

Thermal resistance of the *Bacillus subtilis* microorganism in food processed by preservation

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Summary. In the work lethal lines D-t for a reduction of spores of *Bacillus subtilis* ATCC 6633 by ten times were determined in three types of preserved products with a different pH value: 1. Sterilized Brined Peas (pH 5.8—5.9); 2. Tomato Juice (pH 4.0—4.2); 3. Juice Pirueta (pH 3.2—3.3). The given products with artificially inoculated spore suspension of the given microorganism ($c = 10^{4-5}$ /ml) were warmed up at temperatures of 100, 105, 110, 115 and 120°C during different time intervals of warming in an autoclave. The lethal lines D-t of the investigated microorganism and values z for individual products read from them were obtained by means of a graphic expression of individual mean results of the temperature—time dependence.

Process of heating and pH showed to have a decisive effect on the thermoresistance of *B. subtilis* spores: in the samples of the semisolid non-sour product Sterilized Brined Peas higher D and z values were attained than in the acid environment of the Juice Pirueta which had liquid consistence. The obtained results point to lower D and z values with decreasing pH of samples under the same conditions of warming.

Thermal processes often used in combination with other kinds of preservation have become very important methods of food storage property prolongation. The basic function of thermal processes is to eliminate or at least minimize the number of microorganisms or enzyme content causing food deterioration and endangering the health of consumers. However, an accompanying effect of these processes is a simultaneous destruction of basic nutrients (proteins, minerals, vitamins, etc.) and the transformation of sensorially effective components. As the aim of preservation is to prolong food storage property and simultaneously, to provide its microbiological perfectness and nutritive value, the optimization of sterilizing modes must be a matter of course.

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One of the stages of the entire thermal process optimization is the determination of an optimum temperature—time ratio for individual typical microorganisms and its graphic expression in the form of a lethal line.

For this reason we concentrated on the verification of the technique and method of lethal lines determination in typical spore-forming, medium thermoresistant microorganism *Bacillus subtilis* ATCC 6633 in three types of preserved products (Sterilized Brined Peas, Tomato Juice, Juice Pirueta) in this work. An individual selection of warming temperatures and times combination facilitated to evaluate their effect on the selected microorganism in different microenvironments (different consistence, pH, heat sinking capability, etc.) as well as to master some basic operations in actual conditions of a preservation microbiological laboratory with common equipment.

Basic dependences and concepts [1—6]

The basis of programming and control of preserved thermal processing is the knowledge of some concepts and mathematical procedures coherent with the objectification of this process. The criteria are determined on the basis of an objective comparison of the required and achieved technological effects.

The heat application effect is evaluated most frequently by means of: 1. value D , 2. value U , 3. value z , 4. lethality L , 5. Intensity of thermoinactivating effect W and F .

Materials and methods

A typical spore-forming medium thermoresistant strain *Bacillus subtilis* ATCC 6633 was selected for the study of microorganism thermoinactivating lines in individual preserved products. Nutrient agar No. 2 of our provenience (Imuna, Šarišské Michalany) supplied in powder and prepared as a 4% aqueous solution was applied for cultivation. The inoculation method by rubbing [7—9] 0.2 ml of appropriately diluted suspension on the agar surface was applied and the results were calculated on the number of microorganisms in 1 ml of the investigated sample after their evaluation. To determine the lethal lines of the studied microorganism the following process was applied: the lethal lines at individual temperatures were determined by warming up of selected microorganism cell suspension at different temperatures during different time periods of warming up and a subsequent determination of the microorganism original amount drop. The read values D were laid out against the pertinent

temperatures on a semilogarithmic paper obtaining the thermoinactivating (lethal) line for the reduction of the microorganism original amount by ten times in such a way.

Lines D-t of the same microorganism in the same or similar food have the same gradient. It is most frequently expressed as the so-called z value for practical reasons. It is the temperature difference $t_1 - t_2$ which under the given conditions a shortening or prolongation of period D by one logarithmic cycle corresponds to. The higher the value z the more resistant is the given type of microorganism to the thermal energy effect.

The preparation of spore suspension. The culture *B. subtilis* was left to propagate for 3—5 days in minimum nutrient environment MPB (ten times diluted) at 37°C on a shaking apparatus under aerobic conditions till a concentrated suspension of spores (the concentration of which was determined in advance by a common microbiological method [7]) was created.

