Tribhuvan University Journal of Food Science and Technology
1 (2022) 16-22
DOI: 10.3126/tujfst.v1i1.49932

ORIGINAL RESEARCH PAPER

Kinetics of Lactic Acid Fermentation during *Dahi* Preparation

Arjun Ghimire*[1] and Nisha Raya[2]

[1]*Department of Food Technology, Central Campus of Technology, Tribhuvan University, Hattisar, Dharan-14, Nepal*

[2]*Department of Food Technology, Dharan Multiple Campus, Tribhuvan University, Dharan, Nepal*

Abstract

Dahi with 4.15% fat and 11% solids-not-fat (SNF) was prepared from cow milk, pasteurized at 85℃ for 15 min and cooled to 25℃. Fermentation kinetics was studied to study the effects of sugar concentration (2 to 10%) and starter-level (1 to 3%) on cell growth, lactic acid production and its rate, pH, and lactic acid bacteria (LAB) count which was monitored every 3 h until the total of 15 h fermentation time. The biomass growth rate was very high for 10% sugar at 2% inoculum levels i.e., lnX/X_t = 30.74. Similarly, the lactic acid production rate was maximum (0.17%) for 2% sugar at 1% inoculum level. The overall maximum growth rate was observed at 6% sugar concentration for all inoculum concentrations. The highest growth rate of 0.8 per hour was seen for 1% inoculum at 6% sugar concentration and the pH for the entire process decreased from 6.5 to 4.4 when fermented at room temperature (28℃). The decrease was steep and a gradual fall in pH was obtained at 8 h of fermentation in 2% and 8% sugar levels at all inoculum levels. From the study, inoculation and sugar levels showed a significant effect on the specific growth rate, pH, and the total lactic acid bacterial counts thereby heading one step possibility of commercialization of indigenous product dahi through optimization of the process variables.

Keywords:
Biomass growth rate
Fermentation kinetics
Inoculum levels
Lactic acid bacteria

Article history:
Received: 4 Nov 2020
Received in revised form: 30 June 2021
Accepted: 7 July 2021
Available Online: 02 December 2022

*Corresponding author at:
Central Campus of Technology
Tribhuvan University
Hattisar, Dharan-14, Nepal.
E-mail address: arjun.ghimire@cct.tu.edu.np

Introduction

Fermented dairy foods have been a crucial part of the human diet worldwide since ancient times. Lactic acid bacteria have an essential role in milk fermentation and preservation since lactic acid bacteria display numerous antimicrobial activities in fermented foods. This is mainly due to the production of organic acids. Therefore, Lactic acid bacteria exert strong antagonistic activity against many microorganisms, including milk spoilage organisms and pathogens (Gemechu, 2015). The basic and simple process of making *dahi* is well known to all, but no detailed knowledge of its biochemical and physical changes has been developed so far.

Dahi is a fermented milk product produced through the fermentation process by deliberately adding live, harmless, lactic acid-producing bacteria in the form of culture to milk. The added

lactic acid bacteria grow in milk medium, produce lactic acid, acetic acid, and carbon dioxide through the consumption of lactose in the milk (Narender et al., 2013). The indigenous *dahi* is prepared for sale in earthenware pots at home and local level. More than 50% of the total milk produced in Nepalese households is believed to be processed into *dahi*: only a small quantity of milk produced is sold as such (Joshi and Bahadur, 2015). An important intermediary step in the production of *nauni*, ghee (Kharel et al., 2010), *mohi*, and *chhurpi* (Dewan and Tamang, 2007) is the conversion of milk into *dahi*. The quality of *dahi* in the local market varies from shop to shop as there is no well-described standard for the fermented products. Poor quality milk, unhygienic practices associated with the process involved and the use of "wild type" of starter culture give rise to poor grade *dahi* having six to twelve hours shelf-life under normal room temperature conditions (Younus et al., 2002).

