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The effect of yoghurt culture on the survival of probiotic bacteria in oat-based, non-dairy products

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Abstract

The survival of the probiotic strains *Lactobacillus reuteri* ATCC 55730, *Lactobacillus acidophilus* DSM 20079 and *Bifidobacterium bifidum* DSM 20456, all of human origin, were investigated in three different oat-based, non-dairy products (Adavena M40, MG20 and G40). The products were fermented by the three strains with and without the presence of a commercial yoghurt culture (V2). Samples were stored at 6 °C up to 30 days. In general, the oat-based products were shown to be a suitable support for these intestinal bacteria. *L. reuteri* ATCC 55730 had the highest viability in all of the products investigated. After 30 days the cell viability of *L. reuteri* ATCC 55730 was 10⁸ CFU ml⁻¹ in all three oat-based products. Lower viability was seen when the strains were grown in the presence of the yoghurt culture compared to when they were grown as pure cultures. These products also exhibited a lower pH value in comparison to products fermented with the pure cultures. The utilisation of the main fermentable carbohydrates in the products varied across the bacterial strains. A decrease in β -glucan content was seen for the products fermented by the *B. bifidum* DSM 20456 strain. This work shows that these oat-bases with different mono- and disaccharide composition can be used to support the growth of human intestinal bacteria and also maintain high cell viability during cold storage. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Bacterial survival; Probiotics; Non-dairy; Oats; Adavena[®]

1. Introduction

There is a great interest to increase the consumption of products based on oats that contain both soluble and insoluble fibers due to, among other reasons, the well-documented, positive effects of oats on blood cholesterol levels (Behall, Scholfield, & Hallfrisch, 1997; Wood, 1991). High serum cholesterol concentration levels are strongly associated with an increased risk of ischemic heart disease (Rosengren, Hagman, Wedel, & Willhelmsen, 1997). There is, however, a low consumption of oat-based products, mainly due to the lack of acceptable and suitable food products (Salovaara & Bäckström, 1991). Fermentation processes in combination with tailored oat-bases (Adavena[®]; Ceba Foods AB, Lund, Sweden), rich in e.g. dietary fibers, can be used to increase the interest in oats as a raw material for new, functional food products. These kinds of oat-bases are

entirely made of oat and water using a patented enzymatic process (US patent No. 5.686.123; Lindahl, Ahlén, Öste, & Sjöholm, 1997). One application is a non-dairy, milk substitute (Oatly[®]; Ceba Foods AB, Lund, Sweden). This product has been reported to have both high acceptance among consumers and a cholesterol lowering effect (Önning, Åkesson, Öste, & Lundquist, 1998; Önning, Wallmark, Persson, Åkesson, Elmståhl, & Öste, 1999). It has also been shown that this product can be fermented by various kinds of lactic acid bacteria (Mårtensson, Öste, & Holst, 2000, 2002). Recently a fermented, non-dairy, yoghurt analogue has been formulated using one of these oat-bases (Mårtensson, Andersson, Andersson, Öste, & Holst, 2001).

There has been an increased interest during the last decade to add intestinal *Lactobacillus* spp. and *Bifidobacterium* spp. to fermented food products (Vinderola, Bailo, & Reinheimer, 2000). Food products containing probiotic bacteria, e.g. “probiotic foods”, have recently been defined as: “Foods containing live and defined bacteria, which when given in sufficient numbers, exert beneficial effects by altering the microflora in the host”

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(Schrezenmeir & de Vrese, 2001). Probiotic bacteria are added to take advantage of their health-promoting properties, and to create more diverse products (Klaver, Kingma, & Weerkamp, 1993; Kneifel, Jaros, & Erhard, 1993). It is of great importance that when selecting bacteria for their physiological effects, they should stay viable during the whole shelf-life of the food product and that there is no decrease in their resistance to the acidic environment of the stomach and to bile salts in the small intestine (Kailasapathy & Rybka, 1997; Sarrela, Mogensen, Fondén, Mättö, & Mattila-Sandholm, 2000). It has also been suggested that the food product should contain at least 10^6 colony forming units per gram food product of the probiotic bacterial strain (*Lactobacillus* spp. and *Bifidobacterium* spp.) and that it must be possible to eat approximately 300–400 g per week of the product (Samona & Robinson, 1994).

The aim of this work was to evaluate the survival of two lactobacilli strains and one *Bifidobacterium*, all from human origin, during refrigerated storage in three non-dairy, oat-based products with different carbohydrate composition as a step in the development of new fermented oat-based, non-dairy products.

2. Materials and methods

2.1. Cultures

Three probiotic cultures, *Lactobacillus reuteri* ATCC 55730 (Biogaia Biologics, Stockholm, Sweden), *Lactobacillus acidophilus* DSM 20079 and *Bifidobacterium bifidum* DSM 20456 (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) were used together with a commercial yoghurt culture, V2, which is a 1:1 mixture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* (Visby Tønder A/S, Tønder, Denmark). This yoghurt culture was chosen, because it is a common starter culture in yoghurt products in Sweden.

