Production of ice cream containing probiotic bacteria

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56 **Käsereifung** (hitzeschockbehandelte Zusätze)

Hitzeschockbehandlung von ausgewählten handelsüblichen Zusatzkulturen führte zu einer Abnahme des Zellautolysegrades und der enzymatischen Aktivitäten Die Veränderungen beider Parameter waren abhängig von beim Hitzeschockverfahren verwendeten Temperatur. Je höher die Temperatur, desto mehr sanken Autolyse und Aktivität. Käseaufschwemmungen aus gefriergeschockten Zellen zeigten eine höhere Enzymaktivität und einen stärkeren Protein- und Fettabbau verglichen mit solchen, die mit hitzeschockbehandelten Zusätzen inokuliert worden waren. Dies wird der höheren Freisetzung von Enzymaktivität bei gefriergeschockten Zusatzkulturen zugeschrieben.

Production of ice cream containing probiotic bacteria

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Introduction

Frozen yoghurt has become popular and is continuously reaching new markets. Its popularity is associated with the healthy image of yoghurt, delicate taste and its low fat content. Using the same technology it is possible to produce a probiotic ice cream. This ice cream contains cultures selected for dietary benefits. A probiotic can be defined as "a mono- or mixed culture of live microorganisms which, applied to man or animal, beneficially affects the host by improving the properties of the indigenous microflora" (4). HUIS IN'T VELD and HAVENAAR (4) also describe the possible dietary benefits of probiotic bacteria.

The aim of this study was to produce a probiotic ice cream containing high levels of probiotic bacteria (>10^6 cfu/g, which is the recommended minimum daily intake (5)), and to determine their survival immediately after freezing and during frozen storage. In an attempt to improve the survival of the cells during freezing, one of the batches was added 2% glycerol.

Materials and methods

2.1 *Probiotic strains*

Four different cultures were used. *Lactobacillus reuteri* (LR) obtained from BioGaia Biologics AB, Stockholm, Sweden. *Lactobacillus acidophilus* (LA-5) and *Bifidobacterium bifidum* (BB-12) obtained from Christian Hansen AS, Norway, Oslo, and *Lactobacillus rhamnosus "GG"* (ATCC 53103) obtained from Valio Ltd., Helsinki, Finland.

2.1.1 *Storage of strains*

Concentrated freeze-dried cultures were prepared in the following way. The cells were routinely propagated on 3 successive days, at the rate of 1% in MRS-broth (Oxoid) and incubated at 37°C for 24 h. For the concentrate preparation, the cultures were grown in 300 ml MRS and the cell mass was harvested by centrifugation at ca 1400xg for 10 min (Sorvall RC-5B refrigerated Superspeed centrifuge, Du Pont Instrument). After centrifugation the cell pellets were resuspended in 30 ml UHT (Ultra High Treatment) semi skimmed milk, distributed into 10 tubes, and stored at –80°C until required.

2.2 *Procedure for ice cream manufacturing*

Batches of ice cream mix based on unsalted sweet cream butter and skimmed milk powder (Norwegian Dairies, Oslo) were prepared with 10% fat, 12% milk solids not fat, 12.5% sucrose, 0.8% stabilizer/emulgator (Cremodan SE 38 veg. Danisco Ingredients, Denmark). 0.3% vanilla and 2% glycerol (added to one of the batches). The flow chart is shown in Fig. 1. Water and unsalted sweet cream butter were heated to 75°C. During this heating, skim milk powder and sucrose were added at 40–50°C and the combined stabilizer/emulgator was added at 70°C. The mix was pasteurized at 75°C for 2 min, homogenized at 175 kp/cm² and then aged overnight at 4°C.

