Revista de Microbiologia (1999) 30:163-169
ISSN 0001-3714

THERAPEUTIC PROPERTIES OF WHEY USED AS FERMENTED DRINK

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Submitted: August 05, 1998; Returned to authors for corrections: September 29, 1998; Approved: June 16, 1999.

ABSTRACT

Bioconversion of whey for preparation of beverage was standardized by utilizing yoghurt cultures. The product, wheyghurt drink, made with 4% yoghurt cultures inoculated in deproteinized whey (4.8% lactose, 0.66% ash, 0.46% fat and 0.40% protein adjusted to pH 6.4) and incubated at 42°C for 8h had all the technological requisite and dietetic criteria required in the product. The factors affecting the antibacterial activity of wheyghurt drink against *Escherichia coli*, *Staphylococcus aureus, Shigella dysenteriae* and *Bacillus cereus* were determined. There was a significant variation ($P<0.05$) in the antibacterial activity of wheyghurt drink with different levels of inoculum (1,2,4, and 8%) and concentration of sugar at 37, 42 and 45°C. Incubation at 42°C with 4% culture in whey exhibited highest inhibitory activity. The product stored up to 5 days under refrigeration was of acceptable organoleptic quality and requisite amount of microbial population (10^8 cfu/ml) to be potentially beneficial.

Key words: whey, yoghurt, antibacterial activity

INTRODUCTION

The bioconversion of whey is an interesting process from the view point of human nutrition, especially for therapeutic purposes, in regard to economy, and with advantage for reducing environment pollution. Ancient Greeks as well as Hippocrates, in 460 B.C., prescribed cheese whey for the assortment of human ailments. Use of *Lactobacillus delbrueckii* subsp., *bulgaricus* and *Streptococcus thermophilus* in the manufacturing of yoghurt have been extensively studied throughout the world. Regular intake of this product looks effective both in prevention and treatment of various illness in man viz. gastrointestinal disorders (14), hypercholesterolemia (10), antitumoral (3, 14), reduced protein allergencity, treatment of vaginal discharge, a cure for osteoporosis etc. (10). Although yoghurt bacteria can grow well in whey (5, 23, 27) use of these organisms in the preparation of whey drink is still limited.

The present communication includes a report on the preparation of wheyghurt drink, a fermented whey beverage prepared by using *L. delbrueckii* subsp. *bulgaricus* W and *S. thermophilus* H as culture organisms, assessment of its antibacterial activity as well as its acceptability and survival of the culture organisms in the gastrointestinal segments of wheyghurt drink fed rats.

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MATERIALS AND METHODS

Preparation of whey

Whey was prepared by heating pooled cow milk to 82°C and 2% citric acid solution was added at the rate of 2gm. Per kg of milk. Complete coagulation was effected within one minute and the whey filtered muslim cloth is popularly known as *chhana* whey in India where the coagulum *chhana* is used as a base material for traditional sweetmeats. Whey obtained was adjusted to pH 5.5 using 10% $NaHCO_3$ solution and was heated at 100°C for 10 minute with 0.4% $CaCl_2$ and kept undisturbed overnight at room temperature and filtered to obtain deproteinized whey (20). The product was then polished aseptically through washed diatomaceous earth built up as one half inch cake on a No. 54 Whatman filter paper placed in Buckner funnel (19). The average composition of whey was 4.8% lactose, 0.60% ash, 0.46% fat and 0.4% protein.

Source and Maintenance of Cultures

Lactobacillus delbrueckii subsp. *bulgaricus* W and *Streptococcus thermophilus* H along with the test cultures of pathogenic organisms viz. *Bacillus cereus, Escherichia coli, Shigella dysenteriae and Staphylococcus aureus* were obtained from the National Collection of Dairy Organisms, National Dairy Research Institute, Karnal, India. *Lactobacillus delbrueckii* subsp. *bulgaricus* W. and *Streptococuss thermophilus* H were maintained in sterile deproteinized whey peptone broth (8), with the following composition: peptone, 1gm; sodium chloride, 0.5gm and whey 100ml. pH of the media was maintained at 7.0. This whey medium was transferred to standard corning screw capped tubes (15x125 mm) by filling upto neck and were sterilized by steaming for 30 min on three consecutive days. The stock cultures were activated by three successive transfers at 48 h interval. The pathogenic cultures were maintained on nutrient agar slants (oxoid) and were activated by three successive transfers at 24 h intervals in nutrient broth.

