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Impact of pasteurisation and type of packaging on probiotic bacteria in bio-yoghurts of goats' milk during storage

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Summary

The objective of the research was to determine the impact of the pasteurization process of raw material and of the type of packaging on the survival rate of probiotic bacteria in bio-yoghurts from goats' milk during storage. The bio-yoghurts investigated were manufactured from goats' milk using a container method. The milk under processing was centrifuged and normalized to a fat level of 2%. Next, it was pasteurised at a temperature of 95 °C during 5 min and at 90 °C during 10 min, after which it was cooled to 40 °C. The cooled milk was inoculated with DVS ABT1 inoculants added. The bioyoghurts were thermostated (controlled) in containers at a temperature of 40 °C (+/– 1 °C) until they reached a pH level of 4.7. Then, they were cooled again to a temperature below 20 °C and poured into 4 different packagings made of polypropylene (PP), polystyrene (PS), polyethylene (PEHD), and glass (G), each of a capacity of 200 cm^3. The bio-yoghurts were subsequently stored in the dark for 21 days at temperatures ranging from 2 to 5 °C. The presence of probiotic bacteria in bio-yoghurt was determined 12 h after the bio-yoghurts were manufactured, and on the 7^{th}, 14^{th}, and 21^{st} day of storage. In total 240 samples were analysed. The applied packagings did not statistically significantly differentiated the count of bacteria in bio-yoghurts manufactured from goats' milk, except for the Str. thermophilus bacteria count that changed only on the 14^{th} day after manufacturing. The statistically significant impact of the pasteurisation temperature of raw material was found in the case of the L. acidophilus count immediately upon manufacturing. A lower pasteurisation temperature of goats' milk had a favourable effect on the growth of bacteria, and in such bio-yoghurts the population of all the bacteria was higher. It was found that irrespective of the applied pasteurization temperature of raw material and of the type of packagings, the count of individual bacterial genera was at a level significantly higher than 10^6 cfu/ml, which ensures that the milk drinks studied achieve the therapeutic minimum. Thus, expanding the production of goat milk bio-drinks can enhance the assortment of healthful diary products available in the market.

Keywords: goats' bioyoghurt, pasteurisation, packaging type, probiotic bacteria, viability

Fermented milk drinks can be manufactured from both cow and goat milk. Goat milk is often used interchangeably with cow milk owing to its therapeutic, nutritious, and anti-allergenic properties. Products manufactured from goat milk, in particular fermented milk drinks, possess similar properties (4, 10, 11, 14, 15, 23). Goat milk is naturally predisposed for preparing a yoghurt drink that is light and easily digestible. The curd is soft and may be easily homogenized by being ordinarily mixed (3, 19, 26). However, the research results of many authors (1, 6, 7, 9, 20) prove that goat milk is characterized by high variations in its composition during the period of lactation. Thus, it can be difficult to finally produce goat milk products of similar sensory features. This claim specifically refers to rheological properties which decide, to a large degree, the product texture, and, consequently, also whether or not the prospective consumers appreciate and like such products.

Fermented drinks made of goat milk differ from the drinks made of cow milk, among other things, by a lower content of volatile aroma compounds (and, especially, of acetaldehyde and carbon dioxide (2, 16).

The good quality of all bio-products depends, first of all, on whether or not the selected bacteria strains were appropriate, to what extent their properties are

known, and whether or not the progress of technological processes is correct. Such aspects as: antagonism of bacterial cultures, technological errors, improper packagings, and incorrect storage conditions can cause the count of probiotic bacteria in the product purchased by a consumer to be too low. Bifidobacteria are particularly sensitive to unfavourable environmental conditions, and their count in a product can rapidly drop during storage (17, 22).

According to the standards of IDF/FIL and FAO/WHO, fermented milk products must contain living bacteria showing documented probiotic features, and their count level must not be lower than 10^6 cfu/ml during the entire period of the product's expiry date. On the other hand, in order to achieve a probiotic effect, it is indispensable to consume 10^6 to 10^9 living bacterial cells per day (8, 12, 18, 24).