2.2. Culture media

B. bifidum DSM 20456 was cultured in bifidobacteria medium (BFM-medium) broth (DSM-medium 56) composed of (g l⁻¹) casein peptone, tryptic digest (Oxoid, Basingstoke, UK), 10, meat extract (Difco, Detroit, Michigan, US), 5, yeast extract, 5, K₂HPO₄, 3, glucose, 10 (all from Merck, Darmstadt, Germany). *L. reuteri* ATCC 55730 and *L. acidophilus* DSM 20079 were cultured in MRS broth (Merck) (De Man, Rogosa, & Sharpe, 1960). The three strains were stored as stock cultures containing 20% (v/v) glycerol at -80 °C. Before use, the cultures were propagated twice in cultivation broth supplemented with cysteine-HCl 0.05% (v/v) (Fluka, Buchs, Switzerland) at 37 °C. The transfer

inoculum was a 1% (v/v) culture grown for 24–48 h in a fresh medium under anaerobic conditions using anaerobic jars (85% N₂, 10% H₂, 5% CO₂; Anaerocult® A system; Merck). Solid media were prepared by the addition of 15 g l⁻¹ of granulated agar (Merck), to the broths. The commercial starter culture was maintained according to the manufacturer's instructions.

2.3. Fermentation procedure

Adavena® M40 (M40 product) and Adavena® G40 (G40 product) (20% dry matter) were provided in frozen form by Ceba Foods AB, Lund, Sweden, and analyzed for protein, fat, different carbohydrates, dietary fibers, various vitamins and minerals by an authorized laboratory (AnalyCen Nordic AB, Lidköping, Sweden) (Table 1). The oat-base was diluted to a final dry matter content of 16%, according to earlier formulation developments by Mårtensson, Andersson et al. (2001), and heat-treated at 90 °C with continuous stirring for 5 min, and cooled to fermentation temperature. The MG20 medium was obtained by mixing equal amounts of M40 and G40 during continuous stirring before heat treatment. The V2 culture and the three different bacterial strains (*L. reuteri* ATCC 55730, *L. acidophilus* DSM 20079 and *B. bifidum* DSM 20456) were used as pure cultures. These three bacterial strains were also inoculated together with the V2 culture (*L. reuteri* ATCC 55730 + V2, *L. acidophilus* DSM 20079 + V2, *B. bifidum* DSM 20456 + V2). Before inoculation the bacterial cells were centrifuged, and the cell pellet was washed twice

Table 1
Chemical composition (g/100 g) of three different oat-based products (20% dry matter), M40, MG20 and G40

Component	M40	MG20	G40
Protein (g)	2.2	2.2	2.2
Fat (g)	1.6	1.6	1.6
Glucose	ND ^a	4.4	8.8
Maltose (g)	8.4	4.2	ND ^a
Maltodextrin (g)	5.4	5.4	5.4
Total fiber (g)	1.6	1.6	1.6
Beta-glucan (g)	0.8	0.8	0.8
α-tocopherol (mg)	0.2	0.2	0.2
Thiamin (mg)	0.08	0.08	0.08
Riboflavin (μg)	19.2	19.2	19.2
Niacin (mg)	0.2	0.2	0.2
Folic acid (μg)	6.6	6.6	6.6
Pyridoxine (mg)	0.02	0.02	0.02
Iron (mg)	0.2	0.2	0.2
Magnesium (mg)	9.4	9.4	9.4
Manganese (mg)	0.2	0.2	0.2
Phosphorus (mg)	54	54	54
Sodium (mg)	22	22	22
Zinc (mg)	0.2	0.2	0.2

^a ND, not determined.

with peptone water containing (g l⁻¹): peptone, 1 (Difco), sodium chloride, 7 (Merck). The three strains (*L. reuteri* ATCC 55730, *L. acidophilus* DSM 20079 and *B. bifidum* DSM 20456) were inoculated with 5% (v/v) to the products. The commercial yoghurt culture (V2) was inoculated (0.02%, w/v). All samples were incubated at 37 °C for 16 h. After incubation the fermented samples were stored at 6 °C for 4 weeks. Cell counts and pH measurements were performed every fifth day for 30 days.

2.4. Cell counts

Samples of 1 ml of the different products were decimally diluted in sterile peptone water and 0.1 ml aliquots dilutions plated on the culture media. MRS agar was used for the enumerations of the two lactobacilli strains when present as pure cultures. M17 agar (Merck) (Terzaghi & Sandine, 1975) was used for the enumerations of the *S. salivarius* subsp. *thermophilus* present in the V2 culture. BFM agar was used for the enumeration of *B. bifidum* DSM 20456. MRS supplemented with bile salt No3 (0.5 g l⁻¹) (Difco) (Klaver et al., 1993) together with anaerobic incubation was used for the enumeration of *L. reuteri* 2112 and *L. acidophilus* DSM 20079 when grown in the presence of the yoghurt culture (V2). LP-MRS agar, MRS agar supplemented with lithium chloride (2 g l⁻¹) (Merck) and sodium phosphate (3 g l⁻¹) (Fluka) (Lapierre, Undeland, & Cox, 1992), together with anaerobic incubation, was used for the enumeration of *B. bifidum* DSM 20456 in the presence of the yoghurt culture (V2). Prior study these two medias inhibition of the yoghurt culture (V2) was confirmed.

