STANDARDIZATION OF A METHOD FOR PREPARATION OF PROBIOTIC ACIDO-BIFIDO-YOGHURT

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ABSTRACT

The present investigation was undertaken with a view to study the growth and biochemical activity of normal Yoghurt cultures with probiotic Acido-Bifido-Yoghurt with various combination of the ingredients and cultures and to investigate the sensory quality profiles of this product by comparing this value-added product with its traditional counterpart viz. plain yoghurt. Buffalo milk was used to prepare this value-added product. A human vaginal isolate of \textit{Lb. acidophilus}-LBKV3 was used as a proven probiotic organism and a human strain of \textit{Bifidobacterium bifidum} as another organism. A method was standardize the probiotic Acido-Bifido-Yoghurt. Product was assessed for its sensory attributes through a panes of judges. It was observes that incorporation of probiotic cultures do not affect the sensory characteristic of the product.

INTRODUCTION

The term “Probiotics”, introduced by Parker in 1974 derived from Greek word meaning “for life”. The probiotic foods are defined as “food containing live microorganisms, which actively enhance health of consumers by improving ecological balance of microflora in gut when ingested in sufficient numbers”(Fuller, 1992). Probiotics can also be defined more fully as ‘live supplements, which affect the host animal by improving its intestinal microbial balance’. The most widely used probiotic lactic acid bacteria are lactobacilli and bifidobacteria, which have been isolated from the human gastrointestinal tract. Extensive studies have been conducted on the beneficial effects on human health of these species. Lactic acid bacteria play an essential role in manufacturing many fermented milk products. Applications of these organisms are now being extended to the area of health improvement, which is known as probiotic activity.

Dairy products appear to be preferred delivery medium for probiotic and such products should be viewed as part of overall healthy diet. The viability and quality of probiotic culture is taken in account while incorporating it in the Yoghurt. It is a very well known fermented milk product consumed by large sections of the population throughout the world. It is a coagulated milk product obtained by lactic acid fermentation through the action of \textit{Streptococcus salivarius} ssp. \textit{thermophilus} and \textit{Lactobacillus delbrueckii} ssp. \textit{bulgaricus} from milk (pasteurized/concentrated milk) with or without optional addition (milk powder, skim milk powder, whey powder, etc).

Fermented milks have been an important and highly valued component of man’s diet. Yoghurt is traditionally manufactured using starter bacteria like \textit{Streptococcus salivarius} ssp. \textit{thermophilus} and \textit{Lb. delbrueckii} ssp. \textit{bulgaricus}. Neither of these organisms inhabits the intestinal tract of humans and animals, nor do they survive gastric passage in large numbers (Holdeman \textit{et al.}, 1976). Consequently, incorporation of beneficial intestinal microorganisms viz., \textit{Lb. acidophilus} and \textit{Bifidobacterium} species into Yoghurt has been an area of great interest. The present investigation was undertaken with a view to study the growth and biochemical activity of normal Yoghurt cultures with probiotic strains of \textit{Lb. acidophilus} and \textit{Bif. bifidum} as probiotic organisms and to standardize a method for preparation of probiotic Acido-Bifido-Yoghurt with various combinations of the ingredients and cultures and to investigate the sensory

*Division of Dairy Technology, National Dairy Research Institute, Karnal, 132 001 (Haryana)*
RECEIVING FRESH BUFFALO MILK
FILTRATION/CLARIFICATION
STANDARDIZATION (5% FAT and 10% TS)
HEAT TREATMENT (95°C/15 MIN)
COOLING (37°C)
INOCULATION (1% BIF. BIFIDUM)
INCUBATION AT 37°C FOR ONE H
INOCULATION (1% LB. ACIDOPHILUS)
INCUBATION AT 37°C FOR ONE H
INOCULATION (1% MIXED YOGHURT CULTURE)
INCUBATION AT 40°C TO OBTAIN 0.7% (LA)
PROBIOTIC ACIDO-BIFIDO-YOGHURT
COOLING AND STORAGE

**FIG. 1: FLOW DIAGRAM FOR MANUFACTURE OF PROBIOTIC ACIDO-BIFIDO-YOGHURT**

quality profiles of this product by comparing this value-added product with its traditional counterpart viz. plain yoghurt.

**MATERIAL AND METHODS**

Growth patterns of the lactic cultures: It is generally decided by using increased number of viable cell counts and the titratable acidity. To study the patterns of growth of the lactic cultures used in this investigation namely, *Lb. acidophilus-LBKV₃, Bif. bifidum-NCDC255, Lb. delbrueckii subsp. bulgaricus* and *St. salivarius subsp. thermophilus*, sterilized skim milk was inoculated with active culture of these organisms and its viable counts were monitored on the selective media for 24 h at an interval of three h.

