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Full Length Research Paper

Use of genetic algorithms for high hydrostatic pressure inactivation of microorganisms

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Published quadratic equations obtained by response surface methodology (RSM) for high hydrostatic pressure (HHP) inactivation of *Bacillus cereus* spores, *Bacillus subtilis* spores and cells, *Staphylococcus aureus* and *Listeria monocytogenes*, all in milk buffer, were used to demonstrate the utility of genetic algorithms (GAs). It was possible to obtain several optimum pressure (P), temperature (T) and time (t) combinations for 5 $\log_{10}$ reductions of the mentioned microorganisms in milk buffer by using GAs. Depending on the properties of HHP equipment (maximum operating pressure) or the type of the food product (heat-sensitive), it could be possible to select the suitable *P-T-t* trio among the alternatives. This study reveals that GAs could be used to optimize HHP inactivation of microorganisms in foods.

Key words: Genetic algorithms, high hydrostatic pressure, inactivation, optimization.

INTRODUCTION

High hydrostatic pressure (HHP) has been proposed as an alternative technique to the thermal processing to destroy microorganisms in foods in order to enhance safety and shelf life (Buzrul et al., 2005a, b). HHP is a three-variable process consisting of pressure, temperature and time. For effective use of this method in food preservation, it is necessary to study the interaction of these factors and determine the optimum conditions to obtain desirable levels of microbial destruction while maintaining a maximum degree of sensory and nutritional quality (Avsaroglu et al., 2006; Buzrul et al., 2008).

Genetic algorithms (GAs) are stochastic optimization techniques. They are simple, powerful, general purpose, and derivative free stochastic global search algorithms inspired by the laws of natural selection and genetics. They follow Darwin's theory of evolution, where studied individuals are likely to survive in a competing environment. These algorithms do not need functional derivative information to search for a set solution that minimizes (or maximizes) a given objective function. The property of GAs reduces the computational burden and search time and also enables them to solve complex objective functions (Butun et al., 2006).

The objective of this communication was to demonstrate the utility of GAs for the optimization of process parameters of HHP during microbial inactivation in foods. Published quadratic equations obtained by response surface methodology (RSM) for HHP inactivation of several microorganisms were used to apply GAs to obtain optimal HHP processing conditions.

MATERIALS AND METHODS

Published quadratic equations obtained by RSM for HHP inactivation of *Bacillus cereus* spores (Ju et al., 2008), *B. subtilis* spores (Gao et al. 2006a) and cells (Gao and Hiang, 2005), *S. aureus* (Gao et al., 2005) and *L. monocytogenes* (Gao et al., 2006b), all in milk buffer, served as the databases of this study. The original experimental data to structure RSM and goodness-of-fit ($R^2_{adj} \geq 0.94$) of the models obtained were given in detail in the earlier mentioned studies.

Use of GAs

In this section, the various steps involved in a GA based approach for reduction of the microorganisms by HHP explained and

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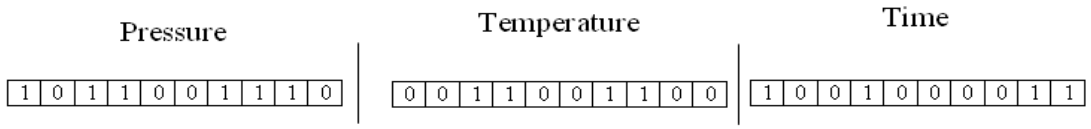

Figure 1. Structures of the genes (note that each process parameter is called a gene, which is represented by 10 binary numbers. Pressure, temperature and time are the parameters for a HHP process) and a chromosome (a chromosome consists of all the genes, that is, there are three genes in one chromosome).