2.5. pH

pH measurements were carried out every fifth day by means of a digital pH meter, MA235 model (Mettler Toledo, Hightstown, US).

2.6. Determination of glucose, maltose and β -glucan

Glucose and maltose were analysed by high pH anion exchange chromatography (HPHEC) using a CarboPac PA 10 column (Dionex, Jouy-en-Josas, France) with 0.2 M NaOH as mobile phase at a flow rate of 1.4 ml min⁻¹. The β -glucan content was analysed by using a commercial enzymatic kit (Megazyme Ltd, Wicklow, Ireland).

2.7. Statistical analysis

Values were expressed as means and standard deviation. Mean values of treatments were compared by Student's *t* test. Differences were considered significant at *P* < 0.05.

3. Results

3.1. Changes in viability and pH in three different oat-based products fermented by different bacterial strains and a yoghurt culture

Viability and pH for the three different strains, *L. reuteri* ATCC 55730, *L. acidophilus* DSM 20079 and *B. bifidum* DSM 20456, are shown in Fig. 1a–c. The highest viability was obtained by *L. reuteri* ATCC 55730 in all of the three products during the storage period. After 30 days the viability was still high (10⁸ CFU ml⁻¹) for the *L. reuteri* ATCC 55730 strain in all of the products used. *L. acidophilus* DSM 20079 showed a higher viability in the MG20 product in comparison to the M40 and the G40 products. After 30 days a viability of 10⁶ CFU ml⁻¹ was observed in the MG20 product. *B. bifidum* DSM 20456 had a greater decrease in viability during the storage period in all of the products in comparison to the two other strains. The highest viability (10⁵ CFU ml⁻¹) after 30 days of storage was found in the M40 product (Fig. 1a). The pH value of the M40 product fermented with the *B. bifidum* DSM 20456 strain decreased during the storage and was also evidently higher in comparison to the other fermented products.

Fig. 2 a–c show the viability of the V2 culture, consisting of *S. salivarius* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. There was no major change in viability of the two bacterial strains during the storage period except for the *S. salivarius* subsp. *thermophilus* strain in the G40 case. The *L. delbrueckii* subsp. *bulgaricus* strain showed a somewhat higher viability in the MG20 and G40 case in comparison to the *S. salivarius* subsp. *thermophilus* strain.

3.2. Changes in viability and pH in three different oat-based products fermented by different bacterial strains in the presence of a yoghurt culture

Viability and pH for the three different strains grown in the presence of a yoghurt culture in three different oat-based media are shown in Fig. 3a–c. The highest viability during the storage period is shown for the *L. reuteri* ATCC 55730 strain with cell counts of 10⁶ CFU ml⁻¹ after 25 days of cold storage in the M40 and MG20 products. The cell counts in the M40 product were slightly less (10⁵ CFU ml⁻¹) at the same time period during the storage. The cell viability of *L. acidophilus* DSM 20079 decreased faster during storage in comparison to *L. reuteri* ATCC 55730. The highest viability for the *L. acidophilus* DSM 20079 strain was observed in the M40 case with a count of approximately 10⁵ CFU ml⁻¹ after 15 days of storage.

The pH values were considerable lower, ranging from 3.8 to 4.4, in all products when they were co-fermented with the yoghurt culture.

