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EFFECT OF HIGH PRESSURE ON MICROFLORA AND SENSORY CHARACTERISTICS OF YOGHURT

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Key words: high pressure, yoghurt, preservation

The effects of the high pressure treatment (400–600 MPa) on microflora and sensorial characteristics of yoghurts was investigated. The experiment indicated that the pressures employed had no significant effect on the acidity of yoghurts. Changes in microbial survivability were related to the applied pressure and to the acidity of pressurized yoghurts. It was confirmed that *Lactobacillus delbrueckii* ssp. *bulgaricus* was more sensitive to pressurisation than *Streptococcus thermophilus*. Yoghurt processed at a pressure of 550 MPa maintained its beneficial sensorial characteristics longer than the non-pressurized yoghurt, during storage for 4-weeks at refrigerating (4°C) and room (20°C) temperatures. The pressure prevented the post-acidification in the yoghurts examined. The number of bacteria in the pressurized yoghurt stored at 4°C maintained under the therapeutic minimum level of 10^6 cfu/mL. The addition of fruit preparation beneficially affected the consistency of the pressure-treated yoghurt. The survivability of bacteria was higher about one order of magnitude in the pressurized fruit yoghurt than in the plain yoghurt.

INTRODUCTION

The beneficial effect of yoghurts on human health and long life span has been known for hundreds of years. Nevertheless the industrial production of yoghurt has been increasing since the 1960s.

Yoghurt is a fermented beverage made from milk using the co-operation of two homofermentative bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. These microorganisms ferment milk sugar – lactose – into lactic acid and small amounts of by-products. The by-products contribute to the natural appearance of yoghurt, its fresh and acidulated taste and its nutritional, prophylactic and therapeutic properties [Tamine & Deth, 1980; De Brabandere & De Baerdemaeker, 1990].

All the microorganisms added to milk with starter cultures, to fulfil their specific function, should be present in fermented beverages in the required number of living and active cells [Bulletin FIL/IDF 1983]. The minimum content of bacteria is different in particular countries, as it is established by legislation. In order to produce therapeutic benefits, the recommended minimum level for bacteria in yoghurt accounts for 10^6–10^8 of viable cells/mL. The presence of viable lactic acid bacteria in high concentrations in yoghurt has been correlated with many benefits for consumers, but it has also led to the appearance of post-acidification in the product, particularly with deficiencies in the cold chain distribution. This problem causes an excessive decrease in pH and modification of the viable lactic microflora in the product [Birollo *et al.*, 2000].

Thermal processing employed after fermentation in order to preserve yoghurt, markedly reduces microflora and thus the nutritive value of the product. That is why among a number of novel methods for food preservation and processing, the non-thermal devices are the most appropriate. This technology involves the application of pressure ranges of 100–1000 MPa at a room temperature [Cheftel, 1995]. The application of adequate pressure to food does not modify its sensory properties, and causes no changes in its colour or vitamin loss.

Research carried out to date have indicated that high pressures can be exploited for used food preservation, as they inactivate or change the metabolism of bacteria and affect their enzymes [Cheftel, 1995; Hayakawa *et al.* 1994].

In yoghurt technology, high pressure may be applied at the stage of curd formation, as well as a means of product preservation [Tanaka & Hatanaka, 1992; De Ancos *et al.,* 2000].

Several studies into the effects of pressures on the acid coagulation properties of milk have been reported so far. These fall into two categories: acid coagulation after pressurization, or acid coagulation under pressure [Johnston *et al.,* 1992, 1993, 1995; Ferragut *et al.* 2000; Needs *et al.,* 2000]. In Japan, high pressure preservation of yoghurt on an industrial scale has been successfully applied for several years [Suzuki, 2002].

Earlier studies have shown that the effect of the high pressure on yoghurt microflora varies considerably; strains of *Lactobacillus delbrueckii* ssp. *bulgaricus*, are more sensitive to processing than strains of *Streptococcus thermophilus* [Reps *et al.,* 2001].

