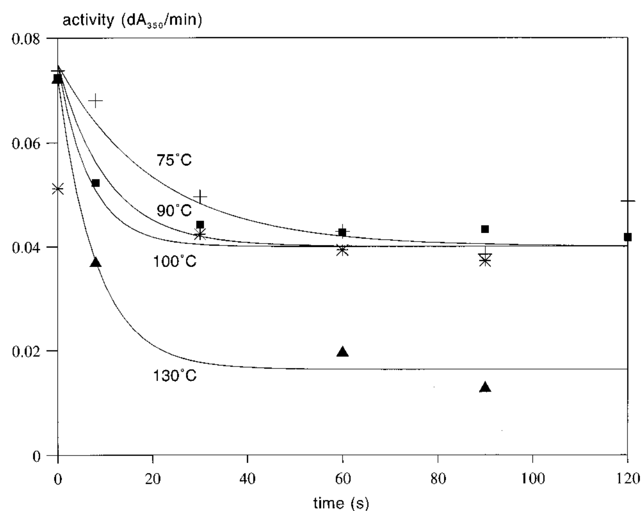



**Figure 2.** Lipase activity in rapeseed as a function of time at different temperatures. (+) 75 °C; (■) 90 °C; (*) 100 °C; (▲) 130 °C. Simulation results are indicated with lines with $k_1 = 0.096$ s$^{-1}$ and $E = 49$ kJ·mol$^{-1}$ for $t \leq 100$ °C; and $k_2 = 0.124$ s$^{-1}$ for $t = 130$ °C (Table 1).

variables using GENSTAT (Rothamsted Experimental Station, U.K.). The inactivation parameters $k_{ref}$, $E$, and $C_{min}$ were estimated simultaneously.

**Heat Inactivation Kinetics—Dynamic Conditions.** The reference inactivation constant ($k_{ref}$, with $T_{ref} = 90$ °C) and the activation energy of the inactivation ($E$) estimated from the static heating experiments were used to predict and compare lipase inactivation during dynamic microwave and steam heating. In this case, the parameters were applied in a dynamic scenario, using the inactivation model described in the differentiated form of eq 8 in combination with the Arrhenius equation for $k$. In the dynamic situation, however, temperature is not constant but increasing with time. This dynamic situation was implemented in the model by replacing a constant temperature by the equation $T = at + T_0$ and by numerical integration. Heating rates ($a$) and initial temperature $T_0$ were measured both for the steam heating and for microwave heating and implemented in the model.

## RESULTS AND DISCUSSION

**Heat Inactivation of Lipase.** *Heat Inactivation Kinetics—Static Conditions.* Regression analysis of the lipase inactivation data for each temperature series separately resulted in a percentage variance that accounted for >90%. Nonlinear regression analysis of the combined data was carried out with time and temperature as explaining variables.

*Lipase in Aqueous Extract.* Analysis of the data with the single exponential model described in eq 8 resulted in a fairly good explanation of experimental variance (89%), with a $k_{ref}$ of 0.67 s$^{-1}$ and $E$ of 166 kJ·mol$^{-1}$ (simulation not shown). Combined analysis of the two lower temperatures (45 and 50 °C) and the two higher temperatures (65 and 75 °C) with the same input value of 150 kJ·mol$^{-1}$ for the inactivation energy, but different estimates for $k_i$ and $C_{min}$, resulted in a very good percentage of variance accounted for the total data set of (98.0%). To carry out the analysis, the value for $E$ was fixed at 150 kJ·mol$^{-1}$. This value was estimated from preliminary analysis of temperature series separately. The activity of free lipase during heating at different temperatures is shown in Figure 1. Estimated values of $k_i$ and $E_i$ are given in Table 1. The results from this free enzyme inactivation are not used for further analysis in this study.