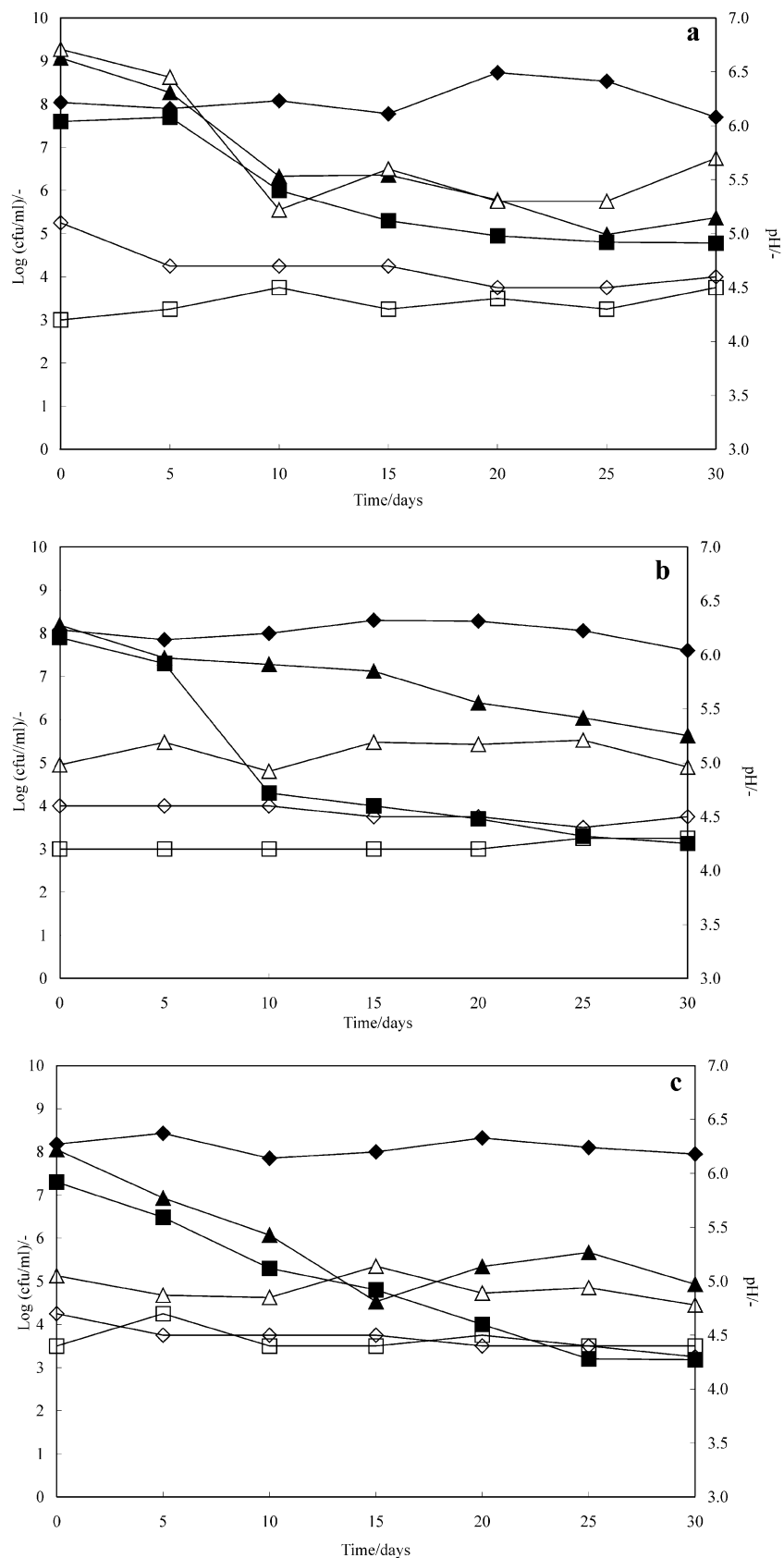


Fig. 1. Survival (closed symbols) and pH (open symbols) of *Lactobacillus reuteri* ATCC 55730 (◆,◇), *Lactobacillus acidophilus* DSM 20079 (■,□) and *Bifidobacterium bifidum* DSM 204 56 (▲,△) in three oat-based media M40 (a), MG20 (b) and G40 (c) during 30 days of storage at 6 °C. The results are the mean values from three determinations.