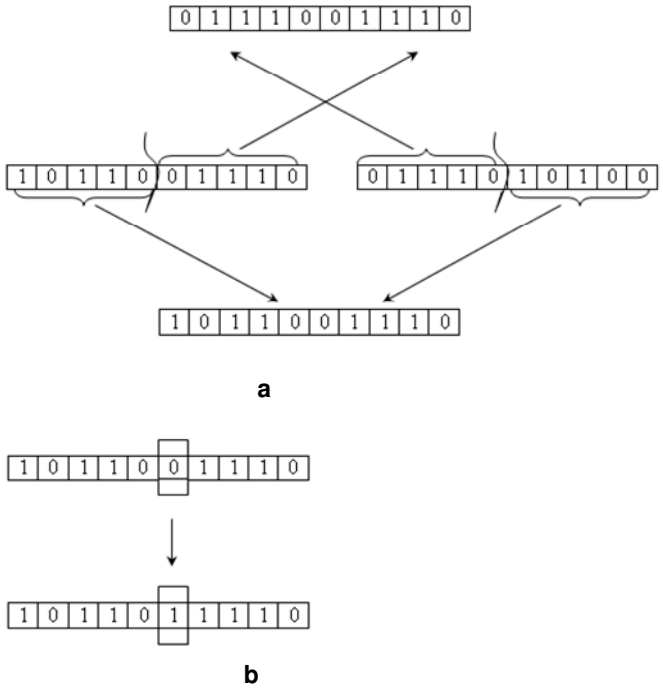

Figure 2. Schematic view of crossover (a) and mutation (b) processes [adapted from Bianchi and Bolognani (1998)].

described how the GA is designed and applied to the present problem. Various components of GAs such as chromosomes, fitness function, reproduction, crossover and mutation are illustrated as applied to the work.

Fitness function

First step is to define the fitness function *F*. The degree of 'goodness' of a solution is qualified by assigning a value to it. This is done by defining a proper fitness function for the problem. In the present problem, the microorganisms are to be reduced by the use of HHP, hence the following fitness function, *F*, is used:

$$F = \frac{1}{5 - Y} \quad (1)$$

Where, *F* is the fitness function as mentioned earlier, and *Y* is the quadratic equation obtained by RSM from literature. The aim is to obtain the optimum HHP process parameters for 5 $\log_{10}$ reduction of microorganisms in milk buffer, therefore in the denominator, (5 – *Y*) is implemented. When the denominator (5 – *Y*), that is, error is minimum, the fitness function (*F*) is maximum.

Initial population and solution

The solution to the problem is three numbers of optimum process parameters (pressure, temperature and time) that are accountable for the reductions of the mentioned microorganisms in milk buffer by HHP. For this purpose, randomly selected initial values of each process parameters are required in the first stage. Each process parameter is called a gene, which is represented by 10 binary numbers. A chromosome consists of all the genes, and in this instance, there are three genes in one chromosome (Figure 1). Thus, each chromosome represents a solution to the problem. GAs start with a set of solutions rather than a single set. The population consists of a set of chromosomes.

Offspring

Offspring is a new chromosome obtained through the steps of selection, crossover and mutation (Bianchi and Bolognani, 1998) (Figure 2). After the fitness of each chromosome is computed, parent solutions are selected for reproduction. This process emulates the survival of the fittest mechanism in nature. Roulette wheel selection is the most common and easy to implement selection mechanism. A virtual wheel is implemented for this