Preparation of probiotic Acido-bifido-yoghurt: The probiotic Acido-bifido-yoghurt was prepared from buffalo milk standardized to 5% fat and 10% SNF by inoculating *Lb. acidophilus-LBKV₃* (1%), *Bif. bifidum-NCDC255* (1%), *Lb. delbrueckii subsp. bulgaricus* (0.5%) and *St. salivarius subsp. thermophilus* (0.5%) using the method given in Fig. 1.

Sensory evaluation of the products: The products were evaluated for their sensory characteristics by a panel of eight judges. The nine-point Hedonic scale was adapted for judging the relative acceptability of the plain yoghurt, Acidophilus milk, bifidus milk and the probiotic Acido-bifido-yoghurt.

**RESULTS AND DISCUSSION**

Criteria employed in culture selection: The basic consideration in selecting the probiotic strain of *Lb. acidophilus-LBKV₃* was the fact that the organism is an isolate obtained from human vaginal surface swabs and it was earlier extensively studied for its probiotic characteristics since last one and half
J. DAIRYING, FOODS & H.S.

Table 1. Comparative appraisal of the growth rates (viable counts) of lactic acid bacterial cultures in milk*

<table>
<thead>
<tr>
<th>CULTURES (T)</th>
<th>INCUBATION PERIOD (IN HRS.)</th>
<th>MEANS (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Lb. delbrueckii sp. bulgaricus</td>
<td>2.10</td>
<td>2.99</td>
</tr>
<tr>
<td>St. salivarius sp. thermophilus</td>
<td>3.28</td>
<td>5.59</td>
</tr>
<tr>
<td>Lb. acidophilus</td>
<td>2.66</td>
<td>2.98</td>
</tr>
<tr>
<td>Bil. bifidum</td>
<td>2.20</td>
<td>2.44</td>
</tr>
<tr>
<td>MEANS (T)</td>
<td>2.56</td>
<td>3.5</td>
</tr>
</tbody>
</table>

*Values are the averages of five replications.

Table 2. Comparative appraisal of the rate of acid production by lactic acid bacteria in milk*

<table>
<thead>
<tr>
<th>CULTURES (T)</th>
<th>INCUBATION PERIOD (IN HRS.)</th>
<th>MEANS (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Lb. delbrueckii sp. bulgaricus</td>
<td>0.24</td>
<td>0.39</td>
</tr>
<tr>
<td>St. salivarius sp. thermophilus</td>
<td>0.26</td>
<td>0.34</td>
</tr>
<tr>
<td>Lb. acidophilus</td>
<td>0.21</td>
<td>0.28</td>
</tr>
<tr>
<td>Bil. bifidum</td>
<td>0.20</td>
<td>0.24</td>
</tr>
<tr>
<td>MEANS (P)</td>
<td>0.23</td>
<td>0.31</td>
</tr>
</tbody>
</table>

*Values are the averages of five replications.

decade (Khedkar, 1988). It was used in monocultures for various probiotic attributes including hypocholesterolaemic activity, implantation ability, therapeutic effects on gastrointestinal and related ailments, antibacterial activities, etc. It was observed that the quality of fermented milk products/dairy beverages prepared by inoculating this organism do not give readily acceptable sensory attributes and hence it is necessary to use this versatile probiotic strain in combination with the cultures like yogurt (Khedkar et al., 2003). As far as Bil. bifidum-NCDC 255 is concerned the same problem of body and texture characteristics of bifidus milk was reported. Both of these probiotic cultures used in the study are natural inhabitants of human body sources, as has been suggested by Gilliland et al., 1975, Moore and Holdeman, 1974 and Mutai et al., 1971. The additional positive merits were their resistance to phenol, bile salts and low pH which enable their viable passage through the gastrointestinal tract and allow them to establish and multiply there in the existing nutritional and ecological conditions (Wheter, 1955 : Rogosa and Sharpe, 1959; Muelhens, 1967; Rasic and Kurmann, 1978; Gilliland, 1979 and Sarra and Dellaigio, 1984).

Growth patterns of the lactic cultures

The growth patterns in terms of viable counts are shown in Table 1 intensifies that the culture L. acidophilus-LBKv, remained in exponential phase up to 9 h at 37°C, giving 5.54 log_{10} cells/ml. The viable counts have shown steady increases in the viable counts giving the log viable counts of 7, 8.4, 9.9 during 12, 15 and 18 h of incubation. It was followed by its entry in stationary phase of three h after 21 h of incubation. It was followed by a phase of decline giving the log_{10} counts of 9.12. It was observed that Lb. delbrueckii subsp. bulgaricus showed a comparatively short lag phase which was followed by log phase and the culture attained viable counts of 6.4, 9.2, 9.9, and 10.4 during 9, 12, 15 and 18 h, respectively. It was observed that the culture entered in stationary phase at 21 h and exhibited the death phase after 24 h. However, culture Bil. bifidum-NCDC-255 remained in exponential phase up to 9 h at 35°C giving log_{10} of around 3 cfu/ml. Thereafter it has shown a gradual but steady increase in viable counts. Rate of growth of this organism observed in the present investigation was very slow and the log of viable counts recorded after 24 h of incubation were just 7.22. The only cocci shaped organisms used as a
Table 3. Comparative appraisal of the biochemical analysis and compatibility of fermented milk products obtained from buffalo milk.

