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Functional Lactic Beverage Supplemented with Commercial Lactic Acid Bacteria

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Probiotic dietary intervention is considered as judicious and sustainable approach for integrated health, as well as, to cope with chronic diseases. In current exploration, lactic beverage was formulated by inoculating different concentrations of commercial lactic acid bacteria (LAB) in whey protein concentrate with addition of fresh mango juice, stored (5 °C) and the product was subjected to physicochemical analysis and hedonic response, periodically. Four treatments were prepared by using different concentration of lactic acid bacteria and stored for stability check and compared with control. The functional lactic beverage showed significant ($p < 0.05$) differences among pH, acidity, protein and lactose contents during storage period as well as within treatments. The hedonic response revealed that lactic beverage can be stored best for 10 days at refrigeration temperature.

Keywords: Lactic beverage, Probiotics, Lactic acid bacteria, Whey protein concentrate.

INTRODUCTION

In recent era, growing medical costs have strained people to find low-cost and efficient means of protecting their health, therefore interest in functional food products has increased. Consumers are now more aware of links between health and nutrition due to popular media. Additionally consumers are also taking proactive role in optimizing individual health and wellbeing without fully relying on pharmaceuticals, to enhance their health span along with life span. Moreover, elderly populations in Western society and increased scientific evidences for the effectiveness of functional foods are among the factors that have triggered rapid development of functional food markets [1]. Amongst functional foods, dairy-based functional products account for nearly 43 % of the market, which is almost entirely made up of fermented dairy products [2]. Lactic beverages serve as a good way for the proper functioning of probiotics. They are living microorganisms if ingested in sufficient amount provide health advantages on host and enhance the balance of microbial flora in host gut against pathogens. Elevated cholesterol level and cancer can also be prevented by probiotics. Due to the health related advantages of *Lactobacillus acidophilus* it is known as a probiotic starter in food. Naturally this strain is present in human intestinal tract and known to be used in fermented foods for many years. The reason of giving importance of this strain is due its appropriateness in general aspects (source, safety, uniqueness and acid and bile resistance), ability to remain viable under laboratory environment and processing and functional and valuable aspects [3]. Whey protein concentrate (WPC) and whey protein isolate (WPI) are frequently used as ingredient in beverages and difference is in their composition. Different technologies are used for manufacturing of these ingredients *e.g.* whey protein isolate and whey protein concentrate are manufactured through ultrafiltration and microfiltration techniques. They occurs clear in solution when added into ready to drink beverages [4].

Keeping in view the importance of human health and useful aspects of whey as well as probiotics the present study was undertaken to formulate a novel functional lactic beverage using whey protein concentrate and a probiotic *i.e.* lactic acid bacteria and to check maximum shelf life of product at room temperature as well as at refrigeration. As fermented products could not be sustained at room temperature due to over fermentation hence second parameter was used only for stability estimation.

EXPERIMENTAL

Culture (lactic acid bacteria) and whey protein concentrate (80 %) was used as test material, supplied from collection centre of Nestle (PVT) Ltd. Culture was stored under refrigerated temperature. The fresh mangoes were collected from the local retailer (Metro cash and carry, Faisalabad 38000, Punjab, Pakistan). All other chemicals and reagents used were of analytical grade.

Product preparation: Lactic beverage was prepared with slight modifications as described earlier [5]. For the purpose, whey protein concentrate was reconstituted in distilled water 10 % (w/v). After that reconstituted whey protein concentrate was heated at 116 °C for 20 min. Commercial lactic acid bacterial cultures *i.e.* YO MIX-495-LYO-250-DCO, that was previously stored at refrigerated temperature, used for fermentation. The cultures were transferred in whey protein concentrate with the ratio of 0.5, 1, 1.5 and 2 % resultantly incubated at 37 °C for 24 h (Table-1). After the completion of fermentation period, whey protein concentrate were cooled down with ice cubes to stop the fermentation. Meanwhile fresh mangoes were peeled off and pulp was taken after that diluted with water for the preparation of mango juice. Whey protein concentrate was diluted with mango juice in ratio *i.e.* 1:2. After that beverage filled in plastic bottles, sealed and stored at 5 °C for further analysis.

