

Production of concentrated yoghurt culture using whey based media

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Abstract

In this investigation, growth performance of yoghurt culture was studied in a developed whey based medium (WBM) for production of cultures biomass and preservation in concentrated dried form. Growth performances (K) of yoghurt cultures in WBM were 0.765 and 0.723 for *S. thermophilus* and *L. bulgaricus*. Culture biomass produced was harvested by centrifugation, re-suspended in freeze-drying medium followed by freeze drying. Viable counts of freeze dried cultures were 11-12 log cfu/ g. Dry culture was packed in storage vials and stored at $-20\pm 1^{\circ}\text{C}$ and viable counts were in the range of 11 to 12 log cfu/g. Physiochemical, microbiological and sensory qualities of yoghurt prepared using concentrate preserved culture and fresh propagated culture were comparable under refrigeration conditions till 5 days of study.

Keywords: Yoghurt culture, DVS culture, whey based media, freeze drying

Yoghurt is a smooth, fermented milk product that evolved empirically some centuries ago and nowadays, it is a widely consumed fermented milk product popular in all parts of world. Yoghurt is produced through lactic acid fermentation of milk by thermophilic lactic cultures i.e. combination of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, fat content ranges from 0 to over 4% and is also a means of pre-serving the nutrients in milk (Chandan, 1989; Hui, 1992). It has a characteristic acidic taste possessing 0.95-1.50% lactic acid and pH ranging from 3.7-4.2 with viable and abundant fermenting microorganisms. It is one of the most popular fermented milk products in the world and produced commercially as well as home (Willey *et al.* 2008). In its commercial production, non fat or low fat milk is pasteurized cooled to 43°C and are inoculated with known cultures of

microorganisms referred to as starter cultures i.e. mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in a 1:1 ratio. The coccus which is the *Streptococcus thermophilus* grows faster than the Rod which is the *Lactobacillus bulgaricus* and is primarily responsible for acid production while the rod adds flavor and aroma. The growth of these microorganisms causes the transformation of milk's sugar, lactose into lactic acid. This process gives yoghurt its texture. The associative growth of the two organisms results in acid production at a rate greater than that produced by them individually (Robinson, 2002).

Streptococcus thermophilus grows more rapidly than *Lactobacillus bulgaricus* initially and begins to produce lactic acid. Lactic acid production results in a decrease in the pH of the medium. While *Streptococcus thermophilus* grows, it releases CO₂ from the breakdown of urea and formic acid. *Streptococcus thermophilus* depletes the oxygen in the medium and this causes the oxidation-reduction potential more favorable for the growth of *Lactobacillus bulgaricus*. The increased acidity, CO₂, formic acid and depletion of O₂ stimulates the growth of bacilli which is more acid tolerant than *Streptococcus thermophilus*. Besides having a stimulatory effect on bacilli, the growth of *Streptococcus thermophilus* depends on the growth of *Lactobacillus bulgaricus*. *Lactobacillus bulgaricus* has higher proteolytic activity than *Streptococcus thermophilus*. The proteolytic enzymes of *Lactobacillus bulgaricus* degrade casein with the liberation of low molecular weight peptides and amino acids which have stimulatory effect on the growth of *Streptococcus thermophilus* (Rajagopal and Sandine, 1990). Both two microorganisms can grow at a temperature of 42-43°C, and the optimum temperature for symbiotic growth is 42°C. Since the optimum growth temperature for *Streptococcus thermophilus* is 37°C and 45°C for *Lactobacillus bulgaricus*, increasing the temperature above from 42°C, the growth of lactobacilli will be favored while the temperatures below 42°C results in increased growth of streptococci. Either case is resulted in a deviation in the ratio of cocci to bacilli, for the optimum yoghurt the ratio should be 1:1 (Shah, 2003).