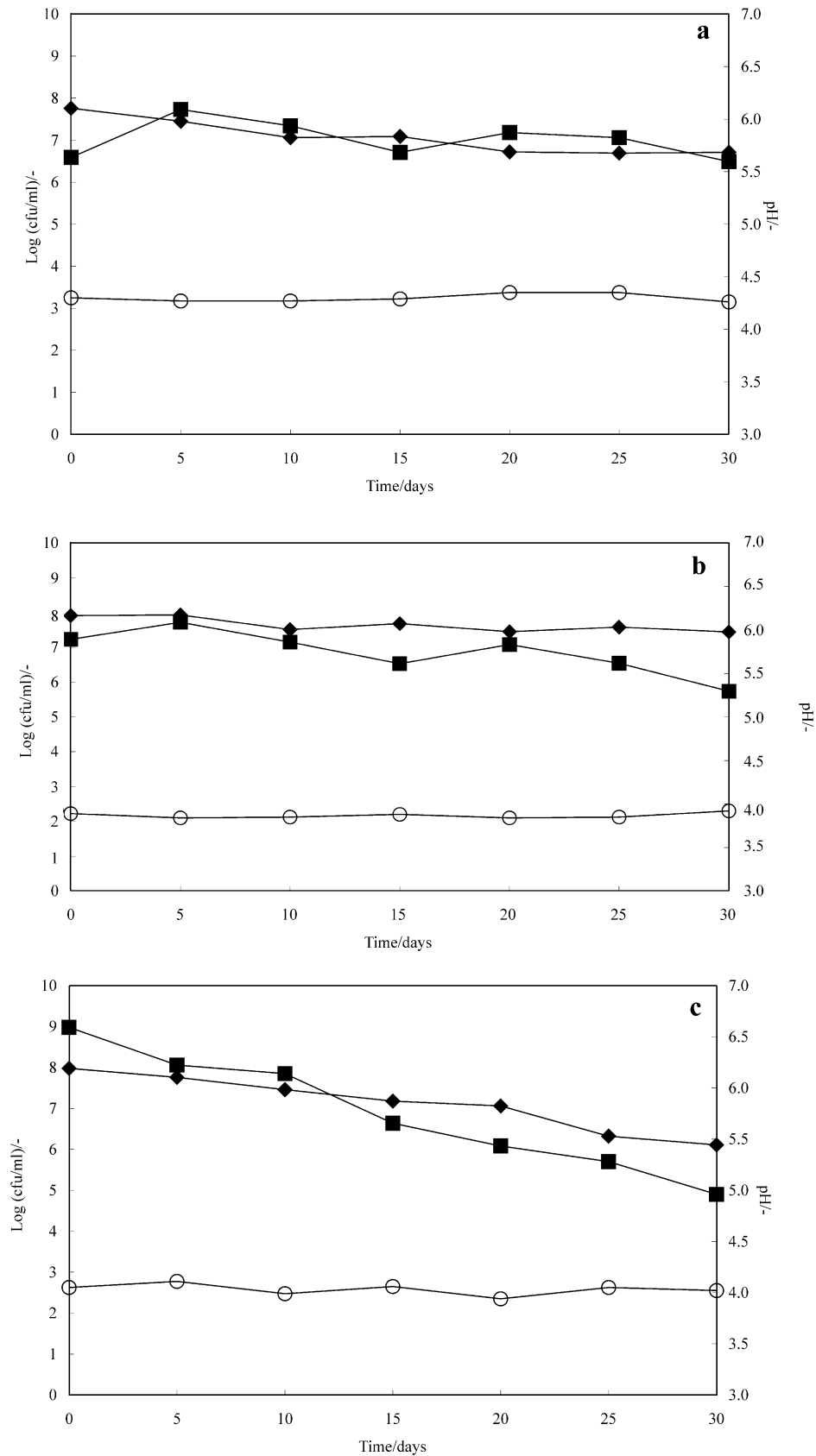


Fig. 2. Survival (closed symbols) and pH (○) of a commercial yoghurt culture (V2) consisting of *Lactobacillus delbrueckii* subsp. *bulgaricus* (◆) and *Streptococcus salivarius* subsp. *thermophilus* (■) in three oat-based media M40 (a), MG20 (b) and G40 (c) during 30 days of storage at 6 °C. The results are the mean values from three determinations.