The objective of the research was to determine the impact of the pasteurization process of raw material and of the type of packaging on the survival rate of probiotic bacteria in bio-yoghurts from goat milk during storage.

Material and methods

Manufacturing the yoghurt. The bio-yoghurts investigated were manufactured from goat milk using a container method. The milk under processing was centrifuged and normalized to a fat level of 2%. Next, it was pasteurised at a temperature of 95°C for a duration of 5 min and at 90°C for a duration of 10 min, after which it was cooled to 40°C. The cooled milk was inoculated with DVS ABT1 inoculants added (0.2% addition), a product of the Chr. Hansen Company. The inoculants applied contained the following 3 bacterial strains showing documented probiotic features: *Lactobacillus acidophilus*, *Streptococcus thermophilus*, and *Bifidobacterium bifidum*. The bioyoghurts were thermostated (controlled) in containers at a temperature of 40°C (+/– 1°C) until they reached a pH level of 4.7. Subsequently, they were cooled again to a temperature below 20°C, and poured in 4 different packagings made of polypropylene (PP), polystyrene (PS), polyethylene (PEHD), and glass (G), each of a capacity 200 cm^3. The bio-yoghurts were then stored for 21 days at temperatures ranging from 2°C to 5°C and without access to light. The presence of probiotic bacteria in bio-yoghurt was determined 12 h (T_0) after the bio-yoghurts were manufactured, and on the 7^{th} (T_1), 14^{th} (T_2), and 21^{st} (T_3) days of storage.

The investigation cycle was repeated six times to ensure that the results obtained were reliable and repeatable. In total 240 samples were analysed.

Microbiological analysis. The quantitative determination of probiotic bacteria was carried out as indicated in the relevant instructions. An average laboratory sample was prepared from one batch.

In order to achieve a homogenous consistency of the material analysed, each of the samples examined was thoroughly shaken prior to its opening. From a properly shaken sample, 1 ml was taken and placed in a test-tube, which was centrifuged for 1 minute in a „Wortex" centrifuge to obtain a homogenous suspension. Next, suitable selective culturing substrates (for *Lactobacillus acidophilus* – an MRS substrate [Oxoid Co.]; for *Streptococcus thermophilus* – Mueller-Hinton substrate; and for *Bifidobacterium* – Agar BL) were inoculated with 1 ml of each individual dilution (from dilutions ranging from 10^{-1} to 10^{-10}). Next, the laboratory tiles were incubated at a temperature of 37°C, under anaerobic conditions, for 72 hours. After incubation, the initial densities of bacteria from *Lactobacillus*, *Streptococcus*, and *Bifidobacterium strains* were counted (5, 13, 21). Further, the bacteria grown on the culturing substrata were identified with regards to their genus; the identification of bacterial species was performed using the following tests: API 50 CHL test to identify *Lactobacillus acidophilus*; API 20A test to identify *Bifidobacterium.* In addition, a special test to detect a fructose-6--phosphate phosphoketolase (F6PPK) enzyme was applied, and the API STREP test to find *Streptococcus thermophilus*.

Statistical analysis. The computation was performed using procedures from a Statistica 7.0 PL computer software package. The hypotheses on the impact of pasteurisation temperature and type of packaging were verified using a two-factor analysis of variance. Null hypotheses on the non-occurrence of any significant impact of a given factor (pasteurisation temperature, type of packaging) and on the lack of significant interaction between factors were rejected in the case of the calculated value of F characteristic, since it was higher than the boundary value at a significance level of $\alpha = 0.05$. The levels of individual quality parameters of goat milk bio-yoghurts were determined in four points: T_0, T_1, T_2, and T_3, and, next, compared.

Results and discussion

The impact of packaging on the count of probiotic bacteria in the goat milk yoghurts at a time point assumed as the starting point (12 hours after manufacturing) was analysed. Based on the results of this analysis, it was proved that regardless of the applied temperature of raw material pasteurisation, the packaging had no statistically significant impact thereon (although differences were found in the counts of probiotic bacteria in individual packagings). The reason for this was that the fermented milk drinks were manufactured using a container method and simultaneously poured into all types of packagings analysed (thus, the time of the product contacting the packaging was too short). In contrast, a statistically significant impact of pasteurisation temperature was found on the count of *L. acidophilus* cells (tab. 1).

