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ScienceDirect
Food Chemistry 103 (2007) 444–453
Food Chemistry
www.elsevier.com/locate/foodchem

Examining for possible non-thermal effects during heating in a microwave oven

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Received 12 March 2006; received in revised form 21 June 2006; accepted 22 August 2006

Abstract

The assumption that the thermal effect (heating) is the sole factor that should be considered when a microwave source is applied has been debated by many reports, often claiming that athermal (non-thermal) effects exist as well. Such effects are claimed to change the chemical, biochemical, or the physical behaviour of some systems while the temperature and all other parameters remain unaltered. The possibility of an athermal effect was tested in a number of chemical, biological and physical systems in a very well controlled, high radiation intensity system (2.45 GHz, up to 1000 W/kg, with continuous radiation up to 48 h). The systems that were tested included: Maillard reaction, protein denaturation and polymer solubility, mutagenesis of bacteria, mutarotation equilibrium of α/β-D-glucose, and saturation solubility of NaCl. All data failed to show any significant athermal effects. The results of this study are in contrast to what has been previously reported for some of the tested systems.

Keywords: Microwave; Athermal; Non-thermal; Heating

1. Introduction

Microwave radiation is extensively used for domestic microwave ovens, radio communication and radar. In many countries, people are exposed to such radiation on a daily basis, and are often concerned about possible negative health impacts. Moreover, food is subjected to relatively high radiation intensity during its preparation in a microwave oven. It is well recognized that, occasionally, adverse effects do occur during microwave heating of food. Due to the relatively low energy of single photons in the microwave range (0.1–3 GHz, 10^{-3}–10^{-4} eV), it has been commonly assumed that energy absorption in that range involves only dissipation by heat, and therefore only thermal effects should be considered. On the other hand, there are many reports that claim the existence of athermal (non-thermal) effects, and these challenge the assumption that the thermal effect (heating) is the sole factor to be considered when microwave radiation is absorbed (Bohr & Bohr, 2000; de Pomerai et al., 2003; Jacob, Chia, & Boey, 1995; Pagnotta, Pooley, Gurland, & Choi, 1993; Porcelli et al., 1997; Velizarov, Raskmark, & Kwee, 1999). Extensive research has been conducted to determine whether athermal effects are associated with microwave radiation, and to measure the extent of the phenomenon if it exists.

An extensive review reflecting this debate about athermal effects was published by the Royal Society of Canada (Byus et al., 1999). This review focusses on the potential health risks of radio frequency fields from wireless telecommunication devices. It reflects the controversy of the topic of mutations in microorganisms due to microwave radiation, and lists 11 scientific publications indicating athermal effects, as compared to eight cases in which no such effects were observed. These investigations have used a wide range of microwave energy absorption or intensities. Thus, the comparison of these studies is difficult, if not impossible, due to the lack of common experimental conditions.

Since biological systems are complex, simpler processes were also tested for the effects of microwaves on their

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doi:10.1016/j.foodchem.2006.08.024

outcome. It was suggested that microwave irradiation could athermally affect the kinetics of the β-lactoglobulin folding process (Bohr & Bohr, 2000). In that study, the protein was heated by microwave, or conventionally in water bath, to a specific temperature and the protein folding was measured by an offline polarimeter. In addition, another study reported that exposure to microwave radiation, which resulted in even a relatively low absorption of energy, of 15–20 mW/kg, was sufficient to athermally enhance the aggregation process of bovine serum albumin, in vitro in a time- and temperature-dependent manner (de Pomerai et al., 2003). An athermal effect was also reported for the mutarotation of α-D-glucose in ethanol–water mixture (Pagnotta et al., 1993). This effect is expressed by an increased reaction rate and a change in the relative amounts of α- and β-D-glucose over time (only in the presence of ethanol). However, many other studies could not detect any athermal effect, e.g., in thiamine degradation kinetics (Welt, Steet, Tong, Rossen, & Lund, 1993) or in early formation of Maillard reaction products (Meissner & Erbersdobler, 1996). In light of the controversy, the study of Gedye (1997) is of great interest. In his study, Gedye (1997) tested a series of organic reactions that were previously reported to be significantly accelerated by microwave radiation. In his experiments, which had a very careful temperature control, Gedye did not detect any microwave athermal effects.

The cumulating studies of microwave effects, present an unclear picture as to the existence or the source of possible athermal effects. Thus, there is a need for reliable data that should be obtained in well-controlled systems. One of the possibilities is that athermal effects are infrequent, and thus, high microwave intensity should be used to allow these effects to be detected over short time periods. In addition, experiments on athermal effects should include very careful and accurate temperature control. In the case of domestic microwave heating, it is also important to minimize or eliminate hot spots. Moreover, one should be able to compare the results of such a system with one that goes through the same thermal history under similar conditions, but with a conventional source of heating energy.

In the present study, we have undertaken to build a system that will allow the output of high microwave energy, with good temperature control, while in parallel accurately performing a similar heating procedure, having the same time–temperature profile, in a conventional non-microwave heating apparatus. Using this layout, we studied five systems, including the Maillard reaction, protein denaturation, mutagenesis of bacteria, glucose mutarotation, and saturation solubility of sodium chloride.