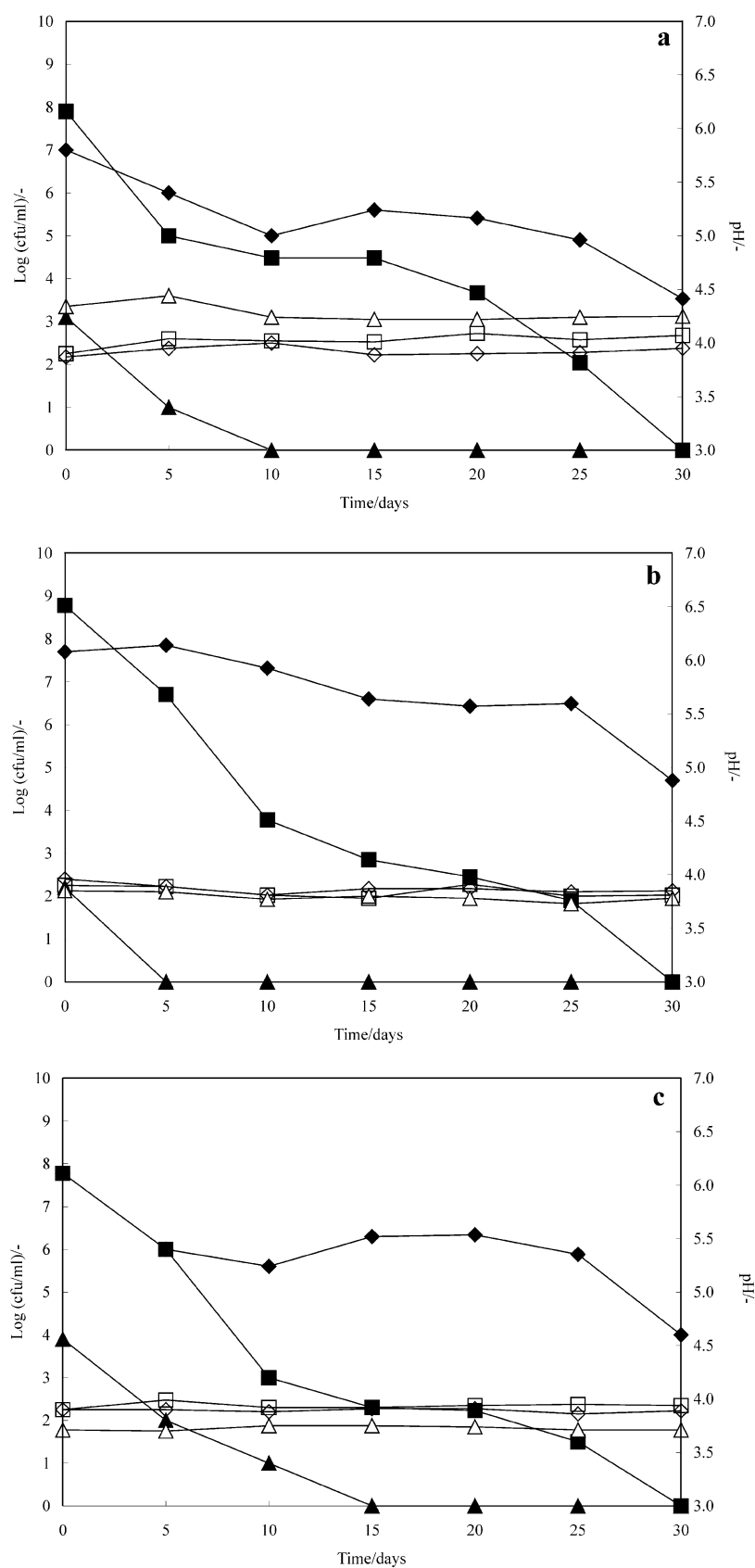


Fig. 3. Survival (closed symbols) and pH (open symbol) of *Lactobacillus reuteri* ATCC 55730 (◆,◇), *Lactobacillus acidophilus* DSM 20079 (■,□) and *Bifidobacterium bifidum* DSM 204 56 (▲,△) in three oat-based media M40 (a), MG20 (b) and G40 (c) during 30 days of storage at 6°C after growth together with a yoghurt culture (V2). The results are the mean values from three determinations.

3.3. Changes in mono- and disaccharide concentration after fermentation

The major mono- and disaccharides in the products, maltose and glucose, were analysed in the different oat-based products before inoculation (control) and after 16 h of fermentation (Fig. 4a–c). All strains were able to utilize a significant ($P < 0.05$) amount of the maltose content in the M40 product (Fig. 4a). A similar amount of the fermentable carbohydrates (maltose and glucose) was also determined after the fermentation period in the MG20 case (Fig. 4b). In the G40 case, there was only a small decrease in the glucose content in the products fermented with *L. reuteri*, *L. acidophilus* and *L. reuteri*+V2 in comparison to the control. In the other products there were a significant ($P < 0.05$) decrease in the glucose content compared to the control (Fig. 4c).

3.4. β -Glucan concentration after fermentation

The β -glucan content in the M40 product is shown in Fig. 5. There was an obvious decrease in the β -glucan content in the M40 product after fermentation with a pure culture of *B. bifidum* DSM 20456. No effect of the β -glucan content was seen when the product was fermented with *L. reuteri* ATCC 55730 and *L. acidophilus* DSM 20079. There was no change in the β -glucan content when the product was fermented with a mixed culture of the V2 culture and *B. bifidum* DSM 20456.

4. Discussion

With the long-term goal to develop new fermented, non-dairy food products based on oats, the addition of probiotic bacteria, such as *B. bifidum*, *L. reuteri* and *L. acidophilus*, may both widen the variety and enhance the physiological effect of these non-dairy, oat-based food products. In this study we have measured the viability of three different probiotic microorganisms that are commonly used in fermented dairy products today, during 30 days of storage with and without the presence of a yoghurt culture.

The suggested viability level for probiotic adjunct cultures included in a product is 10^6 CFU gl^{-1} (Micanel, Haynes, & Playne, 1997). *L. reuteri* had a viability of 10^8 CFU ml^{-1} in all of the products tested after 30 days of storage. It has been reported earlier that oat-based products, e.g. as an oatmeal soup, can be used as a vehicle for *L. reuteri* strains (Johansson, Molin, Jeppsson, Nobaek, Ahrne, & Bengmark, 1993). The *L. acidophilus* DSM 20079 strain exhibited a viability of 10^6 CFU ml^{-1} after 30 days in the MG20 product during cold storage. This shows that it is possible to tailor-

make products suitable for supporting the viability of *L. acidophilus* strains in non-dairy products based on oat. High values of viability have been reported for the *L. acidophilus* strains in milk (Rybka & Kailasapathy, 1995). Results supporting the fact that milk would be a poor support concerning the viability of *L. acidophilus* have also been reported (Gilliland & Speck, 1977). However, it has also been suggested that this variation could be strain dependent (Nighswonger, Brashears, & Gilliland, 1996). There was a difference in viability of the *B. bifidum* DSM 20456 depending on the oat-based product used. The maltose rich product (M40) and the glucose rich product (G40) did not support the viability of *B. bifidum* DSM 20456 to any greater extent. Highest survival was seen in the MG20 product, where a cell count level of 10^6 CFU ml^{-1} was maintained up to 25 days of storage. This shows that there is a potential to have high numbers of a *B. bifidum* strain in a fermented non-dairy product based on this kind of oat-base. Some reports support that the bifidobacteria survives well in milk (Medina & Jordano, 1994; Shin, Lee, Pestka, & Ustunol, 2000) but there are also a number of reports that support the finding that the survival of *Bifidobacterium* in milk is poor (Dave & Shah, 1997; Klaver et al., 1993; Shah, Lankaputhra, Britz, & Kyle, 1995).