The objective of this study was to determine the effect of high pressure treatment on yoghurt quality, with regard to its sensory characteristics and the survivability of microflora.

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MATERIALS AND METHODS

The yoghurt production process. Yoghurt was manufactured with the use of the commercial mixed culture of selected strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*, obtained from Rhodia Food Biolacta in Olsztyn. The mixed culture was used as an indirect inoculation. To this end, 1000 mL of heat-treated milk (15 min/90°C) was inoculated and incubated at 42°C till curd formation and then was kept for two hours at this temperature. Yoghurt was produced with milk – 2.0% v/v fat content, with the addition of skimmed powdered milk.

Pressurization. The yoghurt was poured into sterile Teflon vials and subjected to pressure treatment for 15 min at a pressure range of 400–600 MPa. The pressurization was conducted at an ambient temperature in a hydraulic pressure generator produced by Unipress Equipment (Warsaw). The compression and decompression times were fixed at 2 and 0.5 min, respectively.

Experimental procedures. The experiment was divided into three parts. (1) In the first part of the experiment, the influence of yoghurt acidity on the effect of pressurization was determined. To this end, yoghurt was incubated successively until its acidity reached 4.2, 4.4 and 4.6 pH, and then subjected to pressure treatment in a pressure of 400–600 MPa/15 min at 50 MPa/15 min intervals. The analyses were conducted before and immediately after pressurization. (2) To determine the possibility of applying pressurization for yoghurt preservation, in subsequent parts of the experiment, yoghurt pressurized at 550 MPa/15 min was stored for 4 weeks at temperatures of 4 and 20°C. The analyses were conducted in pressurized and non-pressurized (control) samples of yoghurt at the beginning of the experiment and every seven days of storage. (3) The influence of the addition of fruit preparation on the effect of yoghurt pressurization was examined in the third part of the experiment. Strawberry jam, in the amount of 15%, was mixed with plain yoghurt, manufactured beforehand, and pressurized at 550 MPa/15 min. The analyses were conducted for both pressurized and non-pressurized (control) yoghurt, with and without the addition of fruit preparation.

Microbiological analyses. One mL of a yoghurt sample was diluted with 9 mL of 0.9% physiological saline and mixed uniformly with a vortex mixer. Subsequent serial dilutions were prepared and viable numbers enumerated using the pour plate technique.

The counts of *Streptococcus thermophilus* were enumerated on M 17 agar by incubating the plates aerobically at 30°C for 72 h. To enumerate of *Lactobacillus delbrueckii* ssp. *bulgaricus*, MRS agar and anaerobic incubation at 37°C for 72 h were used.

Acidity measurement. Acidity was expressed in both pH value and in °SH titratable acidity of yoghurt.

The pH values of the yoghurt samples were measured at 17–20°C using an HI932T Hanna Instruments pH meter.

The titratable acidity was determined after mixing a one-gram yoghurt sample with 9 mL of distilled water and titration with N/4 NaOH using 0.5% a phenolphthalein indicator [Bylund, 1995].

Sensory analyses. The sensory analysis (appearance, colour, flavour, aroma, consistency) of the yoghurt samples was conducted by 8 trained panellists according to Polish Norm-A-860434-15:1998.

RESULTS AND DISCUSSION

The first stage of the experiment showed that the rise in yoghurt acidity was accompanied by an increase in the number of lactic bacteria.

A pressure of 400 MPa/15 min reduced the number of bacteria by roughly 4–5 orders of magnitude from the initial number of $\sim 10^8$–10^9 cfu/mL. An increase in the pressure to 500 MPa/15 min did not affect markedly the degree of bacteria inactivation, whereas higher pressures applied, up to 600 MPa/15 min, resulted in a significant rise in the degree of yoghurt microflora inactivation to $\sim 10^2$–10^3 cfu/mL (Figures 1a, b). *Str. thermophilus* was observed to be slightly more resistant to the pressure treatment than *Lb. bulgaricus*. It should be mentioned that lactobacilli were more sensitive to pressurization. Similar changes in the survival of lactic acid bacteria were observed during studies on the effect of high pressure on kefir microflora [Krzyżewska *et al.*, 2000].