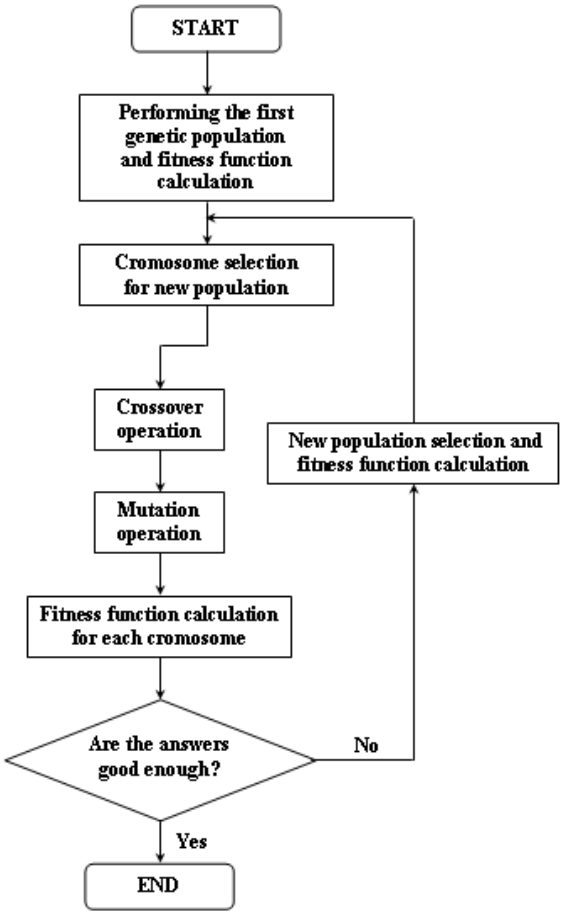

Figure 3. Genetic algorithm flow chart [adapted from Bianchi and Bolognani (1998)].

selection process. Each chromosome with the largest fitness value will occupy the largest area, while the chromosome with a lower value takes the slot of a smaller sector. Crossover is the operation by which a certain number of bits are exchanged between parent chromosomes. In this work, multi-point crossover is adopted for increased efficiency, since three numbers of variables are embedded in one chromosome. Mutation is another genetic operation by which a bit within a chromosome may toggle to the opposite binary. The crossover and mutation are performed based on the probabilities of crossover and mutation, and these values are selected here as 0.6 and 0.03, respectively [preliminary tests were performed for different crossover (mutation) probability values by starting from 0 and reaching 1 (0.5) by steps of 0.1 (0.005)]. Current population was replaced with the new population and this process was repeated until termination criterion was reached (Hasanzadeh et al., 2003) (Figure 3).

Software

Dedicated software was developed in Matlab™ (TheMathworks Inc., Natick, Mass., U.S.A.) for the application of GAs for inactivation of microorganisms by HHP. The parameters of GAs such as crossover and mutation probability, population size and the number of generations are usually selected as common values given in literature, or by means of a trial and error process to achieve the best solution set. The selection of optimum sized population requires some experience in GAs. In this work, the authors varied the parameters and studied the impact. It was observed that a value of 60 was ideal for population size; a lesser value increases the number of iterations.

In this application, the number of variables was the number of controllable optimum process parameters, so every chromosome had 30 binary numbers (10 for each process parameters) (Figure 1). In most of the operating points, an optimum solution was obtained after around 40 to 70 iterations. The number of generations and termination criterion was selected as 100. The parameters selected for the implementation of the GAs are listed in Table 1.

RESULTS

Ju et al. (2008) obtained Equation (2) by using RSM technique for HHP inactivation of *B. cereus* spores in milk buffer.

$$Y = 5.42 + 1.54 \cdot \left(\frac{P-500}{100}\right) + 0.30 \cdot \left(\frac{T-70}{10}\right) + 0.25 \cdot \left(\frac{t-15}{5}\right) - 0.11 \cdot \left(\frac{P-500}{100}\right)^2 + 0.17 \cdot \left(\frac{T-70}{10}\right)^2 - 0.23 \cdot \left(\frac{t-15}{5}\right)^2 - 0.23 \cdot \left(\frac{P-500}{100}\right) \cdot \left(\frac{t-15}{5}\right) \quad (2)$$

Table 1. Selected parameters of GAs.

Parameter	Value
Population size	60
Coding	Binary
Number of generations	100
Selection scheme	Roulette wheel
Crossover operator	Multipoint crossover
Crossover probability	0.6
Mutation probability	0.03
Termination criterion	100 iterations

Where, P is pressure in MPa, T is temperature in °C and t is time in min.

$$Y = \log_{10} N_0 / N(t) \quad (3)$$

Where, $N(t)$is the number of survivors after an exposure time t, and N_0 is the initial number of microorganisms.

