The effect of high pressures on the yoghurt from milk with the stabilizer

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Abstract. The effect of high pressures on the microbiological and physico-chemical properties of yoghurt was investigated. The best results were obtained when the yoghurt was manufactured from milk with the addition of MYO 752 stabilizer (starch, gelatin, pectin) selected from 10 stabilizers. Yoghurt manufactured with the addition of 0, 6% MYO 752 stabilizer was processed at the pressure of 400-600 MPa/15 min. in the range of 50 MPa. Pressurization caused a total reduction of number of Lactobacillus delbrueckii ssp. bulgaricus and reduced the number of Streptococcus thermophilus by 1-2 orders of magnitude. Pressurized and non-pressurized yoghurts characterized of a homogenous consistency and typical plain yoghurt taste. The decrease of the number of living bacteria was observed in yoghurts during the storage. The acidity of pressurized yoghurts remained on the same level at the temperature of 4°C and 20°C. The more intensive antibacterial activity of microflora was observed in yoghurts stored at 20°C in comparison with yoghurts stored at 4°C. Disadvantageous changes of the pressurized yoghurts consistency were not found. The taste and aroma of yoghurts remained without any changes.

1. Introduction
High pressure can be applied in yoghurt technology to preserve milk intended for the production of yoghurt and to preserve a final product. Yoghurt preserved by means of high pressure can be also a good carrier of probiotic bacteria [1]. One of problems that the producers of yoghurt must face, particularly in the case of low-fat yoghurt, is to obtain its proper texture. It has been found that the application of high pressure in the pre-treatment of cow milk intended for yoghurt improves the firmness of yoghurt curd and reduces its syneresis. The best results have been obtained under the pressure of 350 or 500 MPa applied for 15 minutes, at 25 or 55°C [2, 3]. The pressure above 200 MPa significantly inhibits the souring activity of yoghurt bacteria. The application of pressures of 300-400 MPa significantly reduced the number of living cells of Lactobacillus delbrueckii ssp. bulgaricus, whereas the number of Streptococcus thermophilus was only slightly lowered [1]. Pressurization of yoghurt in the pressure range of 400-600 MPa has a negative effect on its consistency; increased whey leak was observed. The objective of the study was to determine the impact of the addition of stabilizing agents used in the industrial production of yoghurts on the quality of pressurized yoghurt. Experiments were additionally aimed at determining whether the pressurization process allows decreasing the dose of stabilizing agent added to yoghurt. The most advantageous of the examined stabilizers was selected for the further part of the research. Yoghurts produced from milk with the addition of the experimentally determined amount of a stabilizer, one from ten, were subjected to the pressure of 400-600 MPa, and afterwards they were stored for four weeks at 4 and 20°C.

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2. Research materials and methods
The research material was yoghurt obtained from commercial yoghurt inoculum: YC-X16 (Chr. Hansen). Yoghurt was prepared with the addition of the following stabilizers, in the following amounts: YO 752 1.2%, YO 747 1.0%, YO749 0.2% (Danisco Biolacta sp. z o. o., Poland); IMG Super 1.8%, MPG Super 1.7%, KBM 100-Super 1.45%, MGP-2 1.80% (Superior, Poland); JMB1 1%, Novation Starch 1.5%. (Dairy Science Institute, Poland).

2.1 Pressurization of yoghurt with the addition of selected stabilizers
Yoghurt (a 100-ml portion) with the addition of selected stabilizers was pressurized in hydraulic pressure generator UNIPRESSEQUIPMENT (High Pressure Research Centre Polish Academy of Science, Warsaw) at 550 MPa/15 min/18°C; the temperature of pressurization was constant. Active acidity (pH) was determined and an organoleptic assessment was performed before the pressurization and directly after the treatment, as well as after four weeks of storage at 4°C. Additionally, the viscosity of yoghurts was tested before and directly after the pressurization, by means of rotating rheometer Rheostress 1 (Haake Gm BH), with the use of Rheo Win Pro Job Manager Archema 2000 software, Edition Version 2.67. The consistency factor was determined, as well as flow behaviour index. Based on the results obtained, YO 752 stabilizer was selected for the subsequent part of the research.

2.2. Pressurization of yoghurt produced from milk with the addition of YO 752 stabilizer.
Yoghurt (a 100-ml portion) prepared from milk with the addition of 0.6% YO 752 was pressurized in the pressure range of 400-600 MPa/15 min. every 50 MPa/18°C; the temperature of pressurization was constant. The following values were determined in yoghurt before pressurization, directly after the treatment and after each one of the four weeks of storage at 4 and 20°C: the number of bacteria, using a colony-count method Lactobacillus delbrueckii ssp. bulgaricus in MRS broth (Merck) and Streptococcus thermophilus in M17 broth (Merck); active acidity (pH) and titratable acidity (°SH); antibacterial properties, using a modified well-diffusion method, the size of the inhibition growth zones of eleven test strains was measured (mm); an organoleptic assessment was performed, according to the Polish Standard PN- A – 86086:2002.