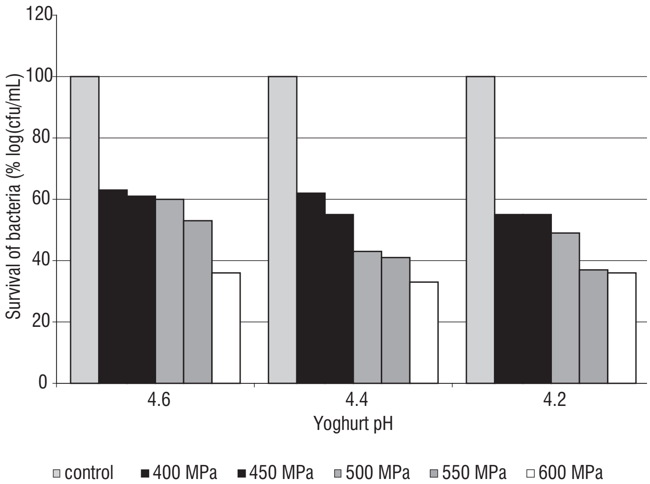

FIGURE 1a. Effect of the high pressure on the survival of *Streptococcus thermophilus.*

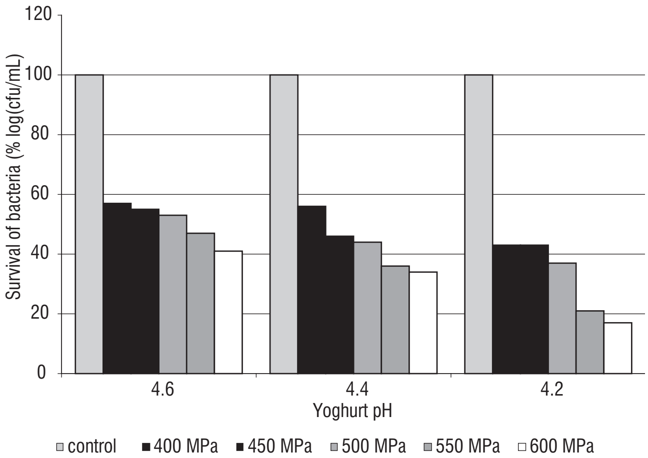

FIGURE 1b. Effect of the high pressure on the survival of *Lactobacillus delbrueckii ssp. bulgaricus.*

The effect of pressurization on milk mineral balance may alter its pH. Johnston *et al.* [1992] reported no significant effect of pressure up to 600 MPa on the pH of skim bovine milk, whereas other authors have observed increases in the pH of pressurized, UHT-treated bovine milk [Schrader & Buchheim, 1998].

In the current experiment, pressures employed were found to have no significant effect on the acidity of yoghurts. On the other hand, the acidity of the yoghurts affected the degree of lactic acid bacteria inactivation, particularly of lactobacilli.

The acidity of the medium enhanced increased the effect of pressurization [Oxen & Knorr 1993]. The highest degree of bacteria inactivation was found in yoghurt with acidity equal to pH 4.20. However, the initial number of bacteria was the highest in this yoghurt, compared to the two other yoghurts.

Changes in the consistency of pressurized yoghurts were also found. The flocculles in the curd and turbidity were observed after treatment pressures in the range of 400–500 MPa. These changes were noticeable to a lesser degree in yoghurts pressurized at 550 and 600 MPa and were connected with the structure of milk proteins after pressure treatment, which depended on the pressure applied.

While studying the effect of pressurization on milk appearance, Desbory-Banon *et al.* [1994] found that pressure of 200 MPa/5 min caused an increase in transparency and the viscosity of milk and decreased the possibility of light dispersion, which was higher after treatment at 400–500 MPa [Schrader & Buchheim, 1998; Needs *et al.*, 2000].

