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MODELING THE ACTION OF MICROWAVES ON *ESCHERICHIA COLI*

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Abstract

Treatment with microwaves of *Escherichia coli*, a pathogen usually used as test food contaminating strain, gives generally a reduction of the microbial population. In this paper, the action of microwaves on *E. coli* on solidified and liquid media was investigated, as function of microwaves power x 1 (varying from 180 to 540 W), treatment time with microwaves x 2 (from 10 to 30 seconds) and inoculum concentration x 3 (from 102 cell/mL to 104 cells/mL) on both solidified and liquid substrate, after treatment and one week after treatment.

The strain *E. coli* ATCC 11775 was used. The inactivation rate (IR) was established as function of the three parameters, by using a 2^3 full factorial design. The data obtained were used to build polynomial models for IR: $IR_{solidified\ substrate} = 28.93 + 18.14 \cdot x1 + 16.18 \cdot x2 + 11.28 \cdot x1 \cdot x2 + 2.45 \cdot x2 \cdot x3$; $IR_{liquid\ substrate} = 13.19 + 4.86 \cdot x1 + 3.01 \cdot x2 + 3.47 \cdot x1 \cdot x2$.

Results showed that only two from the three analysed parameters influence IR of *E coli*, namely the microwaves power x1 and the treatment time with microwaves x2, on solidified or liquid substrate; both of them had a positive influence on IR, as individual or in combination. The inoculum concentration x3 seemed to not influence IR.

The free coefficient in the models was high, indicating that other factors as those investigated could have a positive influence on IR. It was difficult to establish whether action on solidified or on liquid media is more effective. An increase of inactivation was observed in all samples after one week of treatment with microwaves, indicating a possible residual action of microwaves. In the tested conditions, the better treatment with microwaves of liquid or solidified substrates contaminated with *E. coli* was at using microwaves power 540 W for 30 seconds.

***Key words**: Microwaves, Escherichia coli, Full factorial design, Model.*

1. Introduction

Microwave treatment is frequently used in home cooking and by the food industry for different processes as: heating, thawing, frying, precooking, tempering, roasting, grilling, baking, toasting [1, 2, 3]. Microwave treatment has some practical advantages as short treatment time with high heating rates, relatively uniform heating (compared with gas), safe handling, ease of maintenance, easy clean-up [1, 4]. Due to these considerations, microwaves became very popular in home cooking and a large number of food products were developed by the food industry to be prepared at home by using microwaves oven [4].

Besides actions as warming, drying, thawing, microwaves show positive action on the food quality, also. For example Moza *et al.*, [5] reported microwaves treatment as being more effective on degrading the starch granule than the thermal action; microwaves seem to have positive impact in keeping nutrients in food [6], or on formation of derivatives of phenolics with enhanced antioxidant activity [7]. Another important actions of microwaves are pasteurisation and sterilisation, with reduction or total destruction of microorganisms, especially pathogens [8, 9].

Many studies on the destructive action of microwaves were realised by using *Escherichia coli*, a pathogen usually used as test food contaminant strain in different food beverages as juices [10, 11] or milk [12]; all researches indicated reduction of the microbial population, the destructive action of microwaves on microorganisms depending on many factors, as field strength, frequency, form and modulation of waves, duration of exposure [13].

Also, a lot of researches were made to model the action of microwaves on *E. coli*, a typical coliform bacteria; many of them investigates the temperature and moisture distributions during microwave heating of food materials [1, 14] used factorial design to model the combined effects of microwaves and H_2O_2 on *E. coli*.

The domain is remaining challenging because of the new products developed for cooking with microwaves and of the demand for minimal microwaves processing [15].

This research has as goal to obtain models to describe the inhibition rate of *E. coli* on liquid and solid substrates in a consumer microwaves oven as function of low radiation power, short duration and reduced contamination, in order to identify the sterilising effect of microwaves in solid or liquid foods in conditions of minimal processing with microwaves of solid or liquid food samples contaminated with bacteria in small concentration.

