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Research Article

EFFECT OF WHEY PROTEIN CONCENTRATE ON MANUFACTURING OF NONFAT FERMENTED MILK

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Abstract- Consumer perception of food gave a golden opportunity to synbiotic fermented dairy products, since that have been playing a major role in the part of the diet of people throughout the world for their taste, nutritive value and therapeutic properties. "Synbiotic food" contains probiotic microorganisms and prebiotic substances. Thus, it is essential to determine the composition, titratable acidity and textural properties to provide the best synbiotic fermented product. Different combination of *Lactobacillus acidophilus* and *Lactobacillus casei* were studied to cull the best combination on the basis of acid production for preparation of nonfat fermented milk. Keeping in view of the above said observations of the best inoculums percentage (1%) of *Lactobacillus acidophilus* and *Lactobacillus casei* at 1:1 ratio was selected and further planned and conducted to examine the effect of various level of whey protein concentrate in fatless fermented synbiotic fermented milk for optimizing the best combination ratio of total solids which would give the maximum sensory grade. The titratable acidity of nonfat fermented milk was measured at 2h time interval during the incubation period. After reached to the desire acidity level the different combination of products was subjected to sensory evaluation. Synbiotic Nonfat fermented milk added with 3% whey protein concentrate level prepared with 1% inoculum of *Lactobacillus acidophilus* and *Lactobacillus casei* at 1:1 ratio was observed the best and showed highest overall grade for acceptance of the product.

Keywords- Fermented milk, whey protein concentrate, Synbiotic.

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Introduction

The synbiotic fermented dairy foods namely symbiotic dahi are inherent part of human diet. The potential benefits of synbiotic-dahi are increased safety, economic value of the product, nutritional value, digestibility, making the food more suitable for subsequent processing, acceptability, enhancing the flavour and palatability, ease of storage and transportation, shelf life with decreased toxicity, cooking time and processing time. During recent years, an increasing interest has developed in foods that contribute to a positive effect on health beyond their nutritional value. Probiotic foods contain microorganisms or components of microbial cells that have a beneficial effect on the health and well being of the consumer host [1]. Sometimes the probiotic organisms fail to grow properly and to fight against pathogenic organisms due to lack of their foods. This has led to the coining of the term synbiotic food, which includes probiotic microorganisms and their food materials known as prebiotic substances in the system. Yadav, *et al*., (2007) found that *Lactococcus lactis* ssp *diacetylactis, Lactobacillus acidophilus* and *Lactobacilus casei* had the ability to produce oligosaccharides due to transgalactosidal and lactose hydrolysis activities of beta-galactosidase [2]. Champagne, *et al*., (1996) suggested that whey protein concentrate could be successfully used to prepare starter cultures because it gave higher population of bacteria than when milk was used as the medium [3]. Dave & Shah, (1998) found that whey protein concentrate contains amino nitrogen, which was used as a catalator for the growth of microorganisms. They found that supplementation of 2% whey protein concentrate with milk to prepare yoghurt by inoculating *Lactobacillus acidophilus, Biofidobacterium* & *Strptococcus thermophilus (*ABT) culture decreased fermentation time by 4 h to reach pH 4.5 as compare to yogurt preparation having same culture but without whey protein concentrate [4]. Martin-Diana, *et al.,* (2003) suggested that supplementation of 3% whey protein concentrate (35) with goat milk to prepare fermented goat's milk by inoculating ABT culture, decreased fermentation time by 2 h [5]. Martin-Diana, *et al.* (2003) suggested that 3% whey protein concentrate supplementation into goat milk to prepare fermented goat milk increased the Bifidobacterial growth and viability of Lactobacilli [5]. Janer, *et al.,* (2004) reported that addition of 2% whey protein concentrate into skimmed milk to prepare fatless fermented milk by inoculating *Bifidobacterium lactis,* increased the counts of *Bifidobacterium lactis* by 3.5 times at 6th hour of incubation as compare to plain fatless fermented milk [6]. Needs, *et al*., (2000) observed that denaturation of β-lactoglobulin found in whey protein concentrate and its interaction with casein micelles had been shown to highly influence gel fermented milk properties [7]. Shanker, *et al*., (2000) reported that denaturation of whey protein during heat treatment highly influenced viscosity due to an increase of protein binding capacity that resulted in a higher gel viscosity during coagulation [8]. Martin-Diana, *et al.,* (2003) investigated that increase in the content of solid not fat was required in fat less fermented milk to obtain a satisfactory curd tension [5]. Whey protein concentrates might be used as a cheaper and readily available additive to increase viscosity, reduce whey synergism and give impart desirable texture characteristics in fermented milk. This study was planned to evaluate different nonfat fermented synbiotic milk

combinations with different level of whey protein concentrate with different inoculum percentage of *Lactobacillus acidophilus* and *Lactobacillus casei* at different ratio to select the best formulation with highest overall acceptance grade product.

