Effect of level of starter culture on viability of probiotic bacteria in yoghurts

Ljerka Gregurek

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Summary

The effect of level of inoculum on viability of probiotic bacteria (Lactobacillus acidophilus and Bifidobacterium spp.) during yoghurt manufacture and storage at 4 °C was investigated. Yoghurt production was performed using commercially available starter cultures.

The viability of L. acidophilus was maintained at the level of 10^6 cfu/g up to 20-25 days storage. The counts and the stabilities of all three bifdobacteria starter cultures used were higher compared with L. acidophilus.

Postacidification was slightly higher in yoghurts prepared with lower levels of inoculum. Associative yoghurt organisms and pH of yoghurt affected the viability of probiotic bacteria.

Key words: starter culture, viability, probiotic bacteria

Introduction

The nutritional benefits derived by consumption of fermented milks have been well documented (Nakazawa and Hosono 1992.; Robinson 1991.).

Fermented milks have been manufactured with mezophilic and thermophilic lactic bacteria. These bacteria do not survive in large numbers during passage through the digestive tract. As a result, intestinal species have been suggested to have probiotic effects related to the stabilisation of microbial ecology in the gut, reducing risk of colon cancer, increased imune response, reduction in concentration of cholesterol in blood plasma (Gilliland 1990.; Modler 1990.; Shah and Jelen, 1990.).

A suggested minimum level for probiotic bacteria in yoghurt is 10^6 cfu/g (Kurman and Rasic, 1991.). The need to monitor *L. acidophilus* and *Bifidobacterium* spp. survival in yoghurt has often been neglected and a number of products reach the market with few viable bacteria (Shah et al., 1995.). Yoghurt manufacturers mainly rely on starter culture suppliers and blame them for having poor viability of starter cultures especially of probiotic bacteria. Starter culture suppliers may suspect that manufacturers do not follow their instructions concerning incubation temperature and level of starter addition what may lead to poor viability.

In this study the effect of level of starter addition on the viability of probiotic organisms in yoghurt made with three commercial starter cultures was moni-

tored during 35 days of storage at 4 °C. The condition of incubation, pH and storage temperature were according to the recommendations of starter culture supplier.

Materials and methods

Manufacture of yoghurt

Nonfat dry milk (2%) was added to homogenized and pasteurised full fat milk and the mix was heated at 85° C for 30 min and cooled to 40 - 43 °C. The starter cultures in the freeze dried concentrated Direct to Vat Set (DVS) and deep frozen and concentrated Direct to Vat Inoculum (DVI) forms were added in separate containers at the level of 0.5, 1.0, 1.5 or 2.0 g/10 L. The inoculated mix was distributed into 100 mL cups. Incubation was as temperature recommended by the starter culture supplier. The fermentation was stoped at pH 4.5.

The samples were stored at 4° C till the completion of the study.

Starter cultures

Three commercial starter cultures (A_1, A_2, and A_3) were taken for this study.

Cultures A_1 and A_2 (DVS) contained *S. thermophilus* (ST), *L. delbrueckii* spp. *bulgaricus* (LB), *L. acidophilus* (LA) and bifidobacteria (BB), whereas culture A_3 (DVI) contained ST, LA and BB. In all the comercial culture combinations the strains of probiotic organisms (LA and BB) were kept the same. Culture A_3 contained a strain of ST that produces polysaccharides during fermentation. The standard recommended levels of starter culture addition were 2 g for A_1 and 1 g for both A_2 and A_3 cultures per 10 L of yoghurt mix. The temperatures of 42 °C, 40 °C and 37 °C for A_1, A_2 and A_3 starter cultures respectively, were maintained during yoghurt incubation.

Sample preparation

Five yoghurt cups of each of the three products were aseptically emptied into 500 mL sterile glass beakers and mixed. After mixing, a sample was aseptically taken for microbiological analyses. pH values of the yoghurt and yoghurt mix were measured at 20°C using an Metrel MA 5750 pH meter.

Microbiological analyses

A yoghurt sample (1 g) was diluted with 9 mL of 15% peptone water (Oxoid) and mixed. Subseqnent serial dilutions were prepared and viable numbers enumerated using the pour plate technique. The counts of *L. acidophilus* and bifidobacteria were enumerated on MRS-salicin agar and MRS-NNLP agar (Dave and Shah, 1996.).