*Lipase in Whole Seeds.* The decay of lipase activity was described by a Mitscherlich function with a correction for biological age (eq 8). In this model, it is assumed that the level of remaining activity is constant; that is, the inactivation rate ($k_2$ and $k_3$) for the heat-stable lipases is zero. The single-exponential model described in eq 8 resulted in a high $R^2_{adj}$ (89.6%) for the combined temperature series 75, 90, and 100 °C (Figure 2). However, the model was not applicable for the 130 °C data set. The best fit through these data by an arbitrarily chosen exponential decay model is shown in Figure 2. It appears that at temperatures higher than 100 °C the second, heat stable enzyme also decays. This behavior strongly resembles the behavior of lipase activity in extract at lower temperatures. However, the heat inactivation at temperatures >100 °C cannot be analyzed in more detail because of the limited number of experimental data at these temperatures.

**Table 1. Results of Statistical Analysis of Heat Inactivation of Rapeseed Lipase at Static Conditions**

| parameter | lipase extract (SE) | lipase in seeds (SE) |
|---|---|---|
| $k_{1,ref}$ (s$^{-1}$) | 5.27 (1.87) | 0.096 (0.021) |
| $k_{2,ref}$ (s$^{-1}$) | 0.46 (0.06) | 0.124 |
| $E_1$ | — | 49 (33) |
| $E_{1,2}$ (kJ·mol$^{-1}$) | 150 (fixed) | — |
| $C_{lipase2}$ ($\Delta A_{350}$/min) | 0.025 (0.0010) | 0.025 (0.0016) |
| $C_{lipase3}$ ($\Delta A_{350}$/min) | 0.007 (0.0010) | 0.016 (0.0016) |
| $T_{ref}$ (°C) | 90 | 90 |
| $N_{obs}$ | 22 | 31 |
| $T_{lim}$ (°C) | >50 | >100 |
| $R^2_{adj;1,2}$ | 98.0 | — |
| $R^2_{adj,1}$ | — | 89.6 |
| $R^2_{adj,2}$ | — | 98.2 |

The presence of a remaining activity or a heat-stable lipase was reported earlier for cereal bran and soy beans by Vetrimani (1992). It is still inconclusive whether rapeseed contains only one lipase, located in the membrane of the lipid body, or two distinct lipase isoenzymes (Hassanien and Mukherjee, 1986). Hills and Murphy (1988) and Ncube *et al.* (1993) found lipase activity both in purified lipid body membranes and in microsomal fractions. The enzymes had different characteristics with respect to pH optimum, response to substrate concentration, and fatty acid constituents (Hills and Murphy, 1988).

The results show that the inactivation kinetics depend very much on the physical environment. Obviously, the

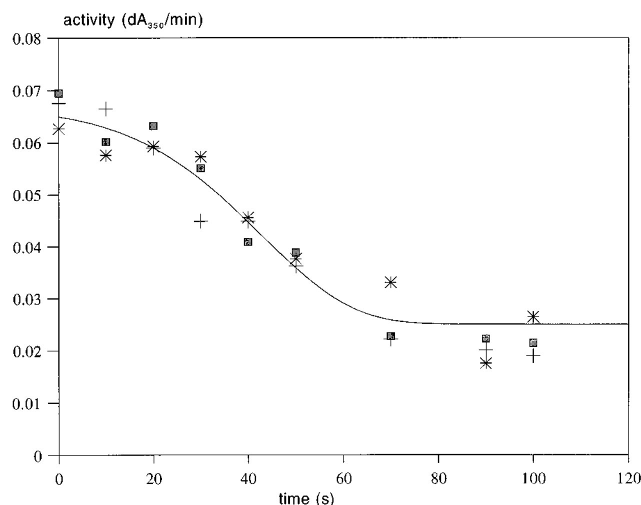



**Figure 3.** Inactivation of rapeseed lipase during microwave heating. Experimental data are shown with symbols and the simulation with the dynamic model is shown with a line ($R^2_{adj}$ = 98.3).