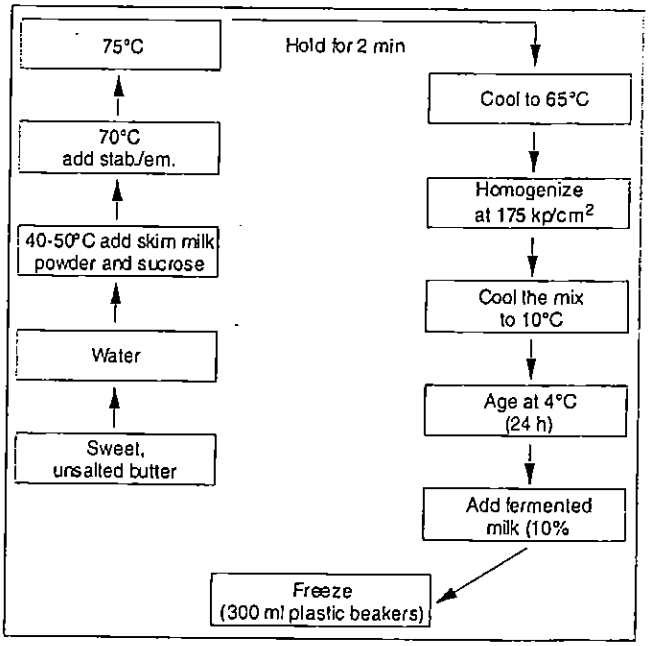

Fig. 1: Production of ice cream

Each strain was cultured for 12 h at 37°C in UHT semi-skimmed milk, fortified by the addition of 1% D-glucose (puriss, Kebo lab AB, 50% w/v filter sterilised solution) and 1% tryptone (Oxoid, 25% w/v filter sterilised solution). This fermented milk was then added

(10% v/v) to the ice cream mix prior to freezing. The freezing was performed in a Carpigiani soft-ice freezer (Bologna, Italy). The ice cream was filled in 300 ml plastic cups and hardened at –20 °C.

2.3 Analyses

2.3.1 Bacteriological

Bacterial counts were estimated after 12 h fermentation, immediately after mixing, after freezing, and after 1, 4, 16 and 52 weeks of frozen storage at –20 °C. The viable numbers were estimated by plating on MRS-agar (Oxoid) and incubating anaerobically for 3 days (37 °C).

2.3.2 Chemical

The concentrations of organic acids and volatile aromatic compounds in the samples were determined according to the methods described by NARVHUS *et al.* (6). Organic acids were determined by HPLC (High Pressure Liquid Chromatography), while HSGC (Head Space Gas Chromatography) was used to determine the amount of volatile aromatic compounds produced by the different strains. pH, organic acids and volatile aromatic compounds were estimated both in fermented milk, mix and frozen ice cream.

2.3.3 Sensory evaluation

The ice cream was organoleptically evaluated after 2 weeks of storage, in accordance with the hedonic scale 1–7 used in the Scandinavian countries, with 7 as the most "intense". The hardened ice cream samples were scored for "probiotic flavour", firmness, chewiness, sourness, off-flavour, iciness and total impression. A commercial frozen ice cream was used as a reference.

All statistical analyses were carried out using SAS (SAS Institute Inc., Cary, USA)

3. Results and discussion

3.1 Viable counts

The fermented milk was added to an ordinary ice cream mix at a rate of 10% v/v and the number of viable bacteria in the complete ice cream mix was thereby decreased by 1 log unit. Figures 2–5 show the bacterial counts in fermented milk (12H; milk fermented for 12 h), mix and ice cream with and without the addition of glycerol.

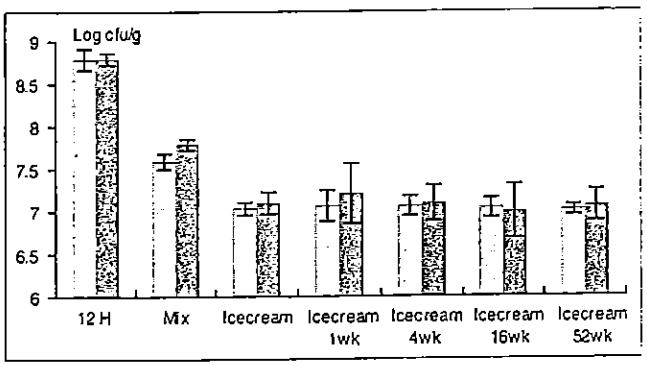

Fig. 2: Viable counts of *B. bifidum* (BB-12); without glycerol, ▨ with glycerol

During freezing, or shortly afterwards, the numbers of viable bacteria decreased by 0.7–0.8 log unit, and the numbers in the frozen ice cream were found to be in the range of 7 log cfu/g for BB12, 7.4 log cfu/g for GG, 7.65 log cfu/g for LR and 6.75 log cfu/g for LA5.