These changes were effected by disintegration of casein micelles [Schmit & Buchheim, 1970]. A slight increase (~9%) in the size of casein micelles in raw skim milk was found after treatment at 200 MPa. The treatment at 250–600 MPa reduced micelle size by 40-50% in raw [Needs *et al.*, 2000] or reconstituted skim milk [Desbory-Banon *et al.*, 1994; Gaucheron *et al.*, 1997].

As a result of pressurization, disadvantageous changes in yoghurt taste and aroma occurred. The most favourable sensory traits were found after their treatment at a pressure in the range of 500–600 MPa/15 min. The taste and aroma were typical of plain yoghurt. The white colour of yoghurts remained unchanged after pressurization.

Taking into consideration the results of the sensory characteristics of pressurized yoghurts, a pressure of 550 MPa/15 min was used for yoghurt preservation in the next stage of the experiment. The number of *Str. thermophilus* was reduced by 3.44 orders of magnitude, *i.e.* from 2.19x10^9 cfu/mL to 7.96x10^5 cfu/mL, and the number of *Lb. bulgaricus* was reduced by 5.02 orders of magnitude, *i.e.* from 7.70x10^8 cfu/mL to 7.10x10^3 cfu/mL, as a result of the pressurization.

Several factors, such as the temperature of storage, oxygen content, concentrations of lactic and acetic acids, *etc*. have been presumed to affect the viability of bacteria in yoghurt [Dave & Shah, 1995].

During storage, there were observed changes in the survivability of microflora in the yoghurts studied.

In yoghurt not subjected to high pressure treatment and stored at 4°C for 4 weeks, the number of *Str. thermophilus* decreased by 0.51 orders of magnitude, *i.e.* from 2.19x10^9 cfu/mL to 6.73x10^8 cfu/mL, and that of *Lb. delbrueckii* also decreased by 1.76 orders of magnitude, *i.e.* from 7.70x10^8 cfu/mL to 1.30x10^7 cfu/mL (Figure 2a).

In pressurized yoghurt stored at a refrigerating temperature, a slight reduction occurred in the number of *Str. thermophilus*, by 1.38 orders of magnitude, *i.e.* from 7.96x10^5 cfu/mL to 3.30x10^4 cfu/mL, and that of *Lb. delbrueckii* by 0.81 orders of magnitude, *i.e.* from 7.10x10^3 cfu/mL to 1.10x10^3 cfu/mL (Figure 2b).

However, in non-pressurized yoghurt stored at 20°C, a decrease in the survivability of *Str. thermophilus* by 0.50, *i.e.* from 2.19x10^9 cfu/mL to 6.90x10^8 cfu/mL, and in the number of *Lb. bulgaricus* by 0.79 orders of magnitude was observed, *i.e.* from 7.70x10^8 to 1.20x10^8 cfu/mL (Figure 3a).

In the case of pressurized yoghurt stored at 20°C, the number of *Str. thermophilus* increased by 1.02 orders of magnitude, *i.e.* from 7.96x10^5 cfu/mL to 8.33x10^6 cfu/mL, and that of *Lb. bulgaricus* by 3.15 orders of magnitude, *i.e.* from 7.10x103 cfu/mL to 9.95x10^6 cfu/mL (Figure 3b).

The observed changes in the number of yoghurt microflora were reflected in variations of pH value as well as titratable acidity (Figure 4).

During 4 weeks of storage at a temperature of 4°C, the pH of the non-pressurized yoghurt decreased from 4.37 to 4.04 whereas its titratable acidity increased from 40 to 48°SH.