2. Materials and Methods

Escherichia coli ATCC 11775, MicroScan biotype 53115012 (Mecconti, Luxemburg) was used as test microorganism, as typical coliform bacteria. The strain was activated on nutrient broth (Merck KGaA, Darmstadt, Germany) at 37 ^{0}C without agitation [16] for 48 hours, with the obtaining of the inoculum. Cell density in the inoculum was determined by using serial dilutions and the spread-plate method [17]; the dilutions were realised with sterile saline solution and MacConkey agar was used at the spread-plate method.

For the treatment with microwaves, the house-hold microwaves oven type MC-9280XC from LG with rotating plate and time control, emitting rated powers from 180 to 900 W at frequency 2450 MHz, was used.

The influence of three factors, radiation power x_1, duration of irradiation x_2 and inoculum concentration x_3, was analysed, based on previous researches showing the importance of these factors on microbial destruction [9, 18, and 19]. Small values for radiation power and duration of irradiation were chosen, in order to simulate the minimal processing with microwaves, as in the case of warming sandwiches or rice milk (solid or solidified substrate) or warming milk (liquid substrate). A 2^3 full factorial design was chosen; the limits for the factors were:

- Microwave power (x_1): minimal 180 W (corresponding to 20% from the oven total power) and maximal 540 W (corresponding to 60% from the oven total power), with an intermediate value of 360 W (corresponding to 40% from the total power). The value of 180 W is recommended by the producers to soften ice cream, butter, cheese or to raise yeasts dough. The value of 360 W is recommended for thawing or melting chocolate and the value of 540 W for reheating or preparing rice or soup [20].

- Treatment time (x_2) from 10 s (minimal value in the factorial design) to 30 s. (maximal value in the factorial design), with 20 s. as intermediate. This interval is recommended by the producers for sandwiches [20].

- Cells density of *E. coli* (x_3) from 10^2 cells/mL (minimal value in the factorial design) to 10^4 cells/mL (maximal value in the factorial design), with 10^3 cells/ml as intermediate. These values were found as being present in raw milk [21].

The inhibition rate (IR) of *E. coli* by microwaves was determined as response, on solidified and liquid substrate. Mueller-Hinton agar plates were used to simulate a solidified substrate, as rice milk and MacConkey broth was used to simulate a liquid substrate.

IR on solidified substrate was determined according to the following steps: Introduction of the inoculum in Petri dishes in sterile condition; Pouring of the cultivation substrate Mueller-Hinton agar melted and tempered at 45 ^{0}C in amounts to fill completely the Petri dish (1 cm high); Cooling; Treatment of Petri dishes with microwaves by using the design presented in Table 1; Incubation of samples for 48 hours at 37 ^{0}C; Analysis of cells multiplication in the Petri dishes. In order to measure the IR, the Petri dishes were photographed and the images where stored as greyscale uncompressed PNG files; the files were batch processed by using the threshold tool from GIMP to select only the pixels with grey levels corresponding to the absence of microorganism growth. The selected pixels where transformed in black pixels. In order to simplify the counting all other pixels from the interior of the Petri dish were transformed to white pixels. IR was calculated as ratio between the number of black pixels and the total number of pixels. Three samples in parallel were analysed for each set of experiments and the respective IRs were determined. The final IR was calculated as average (mean value) between the values obtained for each set of experiments.

IR on liquid substrate was determined according to the following steps: Inoculation of the inoculum in sterile tubes containing 10 mL MacConkey broth; Treatment of tubes with microwaves by using the design presented in Table 1. Incubation of samples was for 48 hours at 37 ^{0}C. Analysis of bacterium multiplication in tubes by using serial dilutions and the spread-plate method, as presented previously [17]. As for the experiments on solidified substrates, three samples in parallel were analysed for each set of experiments; for each experiment, IR was determined as ratio between the number of viable cells after the treatment with microwaves and the number of viable cells before the treatment. Final IR was calculated as average (mean value) between the values obtained for each set of experiments.

The model of the inactivation of *E.coli* cells as a function of three factors: radiation power, treatment time and cells concentration was formulated as a basis for the experimental design and then was fitted to the experimental data and analysed by using ANOVA [22]. The analysis was performed in the R environment for statistical computing and graphics [23].