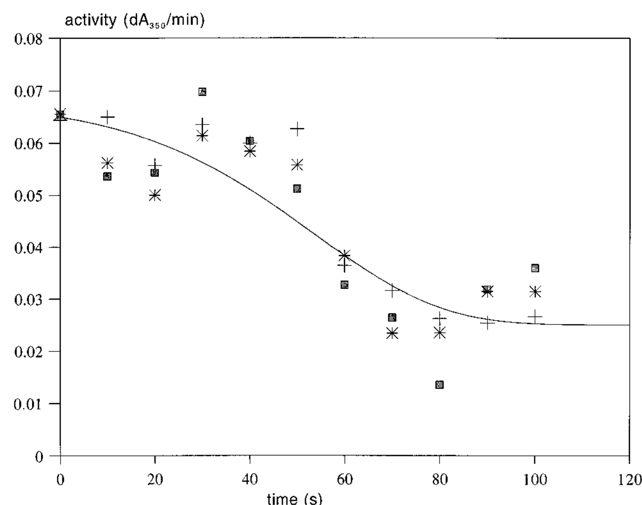



**Figure 4.** Inactivation of rapeseed lipase during steam heating. Experimental data are shown with symbols and the simulation with the dynamic model is shown with a line ($R^2_{adj}$ = 79.2%).

moisture content in the rapeseed extract is much higher than in whole seeds. In aqueous environments, lipase is more easily inactivated by heating. The rate of inactivation at the reference temperature (90 °C) is 5−50 times higher in aqueous media than in the seed matrix (Table 1).

Van Zuilichem *et al.* (1993) also found an increased heat resistance during inactivation of trypsin inhibitor in soy beans with low moisture content (13%) compared with beans with high moisture content (43%). Similar results were obtained for inactivation of phospholipase in soy beans (List *et al.*, 1990) and for myrosinase in Canola seeds (Owusu-Ansah and Marianchuk, 1991). Meerdink (1993) gave an overview of empiric models that describes the dependence of the inactivation constant $k$ of enzymes on temperature and water concentration. Both the activation energy of the enzyme inactivation ($E$) and the rate constant ($k$) increase at higher water contents. As a result, the temperature sensitivity to inactivation of the enzyme increases. However, most models described in the literature are usually not built on fundamental knowledge of enzyme inactivation mechanisms at different water contents.

**Heat Inactivation Kinetics—Dynamic Conditions.** *Effect of Steam/Microwave Heating on Lipase Inactivation.* The activity of lipase in the seeds at various times during a microwave and steam treatment are shown in Figures 3 and 4, respectively. The two heating methods resulted in significantly different inactivation rates ($p = 0.05$). For steam heating, the increase in temperature up to 90 °C was linear in time, with a heating rate of ~0.76 °C·s$^{-1}$. The increase in temperature was linear for the whole microwave heat treatment, with a heating rate of 1.1 °C·s$^{-1}$. A dynamic model based on the differential of eqs 8 and 4 was used in simulation experiments at the same conditions. The dynamic heat treatments were carried out with whole rapeseeds, so the kinetic parameters for whole seeds up to 120 °C ($k_{ref} = 0.096$ s$^{-1}$ and $E = 49$ kJ·mol$^{-1}$) were applied. The $R^2_{adj}$ of the dynamic heat inactivation model is significantly better for the microwave treatment than for the steam treatment (98.3 and 79.2%, respectively).

The heat inactivation during microwave heating followed the expected pattern almost exactly. Consequently, no specific "microwave" effects on lipase inactivation are apparent. During steam-heat treatment, the moisture content of the seeds increased. Therefore, the inactivation constants $k$ and $E$ increase during the treatment. This effect was not included in the model. The "delay" in lipase inactivation by steam heating may also be affected by the way temperatures were recorded.

Thermocouples were placed among the seeds. In contrast, with the direct internal microwave heating, the heat penetration by steam heating may be somewhat slower, causing lower temperatures in the seeds than actually measured.