<table>
<thead>
<tr>
<th>NAME OF THE PRODUCTS</th>
<th>CURDLING TIME (HRS.)</th>
<th>pH</th>
<th>ACIDITY (% LA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidophilus milk</td>
<td>24 H</td>
<td>48 H</td>
<td>72 H</td>
</tr>
<tr>
<td>Bifidus milk</td>
<td>7</td>
<td>4.6</td>
<td>4.4</td>
</tr>
<tr>
<td>Yoghurt</td>
<td>12</td>
<td>5.0</td>
<td>4.7</td>
</tr>
<tr>
<td>Acido-Bifido-Yoghurt</td>
<td>4</td>
<td>4.1</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.0</td>
<td>3.6</td>
</tr>
</tbody>
</table>

*Values are the averages of five replications.

Table 4. Comparative appraisal of acceptability of fermented milk products prepared from the lactic acid bacteria.

<table>
<thead>
<tr>
<th>PRODUCT (T)</th>
<th>RESPONSE RECORDED BY THE JUDGES ON NINE-POINT HEDONIC SCALE</th>
<th>MEANS (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACIDOPHILUS MILK</td>
<td>J1 4.55 J2 5.23 J3 4.18 J4 4.27 J5 3.82 J6 4.30</td>
<td></td>
</tr>
<tr>
<td>BIFIDUS MILK</td>
<td>J1 3.72 J2 3.36 J3 3.44 J4 2.91 J5 3.26 J6 3.21</td>
<td></td>
</tr>
<tr>
<td>YOGHURT</td>
<td>J1 8.24 J2 8.10 J3 8.48 J4 8.98 J5 8.72 J6 9.44</td>
<td></td>
</tr>
<tr>
<td>ACIDO-BIFIDO-YOGHURT</td>
<td>J1 9.00 J2 8.33 J3 8.79 J4 8.64 J5 9.23 J6 8.78</td>
<td></td>
</tr>
</tbody>
</table>

*Values are the averages of five replications.

culture in this study i.e. Str. salivarius subsp. thermophilus showed a very narrow lag phase of less than an hour followed by log phase showing fast increase in the viable counts, reaching to 9.5 log viable counts at 12 h and reaching a maximum log of viable count of around 11 by 18 h of incubation. The graphic representation of the data shows a gradual increase in the viable counts of this organism up to the end of incubation period. The statistical analysis of the data on growth patterns of the four cultures under investigation, as indicated by viable counts was done using complete randomized design indicates that the difference in the rate of growth of three cultures are statistically non significant except for the culture of Bifidobacterium, which was statistically significant. The results obtained in the present investigation are at par with those reported by earlier workers for various lactic acid bacteria (Kohwi et al., 1982, Prajapati et al., 1984, Khedkar 1988 and Shah, 1997). As far as the maximum cell mass production is concerned, the results obtained in the present investigation are in well conformity with those of Patel (1982) and Prajapati (1984) who obtained maximum cell mass at 24 h of incubation.

The growth patterns (in terms of titratable acidity expressed as %LA) of lactic acid bacteria is given in Table 2. To study the rate of acid production of these cultures, sterilized skim milk was inoculated with active culture of the test organisms and its titratable acidity was monitored for 24 h at an interval of three h. It indicates that the culture L. acidophilus-LBKV3 showed a minimum titratable acidity value of 0.21 and a maximum of 0.82% LA at 3 and 24 h, respectively. The maximum acidity value (0.58% LA) in a minimum time of incubation (15 h) indicates that the probiotic culture of Lb. acidophilus-LBKV3 can be suitably used after 15 h incubation. This data is in close conformity with the viable counts obtained in the current investigation. It was observed that the Lb. delbrueckii subsp. bulgaricus showed an acidity value, which is almost sufficient for coagulation within a period of three h. It was swiftly increased to 0.52 in 12 h of incubation followed by 0.60, 0.64, 0.71 and 0.83% at 15, 18, 21 and 24 h, respectively.
The second partner culture of yoghurt i.e. *Salivarius* subsp. *thermophilus* showed a highest initial titratable acidity value (0.26%) within a period of three h of incubation. It was followed by a pattern of steady increase in the acidity ranging from 0.34% to 0.73% LA on sixth and 24 h of incubation. However *Bifidobacterium* NCDC 255 failed to increase any notable acidity in the medium till six h. Thereafter the acidity slowly increased and finally it reached to 0.45% (LA) at the end of incubation period. These observations further strengthen the behavior of this organism seen in case of its viable counts. The statistical analysis of the data indicates that the rate of acid production as a measure to study the growth patterns, is significantly different from the one culture to the another in the present investigation. The standard error value of 3.5289 and critical difference value of 1.0285 confirms the same. The CV value of 1.57937 is indicative of statistically significant difference. Continuous rise in acidity even after the population entered the stationary phase observed in the present study is in agreement with the results of Mutai *et al.* (1971); Rasic and Kurmann (1978); Prajapati (1984). Such rise in acidity could be attributed to the activity of the cells and their enzymes (Rasic and Kurmann, 1978; Gilliland, 1979). One of the aims of this study was to decide the stage of fermentation at which there would be a maximum number of viable cells of the probiotic organisms present per unit volume of the product so that the active cell population of the probiotics should be optimum to exert possible therapeutic benefits to the consumers for appropriate response as suggested by Hawley *et al.* (1959) and Speck (1978).