A higher count of those bacteria was found in drinks produced of goat milk pasteurised at a temperature of 90°C for 10 minutes (fig. 1).

According to Wiatr-Szczepanik and Libudzisz (25), the bacteria of milk fermentation are characterized by high nutritional requirements, among other things, they need amino acids and vitamins to be present. Amino acids and low-molecular peptides are the best source of nitrogen for milk bacteria. As soon as the easily

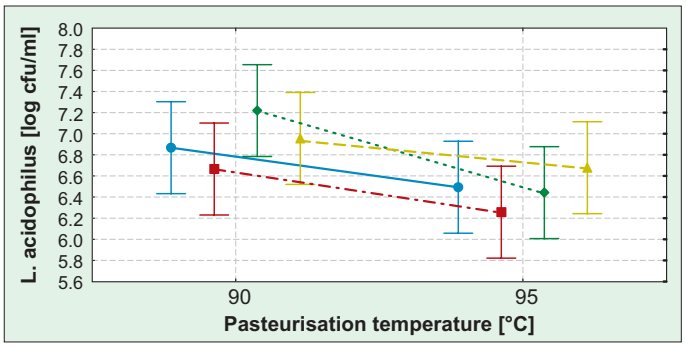

Fig. 1. Impact of the pasteurisation temperature on the count of *L. acidophilus* cells in bio-yoghurts of goats' milk in packagings of various types (● PP, ■ PS, ◆ PE, ▲ G), 12 hours after manufacturing

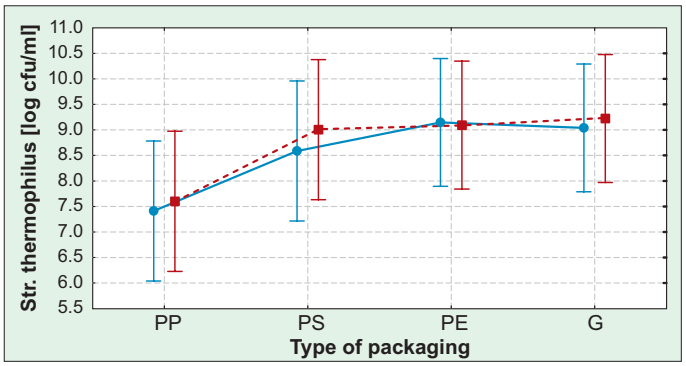

Fig. 2. Impact of packaging type on the *Str. thermophilus* cells in bio-yoghurts of goats' milk produced using different pasteurisation variants of raw material (● Pasteuris. 90°C/10 min, ■ Pasteuris. 95°C/5 min), after 14 days of storage

available nitrogen components contained in milk run out, the further growth of those bacteria depends on the utilization of milk proteins. The count of *L. acidophilus* in the analysed milk drinks, pasteurised at a lower temperature, was higher by ca. 1.2×10^7 cfu/ml, and this fact proves that irrespective of the lower pasteurisation temperature of goat milk, the longer heating time caused proteins to denature, and this produced a better substratum for rod-shaped acidophilic bacteria to develop.

After seven day storing (T_1), again, no statistically significant impact was found of the packaging and pasteurisation temperature on the probiotic bacteria count (tab. 2). In the ensuing second week of storage (T_2), no statistically significant impact of the packaging and temperature of pasteurising of raw material on the probiotic bacteria count was found (tab. 3). But after fourteen days (14) of storing, it was discovered that the packaging had a statistically significant impact on the survival rate of bacteria of *Str. thermophilus* genus (fig. 2). The lowest count of those bacteria was found in bio-drinks contained in PP packagings, whereas the population size thereof was similar in the packagings made of PS, PE, and G. In milk (and, subsequently, in the yoghurt produced from it), there is a limited amount of bacteria growth-enabling substances (25, 27). Therefore, the excessive growth of some bacteria restrains the growth of other bacteria present. In the bio-yoghurts packed in PP packagings at a pasteurisation temperature of 90°C/10 minutes, the decrease in the count of *Str. thermophilus* bacteria by ca. 1.24×10^9 cfu/ml was accompanied by the