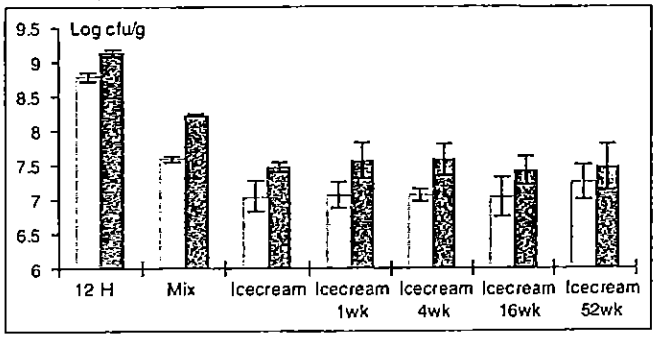

Fig. 3: Viable counts of *Lb.* GG; without glycerol, ▨ with glycerol

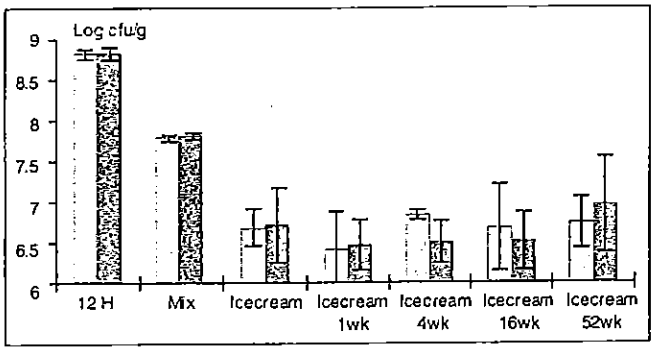

Fig. 4: Viable counts of *Lb. acidophilus* (LA-5); without glycerol, ▨ with glycerol

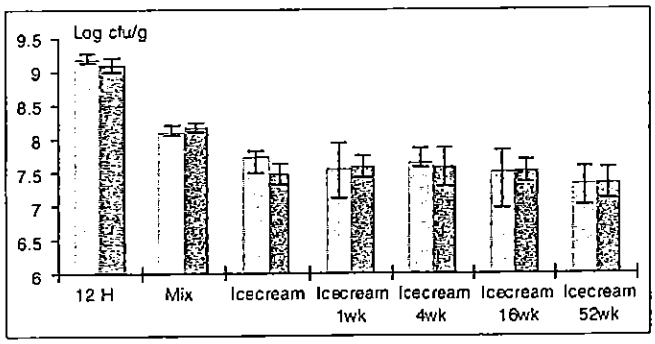

Fig. 5: Viable counts of *Lb. reuteri* (LR); without glycerol, ▨ with glycerol

The decline in bacterial numbers, due to the freezing step, is most likely to be due to the actual freezing of the cells, resulting in the death of some cells. However, the mechanical stresses of the mixing and freezing process and also the incorporation of oxygen into the mix, may have resulted in a further decrease in bacterial count. The viable number did not change significantly ($p<0.05$) throughout 1 year of storage at –20°C and all were above the recommended minimum limit of 10^6 cfu/g. HEKMAT and McMAHON (2) found that both *B. bifidum* and *Lb. acidophilus* were able to survive in frozen yoghurt.

The addition of 2% glycerol did not improve the survival of the strains, but frozen ice cream contains various natural substances with cryoprotective properties. These include for instance casein, sucrose and fat. Glycerol would probably have to be added in the range of 10–12% to have any effect. However, such an amount would then negatively affect the consistency of the ice cream.

Table 1: Sensory evaluation of Ice cream with and without the addition of glycerol

Culture		Probiotic flavour	Off-flavour	Sourness	Firmness	Chewiness	Iciness	Total impression
Ice cream without the addition of glycerol	BB-12	3.07	1.8	2.27	3.67	3.13	1.13	4.93
	GG	2.4	1.4	2.13	3.27	2.47	1.2	4.6
	LA-5	3.27	1.6	2.13	3.67	2.87	1.2	4.87
	LR	4	1.93	2.33	3.87	2.87	1.4	4.8
	Control	3.33	1.47	2.07	3.47	2.73	1	4.93
Ice cream added glycerol	BB-12	3.07	1.47	2.53	2.6	2.53	1.77	4.8
	GG	2.8	1.67	2.4	2.73	2.73	1.6	4.8
	LA-5	3.87	1.8	2.27	2.93	2.73	1.6	4.13
	LR	4.47	1.8	3.13	2.73	2.7	1.73	4.6
	Control	3.73	1.73	2.73	2.47	2.8	1.65	4.87