Samples. Preserved products of liquid and semiliquid consistence were chosen such that each product type was taken from one lot:

1. Pirueta (pH 3.2—3.3) — orange-carrot juice (Slovlik, Trenčín, plant Nové Mesto nad Váhom, Czechoslov. Standard ON 56 9350), weight 420 g, volume 300 ml, made on November 2, 1981.

2. Tomato Juice (pH 4.0—4.2) (Slovlik, Trenčín, plant Nové Mesto nad Váhom, Czechoslov. Standard ON 56 9350), weight 400 g, volume 300 ml, made on September 2, 1981, collected in 1981.

3. Sterilized Brined Peas (pH 5.8—5.9) (Stredoslovenské konzervárne a liehovary, Liptovský Mikuláš, Czechoslov. Standard ON 56 9204), weight 430 g, volume 400 ml, pea size 7.5—9.5 mm, made in 1981.

pH of samples (average 5 values) was measured on a pH-meter of the OP-205 type. The inoculation of a suitable concentration of *B. subtilis* spores into the contents of individual products was performed in dependence on their consistence. In liquid products (Pirueta, Tomato Juice) an opening through which 3 ml of the sample were taken with a sterile syringe and instead of it an aliquot quantity of spore suspension ($c = 10^2/\text{ml}$) was inoculated, was made with a sterile sharp object on a sterile end degreased with acetone and concentrated HCl. The opening was closed by soldering under aseptic conditions and, after 20—30 min of contents shaking on the shaker, the tins were warmed up according to differently selected sterilizing modes in an autoclave. Then the tins were cooled and 0.2 ml of the content was inoculated on Petri dishes. In the semiliquid product Sterilized Brined Peas the inoculation was performed similarly as in the previous cases with the only exception that 10 g of shaken content (the whole parts and the brine) were weighted into sterile Erlenmeyer flasks and 90 ml of physiological solution was added. This decimal shift was taken into account.

Table 1. The reduction of *Bacillus subtilis* spores quantity in the product Sterilized Brined Peas at different temperatures ($c = 10^5/\text{ml}$)

Sterilized Brined Peas						
100°C						
Warming time [min]	0	1	5	15	18	20
$x (n = 4)$	650	345	156	49	43	1
105°C						
Warming time [min]	0	1	10	15	18	20
$x (n = 4)$	286	225	105	69	10	0
110°C						
Warming time [min]	0	1	5	12	15	18
$x (n = 4)$	286	125	56	28	10	0
115°C						
Warming time [min]	0	1	5	15	17	
$x (n = 4)$	255	169	101	10	0	
120°C						
Warming time [min]	0	1	5	12	15	
$x (n = 4)$	255	120	74	9	0	

After putting 0.2 ml of the appropriate investigated sample dilution on the surface of nutrient broth this was spread uniformly with a sterile bent glass rod, and after drying in a thermostat the incubation took place within prescribed time and temperature [7].

Results and discussion

The course of the thermoinactivation of the strain *Bacillus subtilis* ATCC 6633 in 3 types of the preserved products (Sterilized Brined Peas, Tomato Juice and Juice Pirueta) at different temperatures and in different time inter-

Table 2. The reduction of *Bacillus subtilis* spores quantity in the product Tomato Juice at different temperatures ($c = 10^4/\text{ml}$)

Tomato Juice								
105°C								
Warming time [min]	0	1	5	10	13	15	17	18
$x (n = 4)$	495	348	264	153	113	63	13	0
110°C								
Warming time [min]	0	1	5	10	13	15	20	
$x (n = 4)$	381	229	210	114	68	39	11	
115°C								
Warming time [min]	0	1	5	10	13	18		
$x (n = 4)$	481	263	123	68	38	0		
120°C								
Warming time [min]	0	1	5	10	13	15	17	
$x (n = 4)$	571	216	139	78	42	13	0	

vals can be seen in Tables 1—3, the lethal lines D-t of the tested microorganism corresponding to the representation of warming-up temperatures and time dependences are given in Figs. 2—4. Figure 1 shows model of the basic parameters of sterilization course at the temperature of 110°C. Table 1 summarizes the reduction of *B. subtilis* spores quantity in the product Sterilized Brined Peas at temperatures of 100, 105, 110, 115 and 120°C and corresponding warming times. The lethal line D-t of *B. subtilis* (Fig. 2) for the original cell quantity reduction by ten times was obtained by plotting the obtained values into a semilogarithmic coordinate system. Values D and z read from the plot are summarized in Table 4.