Tab. 1. Impact of the pasteurisation temperature and type of packaging at a T_0 time (12 hours after manufacturing) on the bacteria count in bio-yoghurts of goats' milk

Bacteria	Impact of		
	pasteurisation temperature	type of packaging	[pasteur. temp.] × [pack.]
L. acidophilus	9.090*	1.261	0.516
B. bifidum	0.013	0.903	0.529
Str. thermophilus	0.051	1.801	0.085

Explanation: * – calculated value of F characteristic is higher than the boundary value ($\alpha = 0.05$)

Tab. 2. Iimpact of pasteurisation temperature and type of packaging at a T_1 time (7 days after manufacturing) on the survival rate of probiotic bacteria in bio-yoghurt of goats' milk

Bacteria	Impact of		
	pasteurisation temperature	type of packaging	[pasteur. temp.] × [pack.]
L. acidophilus	2.173	0.308	0.368
B. bifidum	0.154	0.187	0.006
Str. thermophilus	0.047	1.934	0.040

Explanation: as in tab. 1

Tab. 3. Impact of pasteurisation temperature and type of packaging at a T_2 time (14 days after manufacturing) on the survival rate of probiotic bacteria in bio-yoghurt of goats' milk

Bacteria	Impact of		
	pasteurisation temperature	type of packaging	[pasteur. temp.] × [pack.]
L. acidophilus	0.198	0.514	1.658
B. bifidum	0.844	0.646	0.227
Str. thermophilus	0.158	2.720*	0.044

Explanation: as in tab. 1

Tab. 4. Impact of pasteurisation temperature and type of packaging at a T_3 time (21 days after manufacturing) on the survival rate of probiotic bacteria in bio-yoghurt of goats' milk

Bacteria	Impact of		
	pasteurisation temperature	type of packaging	[pasteur. temp.] × [pack.]
L. acidophilus	0.040	0.779	0.860
B. bifidum	0.220	0.242	0.315
Str. thermophilus	0.002	1.735	0.080

Explanation: as in tab. 1

increase in the *B. bifidum* count by ca. 3.8×10^6 cfu/ml. Then again, in the drinks pasteurised at 95°C/5 minutes, the *Str. thermophilus* count in the drink packed in a PP packaging was the lowest compared to the drinks packed in all other packaging types. Moreover, it was determined that the decrease in the count of those bacteria by ca. 9.4×10^7 cfu/ml was accompanied by an increase in the *L. acidophilus* count by ca. 6.9×10^5 cfu/ml. The reason for this fact can consist in the fact that the polypropylene packagings, compared to other types of packagings, had a larger area of contact with air (gathered between the surface of the product and the packaging closure) in relation to the volume. This could also impact the survival rate of thermophilic streptococci. Żbikowski (27) has already observed that air present in the space above the product, in particular in plastic packagings, unfavourably impacts the quality of yoghurts.

Three weeks after storing bio-yoghurts of goat milk no statistically significant impact of pasteurisation temperature of raw material, nor of the type of packaging applied, was found on the quality indicators of the bio-yoghurt analysed (tab. 4).

Conclusion

The applied packagings made of: polypropylene (PP), polystyrene (PS), polyethylene (PEHD), and glass (G) did not differentiate the count of bacteria in bio-yoghurts manufactured from goat milk in a statistically significantly way except for the *Str. thermophilous* bacteria count that changed only on the 14th day after manufacturing. A statistically significant impact of pasteurisation temperature of the raw material was found in the case of the *L. acidophilus* count immediately upon manufacturing. A lower pasteurisation temperature of goat milk impacted the growth of bacteria more favourably, and in those bio-yoghurts the population of all the bacteria was higher.

It was found that irrespective of the applied pasteurization temperature of raw material and of the type of packagings, the count of individual bacterial genera was at a level significantly higher than 10^6 cfu/ml, and this ensures that the milk drinks studied may have a therapeutic minimum. Thus expanding the production of bio-drinks from goat milk can make the assortment of diary products with healthy values more attractive to the market. Using goat milk to produce bio-yoghurts may become an important trend of its utilization programme.

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