**Table 2. FFA Content (%) of Rapeseed Oil after Dynamic Steam and Microwave Heating (g of Oleic Acid per g of Oil)**

| treatment | time (s) | % FFA |
|---|---|---|
| untreated | 0 | 0.53 |
| steam | 40 | 0.55 |
| | 90 | 0.55 |
| | 200 | 0.64 |
| mean (SE) | | 0.58 (0.045) |
| microwave | 10 | 0.57 |
| | 20 | 0.66 |
| | 50 | 0.69 |
| mean (SE) | | 0.64 (0.053) |

**Free Fatty Acid (FFA) Analysis.** As a result of lipase activity, FFAs are formed. In intact seeds, lipase and oil are located in different segments of the seed. During the extraction, the oil may be hydrolyzed. The contents of FFA in oil extracted from the rapeseed samples taken during dynamic heating are shown in Table 2. The FFA contents increase somewhat during the initial stage of heating. The mean FFA content of the microwave treated samples is significantly higher than that of the steam-treated samples ($p = 0.05$). The same observation was made in previous studies (Ponne *et al.*, 1991; Pelkmans, 1992). Comparing the activity of lipase during heating with steam or microwave did not reveal any higher levels of activity that could have caused a higher oil hydrolysis during microwave heating. This result implies that the increased FFA content may be a result of an increased time of contact between oil and lipase, caused by earlier rupture of oil droplets by microwave heating. To evaluate this hypothesis, the

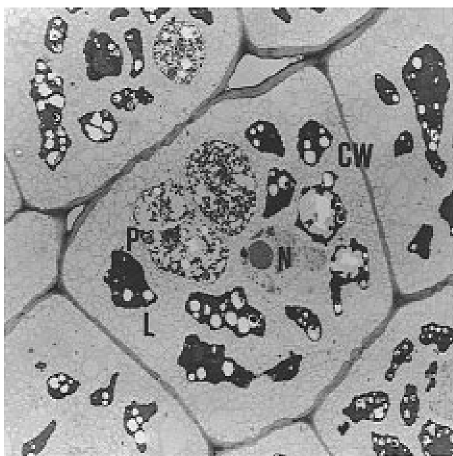
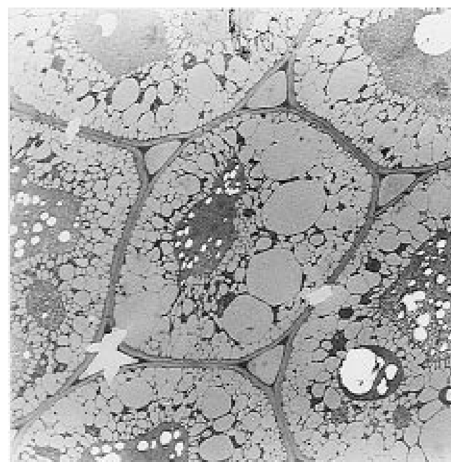
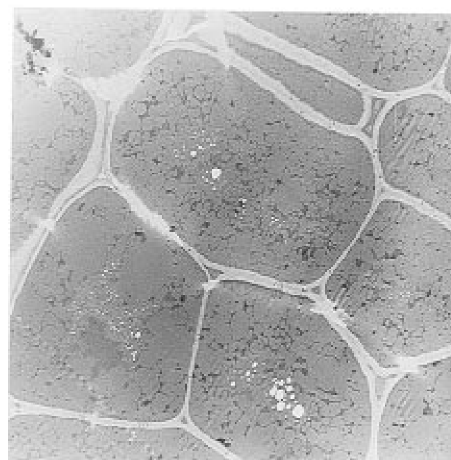



**Figure 5.** TEM picture of intact rapeseed (13% moisture) showing cells with cell wall (CW), protein bodies (P), cell nucleus (N), and oil droplets (L); magnification 2600×. (This figure is reproduced here at 75% of the original.)

structure of the seeds was studied with by electron microscopy.