Time intervals observed

The "O-h" time observations is taken immediately after starter culture addition into cooled yoghurt mix (40 - 43° C). The "O-d" period represents the

analyses after one night storage of samples, and 5-35 d prediods represent the analyses after 5, 10, 15, 20, 25, 30, 35 days storage. The results represented are averages of two replications.

Results and discussion

Counts of *L. acidophilus* gradually decreased during storage up to 15 d and faster thereafter (Table 1). The viability was found to be dependent on the associative yoghurt organism. The viability of A_3 starter culture, in products with higher rate of inoculum during 35 d, was satisfactory (10^6 cfu/g).

Table 1: Changes in viable numbers (log_{10}) of L. acidophilus during production and storage of yoghurt manufactured with various levels of inoculum
Tablica 1: Promjena broja živih stanica (log_{10}) L. acidophilus tijekom proizvodnje i čuvanja jogurta proizvedenog s različitim količinama inokuluma

	Starter culture A_1				Starter culture A_2				Starter culture A_3			
Period[a]	C[b] 0.5	C 1.0	C 1.5	C 2.0	C 0.5	C 1.0	C 1.5	C 2.0	C 0.5	C 1.0	C 1.5	C 2.0
O s	6.15	6.36	6.67	**6.75**	6.15	**6.43**	6.38	6.74	6.34	6.63	6.83	**6.96**
O d	7.18	7.29	7.40	**7.42**	7.23	**7.39**	7.40	7.43	7.43	7.48	7.52	**7.58**
5 d	7.25	7.29	7.40	**6.49**	7.35	**7.40**	7.36	7.31	7.50	7.53	7.63	**7.69**
10 d	6.95	7.22	7.29	**7.31**	7.31	**7.37**	7.25	7.29	7.43	7.50	7.53	**7.62**
15 d	6.65	7.03	7.12	**7.18**	7.07	**7.22**	7.13	7.18	7.25	7.31	7.43	**7.57**
20 d	6.59	6.40	6.48	**6.57**	6.79	**7.00**	6.89	7.02	7.03	7.12	7.35	**7.41**
25 d	4.70	4.89	5.64	**5.80**	6.25	**6.59**	6.87	6.79	6.57	6.69	7.08	**7.20**
30 d	3.92	4.30	4.65	**4.84**	6.11	**6.43**	6.29	6.55	6.02	6.32	6.46	**6.71**
35 d	3.15	3.93	4.09	**4.32**	4.43	**5.17**	5.74	6.00	5.03	5.31	6.03	**6.10**

[a] "Oh", 5 d - 35 d = Observations taken immediately after starter culture addition, after overnight cooling and during 5-35 days.
[a] "Oh", 5 d - 35 d = Vrijednosti dobivene neposredno nakon dodatka starter kulture, nakon hlađenja tijekom noći te tijekom čuvanja od 5 do 35 dana.
[b] C 0.5, 1.0, 1.5, 2.0 = Samples made with 0.5, 1.0, 1.5, 2.0 g DVS/DVI cultures for 10 L of yoghurt mix.
[b] C 0.5, 1.0, 1.5, 2.0 = Uzorci proizvedeni sa 0.5; 1.0; 1.5 i 2.0 g DVS/DVI kulture za dobivanje 10 l jogurtne smjese.
Bold numbers are data for recommended levels of inoculum for the respective starter cultures.
Masno otisnute brojke predstavljaju preporučenu količinu inokuluma za odgovarajuću starter kulturu

At all inoculum levels for A_1 and A_2 starter cultures, the viability was poor. The pH was critical factor for this *L. acidophilus*. Yoghurt prepared with A_1 and A_2 starter cultures showed lower viability of *L. acidophilus* compared with A_3. A possible reason could be the presence of *L. delbrueckii* spp. *bulgaricus* in former starter cultures.

Table 2 represents viable numbers of bifdobacteria in yoghurt prepared from three commercial starter cultures using various levels of inoculum. The type of associative yoghurt organism(s) affected initial multiplication of

bifdobacteria. In yoghurt produced with A_1 starter culture multiplication of bifdobacteria was high despite of starter incubation time needed to reach pH 4.5 which indicate good association between yoghurt and bifdobacteria. In all the yoghurts prepared with A_1 starter culture the number of bifidobacteria remained $\geq 10^6$ cfu/g up to 35 d. For A_2 starter culture the number dropped to $< 10^6$ cfu/g in yoghurts prepared with lower level of inoculum. For the A_1 and A_2 starter cultures the multiplication of bifdobacteria was higher. This could be due to proteolitic activity of *L. delbrueckii* ssp. *bulgaricus* (Shankar and Davies, 1976.) and availability of free amino acids. Klaver and others, (1993.) reported that free amino acids are essential growth factors for bifdobacteria.

