Effect of high pressure on mesophilic lactic fermentation streptococci

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Abstract. The research concerned the effect of high pressure on mesophilic lactic fermentation streptococci, present in two commercial inocula produced by Christian-Hansen. Water solutions of inocula were pressurized at 50-800 MPa, at room temperature, for 30-120 min. Pressurization at 50-100 MPa slightly increased or reduced the number of lactic streptococci, depending on the inoculum and pressurization time. Pressurization at 200 MPa caused a reduction in the number of streptococci by over 99.9%, whereas the pressure of 400 MPa and above almost completely inactivated streptococci. Pressurization also reduced the dynamics of microorganism growth and acidification, to the degree depending on the pressure.

1. Introduction.
High pressure technology (100-1000 MPa) is a modern method of food processing, used for non-thermal preservation of raw materials and food products as well as for modification of their organoleptic and technological properties [1].

Microorganisms demonstrate diverse resistance to high pressure [2] which is a strain characteristic [3]. Resting spores are particularly resistant to high pressure, as they are not completely inactivated even at 1000 MPa [4]. The pressure-caused inactivation of microflora was determined by: the pressure and time of its application [5], temperature [6,7] environmental pH [8, 9] and environmental composition [10].

Potential possibilities of using high pressure technology in the dairy industry seem interesting. Pressurization of milk can replace the process of pasteurization [2], and it can additionally result in the reduction of rennet coagulation time, an increase in the efficiency of a cheese-making process [11] and improvement of organoleptic qualities of produced cheese [2].

Pressurization of ripening cheese can accelerate its ripening [12] and shape its sensory and physicochemical characteristics [13].

It is also possible to apply prior pressurization to inocula in order to obtain attenuated cultures of lower acidifying activity but of preserved lipo- and proteolytic activity [14].

2. The aim of the experiment.
The aim of this experiment was to characterize the effect of high pressures on mesophilic lactic streptococci, present in two commercial cheese-making inocula.
3. Materials and methods.

3.1. The preparation of research material for pressurization.
The subject of the research were lyophilized commercial inocula produced by Christian-Hansen: FD-
DVS Flora Danica and FD-DVS CHN-19, used for direct inoculation of milk in cheese production.
Lyophilized inocula were rehydrated for two hours by shaking water suspensions of cells at room
temperature. Water solutions were prepared in such a way so that they should contain lactic
streptococci in the amount of about $10^8$ cfu/cm$^3$.

3.2. Pressurization of water solutions of inocula.
Inocula solutions were pressurized at 50, 100, 200, 400, 600 and 800 MPa for 30, 60 and 120 min
at room temperature, using U 101 apparatus produced by UNIPRESS EQUIPMENT Warszawa. A
part of inocula was left unpressurized as a control inocula.

3.3. Determination of the number of lactic acid streptococci in the inoculum. The number of
streptococci was determined on M17 agar according to Terzaghi (Merck). Cultures were incubated at
30°C/48 h.

3.4. Comparison of methods used for testing the number of streptococci population: by the colony-
count method and the method of measuring optical density (OD). The usefulness of the OD method
(according to 3.5) for determining the dynamics of microorganism growth was assessed by
determining the optical density of solutions of inocula prepared according to 3.1 and their subsequent
dilutions by sterile distilled water, and determining at the same time the number of streptococci in the
above mentioned diluted solutions according to 3.3.

3.5. Determination of the dynamics of the growth of lactic streptococci and acidification of liquid
nutrient medium. A liquid nutrient medium – M17 broth according to Terzaghi (Merck) – was
inoculated with the same number of living cells. At four-hour intervals, the optical density of the
sample was determined at 600 nm in Beckman DU 650 spectrometer. The cultures were continued at
30°C until the streptococci reached a stationary phase. Parallel to this determinations the active acidity
of the culture was determined by means of pH measurement.

4. Results and discussion

4.1. The effect of high pressures on the number of lactic streptococci.
Pressurization of FD-DVS Flora Danica inoculum at 50 MPa/30 and 60 min increased the number of
lactic streptococci in comparison with the control sample, by 36.8% and 13.2%, respectively. Extending the time of pressurization to 120 min decreased the number of streptococci by 34.2 
Pressurization of the inoculum solution at 100 MPa/30, 60 and 120 min caused a reduction in the
number of streptococci by 15.0%, 32.5% and 37.5%, respectively.
Pressurization of FD-DVS CHN-19 inoculum at 50 MPa/30, 60 and 120 min increased the number of
streptococci by 37.8%, 22.4 and 20.4%, respectively. Pressurization at 100 MPa/30 min also
increased the streptococci population by 27.3% and at 100 MPa/60 and 120 min reduced the number
of streptococci by 37.5% and 43.2%, respectively.

The fact, that lower pressures can cause an increase in the number of lactic acid streptococci, can
be related to the disruption of “chains” of streptococci into smaller fragments, which would cause the
increase in the number of units capable of forming colonies.
Pressurization of FD-DVS Flora Danica inoculum at 200 MPa/30, 60 and 120 min resulted in a
reduction of bacteria population by 94.8%, 98.8% and 99.995%, respectively. Pressurization of FD-
DVS CHN-19 inoculum at 200 MPa/30, 60 and 120 min resulted in a reduction in bacteria population
by 94.8%, 98.5% and 99.965%, respectively. Pressurization of inocula at 200 MPa/120 min had similar effects as pasteurization.