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Materials and Methods

Two strains of lactic acid bacteria in freeze dried ampules form namely *Lactobacillus acidophilus* and *Lactobacillus casei* were obtained from national collection of dairy culture centre, NDRI, Karnal, India. Both the cultures were maintained in sterile litmus milk media and activated by three successive transfers at 24 h interval before use. Skim milk (10% TS) was prepared by dissolving 10.5 gm of skimmed milk powder in 100 ml of distilled water. Whey protein concentrate about 3.17 gm was added into 100 ml Skim milk (10% TS) to maintain 13% TS. Then fatless milk was heated up to 115^{0}C for 15 min and cooled down to 38 – 40^{0}C. 1% Mixed culture of *Lactobacillus acidophilus* and *Lactobacillus casei* having 1:1 ratio was inoculated into the fatless milk and incubated for 7 h at 37^{0}C. The acidity was measured at each 2 h interval during the incubation period of milk as per provision of ISI handbook of food analysis, part XI Dairy products, 1981 [9]. Lactic acid bacteria count at the 7th hour in the incubation period of the samples was carried out by using deMann Rogosa and Sharpe agar (MRS). (As per provision of Manual in Dairy Bacteriology, ICAR-Sub committee on Dairy Education, 1972) [9]. Fatless fermented milk prepared by different combination of whey protein concentrate and skimmed milk powder were judged independently by a selected panel of six experienced members. The products were evaluated in respect to flavour, body and texture, colour and appearance and overall acceptability using a 9-point Hedonic scale evaluation card specially prepared for this purpose [10].

Result & Discussion

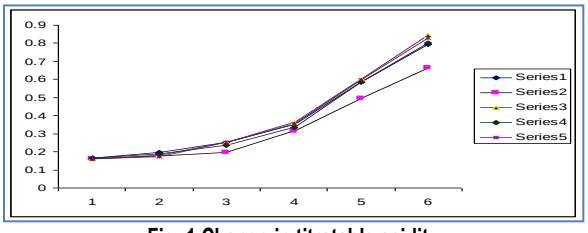
Acid producing ability of different lactic cultures

Two types of single lactic cultures *viz. Lactobacillus acidophilus* and *Lactobacillus casei,* their combinations with different ratios *viz. Lactobacillus acidophilus* + *Lactobacillus casei* (1 : 1, 3 : 1 & 1 : 3), 1% rate of inoculums were studied with a view to select a suitable starter culture or combination for the preparation of fatless fermented milk. The respective data are presented in [Table-1] & [Fig-1]. The acid producing ability of the cultures was measured in term of percentage of titratable acidity (TA %). The results pertaining to the acid producing abilities of above mentioned lactic cultures and combination thereof are presented and discussed as below. It might be observed that the variation in TA values among the type of cultures, the period of incubation and their interaction were significant (P<0.05).

Table-1 *Titratable acidity (%) of fatless fermented milk having 1% inoculum with different periods of incubation*

Combination	Incubation period (h)					
	0	2	4	6	8	10
Lactobacillus acidophilus (C_1)	0.165 ±0.001	0.198 ±0.001	0.252 ±0.002	0.351 ±0.001	0.585 ±0.001	0.792 ±0.001
Lactobacillus acidophilus (C_2)	0.163 ±0.001	0.178 ±0.001	0.198 ±0.001	0.315 ±0.001	0.495 ±0.001	0.666 ±0.002
$C_1 + C_2$ (1:1)	0.162 ±0.001	0.180 ±0.001	0.252 ±0.001	0.360 ±0.001	0.603 ±0.001	0.846 ±0.001
$C_1 + C_2$ (3:1)	0.164 ±0.001	0.189 ±0.001	0.234 ±0.001	0.333 ±0.001	0.587 ±0.001	0.801 ±0.001
$C_1 + C_2$ (1:3)	0.162 ±0.001	0.171 ±0.001	0.252 ±0.001	0.352 ±0.001	0.598 ±0.001	0.828 ±0.001

(Average of three replications)



Fig–1 Change in titratable acidity

Where,

Series -1 is C_1, Series -2 is C_2, Series -3 is $C_1 + C_2$ (1:1), Series -4 is $C_1 + C_2$ (3:1), Series -5 is $C_1 + C_2$ (1:3)

1 stands for 0 h, 2 for 2 h, 3 for 4 h, 4 for 6 h, 5 for 8 h & 6 for 10 h.