The viability for all three strains used in this study was lower in all of the oat-based products tested when the bacterial strains were grown together with a yoghurt culture. These results reflect the poor resistance of these bacterial strains to an acidic environment ($\text{pH} < 4.0$). The concentration of lactic acid is lower at the same pH level in these oat-bases than in milk due to their low buffering capacity (Mårtensson et al., 2000). Low pH level (3.8–4.0) of a fermented oat-based, yoghurt-like product has been proven to increase considerably the general acceptability for this product (Mårtensson, Andersson et al., 2001). Thus, it is important that these strains can survive in oat-based products with this final pH.

L. reuteri ATCC 55730 showed the highest viability during the storage period in comparison to the other strains tested when a yoghurt culture was included as a culture. The highest viability for the *L. reuteri* ATCC 55730 strains was seen in the MG20 product. Both the *L. acidophilus* DSM 20079 and the *B. bifidum* DSM 20456 strain showed a much lower survival level during these more acidic conditions. In fact, the *B. bifidum* strain lost all viability within 10 days of storage in all the products tested. Low viability has also been seen for other *B. bifidum* strains in different commercial dairy products (Shah et al., 1995). This shows that the final acidity in the product has a major impact on the microbial viability during the shelf-life of the product. This relationship between the acidic environment and the effect on cell viability has been reported earlier (Lankaputhra, Shah, & Britz, 1996; Laroia & Martin, 1991; Vinderola et al., 2000). The yoghurt culture, *L. delbrueckii*