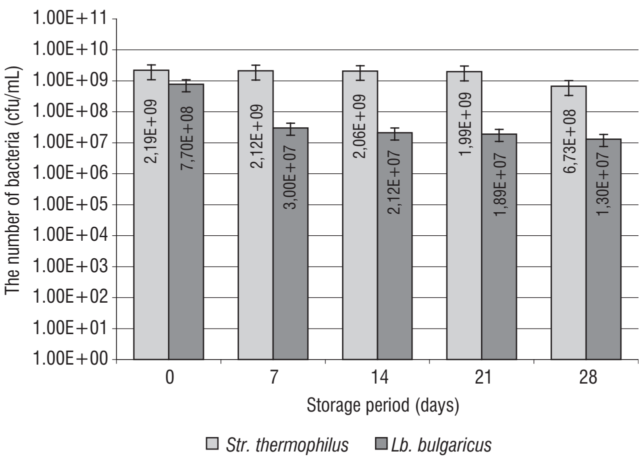

FIGURE 2a. The survival of bacteria in non-pressurized yoghurt stored at 4°C.

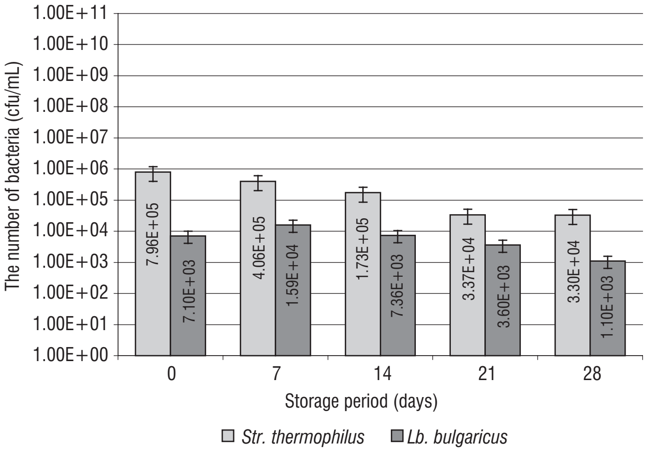

FIGURE 2b. The survival of bacteria in yoghurt pressurized at 550 MPa and stored at 4°C.

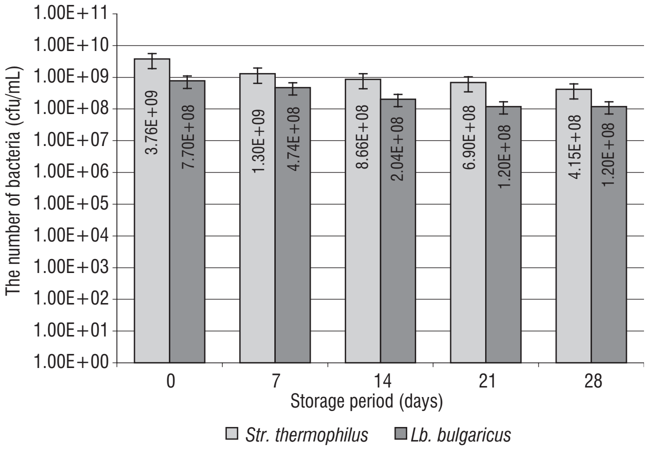

FIGURE 3a. The survival of bacteria in non-pressurized yoghurt stored at 20°C.

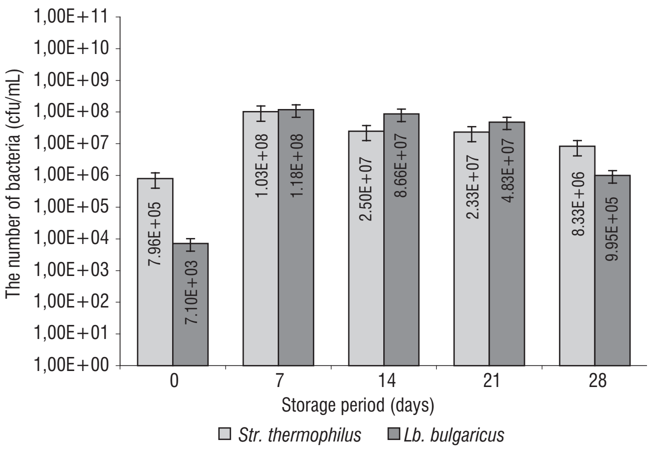

FIGURE 3b. The survival of bacteria in yoghurt pressurized at 550 MPa and stored at 20°C.