Preparation of Wheyghurt Drink

A Schematic diagram conceptualizing the process employed for the production of wheyghurt drink using *L. delbrueckii* subsp. *bulgaricus* W and S. *thermophilus* H cultures for direct consumption is showed in Fig. 1. The effect of some factors such as i) size of inoculum, viz. 1, 2, 4 and 8%. ii) incubation temperature viz. 37, 42 and 45°C iii) concentration of sucrose viz. 0, 6, 8, 10, 12 and 16% and iv) storage at refrigeration temperature (5°C) for 1, 2, 5, 10 and 15 days – on the antibacterial activity of the drink were also examined.

Analysis

Wheyghurt drink was analyzed for titratable acidity (6), volatile acidity (15), lactic acid (4) and β-D-galactosidase activity (13). The antibacterial activity of the product was estimated by the modified cup agar assay technique (7). Culture filtrates (or cell free extracts) were collected by centrifugation at 3000 rpm for 15-20 min. These were passed through Seitz filter separately. Wells of 5 mm diameter were made on solidified nutrient agar (inoculated with the pathogenic test organisms) in each plate, and 50 µl of the cell-free extract introduced transferred to wells. The plates were incubated without inverting at 37°C for 18-24 h and the diameters of inhibition zones were statistically evaluated by analysis of variance (29).

Samples of wheyghurt drink were subjected to sensory evaluation by a panel of 7 judges 9-point hedonic scale (2) and analysed statistically by 2-way classification (29).

For survival of wheyghurt drink organisms in the intestine of rats 10 weanling male albino rats ≥ 21 and ≤ 28 days old were used. Each animal was fed with 15 g of rat feed synthetic ration containing 20% casein, 50% sucrose, 24% hydrogenated vegetable oil, 2% cod liver oil, 4% USP salt mixture, one multivitamin capsule (500 mg Pfizer) per kg. diet and 20 ml wheyghurt drink as preliminary diet for 7 days immediately prior to lights being extinguished. After 16 h, food cups and any remaining food were removed from the cages, the rats fasting for 8 h before being fed again. One the 8th and final day of the experiment, the animals except for one which served as a fasted control were provided with only 20 to 24 g of specific test meal (wheyghurt drink) and given 30 min to consume it. At intervals of 60, 120 and 180 min. after the meal animals were anesthetized with ether, weighed and its abdomen opened and contents of the stomach, duodenum, and jejunum were sampled after injecting and mixing 1.0 c.c. sterile saline (0.85% NaCl) into the clamped-off segments and aspirating with a sterile 5 c.c. syringe and a 22 gauge needle. Serial ten-fold dilutions of the aspirated contents were then prepared with sterile saline, and pour plated in duplicate on Elliker Agar (12).