The most significant fermentation in dairy is the fermentation or souring of milk with lactic acid (De, 2000). Lactose is a sugar found in raw milk that has been fermented by lactic acid bacteria. Therefore, the fermentation of lactose occurs naturally in milk, which is uncontrollable and leads to a spoiled product. To produce desirable and more palatable milk products like yogurt, buttermilk, sour cream, acidophilus milk, kefir, koumiss, bulgaricus milk, the idea of inoculating various selected strains of lactic acid bacteria (pure or mixed) in the milk or the by-products has been developed (Chandan, 2004). The fermentation process improves the nutritional quality, digestibility, and bioavailability of nutrients, while also reducing anti-nutritional factors and enhancing the shelf life and safety of the product (Tamang et al., 2016). Traditional uses of many LB as fermentation agents for foods are considered to be safe for the general population. Lactic acid bacteria cause rapid acidification of food due to the production of acids, primarily lactic acid. Other metabolites associated with LAB include acetic acid, ethanol, aromatic compounds, bacteriocins, exopolysaccharides, and several enzymes. These compounds result in the enhancement of shelf life and microbial safety, as well as the improvement of texture and sensory profile of the fermented products (Leroy and De Vuyst, 2004).

During the fermentation process of preparing *dahi*, numerous physical and chemical changes occur, which have been overlooked by individuals who focus solely on their pattern of consumption. This research, therefore, emphasizes not only the physicochemical aspects but also the parameters of kinetics that are also involved during the *dahi* making process. The main purpose of this research was to understand the effect of concentrations of sugar and culture during the fermentation of *dahi*.

Materials

Milk

Cow milk consisting of 4.15% fat and 6.15% solids-not-fat (SNF) was brought from Laxmi Farm, Itahari, Nepal.

Culture

A previous day *dahi* culture was brought from the same milk farm and serial dilution was carried out followed by spread plating in De Man, Rogosa and Sharpe (MRS) Agar to calculate its microbial count.

Skim milk powder

Skim milk powder testing 95% Milk-Solids- Not-Fat (MSNF) manufactured by Sujal Dairy Pvt. Ltd., Pokhara, Nepal was obtained from Jivan Bikash Dairy Pvt. Ltd., Biratnagar, Nepal.

Fermentation container

Forty-five food grade, odorless, and non-breakable plastic cup (300 ml) was used for fermentation brought from the local market of Dharan, Nepal.

Equipment and glasswares

The following equipment were used in this study:

Electronic balance (Model: HZT-A500, India), Hot air oven (Navyug Udyog, Haryana, India), Gerber centrifuge, Suction pump were taken from Food Technology Department, Dharan Multiple Campus, Dharan, Nepal.

The routine laboratory glasses like petri plates, pipette, burette, and other accessories were taken from Food Technology Department, Dharan Multiple Campus, Dharan, Nepal.

Chemicals required

Sodium hydroxide, sulfuric acid, hydrochloric acid, phenolphthalein indicator, amyl alcohol, and Gerber sulphuric acid procured from Thermofisher Scientific Pvt. Ltd, India, oxalic

acid of laboratory-grade was purchased from Merck Specialties Pvt. Ltd, India.

Methods

Preparation of *dahi*

Dahi was prepared by fermentation as shown in Figure 1. Milk was preheated to 50°C, skim milk powder was added and thereafter, the milk was pasteurized at 85°C for 30 min and cooled to 28°C. The cooled milk was inoculated with various concentrations of local starter culture (1, 2 & 3%), and sugar (2%, 6%, 10%) incubated at room temperature (28°C) for 15 h. The fermentation of milk was carried out in 300 ml plastic cups and stored at 5°C for 24 h for chemical analysis.

