Development of a Probiotics Rich Yogurt Dry Mix

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ABSTRACT

Background.

The probiotic *Lactobacillus acidophilus* NCFM has been scientifically researched to promote health beneficial effects in humans when consumed in sufficient numbers (≈10^7 cfu.mL^{-1}). The incorporation of the NCFM strain in foods has been widely applied world-wide, mainly in liquid fermented milks. Probiotics and other microorganisms can remain viable in high concentration when present in liquid or high water activity products. Products with high water activity have relatively low shelf-life, particularly at ambient temperature. This has generated international interest to investigate the survival of probiotics in low water products. This challenge forms the basis of the current study on the survival of probiotics in dehydrated yogurt mixes. The development of dehydrated food bases, such as yogurt dry mixes has created opportunities for the delivery of probiotics. Such products bring convenience to the consumer as they give flexibility to preparation and the quantities prepared. However, probiotics are sensitive to environmental factors such as water activity, oxygen, and storage temperature; and little is known about their survival mechanisms in dehydrated food systems. Therefore, the aim of this study was to develop probiotic-rich dehydrated yoghurt bases (DYB) with shelf-life of up to 18 months in modified-atmosphere packaging when stored at ambient temperature. The stability of the ready-to-eat (RTE) yogurt during refrigerated storage was also investigated.

Materials and Methods

Milk powder characterization

The degree of whey protein (α-lactalbumin & β-lactoglobulin) denaturation was analysed using the dye-binding method at 615 nm and by HPLC (GF-250 column equipped with UV detector at 280 nm) set at 30 °C. Standard and the NIR methods (800-2500 nm) were used to analyse the levels of fat (gravimetric), moisture (oven drying), and protein (Kjeldahl).

Selection lactic starter cultures and probiotic strain

To determine the suitability of the lactic acid bacteria (*Streptococcus thermophilus* (ST); *Lactobacillus bulgaricus* (LB); probiotic *Lactobacillus acidophilus* (LA) NCFM) used for the development of the DYB, growth kinetics of the cultures were conducted using a 96-well plate reader at 595 nm. Of the freshly prepared (18-24 h) stock cultures, 15 µl (10^{-1} to 10^{-7} dilution) and 135 µl of respective broths were dispensed into the 96 wells and allowed to grow anaerobically at 37°C for 24 h. The lactic acid bacteria (LAB) growth kinetic profiles at various initial inoculation rates (1, 2, 3%) of cultures used in 10% milk medium for 8 h at 43°C were conducted using viable counts. The M17 and MRS+clindamycin agar/broth were used to enumerate ST, NCFM, and the difference between Man de Rogosa (MRS) & MRS+clindamycin medium was used to estimate the levels of LB.

Characterization of DYB

Full factorial 2^3 experimental design was applied to develop twelve DYB formulations containing fat (1.4 & 3.5%), total sugar (15.4 & 14.4%), flavour (natural and strawberry). The DYB formulations were blended using the ribbon-type blender and then packaged in PE/foil/PET & PE/foil/nylon/PET packages. The DYBs were blended thoroughly and packaged under 100% N_2. Viable cell counts the LAB (NCFM, ST and LB), [O_2] and a_w at 20°C were analysed at intervals of three weeks for 9 weeks. Of the 12 formulations initially developed, 3 of them (formulations) with high LAB counts (>10^6 cfu/g), low a_w (<0.15) and [O_2] (<16%) were selected for further characterization and fermentation of yogurt. The first order kinetics was used to monitor the changes in the cell counts of LAB

in the DYB at various storage temperatures (22, 35, 45, 55°C). The results were then used to predict its survival at 4 and 22 °C using Arrhenius law.

Characterisation of liquid yogurts

The selected formulations were high fat high sugar (HFHS), high fat natural (HFN), and low fat low sugar (LFLS). The three formulations were fermented at 43°C for 8 h to produce yogurt and stored at 4°C for 2 weeks , during which analyses of viable cell counts, titratable acidity (% lactic acid), texture (N), viscosity (mPa.s), and syneresis (%) were conducted. pH measurement was conducted in the products and consumer acceptance using the 9-point hedonic scale was also conducted.