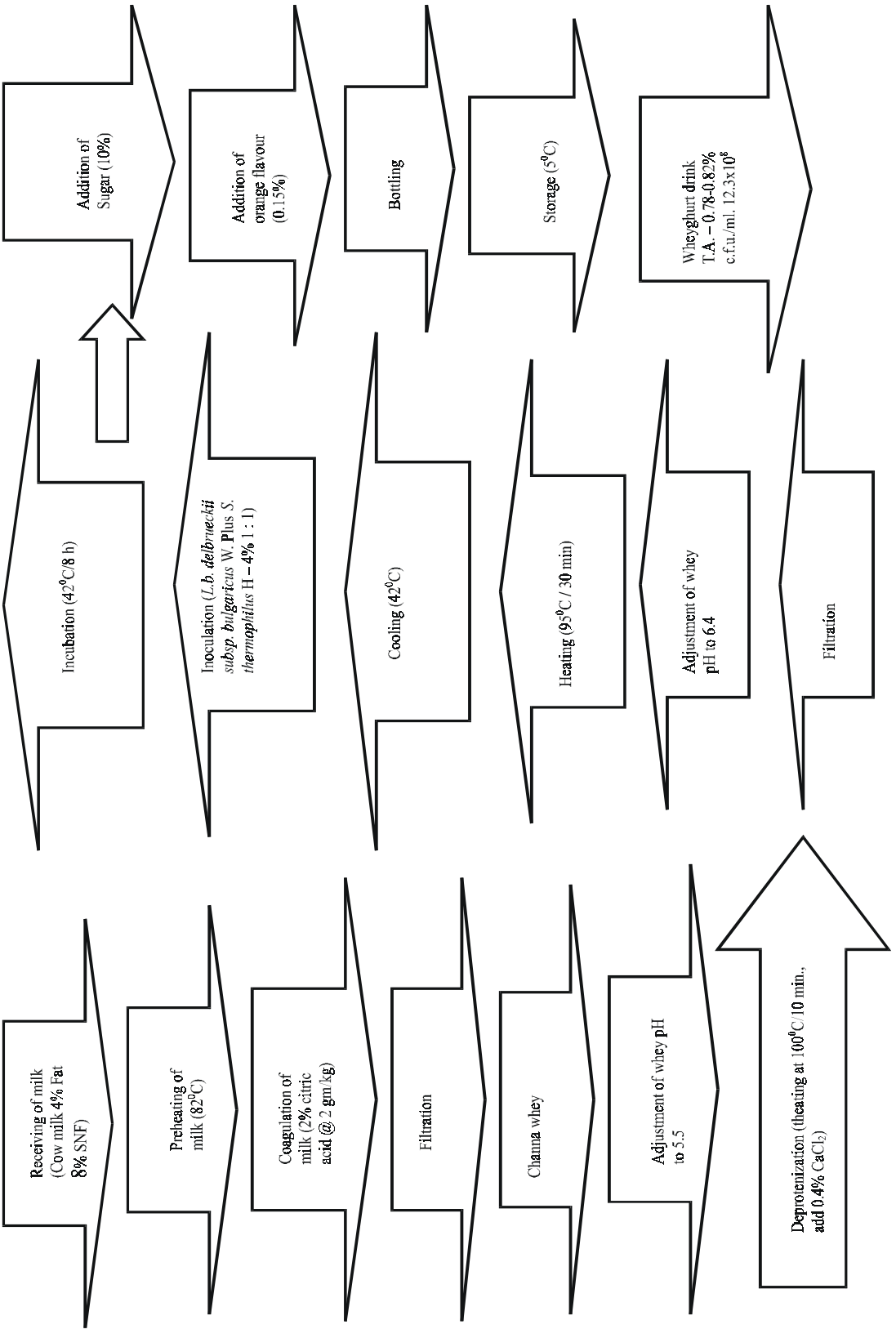

Figure 1. Schematic diagram for the manufacture of wheyghurt drink

RESULTS AND DISCUSSION

Characteristics of the Product

The procedure shown in Fig.1 was adopted for the preparation of wheyghurt drink using 4% mixed culture of *L. delbrueckii* subsp. *bulgaricus* W and *S. thermophilus* H in the ratio of 1:1. The final product had a titratable acedity of 0.78 – 0.82%, 2.0 to 2.4 ml. of volatile acidity, 204-207 µg/ml lactic acid, β-D-galactosidase activity of 2.30 µmol of lactose hydrolysed/gm/h., mild acidic flavour, antibacterial activity against all the four test organisms viz. *E. coli, S. aureus, Shigella dysenteriae* and *B. cereus* (inhibitory zone 8 to 10 mm) and a viable count of 12.3 x 10^8 c.f.u./ml. Rasic and Kurmann, 1979, recommended acidity level of 0.78 to 0.85% for yoghurt preparation (25). Considering that the minimum acidity of 0.7% is specified for yoghurt by the International Dairy Federation (1969), the product showed a desirable acidity level (16). Tramer, 1973 (31); Rasic and Kurmann, 1979 (25) and Singh, 1983 (28) recommended an inoculum of 1-3% for the preparation of yoghurt, but in the present study 4% inoculum was used due to low total solid content in whey. The use of high inoculum ensures a normal course of lactic acid fermentation and restrict unfavourable growth conditions as residual antibiotic, lack of growth substances etc. (22). Viable lactic acid bacteria population in the range of 10^8 to 10^9 cell/ml. of the fermented product causes successful seeding in intestine during consumption (17, 21, 30) and the product prepared according to the schematic chart (Fig. 1) satisfied the condition.

Effect of the Levels of Inoculum

The effect of 1, 2, 4 and 8% inoculum of *L. delbrueckii* subsp. *bulgaricus* W and *S. thermophilus* H (1: 1) on the antibacterial acitivity against four test organisms is depicted in Table 1. There was a significant variation ($P<0.05$) in the antibacterial activity due to change in level of inoculum. A 4% inoculum showed maximum antibacterial activity against *S. aureus* (10 mm.) and *B. cereus* (8 mm.), although antibacterial activity against these two organisms decreased at inoculum level of 8% (9 mm. for *S. aureus* and 7 mm. for *B. cereus*). Pette and Lolkema (24) reported that higher inoculum level increases the *Lactobacillus* content of yoghurt. Single strain culture of *L. delbreueckii* subsp. *bulgaricus* W showed lower antibacterial activity against *S. aureus* and *B. cereus* in comparison to *S. thermophilus* H in *channa* whey. The product exhibited similar antibacterial activity against the other two test organisms viz. *E. coli* and *Shigella dysenteriae* at all inoculum level.