Table 1. Experimental matrix for the factorial design used in the experiments

No. experiment	Factors level			Experimental values		
	x_1	x_2	x_3	x_1, W	x_2, s	x_3, $lg_{cells/ml}$
1	1	1	-1	540	30	2
2	1	1	1	540	30	4
3	1	-1	-1	540	10	2
4	1	-1	1	540	10	4
5	-1	1	-1	180	30	2
6	-1	1	1	180	30	4
7	-1	-1	-1	180	10	2
8	-1	-1	1	180	10	4
9	0	0	0	360	20	3
9'	0	0	0	360	20	3
9"	0	0	0	360	20	3

The initial model, constructed using the rsm (response surface model) package, included terms for the main effects of the factors (x_1, x_2 and x_3), terms for binary interaction ($x_1 \cdot x_2$, $x_1 \cdot x_3$ and $x_2 \cdot x_3$) and quadratic terms (x_1^2, x_2^2 and x_3^2). After the significance test, the terms that have not passed it (meaning P-values higher as 0.1) where removed from the model.

3. Results and Discussion

Some pictures obtained after image processing of the Petri dishes treated with microwaves are showed in Figure 1. The results from experiments 1-type are not presented because the inhibition was fast 100% in all these experiments. A pattern could be detected, indicating the dominant destructive action of microwaves at the edge of the Petri dishes; microwaves have sterilising effect mainly at sample edges. This result can be correlated with other researches, which show that for cylindrical samples, the temperature distribution is different at the edges from those in centrum [24]; Geedipalli et *al.*, [25] showed that temperature is higher at the samples edges. Because microwaves sterilising action seems to correlate with heating [9], the results indicate the heating of samples especially at the edges, with destruction of *E. coli* cells as effect.