TABLE-1
TREATMENTS PLAN FOR THE DEVELOPMENT OF FUNCTIONAL LACTIC BEVERAGE

Ingredients/treatments	T_0	T_1	T_2	T_3	T_4
Whey protein concentrate (g)	10	10	10	10	10
Mango juice (mL)	200	200	200	200	200
Culture (lactic acid bacteria) (%)	No culture	0.5	1.0	1.5	2.0

Titratable acidity and pH: pH of the product was measured by pH meter (Extech Instruments, Waltham, MA) following the method given [6].

Lactose: Lactose contents of beverage were measured by following the method explained [6]. This method is based on capability of sugars that how much they reduce copper ions into cuprous oxide. Reagents used were; Fehling solution A: 69.28 g $CuSO_4$. $5H_2O$, 1L distilled water [Qrec (ASIA) SDN BHD, Malaysia].

Protein: Protein was determined by using the method [7]. Sample was digested with conc. sulphuric acid [Qrec (ASIA) SDN BHD, Malaysia] till green colour obtained. Digested matter then distilled with NaOH. Protein % was calculated by using following formula:

$$\text{Crude protein} = \text{Nitrogen (\%)} \times 6.38$$

Sensory evaluation: Sensory evaluation of the lactic beverage was performed in a sensory laboratory at National Institute of Food Science & Technology, Faisalabad, Punjab, Pakistan. Sensory evaluation was done after fermentation at 0 day and during storage periodically. The 60 consumers in the test of lactic beverage were reported to consume lactic beverage at least 1 to 2 times in a week. Consumers were asked to score for five parameters including colour, flavour, taste, texture and overall acceptability of the product on a 9-point hedonic scale, that is, 9 = like extremely, 5 = neither like nor dislike, 1 = dislike extremely.

RESULTS AND DISCUSSION

Specifications of product: The procedure discussed earlier was adopted for the formulation of lactic beverage using different concentrations of lactic culture in 1:2 ratio. After the product formulation it was subjected to analysis after 0, 3, 7, 10 and 14 days of interval, including pH, acidity, fat, ash, lactose, protein and sensory evaluation. It was observed that pH, acidity, lactose, protein and sensory characters were affected by the different concentrations of starter cultures and with time as well. Mean values observed in the product for different parameters were as under. *e.g.* pH values for the product ranges from 6.3 to 4.5 while titratable acidity values includes 0.28 to 0.72. Moreover, ash and fat contents were 0.63 and 0.76 respectively. Range for lactose contents observed was 2.22 to 0.67 whereas 2.9 to 0.77 were for protein contents. Desirable level for taste flavour and overall acceptability parameter in sensory evaluation was observed for almost all treatments but the ratio of overall acceptability during storage was most desirable for T_4 rather those having lower concentrations of culture.

Effect of culture concentration and storage on pH & acidity: The effect of 0.5, 1, 1.5 and 2 % inoculum of lactic acid bacteria in the beverage was seems to be highly significant. The effect of storage days was also found to be highly significant. pH is the major factor which is affected by the action of lactic acid bacteria during fermentation. Usually lactic acid bacteria drop the pH of whey related drinks to 4.0-4.5 [8]. Results obtained indicated a definite change in pH values with a distinct difference with in treatments and with time. Mean values depicted that the treatment in which there was no culture showed a bit decrease in pH with the passage of time as compare to those treatments containing different concentrations of lactic acid bacteria. Statistical results indicated that lactic acid bacteria during 24 h of fermentation decreased the pH of beverage on an average from 6.44 to 4.48. Maximum pH reduction at 0 day was found for the T_4 and T_3 *i.e.* 4.8 and 4.7, respectively (Fig. 1).

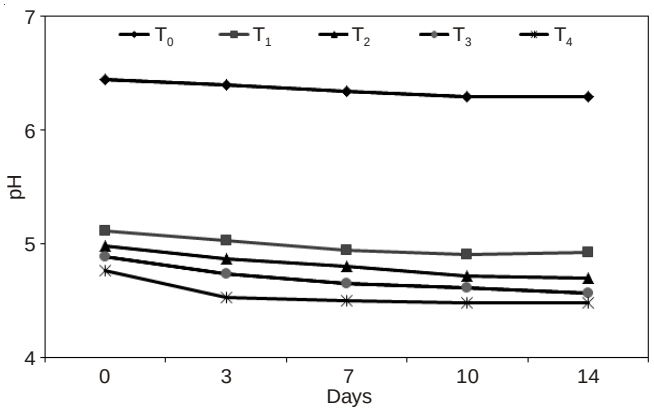

Fig. 1 Effect of storage and culture concentration on pH

The reason could be the more concentration of culture in that treatment. Whereas with the increase in time still difference

was observed *i.e.* reduction in pH was still there although fermentation was stopped, but this reduction was less as compare to the treatments having variation of culture concentration.