Pressurization of FD-DVS Flora Danica inoculum at 400 and 600 MPa/30-60 min resulted in a reduction of the number of streptococci to below 100 cfu/cm$^3$, and after 120 min of pressurization, no presence of living bacteria was found. After pressurization of the inoculum at 800 MPa, no streptococci was observed, regardless of the pressurization time. Pressurization of FD-DVS CHN-19 inoculum at 400, 600 and 800 MPa/30 min reduced the number of streptococci to below 100 cfu/cm$^3$, and in solutions pressurized for 60 and 120 min, no presence of lactic streptococci was found.

A similar sensitivity of bacteria from dairy inocula to high pressures was observed by Krasowska et al. [14] who found that pressurization of lyophilized inoculum, containing lactic streptococci and bacilli at 200-800 MPa/15 min decreased the number of streptococci by 0.67-6.00 logarithmic orders.

Figure 1. The effect of pressurization at (a) 50 MPa (b) 100 MPa (c) 200 MPa on the number of streptococci

4.2. Comparison of the methods of testing the increase in the number of streptococci population by a colony-count method and the method of measuring optical density.

The examined dilutions of FD-DVS Flora Danica inoculum were characterized by OD from 0.0006 to 0.4626, which corresponded to the number of bacteria between $2.10 \times 10^7$ cfu/cm$^3$ and $9.35 \times 10^9$ cfu/cm$^3$; while for FD-DVS CHN-19 inoculum it was between 0.0010 to 0.5041, which corresponded to the number of bacteria between $5.75 \times 10^7$ cfu/cm$^3$ and $9.9 \times 10^9$ cfu/cm$^3$. The relationship between OD and the number of streptococci determined using a colony-count method was directly proportional, linear and the correlation coefficient was very high, (0.9992 and 0.9988, respectively). Therefore, the usage of a faster OD method seems justified.

4.3. Determination of the dynamics of the growth of lactic streptococci in a liquid nutrient medium.

Both control inocula reached a stationary phase after 12 h of incubation and pH decreased with the increase in the streptococci population. For FD-DVS Flora Danica pH in a stationary phase it was 6.09 (in relation to 7.2 at the beginning of culture), while the extension of the culturing time to 20 h lowered pH to 5.76. For FD-DVS CHN-19 pH in a stationary phase it was 6.16 (in relation to 7.2 at the beginning of culture) – while the extension of culturing time to 20 h lowered pH to 5.70.

Pressurization of the inoculum at 50 and 100 MPa, regardless of the time of pressurization, resulted, however, in the extension of time necessary for reaching a stationary phase by 4 h. For FD-DVS Flora Danica pH was lowered during the culturing time from 7.12 to 6.09 and from 7.13 to 6.17, respectively. For FD-DVS CHN-19 pH was lowered during the time of culturing from 7.07 to 5.97 and from 7.12 to 6.17, respectively. It appears to support the thesis that pressurization at low pressures rather causes the disruption of streptococci chains than stimulates the development of lactic streptococci. This increases the number of colonies obtained on M17 agar, but at the same time, it reduces the ability of cells to multiply.

Pressurization of the inoculum solution at 200 MPa, regardless of the time of pressurization, caused an extension of time necessary for reaching a stationary phase to 20 hours of culturing. For FD-DVS Flora Danica pH was lowered during the culturing time from 7.13 to 6.15. For FD-DVS CHN-19 pH was lowered during the time of culturing from 7.14 to 5.87.
Pressurization of the inoculum solution at 400, 600 and 800 MPa, regardless of the time of pressurization, resulted in an almost complete inhibition of streptococci growth and nutrient medium acidification. Pressurization at 400 MPa and higher inactivates or damages bacteria cells to such an extent that within 24 h they are not able to continue growth in a liquid nutrient medium. Impeded functioning and multiplication of pressurized bacteria cells can be to a high extent related to the damage to the cell walls and cell membrane during pressurization [15].

4.4. Comparison of the resistance to high pressures in the case of FD-DVS Flora Danica and FD-DVS CHN-19 inocula.

The inocula under examination were slightly different as regards their resistance to high pressures. FD-DVS CHN-19 inoculum was slightly more resistant, which is proved by the fact that pressurization at 50 MPa (regardless of the time) and at 100 MPa/30 min did not result in a reduction of streptococci in their composition, while 120-minute pressurization at 50 MPa and 30-minute at 100 MPa were sufficient to lower the number of streptococci in FD-DVS Flora Danica inoculum.

There were no significant differences observed in the rate of development of streptococci from various inocula in a liquid nutrient medium, but in most cases the lowering of pH value during the incubation was more obvious in the case of FD-DVS CHN-19.

5. Conclusions

Pressurization of dairy inocula, to the degree depending on the pressure applied, has a significant effect on the number, growth dynamics and acidification of a nutrient medium by lactic acid streptococci present in the composition of both cultures.

A significant reduction in the number of streptococci (over 99.9%) can be obtained by applying a pressure of at least 200 MPa.

Pressurization of inocula at 400 MPa and higher pressures significantly reduces or completely inactivates streptococci present in the inocula.

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6. References

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