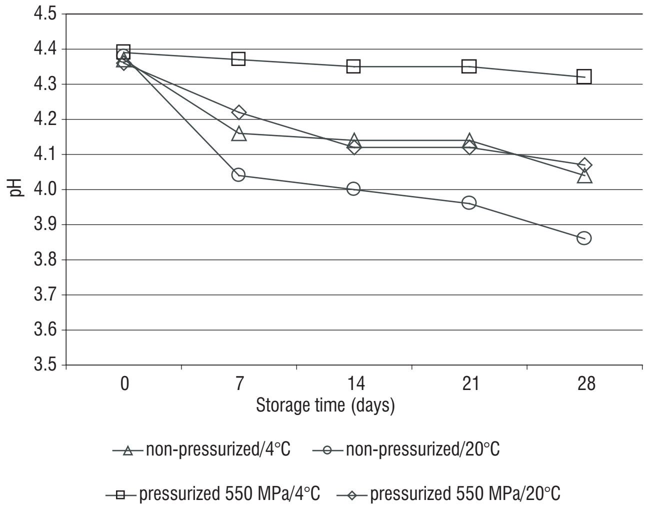

FIGURE 4. The acidity of non-pressurized and pressurized yogurts stored for 4 weeks at 4 and 20°C.

In yoghurt stored at 20°C, pH values varied from 4.37 to 3.86 and titratable acidity changed in the range of 40–53°SH.

Alternations of acidity of the pressurized yoghurts were more stable. For yoghurt stored at 4°C, pH was equal to about 4.38–4.30 pH, and titratable acidity varied from 40–42°SH. In the case of yoghurt stored at 20°C, pH lowered from 4.38 to 4.07, but the value of titratable acidity ranged from 40–47°SH.

The sensory evaluation of both pressurized and non-pressurized yoghurts conducted in the succeeding weeks of storage at a temperature of 4°C, showed minor changes in the consistency and appearance of the samples. However, in non-pressurized yoghurt, unfavourable changes of flavour and aroma occurred. After one week of storage, it had a slightly tart and mealy after-taste.

Detrimental changes in the sensory quality of yoghurt stored at 20°C could be detected even earlier. Unacceptable changes in flavour and aroma were noted in the non-pressurized and pressurized yoghurt after one week and three weeks of storage, respectively.

Finally, plain and fruit yoghurts were subjected to the pressure treatment in the third part of the study.

Pressurization of yoghurt with fruit preparation confirmed results of many reports that among several parameters determining the extent of microbial inactivation, composition and water activity in the dispersion medium play a key role [Cheftel, 1992; Knorr, 1994; Patterson *et al.*, 1995].

In fruit yoghurt processed at 550 MPa/15 min, the number of *Str. thermophilus* decreased by *ca.* 4.17 orders of magnitude, *i.e.* from 8.30×10^8 cfu/mL to 5.60×10^4 cfu/mL, and that of *Lb. bulgaricus* by 6.52 orders of magnitude, *i.e.* from 4.60×10^8 cfu mL^{-1} to 1.30×10^2 cfu mL^{-1}. In pressurized plain yoghurt, the numbers of *Str. thermophilus* and *Lb. bulgaricus* lowered by 5.13 orders of magnitude, *i.e.* from 2.19×10^9 cfu/mL to 1.64×10^4 cfu/mL, and by 7.05 orders of magnitude, *i.e.* from 1.13×10^9 cfu/mL to 1.00×10^2 cfu/mL, respectively.

The acidity of plain yoghurt was 4.16 pH and 47°SH and these parameters remained at the same level after pressurization. In fruit yoghurt, pH accounted for 4.16 and titratable acidity for 46°SH. A slight increase in pH (to 4.18) and a slight decrease in titratable acidity (to 45°SH) were determined after pressurization.