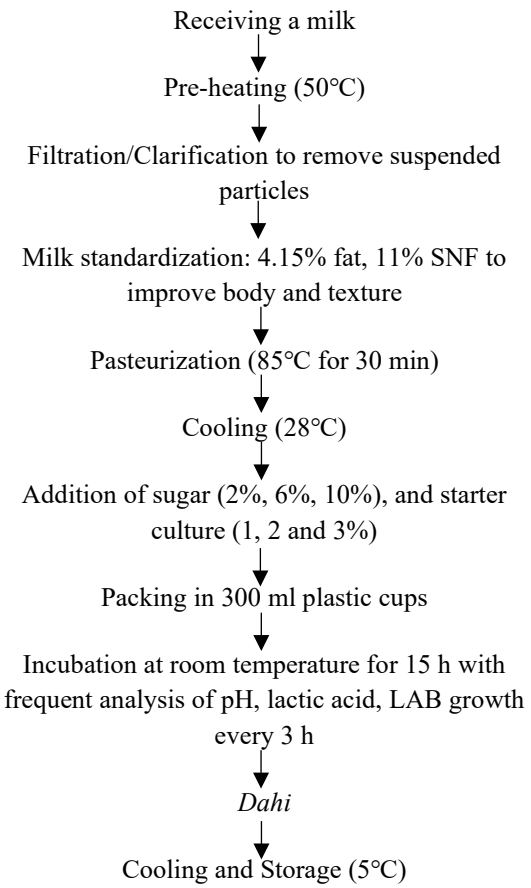

Figure 1
Dahi preparation outline

Fermentation

About 250 g of the standardized milk was put in the respective sterile plastic cup of 300 ml and then the various percentage of sugar and starter culture obtained from the central composite design using Design Expert V7.1.5 was added and covered with aluminum foil. It was then left for fermentation in a room at 28°C for 15 h and the 250 g samples were aseptically taken at every 3 h interval.

Microbial analysis

1 g of sample was weighed in a digital balance and then transferred into the test tube containing 9 ml of distilled water and then vortexed for 2 min. Serial dilution from 10^{-1} g/ml till 10^{-4} g/ml was performed (Maheshwari, 2002).

Analytical procedure

The pH of the sample was determined directly using a digital pH meter (Type 361, Systronics, India) calibrated with standard buffer solutions (Merck) (Ranganna, 1986). Titratable acidity was expressed as a percentage of lactic acid in the sample (AOAC, 2005).

Fat content of *dahi*

Fat was determined by the Gerber method according to the method described in the National Dairy Development Board (NDDB, 2001).

Lactic acid bacteria (LAB) count of *dahi*

LAB count was determined by counting the number of colonies in the Petri plates with the help of a colony counter (Dewan and Tamang, 2007).

Recipe optimization in the preparation of *dahi*

The experimental design (D-optimal, 2 Factors, 3 Levels, and 13 Runs) was done as shown in Table 1 with Design Expert V7.1.5 using 3 levels of sugar (2, 6, and 10 g) and 3 levels of culture (1, 2 and 3 g).

Biomass concentration for sugar and inoculums level variation

The effect of biomass concentration for variation in sugar and inoculums level was studied with the help of a logarithmic graph. Time (h) was plotted on X-axis and logarithmic biomass on Y-axis. The nature of curves demonstrated the fall and growth of the biomass concentration.

Specific growth rate (μ) for variation in inoculums and sugar level

Lactic acid is expressed in percentage and studied based on time for every 3 h intervals. Similarly, the specific growth rate is expressed per hour. The rise and fall of the growth rates can be

illustrated with the nature of the pattern of the graph.

Table 1
Experiment plan generated by Design Expert v7.1.5

Runs	*Factor 1: Sugar*	*Factor 2: Culture*
1	6	1
2	6	2
3	10	3
4	6	2
5	6	2
6	6	3
7	6	2
8	2	2
9	2	1
10	10	2
11	10	1
12	2	3
13	6	2

Results and Discussion

Variation of biomass

The presence of sugar substances as a carbon source in a fermentation medium induces positive increases in biomass during fermentation. In every graph, a short lag period is observed with the maximized log phase but the existence seemed different from the difference in sugar concentrations. The log phase was found to be increased with increasing sugar concentrations at every inoculum level as seen in Figure 2.

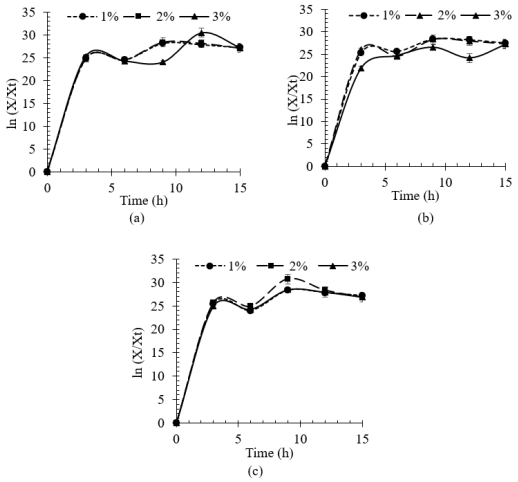

Figure 2
Growth pattern of lactic acid bacteria for (a) 2%, (b) 6%, and (c) 10% sugar at different inoculums level viz. 1%, 2% and 3%