**Curdling time**

Acidophilus milk prepared by inoculating an active culture of *Lactobacillus acidophilus* LBKV 3 @2%, Bifidus milk prepared by inoculating *Bifidobacterium* NCDC 255 @ 3%, Yogurt prepared by inoculating 1% inoculum of *Lactobacillus delbrueckii* and *Salivarius* subsp. *thermophilus* and the probiotic Acido-Bifido-Yogurt prepared by a combination of aforementioned cultures were analyzed for their biochemical characteristics. The results presented in Table 3 indicates that the normal yoghurt showed curdling within a period of four h while probiotic Acido-Bifido-Yoghurt took five h whereas Acidophilus milk took 7 h and the Bifidus milk taken 12 h for curdling. The data indicates that the titratable acidity of Acidophilus milk was 0.82, 0.94 and 1.12% LA, respectively after 24, 48 and 72 h of preparation. The same trend was observed in case of the yoghurt where these values ranged from 0.89, 1.24 and 1.38, respectively whereas for probiotic Acido-Bifido-Yoghurt the values were 0.92, 1.36 and 1.42 respectively during 24, 48 and 72 h. This data clearly shows a reflection of synergistic impact of the symbiotic growth of the multiple starter cultures used in preparing this product. The test strain of *Bifidobacterium* showed a slow rate of growth and the acid development. Its acidity after 24 h was 0.45, which was increased to 0.54 and 0.65 after 48 and 72 h, respectively. The results obtained in present investigation are in close conformity with earlier reports of Moon and Reinbold (1976) and Singh and Sharma (1982).

**Acceptability of fermented milk products**

Various fermented milk products were prepared by using the cultures individually and in combinations as mentioned earlier. The said products were compared for their body, texture and overall acceptability sensory attributes through a panel of six judges. The products were judged for their overall acceptability by employing nine-point Hedonic Scale. The data on the same is presented in Table 4. It could be seen from the data obtained on the acceptability of the four fermented products that there was a wide variation in the response(s) recorded by the six judges. The minimum score...
(3.21) was assigned to Bifidus milk whereas maximum score (9.44) was allotted to probiotic Acido-bifido-yoghurt. It could be seen from the response of judges that Bifidus milk was liked the least (the mean score of six judges 3.31) whereas Acido-bifido-yoghurt was liked the most (mean score of six judges 8.79). Plain yoghurt and Acidophilus milk obtained a mean score value of 8.66 and 4.3, respectively. The differences in the scores received by four products are statistically significant. In order of acceptability the probiotic Acido-bifido-yoghurt was liked the most. This may be attributed to the synergistic impact of the symbiotic growth of four cultures. But as reported by several workers, the Bifidus milk was liked the least. This is due to its unclean flavour, weak body with whey separation. These undesirable characteristics in the product are attributed to a very slow rate of growth and the original habitat of this organism. Acceptability of Acidophilus milk is superior over the Bifidus milk but inferior to the rest of the products under study. But the interaction effect of judges and the four cultures was non-significant. The results obtained in the present investigation are in confirmation with the earlier reports published by several workers (Deeth and Tamime, 1981; Prajapati, 1984; Nahaisi, 1986; Colombel et al., 1987; Khedkar, 1988; Clark and Martin, 1994 and Shah, 1997).

CONCLUSION

It was concluded from this investigation that good quality probiotic Acido-bifido-yoghurt can be prepared from buffalo milk by inoculating mixed cultures of \( Lb. \) acidophilus, \( Bif. \) bifidum, \( Lb. \) delbrueckii ssp. bulgaricus and \( Str. \) salivarius ssp. thermophilus. This product contains the desired viable counts of the probiotic organisms to exert therapeutic benefits. Further research is needed to produce this product on an industrial scale.

REFERENCES