2. Materials and methods

2.1. Experimental system

The experimental system (Fig. 1) consisted of a 250 ml Erlenmeyer flask, containing the reaction solution, that is placed in a microwave cavity, or in an oil bath for the control tests. The reaction solution was continuously circulated through the heating system and the online detector. A high flow-rate was used in order to produce effective mixing and thus eliminate the 'hot spot' problem. Atmospheric pressure was maintained in the flask by venting it to the external surroundings via a short tube. The inlet and outlet (TC_1–TC_2) solution temperatures were measured and recorded by a computerized logger (accuracy ±0.05 °C, resolution 0.01 °C). The microwave (700 W), and the heating bath (1200 W), were controlled by a temperature controller with a temperature profile programming option (Eurotherm-model 2416 a PID controller, accuracy of ±0.1 °C, Leesburg, VA, USA). For the microwave, the feedback changed the feed voltage (0–230 V AC) of the magnetron high-voltage transformer, as described by Gropper, Ramon, Kopelman, and Mizrahi (1998). For the bath, the temperature control was working in an on–off mode. To achieve fast enough temperature response in the bath, the heating oil volume was kept to a minimum of about 1 l, just to keep the flask totally immersed. For experiments involving microorganisms, a sampling port replaced the detector.

The absorption of microwave energy by the test solution was calculated, taking into account the energy needed to make up for heat losses to the surroundings. Stopping the microwave radiation at a certain temperature, and monitoring the temperature drop with time, allowed evaluation of the heat losses within the microwave cavity. The heat losses outside the microwave cavity were evaluated by the temperatures of TC_1 and TC_2, solution flow rate and heat capacity (C_p). When the process involved an increase in temperature, the required energy was also added to these calculations.

2.2. Formation of Maillard reaction products

In order to test the formation of Maillard reaction products, three solutions of D-(±)-glucose and glycine were prepared. The solutions were composed of a glucose solution

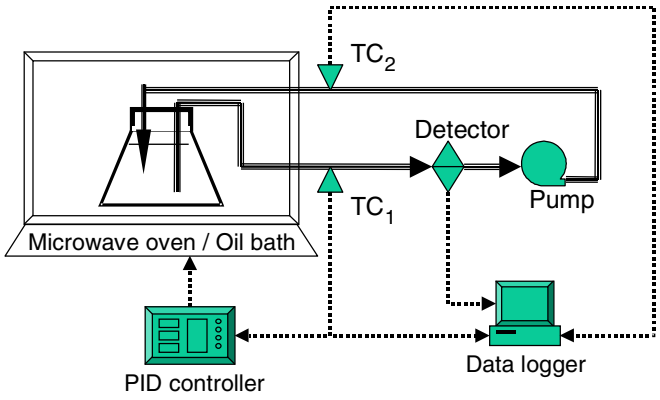

Fig. 1. Scheme of the experimental system (TC_1 and TC_2 are the microwave cavity outlet and inlet, respectively).

and a glycine solution, prepared separately, and mixed for the experiment. In all tests, glucose solution (200 ml) was first placed in the Erlenmeyer flask and heated while circulating through the experimental system until the desired test temperature was reached. At this point, glycine solution (50 ml) at the test temperature was added to the glucose solution. The time of adding the glycine solution was marked as zero. The flow rate of the final solution was 400 ml/min, and the microwave energy input was 31 W for 250 ml of solution. The absorbance (420 nm) was measured online, or at the end of the experiment. Due to the long duration of the test (up to 48 h), we checked for possible evaporation differences between bath and microwave. This difference was found to be negligible, and no more then 0.5%.

The compositions of the solutions were as follows: for test A, 200 ml of a solution of 1.6 M glucose, with H_3PO_4 to give a pH of 5.0 (and sorbate 0.1%w/w as a preservative), were mixed with a 50 ml of 0.5 M glycine, pH 6.7 (sorbate 0.1% w/w), and tested at 64.4 °C; for test B, similar solutions without H_3PO_4 and pH 7.4 were tested at 65.0 °C; for test C, 200 ml of 1.6 M glucose, pH 7.4 (and 0.02% w/w NaN_3 as a preservative), were mixed with 50 ml of a solution of 0.5 M glycine, pH 6.7 (NaN_3 0.02%), and tested at 55.0 °C.

2.3. Protein denaturation and polymer solubility

The experimental solutions were prepared from egg white (spray-dried, Cham Food Israel, Ltd., or from fresh eggs), bovine serum albumin (Sigma A-4503), and ethyl(hydroxyethyl)cellulose (EHEC) (Bermocoll® E-35X, AKZO NOBEL SB, Arnheim, Netherlands). Antifoam (0.01 g/l) was added also to all protein solutions (SIGMA-Antifoam A).

All test solutions were prepared and equilibrated at 4 °C for 14–16 h, and the pH was measured at the end of equilibration. The specific compositions and test conditions for the various solutions are presented in Table 1. Protein or polymer solutions (200 ml) were circulated through the experimental system at a flow rate of 500 ml/min, and heated at a rate of 1.83 °C/min, starting at 37 °C. The turbidity induced by protein denaturation or by crossing the cloud-point of EHEC was detected by an on-line spectrophotometer (accuracy of 1%). Sampling rate was one per 10 s, and the spectrophotometer was calibrated to 100% transmission at 35 °C with each test solution. The microwave absorption rate was 34 W for 200 ml.

In order to determine possible difference between the microwave and conventional heating results, we used the Fermi distribution function (Eq. (1)) to describe the denaturation/solubility curves (Wollny & Peleg, 1994):

$$T = \frac{100}{\left(1 + e^{\frac{t-t_c}{b}}\right)}, \tag{1}$$

where T is the transmission of the spectrophotometer, t is temperature in °C, t_c is critical temperature (constant), and b is the slope indicator (constant). For each test, t_c resembles the temperature at half reaction (transmission) and b is a highly sensitive slop indicator. For optimal estimation of b and t_c we used an Excel solver module with maximum R^2 as the condition.