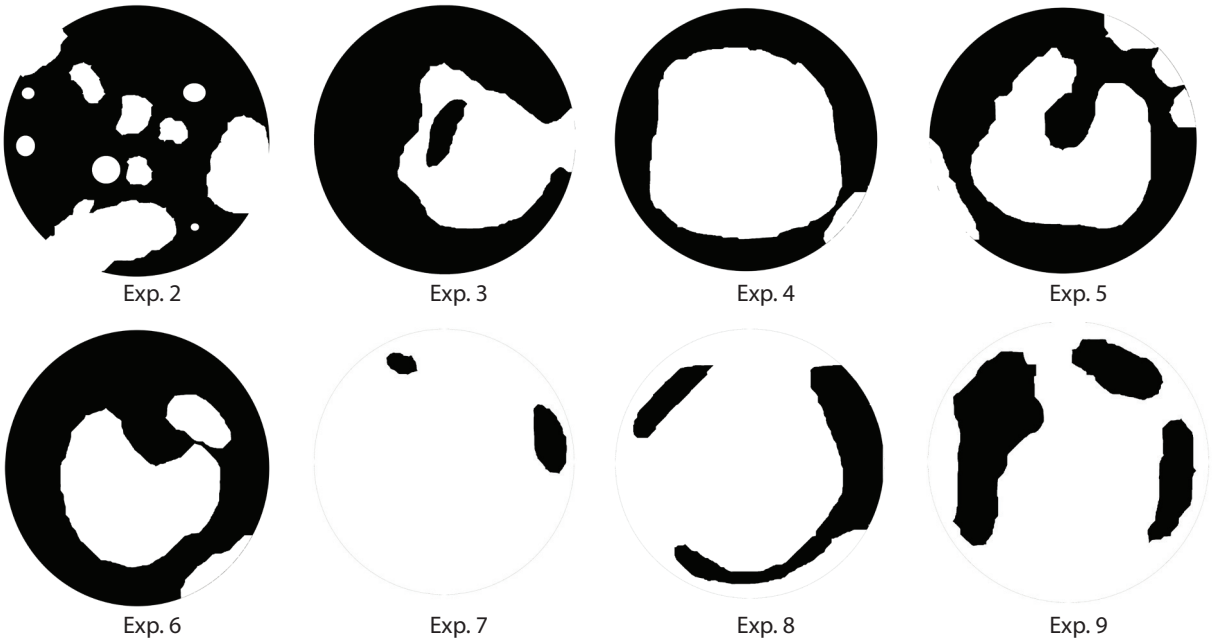

Figure 1. Examples of images obtained from Petri dishes after the treatment with microwaves following the 2^3 full factorial design (at different microwaves powers, treatment time and initial *E. coli* cells concentrations), and incubation for 48 hours at 37 °C; the dark zones indicates total inhibition of bacteria and the white zones the absence of inactivation

Only when the treatment become more aggressive (as in experiments 1- and 2-type, with maximal values for both microwaves power and duration from those tested, meaning 540 W for 30 seconds) the microorganism starts to be inactivated in the middle of the sample, too; the action is not uniform and complete when microorganism is presented initial in higher concentrations (10^4 cells/mL), whereas sterilisation is obtained in samples having initial small concentration of *E. coli* (10^2 cells/mL).

When the treatment is realised at very low microwaves power and for very short time (experiments 7-type), the microwaves have fast no sterilising effect. [19] reported similar results on chicken portions, when short time exposures from 5 to 20 seconds to microwave heating do not have destructive action on the strain *E. coli* O157:H7.

The practical values for IRs are presented in Table 2. The polynomial models were fitted on the IRs mean.

The polynomial model for IR on solidified substrate, in normalised form, is:

$$IR_{solidified\ substrate} = 19.6667 + 16.375 \cdot x_1 + 19.375 \cdot x_2 - 10.125 \cdot x_1 \cdot \mathbf{x}_3 + 33.45 \cdot x_1^2$$

With multiple R squared $R^2 = 0.9551$ and adjusted R squared $R^2_{adj} = 0.9252$, showing a good fit of the model to the experimental data.

The P-values from the ANOVA analysis are given in Table 3. The highest P-value is those of the free factor, indicating an incertitude in the assumption that the intercept is different from null. The removal of intercept har worsened the prediction accuracy (both R^2) so the intercept was kept in the model.

The model shows the very strong positive influence on IR on solidified substrates of the microwaves power (x_1), as singular and quadratic term. The treatment time (x_2) has also a strong positive influence on IR. The initial *E. coli* cells concentration (x_3) has no main effect on the inactivation rate; only its interaction with x_1 has a decreasing effect on IR. The incertitude and the not so high value of R squared show that other factors as those selected in this research influence also, the inactivation rate of the microorganism on solidified substrates and this should be further investigated.

By substituting the experimental values, the model can be rewritten as:

$$IR_{solidified\ substrate} = 21.2499 - 0.48379 \cdot power + 1.9375 \cdot time + 20.25 \cdot lg_{conc.} - 0.0562 \cdot power \cdot lg_{conc.} + 0.001032664 \cdot power^2$$

The model indicates the optimal minimal conditions for inactivating solidified samples contaminated with relatively low concentrations of *E. coli* (10^2 cells/ml), as being: microwaves power 540W and treatment time 30 seconds; in these conditions, IR calculated is 99.057%, very near from those obtained experimentally. This result can be well correlated with the image analysis, which indicates only the conditions $x_1 = 540$ W, $x_2 = 30$ s and initial 10^2cells/mL (experiments 1-type) as being efficient in destruction the pathogenic strain. In the same time, the model can be used to calculate IR for known initial concentrations of *E. coli* in the range 10^2 cells/mL - 10^4 cells/mL. The model has to be validated and then tested with food products, as rice milk.

The polynomial model for IR on liquid substrate, in normalised form, is:

$$IR_{liquid\ substrate} = 21.333 + 18.625 \cdot x_1 + 13.375 \cdot x_1 \cdot x_2 - 8.125 \cdot x_1 \cdot x_3 + 29.5417 \cdot x_1^2$$

The multiple R squared for the model is $R^2 = 0.8926$ and the adjusted R squared is $R^2_{adj} = 0.8209$, showing an acceptable fit of the model to the experimental data. The P-values from ANOVA analysis are presented in Table 3. The P-value of the term associated with $x_1 \cdot x_3$ is the highest, very near to the maximal accepted value, indicating a low significance level.