Table 2: Changes in viable counts (log_{10}) of bifidobacteria during manufacture and storage of yoghurt prepared with various levels of inoculum

Tablica 2: Promjena broja živih stanica (log_{10}) bifidobakterija tijekom proizvodnje i čuvanja jogurta proizvedenog s različitim količinama inokuluma

	Starter culture A_1				Starter culture A_2				Starter culture A_3			
Period[a]	C[b]0.5	C 1.0	C 1.5	C 2.0	C 0.5	C 1.0	C 1.5	C 2.0	C 0.5	C 1.0	C 1.5	C 2.0
O h	5.59	6.27	**6.50**	6.63	6.05	**6.31**	6.40	6.59	5.89	6.29	6.43	**6.60**
O d	7.29	7.49	**7.60**	7.75	6.79	**7.02**	7.04	7.20	6.15	6.69	6.92	**6.93**
5 d	7.17	7.41	**7.53**	7.73	6.81	**6.96**	7.02	7.20	6.09	6.71	6.90	**6.95**
10 d	7.11	7.38	**7.48**	7.71	6.78	**6.88**	7.01	7.09	6.00	6.58	6.79	**6.86**
15 d	7.05	7.29	**7.39**	7.65	6.69	**6.84**	6.96	7.07	5.89	6.44	6.70	**6.75**
20 d	6.89	7.12	**7.25**	7.49	6.45	**6.83**	6.83	6.91	5.49	6.24	6.49	**6.59**
25 d	6.80	7.02	**7.15**	7.35	6.29	**6.78**	6.79	6.93	5.02	6.09	6.33	**6.52**
30 d	6.70	6.70	**6.95**	7.20	6.01	**6.68**	6.78	6.85	4.85	6.04	6.18	**6.45**
35 d	6.05	6.42	**6.89**	7.01	5.43	**6.59**	6.70	6.79	4.31	5.89	6.01	**6.28**

[a] "Oh", 5 d - 35 d = Observations taken immediately after starter culture addition, after overnight cooling and during 5-35 days.

[a] "Oh", 5 d - 35 d = Vrijednosti dobivene neposredno nakon dodatka starter kulture, nakon hlađenja tijekom noći te tijekom čuvanja od 5 do 35 dana.

[b] C 0.5, 1.0, 1.5, 2.0 = Samples made with 0.5, 1.0, 1.5, 2.0 g DVS/DVI cultures for 10 L of yoghurt mix.

[b] C 0.5, 1.0, 1.5, 2.0 = Uzorci proizvedeni sa 0.5; 1.0; 1.5 i 2.0 g DVS/DVI kulture za dobivanje 10 l jogurtne smjese.

Bold numbers are data for recommended levels of inoculum for the respective starter cultures.
Masno otisnute brojke predstavljaju preporučenu količinu inokuluma za odgovarajuću starter kulturu

In table 3 the pH values of yoghurt during storage are shown. For A_1 and A_3 starter cultures the trend of pH decrease was identical and slightly higher compared with A_2, at the recommended levels of inoculum.

Table 3: Changes of the pH values during manufacture and storage of yoghurt prepared with various levels of inoculum
Tablica 3: Promjene pH vrijednosti tijekom proizvodnje i čuvanja jogurta proizvedenog s različitim količinama inokuluma