2.4. Mutagenesis in dark mutants of luminous bacteria

The mutagenic effect of the absorption of high microwave radiation intensity was studied on a dark mutant of the luminescent bacterium *Photobacterium leiognithi (H169)* (kindly contributed by Prof. Ulitzur, 1982). The bacteria were grown on ASW agar plates for 30 h at 27 °C (ASW media composition: NaCl 17 g/l, KCl 0.75 g/l, $MgSO_4 \cdot 7H_2O$ 12.3 g/l, $CaCl_2 \cdot 2H_2O$ 1.45 g/l, Oxide yeast extract 3 g/l [Difco-Sparks MD US], peptone 5 g/l [Difco], agar 18.5 g/l, pH 6.8). Cells were transferred from the plate to a 220 ml salt solution (as above but with no yeast or peptone, to prevent growth by elimination of possible carbon source) to give a concentration of approximately 10^6 cells/ml. The bacterial suspension was circulated through the experimental system at 400 ml/min, and maintained at 31 °C (TC_1 in Fig. 1) by microwave irradiation. In order to obtain a high microwave radiation dose, a 3 m silicon tube coil was added to the tubing system and placed in ice water, to keep the inlet temperature (TC_2) at 28.65 °C. This way, a high intensity microwave radiation of 70 W for 220 ml was required to keep the Erlenmeyer solution at 31 °C. The suspension was sampled through a sampling port that replaced the detector in the system.

Table 1
Composition and test parameters of the protein/polymer aqueous solution

Protein/polymer[a]	Protein/polymer concentration	Salt type and concentration	Solution pH	Spectrophotometer wavelength (nm)
Egg white	Powder, 0.2% w/w	0.05 M $CaCl_2$	6.8	400[b]
Egg white	Fresh, 0.44% w/w	0.5 M KCl	7.4	650
Egg white	Fresh, 0.27% w/w	0.04 M $FeSO_4$ 1 M NaCl	7.1	600
Bovine serum albumin	Powder, 0.2% w/w	0.1 M $CaCl_2$	6.5	650
Egg white	Powder, 0.2% w/w	–	6.7	650
EHEC	Powder, 0.25% w/w	–	6.8	650

[a] In the fresh egg white, protein concentration (w/w) is calculated as 10.9% w/w of protein (Bernice & Annabel, 1963).
[b] Wavelength of 400 nm was also used in attempt to detect denaturation events than those usually detected at 600–650 nm.

Each sampling time included five samples of 1 ml each, immediately placed on ice water.

After completing the sampling procedure, peptone (an essential nutrient for the luminous system of the bacteria) was added to all samples to a concentration of 0.05% w/w. The samples were then transferred for incubation at 25 °C. The light formed by the bacteria was measured by a luminometer at 5, 60, 180, and 900 min after peptone addition (AZUR-Newark model-DELTATOX PS, Delaware, USA). For positive control, mutagenesis was induced in one of the five samples by adding 0.5 μg of proflavine hemisulfate (Sigma p2508) (Ulitzur, 1982).

2.5. Mutarotation of α/β-D-glucose in water/ethyl alcohol solution

For mutarotation experiments, β-D-glucose or α-D-glucose were added to 30 °C water (marked as time = 0) and dissolved by a hand-held mixer for 1 min. Then, a 30 °C ethyl alcohol (95% solution) was added to the glucose solution to the desired concentration. The 200 ml solution at 30 °C was transferred to the experimental system and circulated through the system at a flow rate of 340 ml/min, using an online polarimeter for continuous reading (589.44 nm, 20 cm tube, resolution 0.01°, accuracy ±0.05°). The overall time from the initial mixing to the first reading in the polarimeter was approximately 2 min. The temperature was then raised to 50 °C at a rate of 2 °C/min, and kept at 50 °C to the end. Before each experiment, the polarimeter was calibrated to zero with the test water/ethyl alcohol solution (without glucose) at room temperature. In order to obtain a high microwave radiation dose, a 2 m silicon tube coil was added after the detector, and placed in ice water. This additional tube was also added during the control bath heating experiments. The energy input of the microwave radiation, after reaching 50 °C, was 200 W for the 200 ml solution. For a statistical test, we used a two-tail *t*-test for means (Microsoft Excel software).

2.6. Saturation solubility of NaCl in water

Salt solution saturation experiments were performed by circulating 200 ml NaCl solutions through the experimental system at a flow rate of 400 ml/min. An excess of NaCl crystals was maintained in the system, and a 200 mesh glass filter was added at the outlet in order to prevent pumping out of the NaCl crystals. The refractive index was measured by an on-line refractometer (KRÜSS model AR3-6, accuracy and resolution 0.0001 nD) after reaching equilibrium solubility, usually at approximately 5 min. The absorption rate of the microwave energy for the 200 ml solution ranged from 37 to 62 W (according to the temperature that was selected for the equilibrium). An ANOVA statistical test (JMP-IN software, version 4, SAS Institute Inc.), assuming duplicate results, was used to compare the results of the bath and microwave.

3. Results and discussion

3.1. Formation of Maillard reaction products

The chemistry involved in the Maillard reaction is complex. Therefore, we suggest that, if any of the many stages of this process are athermally affected by microwave radiation, we may expect a detectable effect on the formation of the final colour pigments. Thus, using glucose–glycine solutions, we explored this possibility by heating these solutions in a similar manner, using both microwave and conventional heating. Fig. 2 shows the results of a duplicated test monitored by an online spectrophotometer.