3. Results and discussion
3.1. The effect of pressurization on the quality of yoghurt with the addition of selected stabilizers.
The experiment involved selected stabilizers used in the production of yoghurt in Poland. It is important in yoghurt that the stabilizer should prevent whey syneresis. Unpressurized yoghurt with the addition of stabilizers had a homogenous, firm curd, stringy consistency, white colour and smooth cross section of “porcelain sheen”. Differences were found in pressure-treated yoghurts and they mostly concerned the appearance of curd and the consistency of the product. A slight whey separation was observed with a grainy consistency, and in some cases, a slightly ragged curd. In yoghurts with the addition of YO 752, IMG-Super and MGP-2 Super stabilizers, the curd after the pressurization was homogenous, with slight whey separation, and its taste was refreshing. These yoghurts preserved their organoleptic qualities during the four week storage at 4°C.

As the storage period continued, the deterioration of product quality was observed in the case of some yoghurts. In yoghurt with the addition of YO 747 IMB stabilizer and Novation Starch, after four weeks of storage, a slight wheying off was found. Similar changes were also observed in pressurized yoghurts with the addition of IMB 1 and YO 747.

While assessing taste and smell, it has been established that most unfavourable qualities during four weeks of storage were found for yoghurt with the addition of YO 752 stabilizer.

The acidity of yoghurts with the addition of the analysed stabilizers before pressurization ranged from pH 4.30 – in the case of yoghurt with the addition of YO 749 stabilizer – to pH 4.47 in case of the addition of Novation Starch. After four weeks of storage at 4°C, there was also a slight increase in acidity observed, both in unpressurized yoghurts and in those subjected to the pressure treatment.
The consistency factor and flow behaviour index are the characteristics of the rheological properties of yoghurt. Higher values of consistency factor results in higher viscosity and firmer consistency of yoghurt. The lowest value of the coefficient $k=0.29$ was demonstrated by pressurized yoghurt, without the addition of stabilizer, which indicates a low capacity to water binding. Therefore, the highest degree of wheying off was established in this yoghurt. The value of flow behaviour index $n$ for all yoghurts was within the range of 0.55-0.92. In all yoghurts after pressurization, there was a decline observed in coefficient $K$ and flow behaviour index $n$, which corresponded with the results of the organoleptic assessment, based on which it was found that yoghurts after pressurization were characterized by higher whey syneresis.

3.2. The effect of pressurization on yoghurt produced from milk with the addition of YO 752 stabilizer.

3.2.1. Viability of yoghurt bacteria.
In unpressurized yoghurt, directly after production, the presence of $2.57 \times 10^9$ cfu/g *Streptococcus thermophilus* and $1.90 \times 10^6$ cfu/g *Lactobacillus delbrueckii* ssp. *bulgaricus* was established. During four weeks of storing unpressurized yoghurt at 4°C, there was a reduction observed in the number of *Lactobacillus delbrueckii* ssp. *bulgaricus* by one order of magnitude after the first week and of *Streptococcus thermophilus*. While storing yoghurts at 20°C, there was a change observed in the number of *Streptococcus thermophilus* by two orders of magnitude, and the number of *Lactobacillus delbrueckii* ssp. *bulgaricus* was reduced by three orders of magnitude to $8.00 \times 10^3$ cfu/g. After pressurization and during the storage of yoghurts pressurized in the pressure range of 400-600 MPa/15 min., no presence of *Lactobacillus delbrueckii* ssp. *Bulgaricus* was found. The pressure of 400 MPa had no significant effect on the change in the number of *Streptococcus thermophilus*. There were no significant changes established as regards changes in the number of these bacteria in the first week of storage at 4°C. In the subsequent week of storage, there was a decrease observed in their number by about two orders of magnitude. In the third and fourth week of storing yoghurts pressurized at 400-500 MPa at 4°C, no presence of *Streptococcus thermophilus* were found, except from the yoghurt that was subjected to the pressure of 500 MPa. At 20°C, the presence of streptococci was observed only in the first week of storage. The pressure of 550 and 600 MPa resulted in a decrease in the number of yoghurt streptococci by one order of magnitude. While storing at 20°C, yoghurts subjected to the pressure of 550 and 600 MPa, the number of streptococci was reduced to the level below $10^3$ cfu/g within a week. During the subsequent storage, no presence of *Streptococcus thermophilus* was established in the analysed yoghurt. The established high sensitivity of *Lactobacillus delbrueckii* ssp. *bulgaricus* to pressures over 400 MPa corresponds with the results of research conducted by de Ancos et al. [1]. It was found that the addition of stabilizer, as opposed to fruit additives [4], does not have a protective effect on the bacilli of *Lactobacillus delbrueckii* ssp. *bulgaricus* during the pressurization and the storage. The higher the storage temperature, the more intense was the reduction of bacteria. Similarly, Canganella et al. [5] observed a significant drop in the number of *Streptococcus thermophilus* after 25 days of storage at 12°C, both in natural yoghurt and yoghurt with the addition of fruit.