Results and Discussion

The protein, fat, and moisture contents of skim milk powder (SMP) were ±36%, <1%, and <4%; while for whole milk powder (WMP) were ±26%, ±28, and 2.9%. The undenatured whey protein was <2% using HPLC and <3 mg/g using dye-binding method for both powders.

The three strains of bacteria grew appreciably in milk and broth media, which followed sigmoidal growth in the latter medium. The growth profile of NCFM during fermentation in the absence and presence of ST and LB was comparable in broth and in reconstituted milk media indicating that the bacteria could be used together.

The a_w and $[O_2]$ in selected DYB formulations were <0.15 and <16% respectively, which may play a crucial role in maintaining the NCFM, ST, and LB at $>10^8$, $>10^6$, and $>10^7$ cfu/g. No significant difference ($p>0.05$) between packages was observed during storage as shown by comparable $[O_2]$ throughout storage. In its liquid form, concomitant increase of sigmoidal LAB growth (up to 4 logs) and acidity (pH 6.5 to 4.4) was observed during fermentation. The texture, viscosity and syneresis index were comparable during 2 weeks storage at 4°C; where low fat yogurt performed better than yogurt containing higher fat contents. Meanwhile, the loss of LAB counts as a result of acid accumulation (pH 4.55-4.2; lactic acid 0.7-1.5%) throughout refrigeration storage was observed. The LAB cell counts however were still maintained at $>10^7$ cfu/mL after 2 weeks.

Flavour, sweetness, and sourness were the main descriptors that drive consumer acceptance using Principal Component Analysis (PCA). Based on the cluster analysis, 62% (n=77) of consumer panellists showed clear differences in sample acceptability with 57% of the panellists indicating their likeness for samples HFHS, LFLS, and HFN. The shelf life of selected DYBs was >18 months at 4°C. The LAB survival, particularly ST, was markedly reduced at elevated temperature showing survival rates of $\geq 10^5$ cfu/g after 6, 10, and 14 months for LFLS, HFN, and HFHS, respectively at 22°C.

Conclusion

The cultures used in the current study were stable in the DYBs and liquid yogurts for the formulations used. The products were liked by consumer panellists with predicted storage life of up to 14 months at 22°C.

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LIST OF ABBREVIATION

ANOVA	Analysis of variance
ATP	adenosine triphosphate
A_w	Water activity
B	Bifidobacterium
cfu	Colony forming units
CO_2	Carbon dioxide
DE	Dextrose equivalent
DNA	Deoxyribonucleic acid
DYB	Dehydrated yogurt base
EC	European Committee
EFSA	European Food Safety Authority
ETC	Electron Transport Chain
EU	European Union
FDA	Food and Drug Association
FOSHU	Food for Specialized Health Use
FSANZ	Food Standard Australia New Zealand
GI	Gastrointestinal
GLM	General linear model
GMP	Good manufacturing practice
h	Hour
H_2O_2	Hydrogen peroxide
HACCP	Hazard analysis critical control point
k	Rate constant
IBS	Irritable Bowel Syndrome
LA	*Lactobacillus acidophilus*
LAB	Lactic acid bacteria
LB	*Lactobacillus bulgaricus*
MRS	Man de Rogosa
MSNF	Milk solid non-fat
NCFM	North Carolina Food Microbiology
NZFSA	New Zealand Food Safety Authority

N_2	Nitrogen
NADH	Nicotinamide Adenine Dinucleotide
O_2	Oxygen
Pa	Pascal
PCA	Principal Component Analysis
RSM	Reconstituted skim milk
RTE	Ready-to-eat
s	Seconds
S.D	Standard deviation
S.E	Standard error
SMP	Skim milk powder
ST	*Streptococcus thermophilus*
WMP	Whole milk powder
WPNI	Whey protein nitrogen index

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