As shown in Fig. 2, there was an insignificant difference between the absorbance readings of solutions heated in the microwave and the bath. There was no consistent pattern to these results; e.g., sometimes the microwave heating seems to produce slightly higher readings and sometimes vice versa. This phenomenon was also detected in the other runs, as well as in solution B. Therefore, the differences observed in this case should be considered as variations produced by the testing method. One possible explanation of these variations was a slow drift that was found in the reading of the spectrophotometer.

Since the drift could not be accounted for quantitatively, another test was conducted where only the final point was measured at the end of the test. In this set-up we calibrated the spectrophotometer just before measuring the final pigment concentration (Table 2). Here too, the results suggest no differences between microwave and bath heating. Therefore, no athermal effect could be attributed to the complex Maillard reaction by this experimental set-up.

The results obtained in these experiments agree with the work of Meissner and Erbersdobler (1996). These authors tested the impact of microwave cooking on the formation of early Maillard products (hydroxymethlfurfural (HMF) and lactulose) using milk as a test system. The tests were carried out at controlled temperatures of 80 and 90 °C and holding times of up to 420 min, and the data were compared with the effect of conventional cooking. All tests failed to produce a significant difference between the microwave and conventional cooking methods.

3.2. Protein denaturation and polymer solubility

Non-thermal effects of microwave radiation on protein denaturation have been reported by Bohr and Bohr (2000) and de Pomerai et al. (2003). Therefore, in this study, we have followed previous experiments, using our well-controlled system. The temperature of the protein solution was increased at a constant rate and the turbidity, induced by denaturation or phase transition, was measured by the online spectrophotometer (Fig. 3). Since protein solubility and denaturation are sensitive to salt concentration (Collins, 1997), different protein-salt solutions were tested. In addition, the cloud point of a hydrophobically modified polymer (EHEC) was also tested (Carlsson, 1989). The

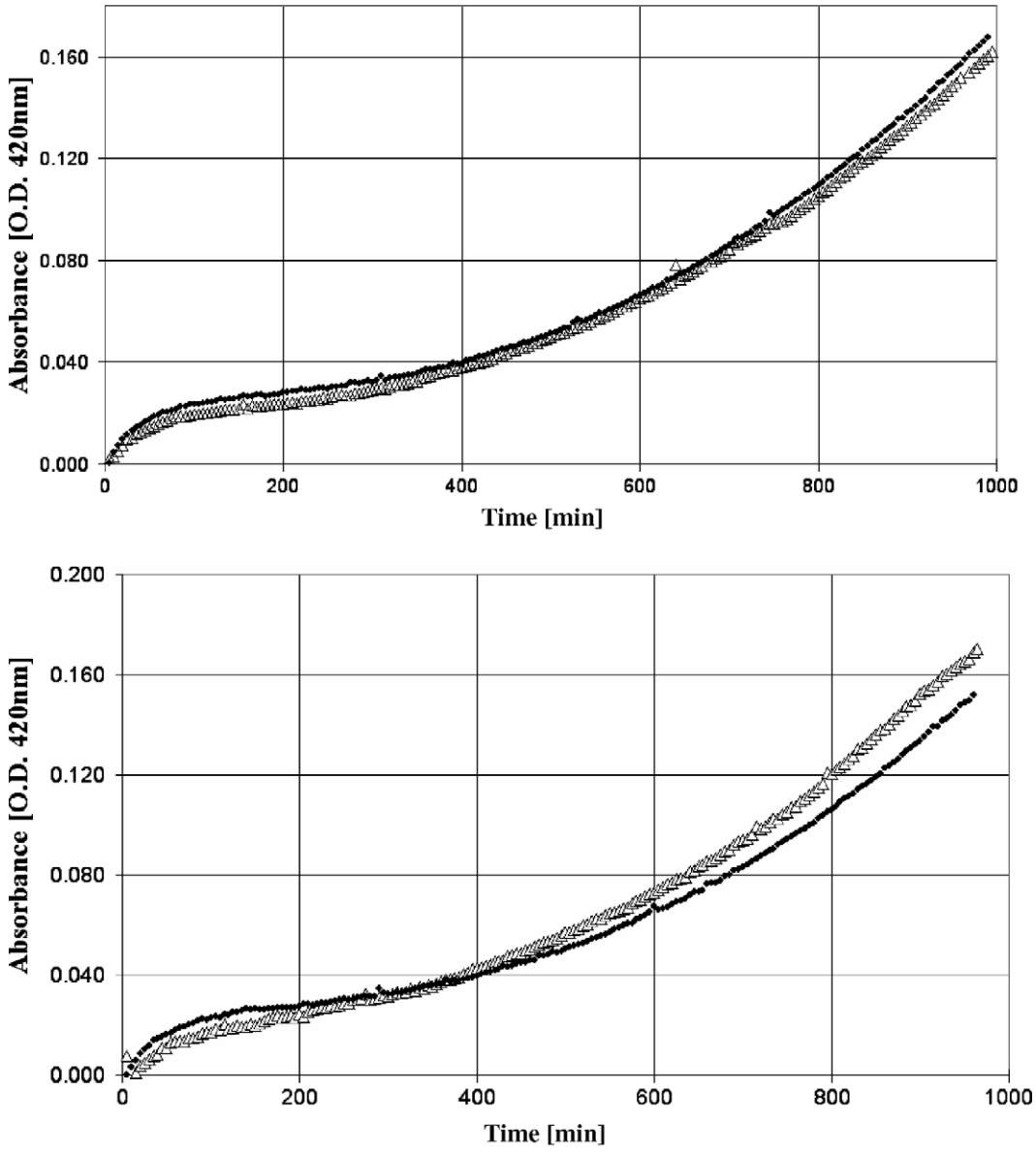

Fig. 2. Development of Maillard reaction pigment products during microwave (●) and oil bath (△) heating. The two Figures describe two independent experiments, where the microwave-heating pattern was precisely repeated by the oil bath. Glucose–glycine (200 ml of 1.6 M + 50 ml of 0.5 M) solution (solution A) was circulated at 64.4 °C. The microwave energy input was 31 W for the 250 ml solution.