It should also be underlined that the consistency of the fruit yoghurt changed favourably after pressurization in

TABLE 1. Sensory evaluation of yoghurts processed at 550 MPa/15min with and without the addition of fruit preparation.

Sample	Appearance	Colour	Taste	Aroma
		Plain yoghurt		
Control	smooth curd, no whey syneresis	white	smooth, with porcelain shine	pure, sour, pleasant, refreshing, slightly aldehyde-like
550	homogenous curd, no whey syneresis	white	smooth, with porcelain shine	pure, sour, pleasant, refreshing
		Yoghurt with fruit preparation		
Control	homogenous curd, no whey syneresis, fruit pieces visible	pink	smooth, pouring	refreshing, strawberry, sour, slightly sweet
550	smooth curd, no whey syneresis, fruit pieces visible	pink	thick, cream-like, stringy, smooth	refreshing, strawberry, sour, sweet

comparison with that of the non-pressurized sample which became thicker, cream-like and stringy (Table 1).

CONCLUSIONS

The sensory traits of yoghurts depended on the applied pressure and not on their varying acidity (pH – 4.2, 4.4, 4.6). The changes in microbial survivability were related to initial number of bacteria and depended on the acidity of the pressurized samples.

During storage at 4°C, yoghurt processed at 550 MPa/15 min kept its favourable sensory properties longer in comparison to the non-pressurized one. Pressure treatment sufficiently reduced the acidifying activity of yoghurt bacteria: the pressurized yoghurt maintained the initial pH after chilled storage at 4°C for four weeks. However, pressures over 400 MPa reduced the number of viable *Lb. bulgaricus* to levels below the legal minimum in many countries.

The addition of fruit preparation had a beneficial effect on the consistency of yoghurt pressurized at 550 MPa/15 min. The results of this experiment indicate that the pressure treatment of yoghurts could be an alternative to the use of additives in yoghurt processing to prolong its shelf life, with the added advantages of improved flavour and texture of products. Further research is needed, however to determine which pressurization conditions would be effective in maintaining the numbers of *Lb. bulgaricus* while improving the consistency of yoghurt.

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WPŁYW WYSOKIEGO CIŚNIENIA NA MIKROFLORĘ I CECHY ORGANOLEPTYCZNE JOGURTU

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Badano wpływ wysokiego ciśnienia (400–600 MPa) na mikroflorę oraz cechy organoleptyczne jogurtu. Nie stwierdzono znaczącego wpływu zastosowanego ciśnienia na kwasowość presuryzowanych jogurtów. Różnice w przeżywalności mikroflory zależały od wysokości zastosowanego ciśnienia i od początkowej ilości bakterii w jogurtach. Potwierdzono wyższą ciśnienioodporność szczepu *Streptococcus thermophilus* w porównaniu do szczepu *Lactobacillus delbrueckii* ssp. *bulgaricus*. Stwierdzono, że jogurt poddany działaniu ciśnienia 550 MPa dłużej zachowywał korzystne cechy organoleptyczne w porównaniu z jogurtem niepresuryzowanym w czasie 4 tygodni przechowywania w temperaturze chłodniczej (4°C) oraz w temperaturze pokojowej (20°C) (tab. 1). Zastosowane ciśnienie zahamowało proces pofermentacyjnego ukwaszania w badanych jogurtach. Liczba bakterii w jogurcie presuryzowanym, przechowywanym w temperaturze 4°C utrzymywała się poniżej zalecanego minimum terapeutycznego tj. 10^6 jtk/mL (rys. 2a i rys 2b). Stwierdzono, również, korzystny wpływ dodatku wsadu owocowego na konsystencję presuryzowanego jogurtu. Przeżywalność bakterii w presuryzowanym jogurcie z dodatkiem wsadu owocowego była o jeden rząd wielkości wyższa w porównaniu z presuryzowanym jogurtem naturalnym.