GA optimization

Once an appropriate approximating model such as Equation (2) is obtained for HHP inactivation, this model can then be analyzed using various optimization techniques (such as GAs) to determine the optimum process conditions for HHP. The search for the optimum conditions for 5 $\log_{10}$ reduction of *B. cereus* spores in milk buffer by HHP was guided by the maximization of Equation (1).

Figure 4 shows the error (5 – Y) values (which are very close to zero) and corresponding pressure, temperature and time values for 5 $\log_{10}$ reduction of *B. cereus* spores in milk buffer. It could be possible to obtain 11 pressure, temperature and time combinations (depending on the equation that describes HHP inactivation of an organism, it may be possible to obtain more than one optimum values by use of GAs). Ju et al. (2008) obtained (only one) optimum value for each parameter (for 6 $\log_{10}$ reduction of *B. cereus* spores in milk buffer) by solving the inverse matrix of Equation (2). GAs do not need functional derivative information to search a set of solution that maximizes a given objective function (Butun et al., 2006).

Figure 5 shows how to obtain optimum process parameters for 5 $\log_{10}$ reduction of *B. cereus* spores in milk buffer. It is apparent from the figure that even if the iteration number was increased, the value (optimum value) obtained by GAs remained constant. Optimum pressure value was obtained at about 40 iterations, while about 70 iterations were needed for optimum temperature and time values.

Table 2 shows the optimum pressure, temperature and time combinations for 5 $\log_{10}$ reduction of each microorganism obtained by the use of GA. It could be possible to select one or two combinations among these values. For example, one could select 444 MPa, 78°C, 18.9 min for 5 $\log_{10}$ reduction of *B. cereus* spores if the maximum operating pressure is 450 MPa for the HHP equipment that is being used or 501 MPa, 67°C, 11.0 min could be used to reduce the adverse effects of temperature (as compared to 78°C) on the food product and also the duration of the HHP treatment. As mentioned earlier, in the databases of this study, optimum pressure (P), temperature (T) and time (t) values for 6 $\log_{10}$ inactivation of the mentioned micro-organisms were also calculated; however, one P-T-t trio for one bacterium was found. GAs could identify more than one optimum value (depending on the equation that is being used) which is an advantage over RSM optimization. Also, it should be noted that obtaining optimum conditions for 5 $\log_{10}$ reduction of micro-organisms is not the only option, it could be possible to apply the same procedure for other log reductions (6, 7 and 8 $\log_{10}$ reductions).

DISCUSSION

The factors that affect the cost of a HHP processing are pressure level (the complexity and the cost of pressure equipment rise more than linearly with the maximum operating pressure), temperature (due to energy supplied to the system by use of a thermostat jacket or laterally surrounding coil), duration of the treatment and filling factor.

The suitable selection of the HHP treatment parameters; pressure, temperature and time can ensure that the processing goal is reached without extensive detrimental effects (Buckow and Heinz, 2008) with minimum cost. When a technology such as HHP is