The rate & increase in titratable acidity increased significantly (P<0.05) in case of culture type $C_1 + C_2$ (1 : 1) than that of single cultures or other combinations because of more synergism effect of this type of culture combination as compare to others, at the same time lactose was more used by $C_1 + C_2$ culture combination. Hence, 1% culture of $C_1 + C_2$ combination with ratio 1:1 was taken as inoculums to prepare fatless fermented milk.

Effect of whey protein concentrate on titratable acidity with different incubation period

The selected combination of culture [$C_1 + C_2$ (1: 1)] @ 1% inoculum was inoculated to prepare fatless fermented milk. The different percentages of WPC were used like 1%, 2%, 3%, and 4%. The SMP percentage was taken for making the fatless fermented milk in such a way that total solids in the fatless fermented milk remained 13%, were studied with a view to select a suitable WPC combination. These data are presented in [Table-2] and in [Fig-2]. The acid production was measured in term of percentage of titratable acidity. It may be observed that changes in TA% with respect to period of incubation, combination of WPC and their interaction were significant (P<0.05). Titratable acidity was increased with increase of percentage of WPC and incubation period. Fatless fermented milk prepared with the addition of 4% WPC showed the highest percentage of TA *i.e.*, 2.265%. It may be due to the most favorable situation of growth to lactobacilli as a result of addition of WPC. Martin-Diana, *et al.* (2003) studied the development of a fermented goat's milk containing probiotic bacteria and found that supplementation of 3% whey protein concentrate increased viability of Lactobacilli. While Janer, *et al.*, (2004) reported that addition of 2% whey protein concentrate into skimmed milk to prepare fat less fermented milk increased the lactic count.

Table-2 *Titratable acidity of fatless fermented milk having varying with different combinations of whey protein concentrates at different incubation periods*

Percentage of WPC	Incubation period (h)					
	0	2	4	6	8	10
1	0.165 ±0.001	0.250 ±0.001	0.360 ±0.001	0.650 ±0.001	1.176 ±0.001	1.716 ±0.001
2	0.168 ±0.001	0.290 ±0.001	0.427 ±0.001	0.725 ±0.001	1.312 ±0.001	1.915 ±0.001
3	0.172 ±0.001	0.331 ±0.001	0.498 ±0.001	0.805 ±0.001	1.457 ±0.001	2.127 ±0.001
4	0.175 ±0.001	0.345 ±0.001	0.520 ±0.001	0.858 ±0.001	1.552 ±0.001	2.265 ±0.001

Culture used @ 1% with 1:1 of $C_1 + C_2$ (Average of three replications)

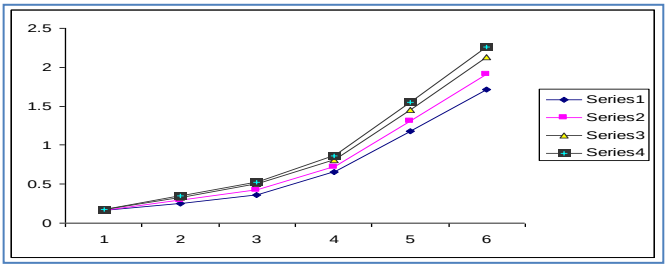


Fig-2 Change in titratable acidity

Where, Series -1 is WPC 1%, Series -2 is WPC 2%, Series -3 is WPC 3%, and Series 4 is WPC 4%

1 stands for 0 h, 2 for 2 h, 3 for 4 h, 4 for 6 h, 5 for 8 h & 6 for 10 h.

Effect of whey protein concentrates on population of Lactic acid bacteria and Titratable acidity at 7th hour of incubation

The selected combination of culture [$C_1 + C_2$ (1:1)] @ 1% inoculum was inoculated to prepare fatless fermented milk. The different percentage of WPC was used like 1%, 2%, 3% and 4%. The SMP percentage was taken for the making the fatless

fermented milk in such a way that total solids in the fatless fermented milk remained 13%. The data related to population of Lactic acid bacteria and TA% at 7th hour of incubation is presented in [Table-3]. Titratable acidity, population of Lactic acid bacteria were increased with increase of percentage of WPC at 7th hour of incubation. The fatless fermented milk prepared with the addition of 4% WPC showed the highest percentage of TA *i.e.*,1.053% and also in case of LAB population *i.e.*, 155 × 10^7 cfu/gm. It may be due to more quantities of nutrition available resulting to more number of Lactose utilizable lactobacilli and more utilization of lactose by microorganisms. Martin-Diana, *et al*., (2003) suggested that 3% WPC supplementation into goat milk to prepare fermented goat's milk increased the bifidobacterial growth and viability of Lactobacilli. Janer, *et al.,* (2004) reported that addition of 2% whey protein concentrate into skimmed milk to prepare fatless fermented milk by inoculating Bifidobacterium *Lactis*, increased the counts of *Bifidobacterium lactis* by 3.5 times at 6th hour of incubation as compare to plain fatless fermented milk.