This was due to the fact that beverage was stored at 5 °C and at that temperature bacteria work slowly [9]. The results obtained in present work are also supported by Pescuma *et al.* [5]. As the lactic beverage was prepared with the addition of yoghurt cultures, so these cultures have ability to drop the pH from 4.9 to 4.5. Even during storage at low temperature they also exhibit their property to reduce pH. As seen from Fig. 1, the maximum pH value for T_0 was found 6.44 at 0 day and with the time it reduced to a value 6.29 at 14th day of storage. This result contain a distinct difference from those values of pH obtained from T_1, T_2, T_3 and T_4, which indicated that if culture concentration is changed it would cause to drop the pH of product with the storage time period. So it was found that product showed a considerable effect of lactic acid bacteria during fermentation as well as during storage at low temperature.

Acidity also exhibited clear changes in product towards culture concentration during storage. Acidity of products having whey as chief component is supposed to be due to the fermentation of lactic acid [10]. The scientific work was related about the compositional analysis of commercial whey drinks. After the research work it was suggested by the authors that whey drinks can be used by those people having low habits for milk consumption. The results represented in this manuscript was also supported by Sampedro *et al.* [11], whom worked on lactic drinks with addition of different forms of whey *i.e.* whey protein concentrate and whey protein isolates. Researcher found that with increase in lactic acid amount after fermentation of lactose acidity level also increased. Maximum increase in acidity level was found for the T_4 at 0 day *i.e.* 0.67 followed by T_3 0.58. Results shown revealed that pH of T_0 were 0.23 at 0 day which increased to 0.33 at 14th day of storage (Fig. 2).

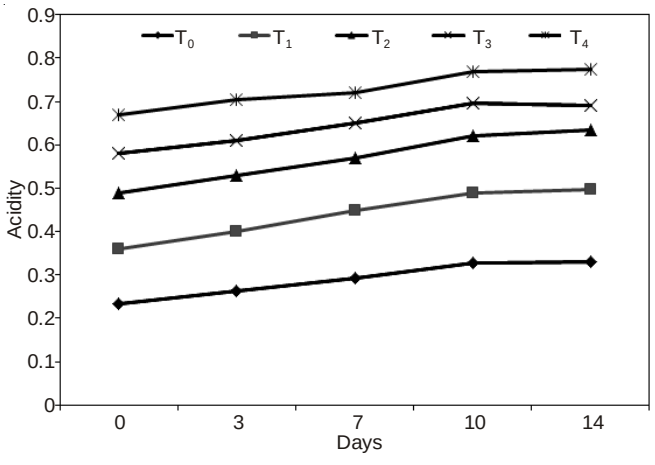

Fig. 2. Effect of storage and culture concentration on acidity

By comparing this value with the acidity rate of T_4 at 0 as at 14th day of storage [5], it was observed that more the concentration of starter culture more will be the rate of fermentation of lactose which results in the formation of organic acids *i.e.* lactic acid, acetic acid, resulting in the high rate of acidity. Although at low temperature cultures slow down their activities but some probiotic species of lactic acid bacteria found to exhibit their activities at low temperature which is known as post acidification [12]. Higher acidification rates are observed along with more consumption of milk sugar (lactose) which overall enhance the acidity of the end product [13].

Acidity rate of T_4 and T_3 at 0 day was 0.67 and 0.58, respectively which increased to the 0.77 and 0.66 respectively at 14th day of storage, this rate of acidity was too high as compare to the T_0 (control). Kar and Masra [14] found somewhat similar results for acidity ranging from 0.68 to 0.78 % in fermented whey drink,whereas results for acidity contents were also supported by Wakil *et al.* [15] which were from 0.50 to 0.62 %.