Table 2: Concentration of aromatic volatile compounds and organic acids in fresh ice cream, with and without the addition of glycerol

Culture		Acetaldehyde	Ethanol	Diacetyl	Acetoin	Citric acid	Lactic acid	Acetic acid
Ice cream without the addition of glycerol	BB-12	1.45	16.55	–	–	2317.52	522.25	839.36
	GG	0.23	15.56	–	–	2263.77	509.64	806.53
	LA-5	1.94	6.34	–	–	2057.75	957.19	695.11
	LR	0.47	1161.88	–	–	2325.30	419.27	653.03
Ice cream added glycerol	BB-12	1.19	20.63	–	–	2285.04	398.35	654.70
	GG	0.29	18.19	0.54	–	2376.37	714.63	660.99
	LA-5	1.91	10.63	–	–	2260.00	794.5	738.11
	LR	0.12	986.91	–	–	2150.67	961.3	839.95

– The aromatic compound was not detected in the sample; all the concentrations are given in ppm

Using this technology, an ice cream with a pH around 6.1–6.3 was obtained. Although the pH of the added fermented milk was between 4.1 (LR) and 5.2 (LA5), the pH of the total mix was high, due to the high buffering capacity of the ice cream mix. To a certain degree, this would suppress the probiotic flavour. HEKMAT and McMAHON (2) found the preferred pH of probiotic ice cream, based on overall acceptance to be 5.5. The pH can be reduced by adding different amounts of fermented milk to the ice cream mix. CHRISTIANSEN *et al.* (1) made batches of experimental ice cream mix by adding from 25 to 50% of commercial cultured milks, fermented with *Lb. acidophilus* and *B. bifidum*.

3.2 Sensory evaluations

A "probiotic" taste was not found to be particularly noticeable. One reason for this could be the high pH of the ice cream. Except for ice cream supplemented with glycerol and the probiotic strain LR, the organoleptic evaluations were not significantly affected ($p<0.05$) by the addition of probiotic cultures. *Lb. reuteri* containing ice cream was judged to be significantly more sour and attained a higher score for probiotic flavour than the other ice cream samples.

All the samples gave a good total impression, were medium sour and did not have any marked off-flavour. As expected, firmness decreased slightly with the addition of glycerol. None of the ice creams were judged to be icy and all had a low score for chewiness (Table 1).

The 4 probiotic strains used in this study produced various flavour compounds in fortified UHT milk which was added to the ice cream mix. However, these compounds were diluted by a factor of 1:10 and therefore did not give any marked influence on the organoleptical quality of the ice cream (Table 2).

4. Conclusions

Probiotic bacteria can be cultured for inclusion in ice cream, in UHT milk fortified with 1% glucose and 1% tryptone.

Viable numbers of the studied probiotic strains remained above 10^6 cfu/g during 52 weeks storage at –20 °C.

The ice cream obtained high scores during the sensory evaluation.

Acknowledgements

The authors gratefully acknowledge the gift of cultures from Ivan Casas, Biogaia Biologics (Lb. reuteri), Valio (Lb. rhamnosus «GG») and Christian Hansen Ltd (Lb. acidophilus LA5 & B. bifidum BB-12).

This work has been partially financed by the Norwegian Research Council.

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6. Summary

HAGEN, M., NARVHUS J.A.: **Production of ice cream containing probiotic bacteria.** Milchwissenschaft **54** (5) 265–268 (1999).

63 Ice cream (probiotic bacteria)

Ice-cream containing probiotic bacteria was produced by mixing fortified milk fermented with probiotic strains with an ice-cream mix, followed by freezing.

Four different strains of probiotic bacteria were used. Each strain was grown (37 °C, 12 h) in UHT semi-skimmed milk fortified by the addition of 1% glucose and 1% tryptone. The fermented milk was added as a 10% addition to an ice-cream mix. The complete mix was frozen in a soft-ice freezer and hardened at ÷20 °C. Ice-cream mixes with and without the addition of glycerol were produced to ascertain whether this had a protective effect against freezing of the probiotic bacteria. Viable counts of probiotic bacteria were made immediately after mixing and after freezing, as well as after 1, 4, 16 and 52 weeks of storage at –20 °C. The ice cream samples were organoleptically assessed for probiotic flavour, firmness, chewiness. sourness, off-flavour, iciness and total impression. During freezing, or shortly afterwards, the viable count declined by 0.7-0.8 log cfu/g. The viable count did not change significantly during 52 weeks of frozen storage ($p<0.05$) and remained above the recommended minimum limit of 10^6 cfu/g. The incorporation of glycerol in the ice-cream did not improve the survival of the strains. All the ice-cream samples received a high score in the organoleptic evaluation; the probiotic taste was not found to be particularly noticeable. *Lb. reuteri* containing ice-cream was significant more sour and attained a higher "probiotic flavour" than the other ice-cream samples.

