

# PROBIOTIC FOOD SUPPLEMENT FORTIFIED WITH CALCIUM, MAGNESIUM, AND ZINC LACTATES

M. Beran, L. Adámek, P. Molík, P. Hanák

Food Research Institute Prague, Radiová 7, 102 31 Prague 10, Czech Republic

## INTRODUCTION

The ingestion of probiotics is associated with various beneficial effects on human health and modifies the physiological homeostasis of the intestinal flora.

Probiotics are microorganisms that block the invasion of human and animal intestinal cells by the enteroinvasive bacteria. Furthermore, they should be able to stimulate and modulate the intestinal immune response, and to protect and stabilize the mucosal barrier. Finally, it has been suggested that such microorganisms can play a role in the prevention of carcinogenesis and of tumor growth. Probiotic food supplements should contain more than  $1 \times 10^9$  CFU.

Calcium with magnesium and zinc is necessary for proper bone development, muscle function, reproduction, immune response, nervous system health, blood pressure, wound healing, and energy production. These elements play essential roles in maintaining proper bone mineralization. The epidemic of osteoporosis has created a major market for calcium and magnesium supplementation.

Lactate metal salts are commonly used in food supplements because of their high bioavailability. Bioavailability of calcium lactate form is comparable to the milk calcium complex.

## EXPERIMENTAL

### Microbial culture

*Lactobacillus acidophilus*, FRIP Collection of Microorganisms

### Fermentation conditions

Bacteria were cultivated in a sterile medium containing 10 or 20 % (w/v) of sweet whey (Kunin Dairy, Czech republic) under anaerobic or microaerophilic conditions at 37°C. Inoculum was prepared by passaging the bacterial culture in 10 times diluted sterile defatted milk during 48 hours and used for inoculation of the sterile whey medium (50 ml per liter of medium). During the fermentation, acidification of the media takes place because of lactic acid formation. The medium pH value was kept at 5.5 by periodical additions of 10 - 20% (w/v) solutions of  $\text{CaCO}_3$ ,  $\text{MgCO}_3$ , or  $\text{ZnO}$ . Lactate salts of the corresponding cations were formed during lactic acid neutralization. Fermentations were finished after 48 - 72 h and the complete medium was dried by spray drying or freeze drying with or without addition of a drying carrier. Dried sugar beet fiber (SINECAL, Czech Republic) and defatted milk were used as carriers and cryopreservative agents. Doses of 300 - 500 g of SINECAL per liter of medium were used for freeze drying and 300 ml of defatted milk per liter of the medium was used for spray drying or freeze drying.

### Storage experiments and determination of bacterial vitality

Dried samples of the fermented media were stored at 5 or 20°C in a refrigerator or thermostat.

Bacterial vitality was determined by a standard method of counting of CFU in test tubes using MRS agar M641 (HiMedia Laboratories).

### Determination of lactate concentration in the dried products

To determine lactate concentration Gel Permeation Chromatography (GPC) was used under following conditions:

TSK gel GMPW column; 7.5 x 300 mm (Supelco); UV-VIS photodiode array detection; eluent: 0.02M  $\text{Na}_2\text{HPO}_4$  + 0.5M NaCl; flow rate: 0.5 ml.min<sup>-1</sup>; injection: 20 l.

Samples of the dried media were extracted by water under standard conditions and filtered by nylon microfilter (TESSEK Ltd., Czech Republic) before injection.

## RESULTS and DISCUSSION

Table 1 shows doses of  $\text{Zn}^{2+}$ ,  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$  added into the 10% whey medium in the form of carbonate salts of calcium and magnesium and zinc oxide to achieve respective lactate salts by neutralization of lactic acid produced during the fermentation. Total inhibition of the lactic acid production was repeatedly observed at Zn concentrations above 35 g.l<sup>-1</sup>. No similar inhibition was observed when  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  cations were used. Dried preparations of the complete media contained up to 255 mg.g<sup>-1</sup> of Zn, 109 mg.g<sup>-1</sup> of Ca, and 78.9 mg.g<sup>-1</sup> of Mg. More than 90% of the cations in the preparations were in the lactate form as determined by GPC method in connection with atomic absorption spectrometry.

Fast declines of bacterial vitality in several days were observed with preparations dried without a carrier or with the sugar beet fiber SINECAL (results unshown).