Direct Vat Set (DVS) starters are used for preparing good quality products, primarily organized dairy sectors over propagated fresh cultures. DVS, concentrated active starters (10¹¹- 10¹² viable cells per gram) are available as freeze dried and frozen forms. These cultures are inoculated directly into the vat without intermediate sub-culturing. Advantages are improved quality fermented milk products, fewer rejections, ease of use and reliability. It avoids investment for Mother cultures lab, bulk starter equipment and laboratory facilities, enable to produce much variety of products. DVS culture also makes it easier to adapt to short term changes in market demand and provides flexibility in production process also. Direct vat cultures are carefully selected strains of frozen concentrated or concentrated freeze-dried cultures which can be added directly to the milk with no intermediate growth step. Direct vat cultures are also known as starter cell concentrates, Direct Vat Set (DVS)

Direct Vat Inoculant (DVI). These are concentrated cell preparations containing cells in the order of 10^{11} - 10^{13} CFU/g. DVS are available in two forms - Freeze dried granular form & Deep-frozen pellets. Present study reports the preparation of yoghurt culture biomass in a developed whey based medium and preservation as concentrated DVS form by freeze drying.

Materials and Methods

The candidate strains of yoghurt culture NCDC 144 i.e. *Lactobacillus bulgaricus* NCDC 09 and *Streptococcus thermophilus* NCDC 74 were procured from National Collection of Dairy Cultures (NCDC), Dairy Microbiology Division, ICAR-National Dairy Research Institute, Karnal as freeze dried ampoules and cultures were activated in Chalk litmus milk 42°C /15-18 h and sub-cultured in skim milk and stored at 5-7°C. Before using for experiments, cultures were activated by 2-3 transfers in MRS, M17 broth at 42°C for 8-12 h, respectively.

Preparation of cell biomass in whey based medium

Lactobacillus bulgaricus NCDC 09 and *Streptococcus thermophilus* NCDC 74 cell biomasses were prepared in a developed whey based medium separately. Whey based medium (two flasks, 200 ml each) was prepared as per the composition standardized in our laboratory and sterilized by autoclaving (121°C for 20 min). *Lactobacillus bulgaricus* NCDC 09 and *Streptococcus thermophilus* NCDC 74 was inoculated (@1% active culture) in whey based medium flasks separately followed by incubation 42°C for 10 h. Cell biomass was harvested from the cultured medium by centrifugation (10,000 rpm, 10 min, 4°C).

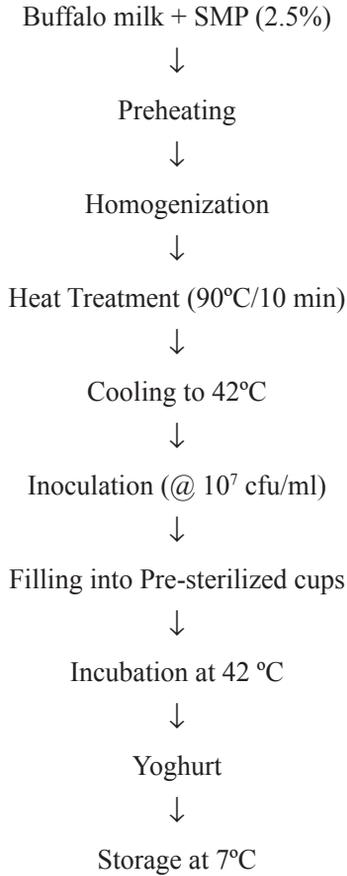
Preparation of DVS yoghurt cultures by freeze drying

Cell pellet was re-suspended in a freeze drying medium was preserved by freeze drying. Cell pellets harvested separately were suspended in 10 ml of cryo-protective medium (2% sodium glutamate in skim milk). Then, the contents were mixed, poured in sterile petridish and kept at -20°C over night for freezing. The frozen content in petridish was dried by freeze drying at -45°C under vacuum. The dried material was crushed with sterile spatula to convert into powdered form, packed in cryovials and stored at -20°C.