Effect of culture concentration and storage on lactose & protein: Prepared lactic beverage was analyzed for lactose contents. Lactose contents of the beverage were found to be variant not only within the treatments but also within the storage time period. It was observed by the results that culture concentration affect the lactose contents significantly.

The lactose contents were found to be similar with the results described [5]. The researcher worked on isolation and identification of lactic acid bacteria and stated about the ability of strain to be viable under extreme conditions of acidity and high bile salt and to degrade the lactose. In research yoghurt cultures were produced and observed for above mentioned characteristics. Furthermore, the lactose degradation was found to be from 48 to 42 % in first 3 h of fermentation. Kar and Misra [14] found the lactose contents to 4.8 % whereas results depicted in present research are also supported [16] whom reviewed about whey beverages and on an average found 4.4 % of lactose contents. It was found that almost 80 % of lactose contents were consumed by the lactic acid bacteria during fermentation especially when combinations of starter cultures were used [8]. Same results were investigated [13]. Yoghurt cultures were used in their study in which they found that in first 12 h of fermentation almost 60 % of lactose was consumed by the bacteria. Maximum lactose contents were 2.3 % for T_0 at 0 day which decreased to 2.14 % at 14th day of storage at 5 °C. While the maximum decrease in value for lactose contents after 24 h fermentation at 0 day found were 0.73 % and 0.78 % for T_4 and T_3 respectively which was further reduced to 0.61 and 0.68 % at 14th day of storage (Fig. 3).

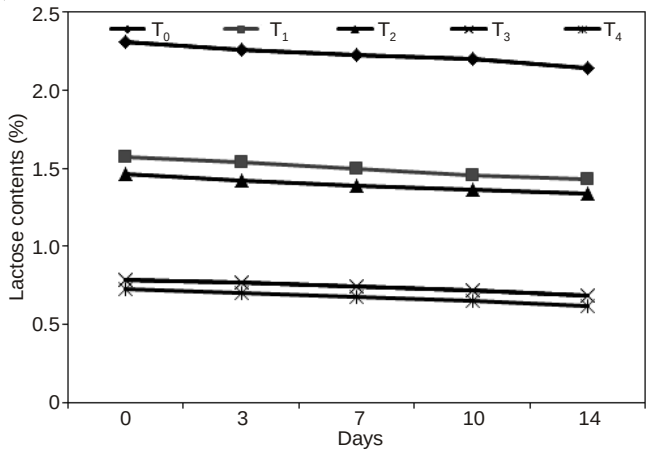

Fig. 3. Effect of storage and culture concentration on lactose contents

Prepared product was analyzed for protein contents. It is observed that effect of treatments (starter culture) was found

to be highly significant. The effect of days was also found to be highly significant. Whereas, interaction of days and culture dose (0.5%, 1 %, 1.5 %, 2 %) was depicted to be non-significant. Lim *et al*. [17] worked on whey protein concentrates in which influence of high hydrostatic pressure was evaluated on hydrophobicity of whey protein concentrates. During research work 6.2 % of protein contents were found in whey protein concentrate having 80 % of protein. Yoghurt cultures when used in combination are considered to be involved in the breakdown of protein contents especially β-lactoglobulin, an allergen [5]. Lactic acid bacterial cultures decreased whey proteins significantly to reduce the allergy problems related to the β-lactoglobulin. Almost 80 % of protein degradation occurs in first 12 h of fermentation which causes to reduce the protein contents of fermented drink [5]. Maximum protein contents for T_0 at 0 day were 2.95 which become decreased to 2.84. Whereas for T_4 after fermentation it was found to decrease to 0.846 at 0 day which further reduce to 0.710 till the 14th day during storage (Fig. 4).