Effect of Incubation Temperature

The data on the effect of different incubation temperature viz. 37°C, 42°C and 45°C on the antibacterial activity of wheyghurt drink is presented in Table 2. At 45°C weak (6 mm. inhibition zone against *E.coli, S. aureus* and *Shigella dysenteriae*) or no antibacterial activity (against *B. cereus*) was visible, in despite of maximum titratable acidity (0.82% against 0.74% and 0.80%, respectively, at 37 and 42°C) was reported at this temperature. The data indicated that production of antibacterial substances was not related to titratable acidity (9, 26). Maximum antibacterial activity of the product was obtained at 42°C, probably due to increased total cell count of 12.5 x 10^8 c.f.u./ml. promoted by temperature, leading to increase in the production of antibacterial substances.

Table 1. Effect of level of inoculum on the antibacterial activity of wheyghurt drink

Culture Combination: *Lactobacillus delbrueckii subsp. bulgaricus* W and *Streptococus thermophilus* H (1: 1)

Percent Inoculum	Titratable Acidity (LA %)	Total cell count (c.f.u./ml)	Dia. of zone of Inhibition (mm.)*			
			E. coli	*S. aureus*	*Shigella dysenteriae*	*B. cereus*
1	0.64	2.89 x 10^7	8.5	9.0	8.5	7.5
2	0.74	3.47 x 10^7	9.0	8.5	9.0	7.0
4	0.80	12.30 x 10^8	9.0	10.0	9.0	8.0
8	0.90	21.0 x 10^8	9.0	9.0	9.0	7.0

* Included dimateter of well (5 mm.) (amount of supernatant in well 0.05 ml).

Effect of Sugar Concentration

When sweetened wheyghurt drink was prepared using different concentrations of sugar (0, 6, 8, 10, 12 and 16%) it was observed that as the level of sugar addition increased there was very slight change in the titratable acidity, total viable count and antibacterial activity of the product upto 10% level of sucrose (Table 3) but at 12% sucrose level the changes were significant ($P<0.05$). Addition of 16% sucrose exhibited no antibacterial activity against any of the four test organisms with a low acidity (0.68%) and viable count (3.2 x 10^8 c.f.u./ml). Tramer (31) also reported that during preparation of yoghurt addition of sugar should not allow total solids to exceed 22% to avoid severe inhibition of yoghurt starters. However, it was observed that wheyghurt drink with 10% level of sucrose was excellent in taste with optimum titratable acidicy (0.78%) and recommended viable count (12.1 x 10^8 c.f.u./ml).

Effect of Storage at Refrigeration Temperature

Refrigerated storage (5^{o}C) of wheyghurt drink for 15 days indicated that the storage time increased beyond 5 days caused decrease in the antibacterial activity against the four organisms tested and with sharp decline after 10 days (Table 4). The total viable count decreased from 12.5 x 10^8 c.f.u./ml. to 54 x 10^6 c.f.u./ml. after 15 days of storage. The product was very sour in taste after 10 days of storage and was not liked by the consumers (sensory score 4.90). Kumar *et al*., (18) also reported a highly acidic product from fermentation of whey with yoghurt culture. Average sensory evaluation of wheyghurt drink by a panel of seven judges showed that maximum average sensory score of 6.50 in nine point hedonic scale was obtained after 24 h. of storage. The product was acceptable on the basis of mouthfeel, overall appearance and optimum level of acidity up to 5th day of storage (sensory score 6.00).

Survival of Wheyghurt Drink Microflora in the Rat Intestine

The total viable cell counts of the gastrointestinal segments (stomach, jejunum and duodenum) of wheyghurt drink fed rats at intervals of 60, 120 and 180 min. after meal are presented in Table 4. The count remained elevated until 2 to 3 h after ingestion of wheyghurt drink thereby demonstrating significant survival and potential metabolic activity in the upper gastrointestinal tract of the animals. Highest count

Table 2. Effect of incubation temperature on antibacterial activity of wheyghurt drinks

Incubation Temperature (0^0C)	Acidity (LA %)	Total cell count (c.f.u./ml)	Dia. of zone of Inhibition (mm.)*			
			E. coli	*S. aureus*	*Shigella dysenteriae*	*B. cereus*
37	0.74	11.2 x 10^8	9.5	9.5	8.5	7.5
42	0.80	12.5 x 10^8	9.0	10.0	9.0	8.0
45	0.82	6.8 x 10^8	6.0	6.0	6.0	-

* Included well dimateter of well (5 mm.) (amount of supernatant in well 0.05 ml).