3.2.2. The effect of pressurization and storage conditions on the acidity of yoghurt.
The acidity of yoghurt directly after preparation was 4.40 pH and 44°SH, respectively. As the result of pressurization, depending on the operating pressure, there was a drop observed in acidity by 0.01-0.06 pH. Changes in yoghurt acidity could be caused by the effect of high pressure on the structure of albumen and mineral components of milk. [6]. The acidity of unpressurized yoghurt during the four-week storage at 4°C gradually increased from 4.40 to 4.21 pH and from 44°SH to 50°SH, and at 20°C – to 3.91 pH and 58.5°SH. However, in pressurized yoghurts, during storage at 4°C and 20°C, the pH value was maintained at a similar level, with a slight growing tendency.

3.2.3. The effect of pressurization and storage conditions on the antibacterial activity
Milk fermentation bacteria synthesize many substances of antibacterial properties. This bacillus is also capable of producing large amounts of \( \text{H}_2\text{O}_2 \), to which bacteria of the *Clostridium* species are particularly sensitive. *Streptococcus thermophilus* demonstrates wide spectrum of activity by producing bacteriocins of antibacterial properties in relation to Gram-positive and Gram negative bacteria [7]. Unpressurized yoghurt demonstrated the best antibacterial properties, both directly after production and during the storage. As regards 11 test strains, unpressurized yoghurt revealed the best antibacterial proprieties in relation to four strains. In the yoghurt stored at 20°C, unlike yoghurt stored at 4°C, a stronger antibacterial activity was observed in relation to all test strains. Under high pressure, a weakening of the antibacterial activity in pressurized yoghurt was observed. Based on the results obtained, it can be stated that the amount of pressure applied influences the antibacterial properties of yoghurt. The amount of pressure was inversely proportional to antibacterial properties demonstrated by yoghurt directly after pressurization and during the storage, which is also confirmed by [8].

### 3.2.4. The effect of pressurization and storage conditions on the organoleptic qualities of yoghurt.

Yoghurts subjected to the pressure of 400 and 450 MPa were characterized by white colour, homogenous, firm curd with slight whey separation. The consistency of yoghurts was thick and stringy. While stored at 4°C, yoghurt was characterized by a typical aldehyde taste, was slightly sour, without any strange aftertaste, and at 20°C no clear yoghurt taste could be sensed, but it was slightly sour with noticeable sweetish aftertaste. Yoghurt was subjected to pressures of 500, 550 and 600 MPa/15 min. At both storage temperatures, it preserved white colour and homogenous curd with light leak of whey. At 4°C, yoghurts characterized by homogenous consistence, slight yoghurt taste, were sweetish and slightly sour. Yoghurts stored at 20°C were characterized by runny, but homogenous consistency. However, no typical yoghurt taste could be sensed. The addition of a stabilizer had a favourable effect on the improvement of the consistancy of the examined yoghurts, by significantly reducing syneresis. The most favourable organoleptic qualities were found in yoghurt subjected to the pressures of 400 and 450 MPa.

### 4. Conclusions

*Streptococcus thermophilus* present in the composition of yoghurt inocula are characterized by greater sensibility to high pressures, as compared with the bacillus of *Lactobacillus delbrueckii ssp. bulgaricus*. The amount of pressure applied has an effect on the viability of yoghurt bacteria. The addition of a stabilizer has a favourable effect on the consistency of pressurized yoghurt by reducing syneresis. The stabilizer applied does not have a preserving effect on the viability of yoghurt bacteria during pressurization. High pressure has a negative effect on antibacterial properties of yoghurt. Pressurization impedes the process of yoghurt souring during storage. The addition of stabilizer helps to obtain yoghurts of good organoleptic characteristics and significantly extended durability. Improvement of antibacterial properties of yoghurt after pressurization is feasible upon the addition of the probiotic bacteria *Lb. acidophilus* and *Bifidobacterium sp.*

### 5. References

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