**Transmission Electron Microscopy (TEM) Determination of Seed Microstructure.** Fornal *et al.* (1992) showed, in raw rapeseed material, that protein bodies (concentrated in aleuron cells) have a regular spherical shape and are immersed in the cell matrix. Oil droplets among the protein bodies are separated by fine membranes. Bengtsson *et al.* (1976) and Appelqvist (1976) describe the same structures with different components separated into compartments. This segmentation implies that lipolytic enzymes, such as lipase and lipoxygenase, will normally not make contact with the lipid substrate. In Figure 5 (untreated), Figure 6 (microwave heated), and Figure 7 (steam heated), pictures are shown of cotyledon cells of rapeseed at various times of dynamic heating. In the intact seeds (Figure 5), cells with densely packed oil droplets in the cytoplasm surrounded by a thin membrane are clearly visible. Isolated protein bodies and a cell nucleus can also be distinguished. During both heat treatments (Figures 6 and 7), a remarkable swelling of the oil droplets and coalescence of the oil can be observed. Membranes of the oil droplets rupture and oil accumulates near the cell wall. At the same time, protein structures agglomerate into larger complexes. These observations are in agreement with those reported by Fornal *et al.* (1992), who observed similar structural changes of cotyledon cells in rapeseed after 3 min of steaming. Longer times of steaming resulted in a continuation of the structural changes. However, the structural changes differ for microwave and steam heating (Figures 6 and 7). For microwave heating, the rupture and confluencing of small lipid bodies into large oil complexes occur in an earlier stage of heating (already evident after 10 s). Even when the difference in heating rate is taken into account, the effect of 40 s of steam heating on rupture of oil bodies is not as dramatic as 10 s of microwave heating. It is likely that the microwave energy itself dissipates within the seed material and causes a direct heating and expansion of the oil droplets. This efect of microwave energy could explain the slight but significant difference observed in FFA content of microwave-heated seeds; that is, the early breakdown of oil droplets allows lipase, which is not inactivated during the initial stage of heating, an increased opportunity to react with lipids. The effect of microwave heating on seed structure may also result





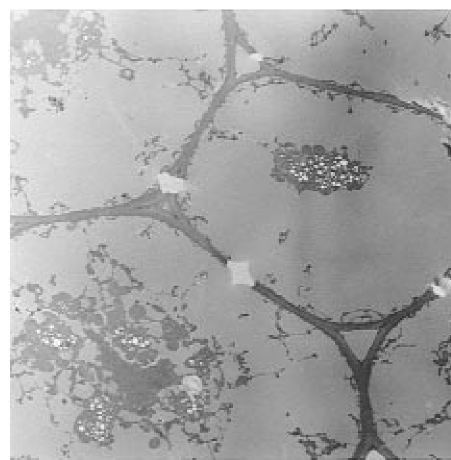


**Figure 6.** TEM picture of microwave-treated rapeseed after different heating times: (A, top) 10 s; (B, middle) 20 s; (C, bottom) 50 s. 2600×. (This figure is reproduced here at 75% of the original.)

in an increased oil extraction efficiency for short heating times because of the higher accessibility of the lipids. This effect has already been reported by Ponne *et al.* (1991).

**Conclusions.** Nonlinear regression based on model formulation provides a powerful tool to estimate characteristic properties of rapeseed lipase activity and its denaturation. The heat inactivation of lipase could be described with a single-exponential model with a constant remaining activity and a correction for biological age. The rate of inactivation is primarily determined by the physical environment of the enzyme (aqueous