Biomass development was less comparable for the 2% sugar level with different 1, 2 & 3% inoculums. Because of the rising metabolite rate, a huge proportion of biomass growth was observed in 10% sugar followed by 6% and 2% sugar concentrations. The maximum biomass was observed in 10% sugar with a 2% inoculum level ($\ln X/X_t$ = 30.74). A similar type of growth pattern was observed by Ginovart et al. (2002) in the growth rate of *S. salivarius* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in yogurt where the log phase was observed up to 6 h keeping lactose as the only substrate. It is because in the initial phase rising substrate concentration allows a feasible environment for lactic acid bacteria to multiply rapidly to their peak level and then decreased subsequently. The rate of growth progressively decays as a result of the increase of lactic acid in the medium (Ginovart et al., 2002).

Lactic acid formation kinetics

For various sugar levels, the inoculum levels were kept constant, i.e., 1, 2 and 3%. During fermentation, the rate of growth and the rate of lactic acid biosynthesis were both affected, in addition to the use of sugar. The synthesis of lactic acid during fermentation is shown in Figure 3 for 2, 6 and 10% sugar level and inoculum portion of 1, 2 and 3%.

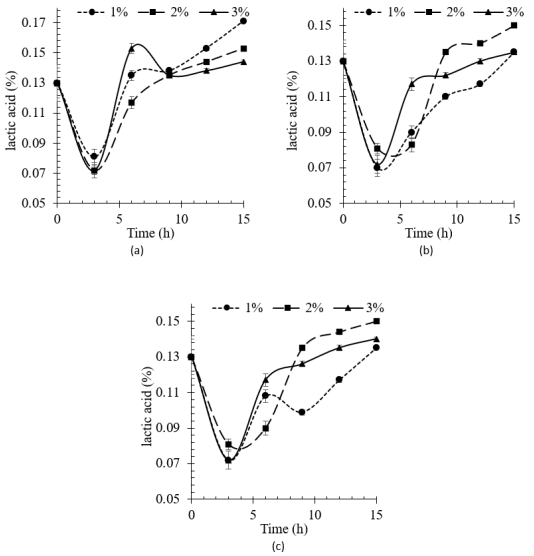

Figure 3
Lactic acid production for (a) 2%, (b) 6%, and (c) 10% sugar during fermentation

The rate of production of lactic acid appears to be higher for 2% sugar at a 1% inoculum than for 6% and 10% at all inoculum levels, respectively. It is because, as long as sugar is available, lactic acid bacteria do not use lactose for the same 15 h

of fermentation for acid production. With a limited supply of sugar, lactose must be used by bacteria for the production of acid. During fermentation, the data showed the maximum lactic acid percent was 0.171% for 2% sugar level at 1% inoculum while the maximum values approached the same i.e., 0.15% lactic acid for 6% and 10% sugar at 2% inocula, respectively.

Different mixture of culture from *S. thermophilus* (HST, 197) and *L. bulgaricus* (RTS, Yb) was studied by Walia et al. (2013) for total acid production for 2 to 10 h of incubation in cow's milk and buffalo milk. It was observed that with the increase in incubation time, a combination of different strains in both types of milk increased the production of acid in the culture. Earlier studies by Mocquot and Hurel (1970) and Tramer (1973) also showed that there was a rise in acid production in the cultivation combinations of different strains in both cow and buffalo milk from 10 to 12 h of incubation with the rise in fermentation period.

Variation of specific growth rate (μ)

The inoculum levels for different sugar levels were kept constant i.e., 1, 2 & 3% while the substrate concentration changed frequently for the study. The change in the growth rate for 2%, 6%, and 10% sugar is described in Figure 4.