Table 3. Effect of concentration of sugar on antibacterial activity of wheyghurt

Concentration of Sugar (Percent)	Acidity (LA %)	Total cell count (c.f.u./ml)	Dia. of zone of Inhibition (mm.)*			
			E. coli	*S. aureus*	*Shigella dysenteriae*	*B. cereus*
0	0.80	12.5 x 10^8	9.0	10.0	9.0	8.0
6	0.80	12.5 x 10^8	9.0	10.0	9.0	8.0
8	0.80	12.3 x 10^8	9.0	9.5	9.0	8.0
10	0.78	12.1 x 10^8	9.0	9.5	9.0	8.0
12	0.74	10.8 x 10^8	8.0	9.0	8.0	7.5
16	0.68	3.2 x 10^8	-	-	-	-

* Well diameter included (5 mm.)
- :No inhibition observed.

Table 4. Effect of storage at refrigeration temperature (5°C) on bacterial growth, antibacterial activity and sensory score of wheyghurt drink

No. of Days of Storage	Total cell count (c.f.u./ml)	Dia. of zone of Inhibition (mm.)*				Average Sensory score.
		E. coli	*S. aureus*	*Shigella*	*B. cereus dysenteriae*	
1	12.5 x 10^8	9.0	10.0	9.0	8.0	6.50
2	11.2 x 10^8	9.0	10.5	9.0	8.5	6.35
5	9.6 x 10^8	8.5	9.0	8.5	8.0	6.00
10	32 x 10^7	7.0	8.0	7.5	7.0	4.90
15	54 x 10^6	6.0	8.0	7.0	7.0	3.00

* Well diameter included (5 mm.)

Table 5. Viable cell counts of gastrointestinal segments of rat given special dietary treatment.

(Treatment = Rat feed (sucrose) + 20 ml. Wheyghurt Drink as preliminary meal and Wheyghurt Drink as test meal)

Gastrointestinal Segements	Log Counts of Viable Cells per ml.		
	60 min.	120 min	180 min
Stomach	7.83	6.60	3.41
Jejunum	4.90	7.45	5.20
Duodenum	4.20	5.17	5.58

was observed in the stomach whereas lowest count was observed in the duodenum. This may be due to the effect of bile salt in the duodenum which altered permeability of the bacterial cells and thereby resisted the growth of the organisms. Acott and Labuza (1) have shown that yoghurt microflora were capable of surviving simulated gastric digestion where Goodenough and Kleyn (13) have demonstrated gastrointestinal survival of yoghurt organisms in vivo up to 3 h. after feeding.

CONCLUSION

Wheyghurt drink made with yoghurt cultures showed potential therapeutic properties, and optimum sensory qualities with a shelf life of 5 days. The yoghurt microflora survived in the gastrointestinal tract, and the mass effect combined with the antagonistic activity against undesirable organisms represents an important factor for the utilization of fermented whey drink preparation with both dietetic and technological properties.

RESUMO

Propriedades terapêuticas de soro de leite usado como bebida fermentada

A bioconversão de soro de leite para preparação de bebida foi padronizada utilizando culturas de iogurte. O produto feito com culturas de iogurte a 4%, inoculadas em soro desproteinizado (lactose 4,8%; cinzas, 0,66%; gordura 0,46% e proteína 0,40%, pH 6,4), incubado a 42°C por 8h, apresentou todos os requisitos tecnológicos e critérios dietéticos requeridos para o produto. Os fatores que afetam a atividade antibacteriana do produto contra *Escherichia coli*, *Staphylococcus aureus*, *Shigella dysenteriae* e *Bacillus cereus* foram determinados. Houve uma variação significativa na atividade antibacteriana do produto contendo diferentes níveis de inóculo (1, 2, 4 e 8%) e concentração de açúcar a 37, 42 e 45°C. Incubação a 42°C com cultura a 4% no soro apresentou a maior atividade inibitória. O produto armazenado até 5 dias em refrigeração apresentou características organolépticas aceitáveis e microrganismos em quantidade adequada (10^8 ufc/ml) para ser considerado benéfico.

Palavras-chave: soro de leite, iogurte, atividade antibacteriana

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