Table-3 *Lactic acid bacteria count at 7th hour of incubation of fat less fermented milk having different combination of WPC by remaining the T.S constant as 13%*

WPC%	Lactic acid bacteria count	Titratable acidity (%)
0	62×10^6	0.501
1	120×10^7	0.801
2	133×10^7	0.873
3	145×10^7	0.954
4	155×10^7	1.053

Sensory evaluation of whey protein concentrate added fatless fermented milk

A sensory panel comprising of six experienced members evaluated the Flavour, Body & Texture, Colour & Appearance and Overall Acceptability of the product by using 9-point Hedonic scale. Sensory evaluation was done for five samples of fatless fermented milk. First one was prepared from only skim milk having 13% T.S. Second one was prepared by adding 12% T.S. from SMP and 1% T.S. from WPC, third one was prepared by adding 11% T.S. from SMP & 2% T.S. from WPC, fourth one was prepared by adding 10% T.S. from SMP & 3% T.S. from WPC and fifth one was prepared by adding 9% T.S. from SMP & 4% T.S. from WPC. The respective observations are depicted in [Table-4]. The effect of addition of whey protein concentrate on the organoleptic characteristics of fermented milk was therefore, studied with a view to assess to their efficiency to improve the acceptability. All five sample of fermented milk exhibited distinct separation of whey on top and the cream coloured was observed on top of samples. Flavour was almost similar in second, third and fourth samples but in fifth one distinct whey flavour was evaluated so obtained less overall score among all samples. Combination-4 (*i.e.*, 3% WPC) secured overall acceptability more among all might be due to good texture, less whey separation and absence of whey flavour, whereas combination-1 (*i.e.*, 0% WPC) obtained least score of overall acceptability due to more separation of whey. Martin-Diana, *et al*., (2003) reported that addition of 3% whey protein concentrate into fatless fermented milk reduced whey synersis and gave impart desirable texture characteristics in fermented milk. The finding of this investigation is therefore, in accordance with the above reports. It is observed that average score of fatless fermented milk prepared with 3% WPC in overall acceptance characteristics was maximum *i.e.*, 9.0. Although the maximum acidity (1.053% TA) was produced by fat less fermented milk prepared with 4% WPC but it failed to be accepted organoleptically. Now the fatless fermented milk prepared with 3% WPC was selected on the basis of overall acceptability by the technical exports or judges.

Table-4 *Sensory evaluation of 6 members on the basis of 9 point Hedonic Scale*

Attributes	Level of whey protein concentrate				
	0%	1%	2%	3%	4%
Flavour	8	8.5	8.5	8.5	7.5
Body & Texture	7	8	8.5	8.5	8.5
Colour & Appearance	8.5	8.5	9	9	8.5
Overall Acceptability	7.5	8.5	8.5	9	8

Liked Extremely – 9, Liked Very much – 8, Liked Moderately – 7, Liked Slightly – 6, Neither Liked nor disliked – 5, Disliked Slightly – 4, Disliked Moderately – 3, Disliked Very much – 2, Disliked Extremely – 1

Conclusion

Two cultures named *Lactobacillus acidophilus* (C_1) and *Lactobacillus casei* (C_2) alone and their combination 1:1, 3:1 and 1:3 were inoculated @ 1% for preparation of fatless fermented milk having 13% TS. C_1 + C_2 with 1:1 ratio culture combination was used to prepare fatless fermented milk by adding whey protein concentrate. Whey protein concentrate 1%, 2%, 3%, and 4% were added into skim milk in such a way that the total solid remained 13% in fat less fermented milk. By increasing the rate of WPC from 0 to 4% the titratable acidity and population of lactic acid bacteria were increased in fatless fermented milk. 4% WPC added fatless fermented milk gave higher TA% and LAB population than 3% WPC added fatless fermented milk. Five samples of fatless fermented milk, varying with different combinations of WPC like 0%, 1%, 2%, 3%, and 4% were judged organoleptically. 3% WPC added fatless fermented milk was scored highest overall grade for acceptance of the product. Therefore, addition of 3% whey protein concentrate level with 1% inoculum of *Lactobacillus acidophilus* and *Lactobacillus casei* at 1:1 ratio was observed the best and showed highest overall grade for acceptance of the product and this can be recommended to the dairy Industry to introduce in the market.

Application of research: This nonfat fermented milk can be used for modulation of gut microbiota as well as ultrapeutic agent for human medical disorder

Research Category: Synbiotic food formulation

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Research project name or number: MSc Thesis

Author Contributions: All author equally contributed

Author statement: All authors read, reviewed, agree and approved the final manuscript

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