HAGEN, M., NARVHUS J.A.: **Herstellung von Speiseeis mit zugesetzten probiotischen Bakterien.** Milchwissenschaft **54** (5) 265–268 (1999).

63 Speiseeis (probiotische Bakterien)

Die Eiskrem mit Zusatz von probiotischen Bakterien wurde durch Mischen fermentierter Milch mit einer normalen Eiskremmischung hergestellt.

Die Milch wurde mit 4 unterschiedlichen probiotischen Bakterienstämmen fermentiert. Jeder Stamm wuchs bei 37 °C 12 h in fettarmerUHT-Milch, der 1% Glukose und 1% Trypton zugesetzt waren. 10% fermentierte Milch wurde der Eiskremmischung zugesetzt, bevor die Mischung in einer Softeismaschine gefroren und bei –20 °C gehärtet wurde. Es wurde Eiskrem mit und ohne Zusatz von Glycerin hergestellt, um zu festzustellen, ob Glycerin die Keime beim Gefrieren schützt. Die Keimzahl wurde sowohl direkt nach dem Mischen als auch nach dem Gefrieren und nach einer Lagerung von 1, 4, 16 und 52 Woche(n) bei einer Temperatur von –20 °C festgestellt. Die Eiskrem wurde sensorisch beurteilt nach den Parameteren "probiotischer Geschmack", Festigkeit, "Kauverhalten", Säuregrad, Beigeschmack, Kälteempfinden und Gesamteindruck. Beim Einfrieren oder direkt danach sank die Keimzahl um 0,7–0,8 log KbE/ml, während der 52 Wochen langen Gefrierlagerung sank die Keimzahl nur unwesentlich ($p<0,05$) und blieb oberhalb des empfohlenen Minimums bei 10^6 KbE/ml. Der Zusatz von Glycerin hatte keinen Einfluß auf die Keimzahl in der Eiskrem. Alle Eiskremproben erhielten keine gute organoleptische Beurteilung; der „probiotische" Geschmack war nicht sehr spürbar. *Lb. reuteri* ergab ein signifikant saureres Eis, mit einem deutlicher "probiotischen Flavour" als die anderen Proben.

A fast and simple method to determine the whey powder to milk powder ratio using spectroscopy in alkali

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1. Introduction

In 1987 LUF and BRANDL (1) proposed a method based on the second derivative UV spectroscopy to determine the whey protein to total milk protein ratio. In 1995 MEISEL developed a method to determine the whey protein and casein in total milk protein using fourth derivative spectroscopy (2). DE JONG and OLIEMAN (3) recently carried out a study on the applicability of second derivative spectroscopy to determine the serum protein to casein ratio. These spectroscopic methods are based on differences in the UV spectrum between tryptophan and tyrosine, as well as on differences in the tryptophan to tyrosine ratio which is 0.19 for casein and 0.59 for whey proteins.

DE BLOCK *et al.* (4) recently presented a method to determine the whey powder to milk powder ratio based on spectroscopy in alkali. Whereas the zero order spectra of tryptophan and tyrosine overlap at neutral pH, the spectrum of tyrosine at alkaline pH shifts towards a longer wavelength and the absorption is duplicated as a result of the ionisation of tyrosine (Fig. 1). Thanks to the enhanced resolution between both spectra, a method was developed based on the zero order and first order spectra. Since a formula is used for the standard curve by which results are presented as a ratio of absorption differences, the results are not influenced by the protein concentration and are affected only to a small extent by background absorption or light scattering. The European Union is planning to introduce a spectroscopic method to determine the whey protein to total milk protein ratio. This paper presents a reliable, simple and fast method based on the method of DE BLOCK *et al.* (4). Essentially, this method has been simplified by changing 3 aspects:

1) the precipitation of proteins by trichloro-acetic acid (with subsequent washing steps) is replaced by a Röse-Gottlieb type fat extraction (5): such a fat-removing step is very important, since fat globules cause undesirable light scattering,