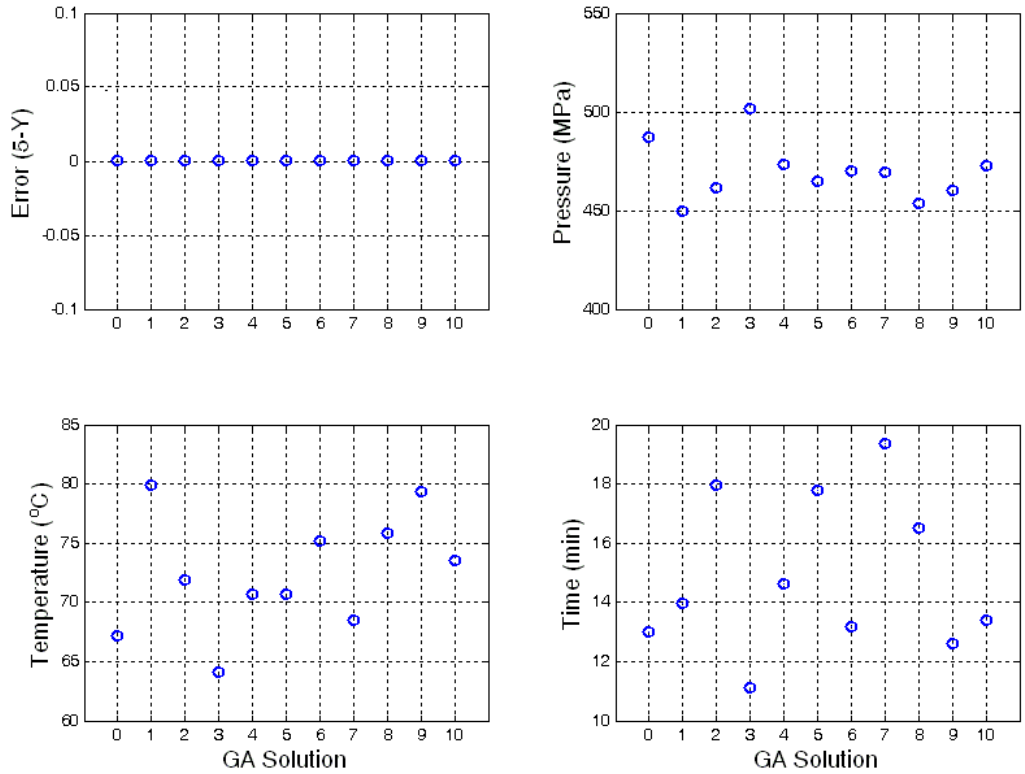

Figure 4. GA solutions for finding optimum HHP process parameters for 5 $\log_{10}$ reduction of *B. cereus* spores in milk buffer. The error values, 5 – *Y* (up left) which are very close to zero (where *Y* is the model equation describing the microbial inactivation of *B. cereus* spores); pressure in MPa (up right); temperature in °C (down left); time in min (down right). Note that GAs produced 11 pressure, temperature and time combinations for 5 $\log_{10}$ reduction of *B. cereus* spores in milk buffer.