Table 2
Formation of Maillard reaction products in glucose–glycine solution (1.6 M/0.5 M) incubated at 55 °C for 48 h (measured by absorbance at 420 nm)

Experiment No.	Bath	Microwave
1	0.244	0.244
2	0.247	0.248

recorded turbidity data were used to analyze the difference between the four runs of each solution. An example of graphic presentations of the test results is presented in Fig. 4.

To analyze the denaturation results, we had to fit the curve of "Fermi distribution function" to our test points (see Section 2). The fitting of results for each test run gave two constants: t_c and b, where, t_c, the critical temperature, resembles the average denaturation temperature. For example, the denaturation in Fig. 4 started at 59 °C and ended at approximately 73 °C, and the average was 66 °C. The constant, b, is a highly sensitive slope indicator. Similar analyses were carried out for all tests and the results are presented in Table 3.

The results for t_c in Table 3 reveal no consistent pattern; e.g., sometimes the microwave heating seemed to have produced slightly higher readings and sometimes vice versa. Therefore, apparently, the differences observed in this case can be considered as variations produced by the testing method. These variations may possibly be attributed to the inconsistency in protein aggregation or even adherence of aggregates to the spectrophotometer cell (cuvette) dur-

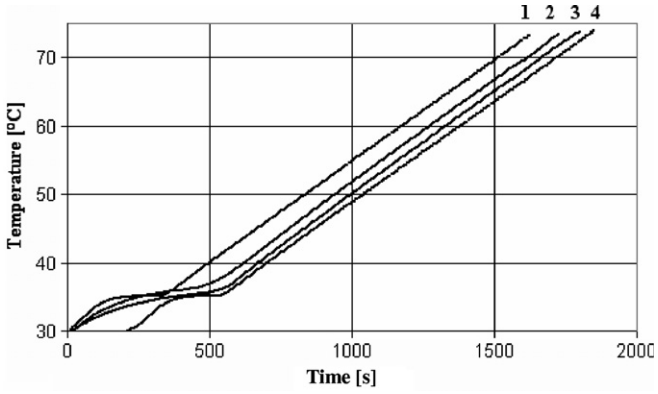

Fig. 3. Heating profile of egg white protein (0.2% w/w) in a $CaCl_2$ solution (0.05 M, pH 6.8) in microwave oven (1 and 4) or oil bath (2 and 3).

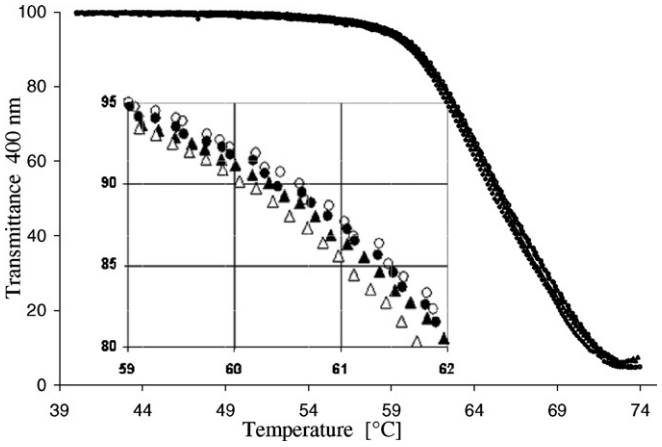

Fig. 4. Turbidity development during heating of egg white solution (0.2% w/w) in $CaCl_2$ (0.05 M, pH 6.8). The Figure contains two runs of microwave (●, ○) and two of bath (▲, △). The inserted graph contains the beginning of the rapid protein denaturation.

ing the heating. Thus, we suggest that the slight differences in the development of turbidity in the microwave, as compared to bath heating, may be well within the experimental error, and cannot be attributed to athermal effects.

With regard to the study of Bohr and Bohr (2000), that reported enhanced folding and denaturation of globular proteins by non-thermal microwave effects, one should note the different experimental method. After heating the protein solution to a certain temperature, they measured the degree of folding with a polarimeter. It should be noted that the rate of heating in their experiment was different in the microwave (compared to bath heating). Thus, we looked at the effect of the time–temperature of a protein solution on its denaturation temperature (Fig. 5). In Fig. 5a, the time/temperature histories of the protein solution in the microwave and the bath heating were not identical and consequently, there were also differences in the denaturation temperature curves (Fig. 5b). As shown before, when the same test was conducted with the same time/temperature profile, the resulting denaturation curves were practically identical (Fig. 4). This phenomenon is also known from the literature where the denaturation point was measured by DSC (Leharne & Chowdhry, 1998).