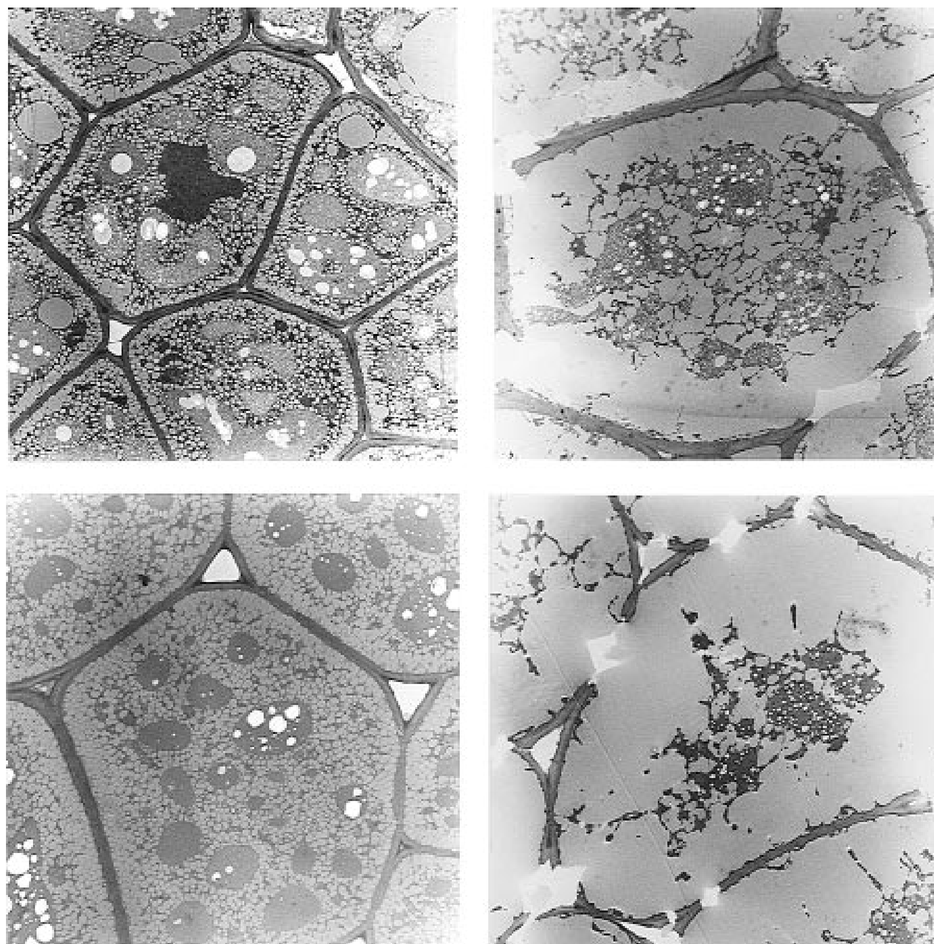


**Figure 7.** TEM picture of steam treated rapeseed after different heating times: (A, top left) 20 s; (B, bottom left) 40 s; (C, top right) 90 s; (D, bottom right) 200 s. 2600×. (This figure is reproduced here at 75% of the original.)

extract versus seed matrix). Apparently, two separate rapeseed lipase species, a heat-sensitive one and a heat-stable one, are present in rapeseed. The heat-stable enzyme seems to denature only at temperatures >100 °C. The heat-sensitive one is completely inactivated at 100 °C. The different action of lipase following steam and microwave heating may be explained by differences in moisture content during heating. For practical use, however, both the microwave heat treatment and the steam treatment are adequate in reducing lipase activity.

The FFA content of oil from steam- and microwave-heated rapeseed was acceptably low, which makes both heating technologies suitable for practical application as a pretreatment. However, microwave heating caused a slightly higher FFA content in the rapeseed oil than did steam heating. Studies of the seed structure showed that this difference is probably due to an increased availability of lipids for reaction with lipase in the early stage of microwave heating.

## ABBREVIATIONS USED

*C*, lipase activity ($\Delta A_{350}$/min); *k*, inactivation rate constant ($s^{-1}$); *E*, activation energy of heat inactivation ($J{\cdot}mol^{-1}$); *R*, gas constant (8.314 $J{\cdot}K^{-1}{\cdot}mol^{-1}$); *t*, time (s); *T*, temperature (K); *N*, number; 0, initial, at $t = 0$; abs, absolute, measured temperature; *i*, number of lipase isoenzyme; inact, inactive, denatured; max, maximal activity; min, minimal activity; obs, observations; ref, reference temperature 90 °C (363 K).

## ACKNOWLEDGMENT

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