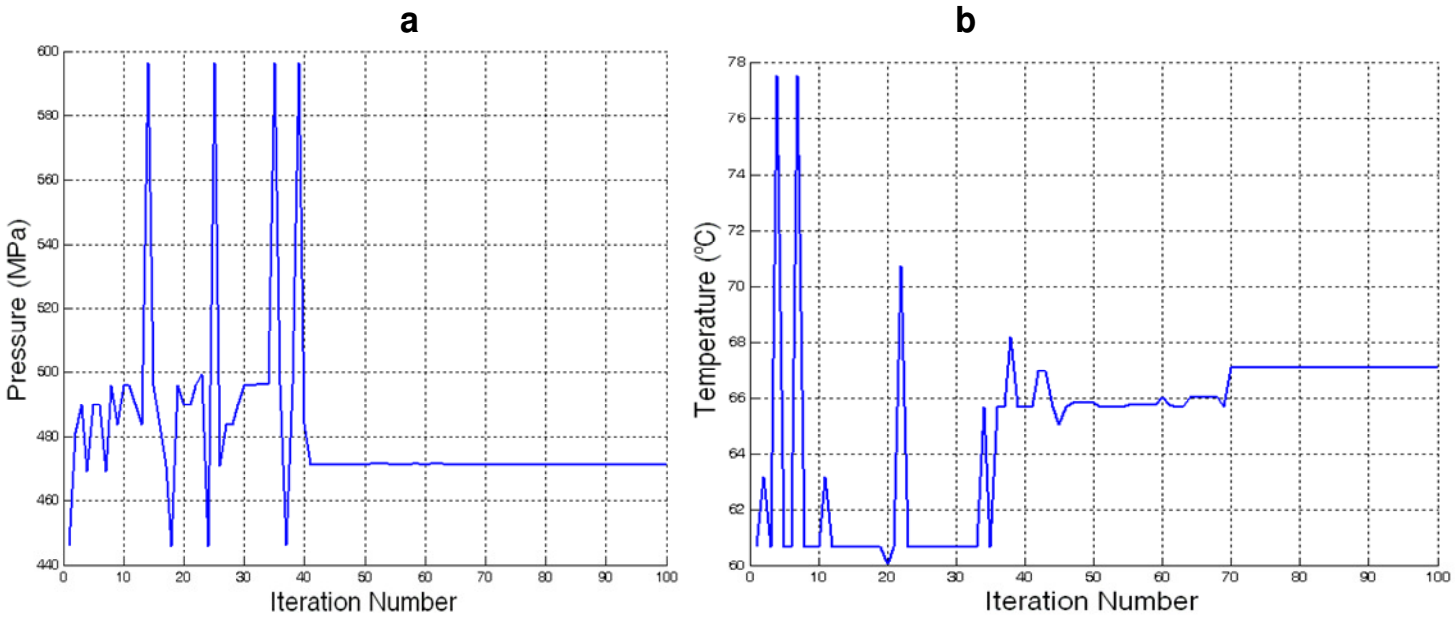

Figure 5. Obtaining optimum process parameters for 5 $\log_{10}$ reduction of *B. cereus* spores in milk buffer: (a) pressure; (b) temperature and (c) time. Pressure, temperature and time values correspond to 473 MPa, 67°C, 17.7 min, respectively. Note that even if the iteration number was increased, the value (optimum value) obtained by GAs remained constant.