3.3. Mutagenesis of luminous bacteria

One of the ways to examine the possibility of carcinogenic potential of microwave radiation is by following mutagenesis of bacteria. The contradicting results of the studies of mutagenic potential of microwave radiation were discussed in the introduction of this paper, and are the basis for this specific set of experiments. These experiments were designed to overcome some of the possible artifacts when testing the mutagenic effect of microwaves, such as hot spots. Despite all efforts to improve the design of domestic microwave ovens, the interaction of the microwave field with the test sample and the wall of the microwave cavity are not uniform. Therefore, if the problem is

Table 3
Fitting parameters for the "Fermi distribution function" describing protein/polymer denaturation during microwave and conventional heating[a]

Solution	Critical temperature (t_c) (°C)		Slope indicator (b)		R (for fitting "Fermi distribution function")	
	Bath	Microwave	Bath	Microwave	Bath	Microwave
Egg white/$CaCl_2$ 400 nm	65.6	65.9	2.54	2.69	0.99	0.99
	65.4	65.9	2.60	2.67	0.99	0.99
Egg white/KCl 650 nm	70.2	70.5	1.06	1.15	0.99	0.99
	70.3	70.4	1.04	0.81	0.99	0.98
Egg white/$FeSO_4$ 600 nm	76.0	74.8	1.69	1.4	0.98	0.95
	75.7	75.9	1.57	1.63	0.99	0.86
Bovine serum albumin 650 nm	70.9	70.6	0.77	0.77	0.99	0.99
	70.5	71.0	0.75	0.8	0.99	0.98
Egg white 650 nm	57.9	57.9	1.00	0.97	0.99	0.99
	57.8	57.8	1.02	0.96	0.99	0.99
EHEC polymer 650 nm	65.6	65.4	1.41	1.34	0.99	0.98
	65.6	65.5	1.41	1.34	0.99	0.99

[a] For each experiment, the two sets of parameters represent two independent experiments.

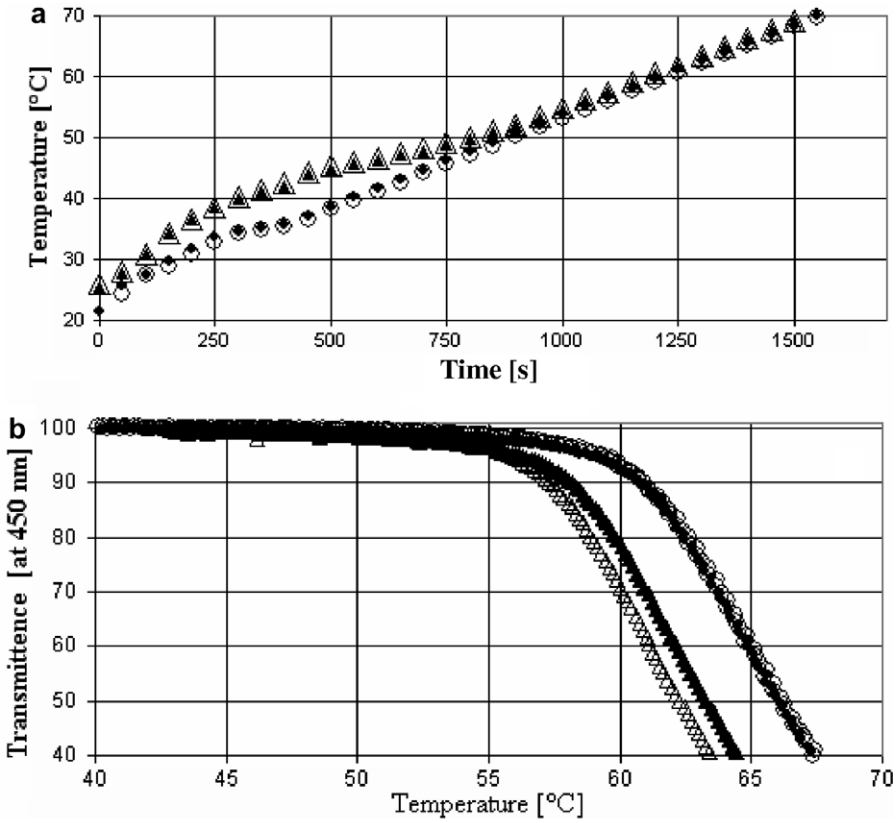

Fig. 5. An example of an experimental artifact as a result of the effect of time–temperature profile on protein denaturation. When heated in a bath (▲, △), the protein solution (egg white + $CaCl_2$) was held at higher temperatures longer than was the solution heated by microwave (●, ○) before reaching the denaturation temperature (a). Consequently, this time–temperature history means that the bath solutions exhibit lower denaturation temperature than do the microwave-heated solutions (b).

not reduced by effective stirring of the test solution, rapid mutagenesis may be induced, simply due to overheating of specific loci. The microorganisms that experience stress at the higher temperature are more likely to respond by increasing the mutation rate (Duwat, Ehrlich, & Gruss, 1999; Rocha, Matic, & Taddei, 2002).

According to the concept of our test method, dark mutants of luminous bacteria should regain their luminescence capability by mutagenesis. Thus, if indeed microwave radiation does induce mutagenesis, we would expect an increase of luminous intensities. However, we could not find such an effect, even after subjecting these bacteria to high intensity microwave radiation, namely 70 W per 220 ml suspension for up to 120 min (Table 4). To ensure that the lack of light-emitting capability is not the result of bacterial death, duplicated bacterial suspension samples were treated with a known mutagenic agent after their exposure to microwave radiation, and these proved to become luminous again (positive control Table 4). It should be noted that the sensitivity of the bioluminescence assay for mutagenic agents (including to UV irradiation) is usually 50–100 times greater than the Ames assay (Ulitzur, 1982). Thus, even if we revert 1–3 bacteria in a sample in our test, we will obtain an increase in the luminous intensity, by 3 times above the background light level. Therefore, the results of this set of experiments suggest that there was no mutagenic effect of the absorbed high power microwave radiation, at least during the time range of this experiment.