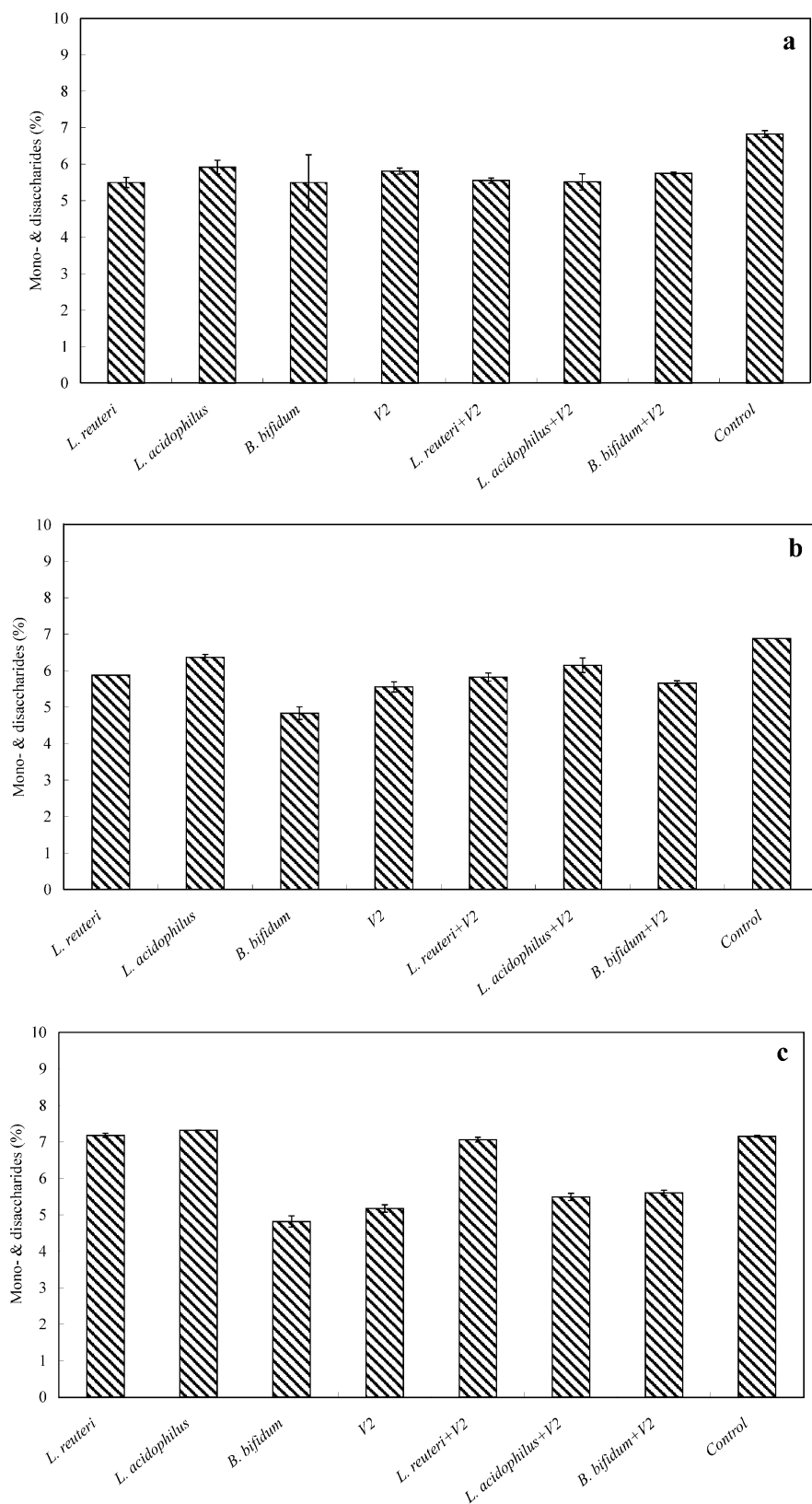


Fig. 4. Main mono- and disaccharide content (glucose and maltose) in the three different oat-based products M40 (a), MG20 (b) and G40 (c) after 16 h of fermentation with pure cultures of *Lactobacillus reuteri* ATCC 55730, *Lactobacillus acidophilus* DSM 20079, *Bifidobacterium bifidum* DSM 20456 and V2 (consisting of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*) and mixed cultures of *L. reuteri*+V2, *L. acidophilus*+V2, *B. bifidum*+V2 and unfermented media (control). The results are the mean values \pm standard deviation from three determinations.

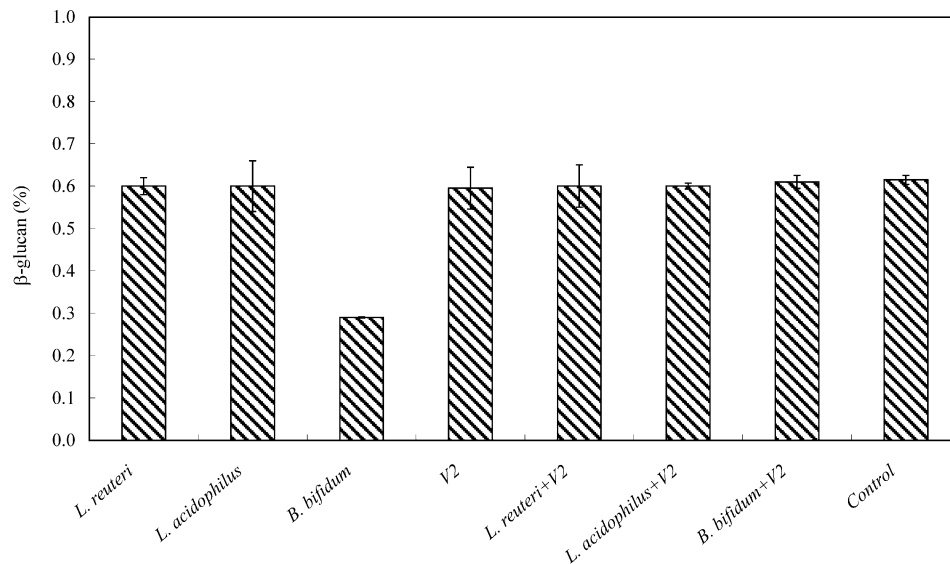


Fig. 5. The content of β -glucans in the M40 product after 16 h of fermentation with pure cultures of *Lactobacillus reuteri* ATCC 55730, *Lactobacillus acidophilus* DSM 20079, *Bifidobacterium bifidum* DSM 20456 or V2 and mixed cultures of *L. reuteri*+V2, *L. acidophilus*+V2, *B. bifidum*+V2 and unfermented media (control). The results are the mean values \pm standard deviation from three determinations.

subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* exhibited high viable cell count in all of the products, which is in accordance with earlier findings by Mårtensson, Andersson, Andersson et al. (2001).

There was a variation in β -glucan content after the fermentation depending on the bacterial strain used. A decrease was seen when using the *B. bifidum* DSM 20456 strain. No change of the β -glucan level was, however, seen for the *L. reuteri* ATCC 55730, *L. acidophilus* DSM 20079 or for the V2 culture. It is likely that a decrease in the final β -glucan concentration is due to β -glucanase activity of the *B. bifidum* DSM 20456 strain. It is of a great concern that the β -glucan level in the products will be unaffected through the fermentation process and storage of the final product as the β -glucan is the main active component for the cholesterol-lowering properties recognised in oats.

This work shows that non-dairy, oat-based products are suitable substrates and can support high cell viability during cold storage for 30 days for different probiotic strains. The results also show the importance of selecting the right culture for the fermentation process to obtain an acceptable fermented product in terms of acidity and unaffected β -glucan content.

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