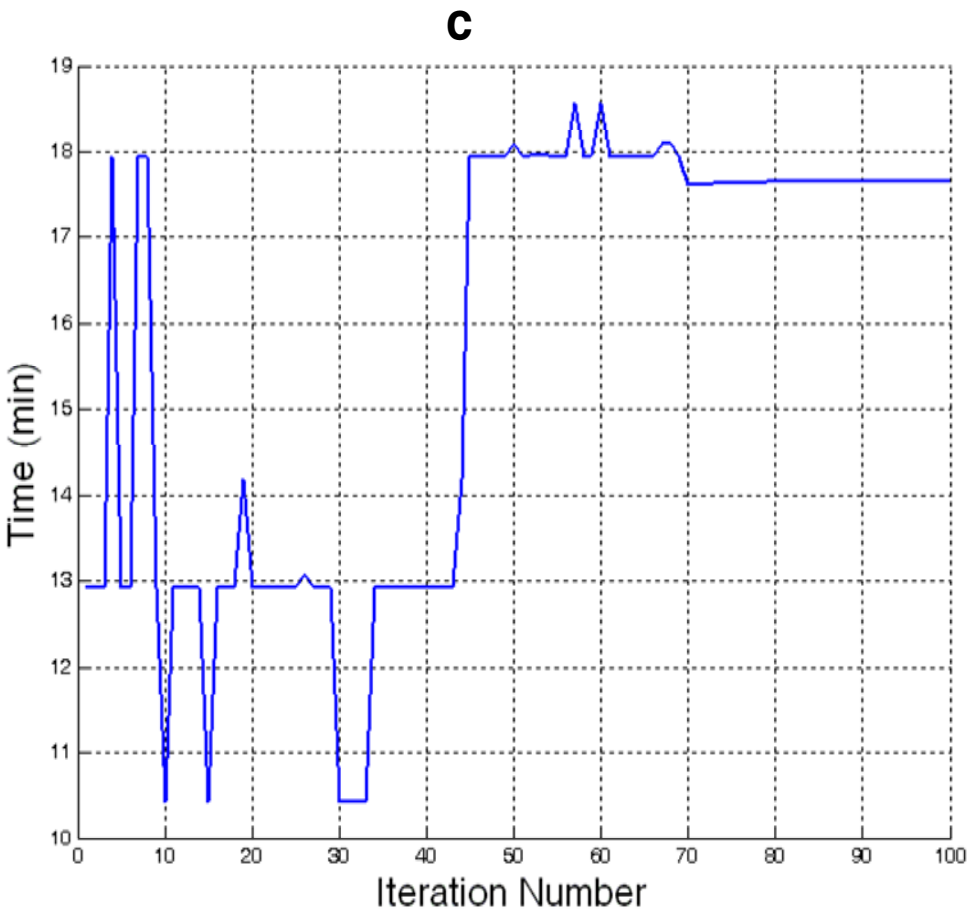

Figure 5. Contd.

investigated for use in the food industry, a food scientist and engineer often face the challenge of developing methods and techniques for validation of the process. For example, the U.S. Food and Drug regulations for pasteurization (21 CFR 131.3 and 21 CFR 1240.61) and sterilization (21 CFR 108, 113 and 114) primarily stipulate minimum temperature and time requirements for processing foods. Such information does not exist for HHP processed products and it is important to establish microbiological criteria for safe production of foods by HPP (Sizer et al., 2002; Balasubramaniam et al., 2004). Use of GAs could be very beneficial to the food industry to select the optimum conditions of HHP parameters since GAs may provide more than one optimum conditions (depending on the equation used) for a given $\log_{10}$ reduction. As mentioned earlier, depending on the type of the HHP equipment or the food that is being pressurized, one could find and identify the best candidate from a collection of alternatives.

The first step to the use of GAs in HHP inactivation of microorganisms in foods is to define the microbial inactivation by appropriate model equation. The necessary equations (obtained by RSM) for this study was found from literature. It should be noted that RSM is not the only option to build a model equation for microbial inactivation by HHP. Other modeling techniques could also be used. The second step is to analyze the model equation by GAs. It is well recognized that GA method is useful when the response hypersurface in which the optimum searched is of high dimension and has many local optima. The simultaneous optimization of several parameters is always quite challenging task when the response surfaces are characterized by several local optima (Leardi, 2001).

One possible drawback in the GAs is the large use of computational effort when compared with the traditional optimization methods (Correia et al., 2005).In conclusion, five model equations obtained by RSM for HHP inactivation of *B. cereus* spores, *B. subtilis* spores and cells, *S. aureus* and *L. monocytogenes* were analyzed by GAs to evaluate the optimum pressure, temperature and time combinations for 5 $\log_{10}$ reductions of the mentioned microorganisms in milk buffer. Although experimental verification is needed, this study reveals that GAs could be used to optimize HHP inactivation of microorganisms in foods.

Table 2. Microorganisms, corresponding equations used and optimum process conditions (pressure, temperature and time) for 5 $\log_{10}$ reductions obtained by GAs.