	Starter culture A_1				Starter culture A_2				Starter culture A_3			
Period[a]	C[b] 0.5	C 1.0	C 1.5	C 2.0	C 0.5	C 1.0	C 1.5	C 2.0	C 0.5	C 1.0	C 1.5	C 2.0
O h	6.55	6.49	6.43	**6.42**	6.56	**6.50**	6.45	6.43	6.61	6.60	6.54	**6.57**
O d	4.36	4.25	4.27	**4.30**	4.28	**4.29**	4.30	4.33	4.33	4.34	4.39	**4.40**
5 d	4.18	4.22	4.27	**4.30**	4.27	**4.29**	4.31	4.33	4.32	4.33	4.30	**4.31**
10 d	4.15	4.21	4.27	**4.29**	4.26	**4.28**	4.31	4.30	4.20	4.25	4.30	**4.25**
15 d	4.13	4.16	4.25	**4.28**	4.25	**4.28**	4.30	4.31	4.19	4.19	4.25	**4.24**
20 d	4.10	4.12	4.20	**4.22**	4.16	**4.22**	4.25	4.30	4.18	4.20	4.22	**4.23**
25 d	4.08	4.11	4.19	**4.20**	4.11	**4.23**	4.26	4.29	4.15	4.16	4.20	**4.20**
30 d	4.07	4.08	4.19	**4.20**	4.11	**4.19**	4.28	4.28	4.11	4.14	4.16	**4.21**
35 d	4.03	4.07	4.18	**4.19**	4.08	**4.18**	4.23	4.27	4.09	4.10	4.14	**4.18**

[a] "Oh", 5 d - 35 d = Observations taken immediately after starter culture addition, after overnight cooling and during 5-35 days.
[a] "Oh", 5 d - 35 d = Vrijednosti dobivene neposredno nakon dodatka starter kulture, nakon hlađenja tijekom noći te tijekom čuvanja od 5 do 35 dana.

[b] C 0.5, 1.0, 1.5, 2.0 = Samples made with 0.5, 1.0, 1.5, 2.0 g DVS/DVI cultures for 10 L of yoghurt mix.
[b] C 0.5, 1.0, 1.5, 2.0 = Uzorci proizvedeni sa 0.5; 1.0; 1.5 i 2.0 g DVS/DVI kulture za dobivanje 10 l jogurtne smjese.

Bold numbers are data for recommended levels of inoculum for the respective starter cultures.
Masno otisnute brojke predstavljaju preporučenu količinu inokuluma za odgovarajuću starter kulturu

The pH drop of yoghurt, made with all three starter cultures, was higher at lower rate of inoculum.

Conclusions

In this study reduced levels of inoculum, of commercially available DVS/DV1 probiotic yoghurt cultures, resulted in slightly higher post acidification. This had an adverse effect on viability of probiotic organisms. The starter cultures (DVS/DV1) can be mixed in certain proportions in order to have required ratios of yoghurt and probiotic organisms. We found in this study that any alteration in the inoculum level and incubation temperature changes this ratio and the viability of probiotic organisms is affected.

L. acidophilus lost its viability more rapridly than bifdobacteria. In most of the products *L. acidophilus* counts were maintained at the level of $> 10^6$ cfu/g for 25 d, and remained ($\leq 10^6$ cfu/g) at higher level in yoghurt prepared from starter cultures containing only *S. thermophilus* as yoghurt organism.

It was found that pH was crucial factor for the viability of *L. acidophilus.* In this study good stability of bifidobacteria in yoghurts prepared with three

starter cultures is observed. Overall, the viability of *L. acidophilus* needs to be improved in two starter cultures. The level of inoculum and incubation temperature should be properly maintained in order to achieve maximum viability of probiotic organisms.

UTJECAJ KOLIČINE DODANE KULTURE NA PREŽIVLJAVANJE PROBIOTIČKIH BAKTERIJA U JOGURTU

Sažetak

U uzorcima jogurta, proizvedenim korištenjem komercijalnih kultura, istražen je utjecaj količine dodanog cjepiva na probiotičke bakterije (Lactobacillus acidophilus i Bifidobacterium spp.) tijekom priprave i čuvanja proizvoda pri +4°C.

Broj živih bakterija L. acidophilus održao se na 10^6 cfu/g do 20 - 25 dana čuvanja, a nakon toga je opao.

Za sve tri kulture, koje su korištene za pripravu uzoraka, broj bifidobakteria bio je zadovoljavajući i vrlo stabilan, stabilniji od broja L. acidophilusa.

Naknadno zakiseljavanje ispitivanih uzoraka nešto je izraženije kod uzoraka jogurta proizvedenih korištenjem manjeg % cjepiva. Bakterije iz sastava kultura za proizvodnju jogurta (jogurtne kulture) i pH vrijednosti jogurta utječu na preživljavanje probiotičnih bakterija.

Ključne riječi: mljekarske kulture, preživljavanje, probiotične bakterije

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Author address - Adresa autora:
Dr. sc. Ljerka Gregurek
Probiotik d.o.o.
Zagreb, Grada Gospića 1

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