Evaluation of quality of DVS culture

Total viable lactic count in DVS culture was enumerated by plating on MRS agar (42°C for 24-48 h).

Buffalo whole milk was used for preparation of yoghurt. The product was prepared according to the method described by Tamine and Robinson, (2004) with suitable modifications.



Flow diagram for preparation of Yoghurt

Analysis of yoghurt

Yoghurt prepared with fresh and DVS culture was analyzed for sensory, physico-chemical and microbial analysis at 0 and 5 day of storage.

pH

pH of the sample was measured at 20°C with the help of pH meter (Thermo pH meter).

Titrateable acidity (AOAC, 2007)

Sample (20ml) was transferred in beaker (100ml) and 3 to 5 drops of phenolphthalein was added to sample and titrated against N/10 NaOH solution with continuous stirring till faint pink color persists. The volume of NaOH solution required was measured and titrateable acidity (% lactic acid) was calculated as follows:

% lactic acid = $\frac{9 \text{ NV}}{\text{W}}$ grams of sample

Where-

V = volume of N/10 NaOH required (ml) and

W = volume of sample taken for analysis (20 ml)

N = normality of alkali used for neutralization

Microbial analysis

Yoghurt was serially diluted with normal saline (0.85% NaCl solution). Further total lactic count, coliform count and Yeast and mould counts were enumerated (log cfu/g). Total lactic counts were enumerated by plating on MRS agar (42°C for 24-48 h). Total coliform counts were enumerated by plating on VRBA (37°C for 24 h). Total Yeast and molds counts were estimated by plating on PDA (25°C for 3-5 days).

Sensory analysis

Yoghurt samples prepared with fresh active and freeze dried concentrate cultures were evaluated for sensory attributes such as color and appearance, body and texture, acidity, flavour and overall acceptability on nine-point hedonic scale. A panel of five discriminative and communicative judges was selected from the faculty of Dairy Microbiology Division, Dairy Technology Division of NDRI, Karnal for sensory analysis.

Results and Discussion

Growth in Whey Based Medium

Growth performance of individual yoghurt cultures was studied in formulated whey based medium for evaluation of suitability in biomass production. Growth performance of culture were studied in whey based medium by inoculating the active culture (1%) and incubated at 42°C for 10 h. The viable counts were reached to approximately 8-9 log cfu/ml. Growth of *S. thermophilus* in whey based medium was higher in comparison to *L. bulgaricus* (Table 1). Cells of *Streptococcus thermophilus* colonies were small and white and *Lactobacillus delbrueckii* ssp. *bulgaricus* colonies were large and white and have a white cloudy zone.

Table 1: Growth of culture in whey based medium

Cultures	Viable counts (cfu/ ml)	
	0 h	10 h
<i>Streptococcus thermophilus</i> NCDC 74	1.30×10^7	2.60×10^9
<i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i> NCDC 09	1.60×10^7	2.40×10^9

Preservation of yoghurt culture

Whey based media (200 ml) was prepared and inoculated with 2% of yoghurt mix culture and then incubated for 10 h at 42°C, biomass were harvested by centrifugation (10000 rpm for 10 min at 4°C). Biomass was re-suspended in cryoprotective solution and then the culture was preserved by freeze drying -40±1°C. Pre-freezing was done at -20°C for overnight. Dried culture powder was packed in cryo-vials stored at -20±1°C. Prepared freeze dried cultures were preliminary examined for total viable count and curd setting time in skim milk (Fig. 1). The total viable count obtained is in range of 11-12 log cfu/ml and the curd setting time was observed at 6-7 h.