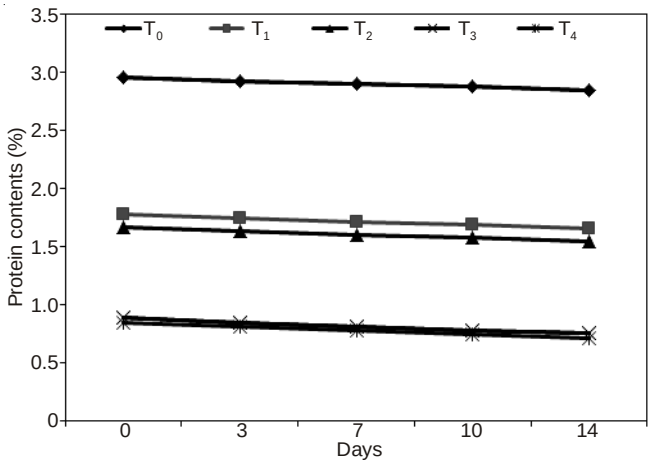

Fig. 4. Effect of storage and culture concentration on protein contents

In the lactic beverage 2.95 % of protein contents were found. The variations in the results could be due to the addition of mango juice whose protein contents were 0.65 %. The results obtained are further supported [18] whom worked on different Indian mango varieties to evaluate the valuable components of those varieties. And during scientific research work almost similar results for protein contents were found as presented in this manuscript.

Sensory evaluation: Lactic beverage was subjected to organoleptic evaluation after fermentation and during the storage at low temperature. Five parameters including colour, flavour, taste, texture and overall acceptability were determined by consumer panel on 9-point hedonic scale. The product was acceptable when used within 10 days of storage.

The main objective of present study was to evaluate the effect of culture with different concentrations on the lactic beverage and to check the maximum shelf life of the product at refrigerated temperature and at room temperature. Product could not be sustained at room temperature more than one day and was unacceptable from consumption point of view so the only refrigerated temperature was selected for research work. Beverage was prepared with different culture dose (0.5 %, 1 %, 1.5 %, 2 %) using whey as functional and chief ingredient in the product. Since a combination of yoghurt cultures was used this considered to exhibit the process of post acidification under low temperature so beverage was found to be sour after 10 days.

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REFERENCES

1. A. Palou, C. Pico and M.L. Bonet, *Nutr. Rev.*, **62**, 169 (2004).
2. H. Lewis, Global Market Review of Functional Soft Drinks - Forecasts to 2014: Market Values and Volumes for Global Functional Soft Drinks, Aroq Limited, Bromsgrove, pp. 4-14 (2009).
3. X. Pan, F. Chen, T. Wu, H. Tang and Z. Zhao, *Food Contr.*, **20**, 598 (2009).
4. L. Makras, G. Van Acker and L. De Vuyst, *Appl. Environ. Microbiol.*, **71**, 6531 (2005).
5. M. Pescuma, E.M. Hébert, F. Mozzi and G. Font de Valdez, *Int. J. Food Microbiol.*, **141**, 73 (2010).
6. W. Horwitz, Official Methods of Analysis of the Association of Official Analytical Chemists, edn 13 (1980).
7. AACC, Approved methods of the American Association of Cereal Chemists, (CD-ROM), Scitech Book News, Ringgold Inc, Portland, edn 10 (2001).
8. M. Koiche and B.A. Dilmi, *Afr. J. Biotechnol.*, **9**, 1635 (2010).
9. R. Coda, C.G. Rizzello, A. Trani and M. Gobbetti, *Food Microbiol.*, **28**, 526 (2011).
10. P. Jelen, R. Currie and V.W. Kadis, *J. Dairy Sci.*, **70**, 892 (1987).
11. F. Sampedro, D. Rodrigo and M. Hendrickx, *J. Food Eng.*, **86**, 133 (2008).
12. C.H. Lee, *Food Contr.*, **8**, 259 (1997).
13. M. Pescuma, E.M. Hébert, F. Mozzi and G. Font de Valdez, *Food Microbiol.*, **25**, 442 (2008).
14. T. Kar and A.K. Misra, *Rev. Microbiol.*, **30**, 163 (1999).
15. S.M. Wakil, O.T. Ayenuro and K.A. Oyinlola, *Food Nutr. Sci.*, **5**, 495 (2014).
16. B.B. Solak and N. Akin, *J. Food Sci. Eng.*, **2**, 129 (2012).
17. S.Y. Lim, B.G. Swanson, C.F. Ross and S. Clark, *J. Dairy Sci.*, **91**, 1308 (2008).
18. C.M. Ajila, S.G. Bhat and U.J.S. Prasada Rao, *Food Chem.*, **102**, 1006 (2007).