Microorganism	Equation	Conditions for 5 $\log_{10}$ reduction (obtained by GA)			Data source
		P (MPa)	T (°C)	t (min)	
B. cereus spores	$Y = 5.42 + 1.54 \cdot \left(\frac{P-500}{100}\right) + 0.30 \cdot \left(\frac{T-70}{10}\right) + 0.25 \cdot \left(\frac{t-15}{5}\right) - 0.11 \cdot \left(\frac{P-500}{100}\right)^2 + 0.17 \cdot \left(\frac{T-70}{10}\right)^2 - 0.23 \cdot \left(\frac{t-15}{5}\right)^2 - 0.23 \cdot \left(\frac{P-500}{100}\right) \cdot \left(\frac{t-15}{5}\right)$	473	67	17.7	Ju et al. (2008)
		444	78	18.9	
		454	79	13.8	
		470	77	12.1	
		474	65	18.4	
		475	66	16.4	
	$400 \leq P \leq 600$ MPa	476	62	17.0	
	$60 \leq T \leq 80$ °C	478	67	15.0	
	$10 \leq t \leq 20$ min	480	65	15.0	
		498	80	18.6	
		501	67	11.0	
S. aureus	$Y = 5.26 + 0.82 \cdot \left(\frac{T-45}{15}\right) + 1.21 \cdot \left(\frac{P-400}{200}\right) + 0.73 \cdot \left(\frac{t-15}{5}\right) - 0.38 \cdot \left(\frac{T-45}{15}\right)^2 - 0.01 \cdot \left(\frac{P-400}{200}\right)^2 - 0.33 \cdot \left(\frac{t-15}{5}\right)^2 + 0.18 \cdot \left(\frac{T-45}{15}\right) \cdot \left(\frac{P-400}{200}\right) - 0.08 \cdot \left(\frac{T-45}{15}\right) \cdot \left(\frac{t-15}{5}\right) + 0.17 \cdot \left(\frac{P-400}{200}\right) \cdot \left(\frac{t-15}{5}\right)$	349	40	19.9	Gao et al. (2005)
		351	39	20.0	
		360	39	19.2	
		377	38	18.4	
		380	38	17.5	
		382	39	17.0	
		388	38	17.5	
		389	36	19.5	
		391	36	20.0	
	$200 \leq P \leq 400$ MPa	398	36	19.4	
	$20 \leq T \leq 40$ °C	400	37	17.2	
	$10 \leq t \leq 20$ min	499	87	19.4	Gao et al. (2006a)
	$Y = 4.123 + 1.557 \cdot \left(\frac{P-500}{100}\right) + 0.347 \cdot \left(\frac{T-80}{10}\right) + 0.248 \cdot \left(\frac{t-15}{5}\right) + 0.266 \cdot \left(\frac{P-500}{100}\right)^2 + 0.339 \cdot \left(\frac{P-500}{100}\right) \cdot \left(\frac{T-80}{10}\right)$	500	90	19.7	
		537	82	18.6	
		541	83	17.1	
		543	82	17.2	
B. subtilis spores		543	84	15.6	
		547	80	17.0	
	$400 \leq P \leq 600$ MPa	556	74	16.9	
	$70 \leq T \leq 90$ °C	566	74	12.7	
	$10 \leq t \leq 20$ min	568	75	11.6	
		572	72	10.9	

Table 2. Continue.

	$Y = 5.42 + 0.46 \cdot \left(\frac{T-45}{15}\right) + 1.764 \cdot \left(\frac{P-400}{200}\right) + 0.254 \cdot \left(\frac{t-15}{5}\right) - 0.218 \cdot \left(\frac{T-45}{15}\right)^2 - 0.645 \cdot \left(\frac{P-400}{200}\right)^2$	295	53	19.5	Gao and Jiang (2005)
		314	55	20.0	
		334	51	16.5	
		335	52	16.3	
		340	52	15.0	
B. subtilis cells		350	52	12.9	
	$200 \leq P \leq 400$ MPa	351	44	17.0	
	$30 \leq T \leq 60$°C	352	47	14.5	
	$10 \leq t \leq 20$ min	357	51	11.9	
		384	43	11.2	
		400	36	13.4	
	$Y = 4.263 + 1.313 \cdot \left(\frac{P-400}{100}\right) + 0.701 \cdot \left(\frac{T-40}{10}\right) + 0.687 \cdot \left(\frac{t-10}{5}\right) + 0.112 \cdot \left(\frac{P-400}{100}\right)^2 + 0.134 \cdot \left(\frac{T-40}{10}\right)^2 - 0.282 \cdot \left(\frac{t-10}{5}\right)^2$	368	49	15.0	Gao et al. (2006b)
		384	50	10.9	
		390	47	14.3	
		415	43	13.2	
L. monocytogenes		435	48	7.5	
	$300 \leq P \leq 500$ MPa	450	42	9.3	
	$30 \leq T \leq 50$°C	453	36	12.4	
	$5 \leq t \leq 15$ min	460	39	9.9	
		465	35	11.3	
		495	34	8.4	
		498	32	8.8	

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