Table 2 shows comparison of vitality of *Lactobacillus acidophilus* culture in probiotic preparations fortified with  $\text{Zn}^{2+}$ ,  $\text{Ca}^{2+}$ , or  $\text{Mg}^{2+}$  and dried by spray drying or freeze drying with additions of defatted milk. No significant declines of the bacterial vitality were observed even after 185 days of storage at 5°C. The results were independent of the drying method. Bacterial vitality in the samples dried with additions of defatted milk and stored at 20°C was also stable at least for 40 days (results unshown).

**Table 1**

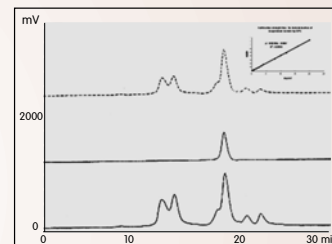
Fermentation of 10% whey medium by *Lactobacillus acidophilus* culture with fortification by  $\text{Zn}^{2+}$ ,  $\text{Ca}^{2+}$ , or  $\text{Mg}^{2+}$  cations

Time of cultivation (h)	Total dose of cation (g / l)		
	$\text{Zn}^{2+}$	$\text{Ca}^{2+}$	$\text{Mg}^{2+}$
7	23.05	1.29	1.17
24	36.70	4.58	4.92
32	36.70	8.26	7.35
48	36.70	12.85	10.85
Bacterial biomass (g of DM / l)	145.70	118.00	137.50
Cation concentration (mg/g of DM)	255	109	78.9

**Table 2**

Comparison of vitality of *Lactobacillus acidophilus* culture in probiotic preparations fortified with  $\text{Zn}^{2+}$ ,  $\text{Ca}^{2+}$ , or  $\text{Mg}^{2+}$  and dried by spray drying or freeze drying with addition of defatted milk

Storage time (5°C) (days)	Dried probiotic preparation fortified with:					
	Zn (68.7 mg/g)		Ca (27.4 mg/g)		Mg (28 mg/g)	
	Drying method:					
	spray drying	freeze drying	spray drying	freeze drying	spray drying	freeze drying
	CFU / g	CFU / g	CFU / g	CFU / g	CFU / g	CFU / g
7	-	-	$6 \times 10^8$	-	$6 \times 10^8$	-
11	-	$1 \times 10^9$	-	$4 \times 10^7$	-	$5 \times 10^8$
42	$2.7 \times 10^9$	$1.6 \times 10^9$	$1.1 \times 10^7$	$1.1 \times 10^7$	$1 \times 10^7$	$1 \times 10^8$
90	$2.8 \times 10^9$	$2 \times 10^9$	$4 \times 10^8$	$1.5 \times 10^7$	-	$1.9 \times 10^8$
117	$2.7 \times 10^9$	$1.1 \times 10^9$	$1 \times 10^7$	$9 \times 10^6$	$1.7 \times 10^8$	$1.2 \times 10^8$
185	$2.8 \times 10^9$	$1.7 \times 10^9$	$4.5 \times 10^8$	$1 \times 10^7$	$1.4 \times 10^8$	$2.1 \times 10^8$



**Fig 1:**

Identification of lactate peak in GPC chromatograms and calibration straight line for magnesium lactate determination

Lower chromatogram: fermented whey medium fortified with  $\text{Mg}^{2+}$  cations

Middle chromatogram: standard sample of magnesium lactate

Upper chromatogram: fermented whey medium fortified with  $\text{Mg}^{2+}$  cations with a standard addition of magnesium lactate



**Fig 2:**

Tablets of the probiotic food supplement fortified with calcium, magnesium, and zinc lactates

## CONCLUSIONS

A simple technological process of production of a probiotic food supplement containing up to 255 mg.g<sup>-1</sup> of Zn, 109 mg.g<sup>-1</sup> of Ca, and 78.9 mg.g<sup>-1</sup> of Mg in the lactate form and more than  $1 \times 10^9$  of CFU of vital bacterial culture *Lactobacillus acidophilus* was described. Bacterial vitality can be preserved during a long - termed storage. The proposed technological process has already been verified by a pilot plant experiment.

The dried product can be processed into tablets, gelatin capsules or added into instant drinks and different food products.

This work was supported from the National Agency for Agricultural Research (project QF3285).