The reduction of *B. subtilis* spores quantity in the product Tomato Juice at temperatures of 105, 110, 115 and 120°C is given in Table 2, and the lethal line of *B. subtilis* in Fig. 3. Table 2 as well as summarizing Table 4 point to a high percentage of surviving spores. A total inactivation of the tested microorganism was attained only at higher temperatures: 115°C (18 min) and 120°C (17 min).

Thermoinactivation course of *B. subtilis* spores in the liquid product Pirueta

Table 3. The reduction of *Bacillus subtilis* spores quantity in the product Pirueta at different temperatures ($c = 10^6/\text{ml}$)

Juice Pirueta						
100°C						
Warming time [min]	0	1	5	10	15	17
$x (n = 4)$	611	396	230	113	21	0
105°C						
Warming time [min]	0	1	5	10	13	15
$x (n = 4)$	529	309	245	29	15	3
110°C						
Warming time [min]	0	1	8	10	14	15
$x (n = 4)$	476	274	134	39	9	5
115°C						
Warming time [min]	0	1	3	8	10	12
$x (n = 4)$	668	330	283	99	19	6

Table 4. D and z values for *Bacillus subtilis* at different temperatures in 3 kinds of preserved products

Product	D_{100} [min]	D_{105} [min]	D_{110} [min]	D_{115} [min]	D_{120} [min]	z [°C]	pH
Sterilized Brined Peas	16.4	13.0	12.2	10.8	8.8	89.0	5.8—5.9
Tomato Juice		15.1	13.7	11.3	10.4	51.5	4.0—4.2
Juice Pirueta	13.5	8.3	7.2	5.2		47.0	3.2—3.3

at temperatures 100, 105, 110 and 115°C is given in Table 3. D values in Table 4 are obviously lower than in the previous product, which may be connected with a considerable reduction of pH of the product. The thermoinactivation line obtained by the depiction of D values in dependence on the pertinent temperatures is shown in Fig. 4.

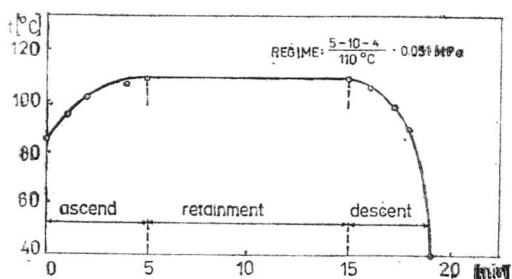
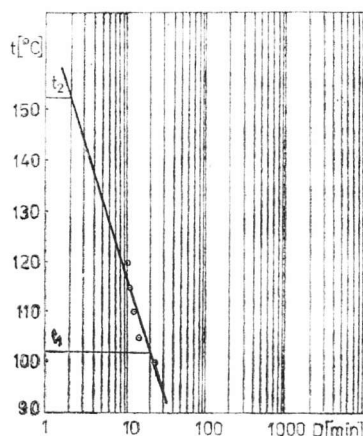
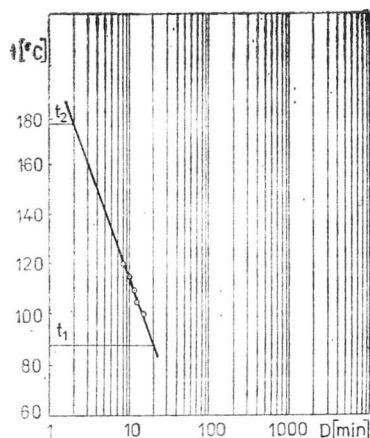


Fig. 1. The typical underheating line at sterilization of products.