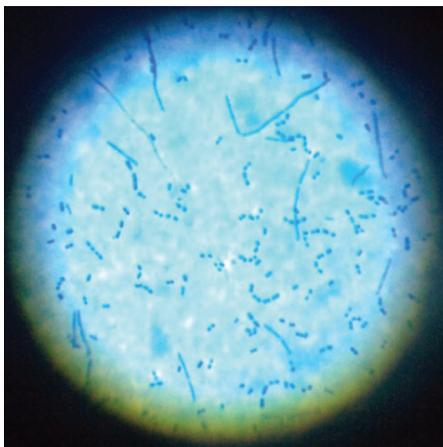


Fig. 1: Morphology of yoghurt culture

Evaluation of preserved yoghurt cultures in preparation of yoghurt

Yoghurts were prepared using fresh culture and freeze dried concentrated culture. The yoghurt samples were stored under refrigeration conditions and were evaluated for sensory, physicochemical and microbiological analysis at 0 and 5day of storage in order to assess the suitability of preserved cultures in preparation of yoghurt as compared to fresh culture.

pH of product prepared form fresh culture was lower (4.80) than DVS culture (4.9) and acidity were 1.20 and 1.10% lactic acid, respectively. There is gradual decrease in pH and increase in acidity during 5 days storage (Table 2).

Table 2: Physicochemical characteristics of yoghurt prepared with fresh and preserved cultures during storage

Parameter	Storage (day)	Fresh culture	Preserved culture
Acidity (%LA)	0	1.20	1.10
	5	1.25	1.15
pH	0	4.80	4.90
	5	4.60	4.50

Yogurt by nature is a high-acid (low pH) product and is therefore inherently protected against defects caused by most contaminating organisms. Yoghurt stored under refrigerated conditions was analyzed for microbial counts such as coliforms, yeast and molds and lactic acid bacteria. Counts on MRS and M17 agar media

reveals that total lactic counts in yoghurt samples were in the range of 8.0 to 9.0 log cfu per gram. The counts in yogurt prepared with preserved concentrated cultures were comparable to yoghurt prepared using fresh propagated cultures. There was a slight increase in counts after 5 days storage for all the yoghurt samples (Table 3). Coliforms; yeast and moulds were less than 10 cfu per gram in all the yoghurt samples. Bulk cultures may be prepared separately from pure strains or frozen concentrates may be added directly to the mix. The latter eliminates the need to maintain culture transfer facilities (Kosikowski and Mistry, 1997). Yoghurt culture which is a mixture of *Lactobacillus delbrueckii* spp. *bulgaricus* and *Streptococcus thermophilus* used for the preparation of yoghurt for consumer acceptance and storage studies.

Table 3: Microbial analysis (Lactic acid bacteria count) of yoghurt samples]

Storage (day)	Fresh culture		Preserved culture	
	MRS	M17	MRS	M17
0	7.0×10^8	8.0×10^8	7.6×10^8	8.5×10^8
5	10.0×10^8	13.0×10^8	11.0×10^8	11.8×10^8

All the yoghurt samples were evaluated for sensory attributes such as color and appearance, body and texture, acidity, flavour and overall acceptability on a nine-point hedonic scale during storage of 5 days. During product storage of 5 days at $7 \pm 1^\circ\text{C}$ color and appearance, body and texture, acidity and flavor of yoghurt prepared from control and DVS culture is in the range of like moderately to like very much over the storage of DVS culture. Product prepared from the control and DVS culture having similar overall acceptability during storage of 5 days (Table 4).

Table 4: Sensory characteristics of yoghurt prepared with fresh and preserved cultures during storage

Parameters	Storage (day)	Samples	
		Fresh culture	Preserved culture
Flavour	0	7.50	8.00
	5	7.50	7.00
Body and texture	0	8.00	8.00
	5	7.00	7.00
Color and appearance	0	7.50	7.50
	5	7.00	7.00
Overall acceptability	0	7.50	8.00
	5	7.00	7.00

Conclusion

Study of growth performance of yoghurt culture in whey based media, production of cultures biomass and preservation in concentrated form and evaluation of preserved cultures for preparation of yoghurt. Yoghurt culture preserved by freeze drying can be used in preparation of yoghurt and it has similar sensory and physicochemical properties. Preserved freeze dried culture can be used in the preparation of good quality yoghurt.

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