Table 2. Experimental values of the inactivation rate IR for all the experiments

No. Experiment	IR on solidified substrate				IR on liquid substrate			
	IR1	IR2	IR3	IR mean	IR1	IR2	IR3	IR mean
1	100	98	99	99	100	99	100	100
2	67	71	68	69	65	66	62	64
3	70	66	67	68	50	53	48	50
4	42	38	45	42	60	66	65	64
5	55	58	58	57	6	7	8	7
6	66	63	66	65	30	25	32	29
7	3	4	4	4	38	36	35	36
8	20	23	20	21	55	59	57	57
9	25	28	23	25	10	18	14	14
9'	17	10	15	14	27	30	29	29
9"	19	15	25	20	21	14	27	21

As the previous model on solidified substrates showed, the model on liquid substrates indicates the very strong positive influence on IR of the microwaves power (x_1), singular and doubled; treatment time (x_2) and initial *E. coli* cells concentration (x_3) alones have no influence on IR; only their combinations with x_1 have positive influence (in the case of x_2) or negative action (in the case of x_3) on IR. Other factors as those selected in this research influence strongly, also, the inactivation rate of the microorganism on liquid substrates.

By using the transformation relation between the normalised and natural scale, the model can be rewritten as:

$$IR_{liquid\ substrate} = 106.9998 - 0.566204 \cdot power - 2.675 \cdot time + 16.25 \cdot lg_{conc.} + 0.0074305 \cdot power \cdot time - 0.045138 \cdot power \cdot lg_{conc.} + 0.000911781 \cdot power^2$$

Table 3. P-values from the ANOVA analysis for the models

Factor	Model of $IR_{solidified\ substrate}$	Model of $IR_{liquid\ substrate}$
x_0	0.05	*0.018561*
x_1	0.0012	*0.003832*
x_2	0.0005144	-
$x_1 \cdot x_2$	-	*0.016867*
$x_1 \cdot x_3$	0.012	*0.093554*
x_1^2	0.0008921	*0.00917*

4. Conclusions

- This research is centred on analysing the action of minimal processing with microwaves at low microwaves powers for very short time on solidified or liquid samples contaminated with small concentrations of *E. coli* (as indicator of the contamination with coliforms), in order to build a polynomial model to describe the inactivation rate of the bacteria and finally to find solutions to sterilise the samples. A solidified cultivation medium for *E. coli* was considered to simulate products as gels or even rice milk, and a liquid medium for *E. coli* was considered to simulate liquid foods. To obtain a model of the behaviour of samples, a 2^3 full factorial design was used, with factors x_1 (microwaves power from 180 W to 540 W), x_2 (time of treatment from 10 to 30 seconds) and x_3 (initial concentration of *E. coli* from 10^2 to 10^4 cells/mL) to describe the sterilisation effect as inactivation rate IR of the microorganism.

- The image analysis of the solidified samples shows the dominant action of microwaves at the edges of the solidified sample, with total destructive action at the treatment at 540 microwaves power for 30 seconds.

- The polynomial models obtained show the very important role of microwaves power, simple or quadratic, on IR on both liquid and solidified substrate and the reduced effect of initial concentration of *E. coli* as factor, which only in interaction with x_1 seems to have an influence on IR. Other factors as those analysed are influencing strongly the inactivation rate of bacteria, also.

- Models can be used to calculate IR on solidified or liquid substrates for known initial concentrations of *E. coli* in the range 10^2 cells/Ml - 10^4 cells/mL at treatments with microwaves at low power for very short time.

- Minimal processing of solidified or liquid samples contaminated with small concentrations of *E. coli* could be a solution to sterilise them, the conditions for complete sterilisation being treatment at 540 microwaves power for 30 seconds in solidified or liquid media. In other conditions from those tested, complete sterilisation didn't' occurs.

5. References

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