3.4. Mutarotation of α/β-D-glucose in water/ethyl alcohol solution

The mutarotation experiment was conducted as a repetition of the experiments of Pagnotta et al. (1993). In their report, these authors claimed that they found a significant non-thermal microwave effect. During their microwave-heating test, the ratio of α/β-D-glucose seemed to reach the same expected equilibrium as in the water bath after 45 min. However, 100 min later, the amounts of α-D-glucose increased by more than 40%. This phenomenon did not appear in the control water bath tests. In our well-controlled study, on the other hand, we could not detect any difference between the mutarotation performances in microwave and bath heatings (Fig. 6).

A typical curve, describing the polarization change with time, may be considered as two separate phases of the process, namely before and after reaching equilibrium. Analysis of both, the first and second phases (first one from 0 to about 50 min), revealed no difference

Table 4
Comparison of microwave-induced mutagenesis with mutagenesis of proflavine hemisulfate, a known chemical mutagen in dark mutant of luminescent bacteria

Experiment No.	Microwave exposure time (min)	Relative luminescence intensity (mean ± SE)	
		Microwave heating	Chemical mutagenesis
1	0	36 ± 4	14740 ± 7200
	10	41 ± 3	15280 ± 4950
	45	35 ± 3	5840 ± 2450
2	0	32 ± 3	6020 + 800
	30	36 ± 3	7270 + 3190
	60	36 ± 2	3290 + 1040
	120	34 ± 3	2920 + 1450

Microwave radiation intensity absorbed by the bacterial suspension was 70 W/220 ml of suspension. The mean is a calculation of light measured at 5, 60, 180, 900 min after adding peptone and four samples.

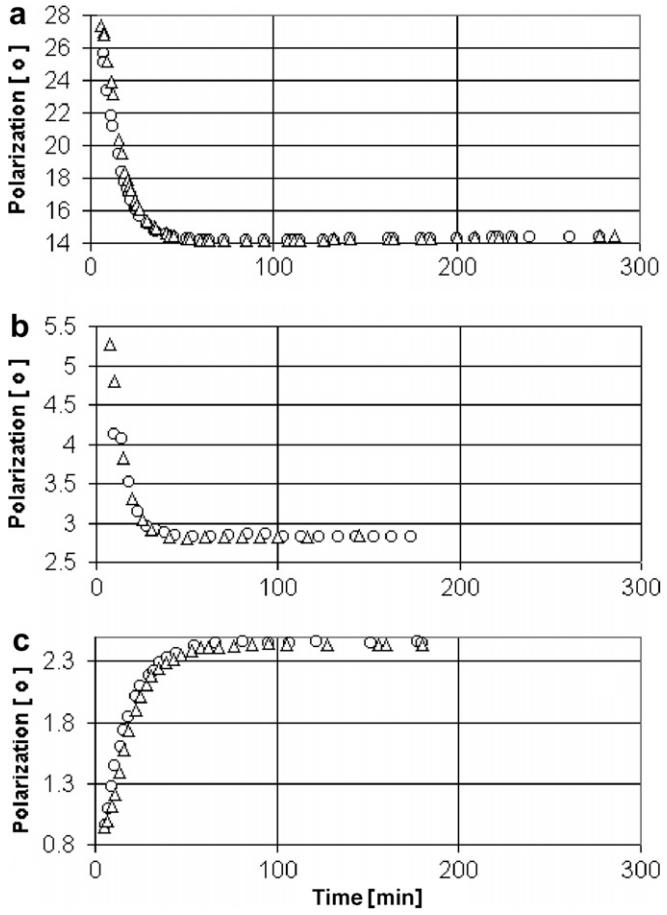

Fig. 6. Mutarotation of α or β-D-glucose solutions during microwave (○) or bath (△) heating. The change in the polarization of: (a) solution of 0.74 M α-D-glucose, (b) 0.139 M α-D-glucose, and (c) 0.125 M β-D-glucose. A pure α or β-D-glucose solution mutarotates to β/α-D-glucose and back, until reaching equilibrium. The microwave intensity was 200 W per 200 ml solution, and the relative amounts of α and β-D-glucose were measured by an online polarimeter.

between the microwave and the bath heating. For the second phase, where equilibrium is reached, we conducted a statistical two-tail *t*-test. For tests A and B (Fig. 6), no statistically significant differences were found between the microwave and the bath heating results. However, a significant difference (between the means of the polarimeter readings) of 0.019° was found in test C. Nonetheless, this difference seems doubtful considering the accuracy of the polarimeter ±0.05°.

With regard to the study of Pagnotta et al. (1993), it is difficult to explain why the extent of mutarotation changed after reaching the expected equilibrium. If there is a substantial microwave athermal effect on the mutarotation, why did it reach the expected equilibrium in the first place? The authors could have provided a possible explanation by transferring the microwave solution, in which the athermal effect was observed, back into the bath, to examine whether an irreversible effect took place or whether the system returned back to the expected value of mutarotation. An irreversible effect may then be attributed to change in composition and formation of new compounds due to unclear reasons. In summary, even though practically similar methods were utilized in the present study (only, in the present study an on-line detector and higher microwave intensity were used), no evidence of any microwave athermal effect was observed.