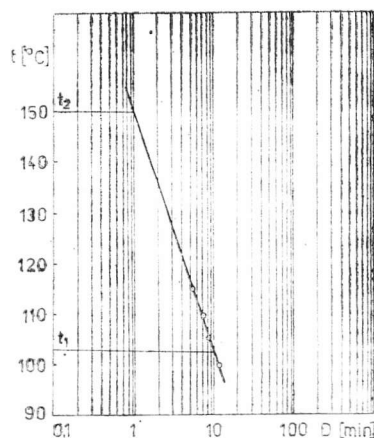
$$z = t_2 - t_1 = 51.5$$

Fig. 3. The thermoinactivation line D-t of *B. subtilis* in Tomato Juice.



$$z = t_2 - t_1 = 89.0$$

Fig. 2. The thermoinactivation line D-t of *B. subtilis* in Sterilized Brined Peas.



$$z = t_2 - t_1 = 47.0$$

Fig. 4. The thermoinactivation line D-t of *B. subtilis* in the Juice Pirueta.

From the obtained results it follows that heat sinking capability and pH have a decisive effect on the thermoinactivation of *B. subtilis* spores, and that under the same warming-up condition the *D* and *z* values are appropriately lower with decreasing pH of the samples.

The presented results give evidence to the fact that data referred to in literature cannot be simply taken over and applied to any similar product without the risk of reducing its storing stability. *D* values for *B. subtilis* in more-com-

ponent meat-vegetable tins differing to certain degree from the values obtained in our experimental work [11, 12] and referred to in literature can serve as an example. Considerably differing values were determined for model systems, too [13]. For this reason it is necessary to investigate systematically and with great attention the thermal resistance of a wide range of microorganisms possibly occurring in individual kinds of food processed by preservation, taking into consideration an even broader scale of warming temperatures and time intervals in the future.

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Термическая устойчивость микроорганизма *Bacillus subtilis* в продуктах, изготовленных на консервных заводах

Резюме

В работе определялись леталитные линии D-т для десятикратного снижения количества спор *Bacillus subtilis* ATCC 6633 в трех типах продуктов консервных заводов с различной величиной pH: 1. Стерилизованный зеленый горошек в соленой заливке (pH 5,8—5,9); 2. Томатный сок (pH 4,0—4,2); 3. Сок „Пируэт“ (pH 3,2—3,3). Упомянутые продукты с искусственно инокулированной суспензией спор изучаемого микроорганизма ($c = 10^4$ -5/мл) нагревались в автоклаве при температурах 100, 105, 110, 115 и 120 °C в течение времени разной продолжительности. Путем графического выражения отдельных средних результатов зависимости „температура—время“ были получены леталитные кривые для изучаемого микроорганизма D-т, а с них считаются значения z для отдельных продуктов.

Решающее влияние на термическую устойчивость спор *B. subtilis* оказывает теплопередача и pH: в образцах полутвердого продукта „Стерилизованный зеленый горошек в соленой заливке“ значения D и z были выше, чем в кислой среде сока „Пируэт“ жидкой консистенции. Из полученных результатов вытекает, что с понижением pH образцов в одних и тех же условиях нагревания величины D и z будут ниже.

Teplotná rezistencia mikroorganizmu *Bacillus subtilis* v konzervárenských spracúvaných potravinách

Súhrn

V práci sa stanovili letalitné čiary D-t pre desaťnásobné zníženie počtu spór *Bacillus subtilis* ATCC 6633 v troch typoch konzervárenských výrobkov s rôznou hodnotou pH: 1. Sterilizovaný hrášok v slanom náleve (pH 5,8—5,9); 2. Rajčinová šťava (pH 4,0—4,2); 3. Džús Pirueta (pH 3,2—3,3). Uvedené výrobky s umele inokulovanou suspenziou spór uvedeného mikroorganizmu ($c = 10^4$ -5/ml) sa zahrievali v autokláve pri teplotách 100, 105, 110, 115 a 120 °C a pri rôzne dlhých zahrievacích časoch. Grafickým vyjadrením jednotlivých priemerných výsledkov závislosti teplota—čas sa získali letalitné čiary D-t pre sledovaný mikroorganizmus a z nich odčítané z hodnoty pre jednotlivé výrobky.

Rozhodujúci vplyv na termorezistenciu spór *B. subtilis* má prestup tepla a pH: vo vzorkách polotuhého nekyslého výrobku Sterilizovaný hrášok v slanom náleve sa dosiahli vyššie D a z hodnoty ako v kyslom prostredí džúsu Pirueta tekutej konzistencie. Z dosiahnutých výsledkov vyplýva, že s klesajúcim pH vzoriek sú pri tých istých podmienkach zohrevu hodnoty D a z nižšie.