3.5. Saturation solubility of NaCl in water

Some of the hypotheses behind athermal effects of microwave heating suggest that microwave radiation may change the solvent properties of water. Saturation solubility of salt is a sensitive and simple method for examining this hypothesis. The effect of microwave radiation was studied on the equilibrium concentration of salt in water in the presence of excess amounts of salt crystals. Fig. 7 reveals no change between the refractive index of the salt solution in the microwave (as compared to bath heating). The ANOVA test also refuted possible differences in the intercept and slope between the microwave and the bath linear regression curve (R^2 Adj. = 0.986, intercept Prob. > |*t*| = 0.86, slope Prob. > |*t*| = 0.96).

3.6. Possible experimental errors and artifacts

The task of detecting athermal effects during thermal processes, and the 'cleaning' of the thermal contributions

to the observed phenomena, may be extremely difficult. Careful examination of the various reports on athermal effects of microwave radiation may suggest that, in some cases, at least some of the results are possibly due to problematic experimental set-ups and the overlooking of some critical points, all of which may lead to experimental artifacts. For this specific reason, much emphasis was placed in this study on experimental setting planned to avoid such pitfalls. Nevertheless, even in the present set-up, we have encountered some possible artifacts, and we believe it is important to report these observations, in particular for the planning of future studies in this field. In the following section we report the two major problems we have encountered, and the way that these have been solved.

In the initial runs of the Maillard reaction tests in our set-up, it appeared that the formation rate of Maillard products in the heating bath was substantially more rapid than in the microwave system. Thus, the difference between the microwave and the bath curves could have been considered as an apparent athermal effect. However, a close look at the Erlenmeyer glass after pouring out the solution from the heating bath experiment revealed a thin brown film on the wall that did not appear in the microwave. Obviously, there is a fundamental difference between microwave and conventional heating. In the microwave oven, the heat is generated in the test solution by direct absorption of the microwave radiation. On the other hand, when conventional heating in an oil bath is used, the heat flows by temperature gradients through a liquid film on the Erlenmeyer glass wall. So, by definition, the solution film that touches the Erlenmeyer glass wall is hotter than is the bulk solution. Therefore, to reduce the problem of different temperature gradient between the microwave oven and the heating bath, we improved the mixing of the solution by placing the inlet pipe at the centre of the Erlenmeyer flask bottom, and the horizontal outlet pipe close to the flask wall. This set-up produced a vortex that considerably improved mixing and that eliminated the effect of the extra browning at the film close to the wall. When using this modified set-up, the difference between the microwave and the heating bath disappeared.

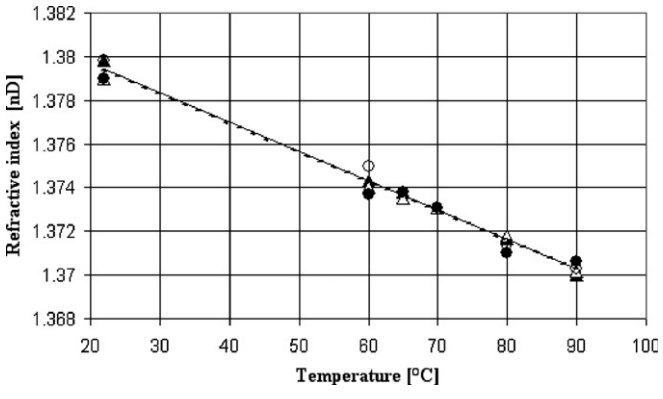

Fig. 7. Equilibrium refractive index of saturated NaCl water solution maintained at different temperatures by either microwave (●, ○) or bath heating (▲, △). Lines represent linear regression of bath heating (——) and linear regression of microwave heating (------). The amount of dissolved sodium chloride in water was measured by an on line refractometer. The energy input of the microwave ranged from 37 to 62 W (according to the temperature) for a 200 ml solution.

In the experiments to study the saturation solubility of NaCl in water, we first boiled water with excess of NaCl at atmospheric pressure in the microwave oven and compared it to heating on a heating plate. Both the microwave and the heating plate were operated so as to perform a delicate heating and boiling processes by using an on/off control. Under these boiling conditions, waiting at least 5 min to reach saturation, we took samples of approximately 50 ml by sucking the solution, via a 200 mesh filter to a vessel, and dried it in a vacuum oven. By measuring the weight before and after drying, we determined the saturation solubility. To ensure that we can compare the data of the microwave oven with that of the heating plate, we used exactly the same procedure and tools. The gravimetric results showed an increase of ~1.4% in the NaCl weight in the heating plate experiment, with statistical significance. We suspected that this result might be an artifact due to the presence of micro-crystals in the system, which had escaped the 200 mesh filter. These crystals might have been produced by temperature fluctuations, during either heating or sampling. Therefore, in order to ensure that we only measuring dissolved NaCl, we decided to use an online refractometer, with the advantage of measuring only soluble NaCl.

4. Conclusion

The present study examined the possibility of athermal effects due to microwave radiation in a number of chemical, biochemical and microbial systems. For this purpose, a special set-up was built in order to eliminate, as much as possible, experimental artifacts. Athermal effects were not detected in any of the tested systems. Interestingly, in this set-up, we could not repeat some of the studies in which the existence of athermal effects were suggested, very likely due to the different set-up. Overall, under the experimental conditions and the accuracy of measurements employed, the results of the present study cannot support the hypothesis of athermal effects induced by microwave radiation.

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