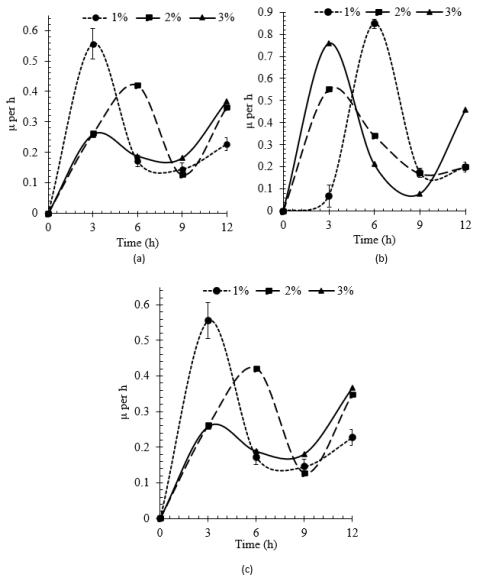

Figure 4
Variation of specific growth rate (μ) for (a) 2%, (b) 6%, and (c) 10% sugar at 1, 2 & 3% inoculums levels

The overall maximum growth rate was observed at 6% sugar concentration for all inoculum concentrations. The highest growth rate of 0.8 h^{-1} was seen for 1% inoculum at 6% sugar concentration. The growth rate pattern during the fermentation process was characterized by a short lag period up to 3 h, followed by rapid biomass growth and simultaneous lactic acid biosynthesis. 2% and 10% sugar fermentation displayed a long duration of slow development i.e., specific growth rate of 0.56 h^{-1} in the first 3 h affecting the fermentation time. Ginovart et al. (2002) also observed the increase in the number of bacterial cells in pure and mixed cultures of *S. thermophilus* (*S.*) and *L. bulgaricus* (*L.*) in yogurt till 4 h of fermentation time. The growth of biomass during the fermentation cycle indicates the active participation in the uptake and consumption of sugar.

Change in pH

The pH decreased dramatically during the time of fermentation which was due to the fast action of the lactic acid bacteria against the metabolite. Until the sugar is completely used for biomass development, lactose cannot be used for the production of acid. The changes in pH during *dahi* fermentation are depicted in Figure 5.

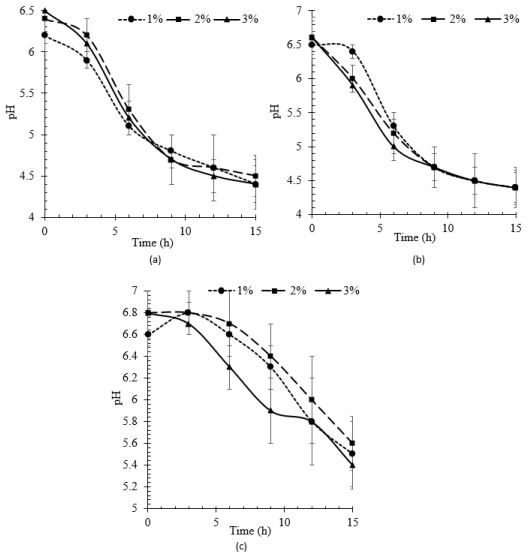

Figure 5
Effect on pH level during fermentation for (a) 2%, (b) 6%, and (c) 10% sugar

During fermentation, the pH for the entire process decreased from 6.5 to 4.4. The decrease was steep and a gradual fall in pH was obtained at 8 h of fermentation in 2% and 8% sugar levels at all inoculum levels. However, the process was

delayed with increased sugar levels and the final pH was found to be 5.5 after 15 minutes of study. The use of lactose in *dahi* continues as long as the external pH enables the lactic acid bacteria to metabolically function. In a study conducted by Varghese and Mishra (2008), the yogurt produced lactic acid and the pH had reached 4.0 where *L. bulgaricus* ceased its metabolic function and no further use of lactose occurred. Further conversion of lactose was possible by redirecting the metabolism of lactic acid bacteria toward the development of more pH-neutral components. The pH decreases faster by 2% sugar level compared to other levels in the samples despite inoculum levels. The LAB used them completely and quickly because of the low percentage of the sugar so that the high amount of lactose usage increased to fall in pH.

Conclusion

During the fermentation, sugar followed by inoculum concentration has a significant impact on the biomass growth, lactic acid formation, specific growth, and pH of dahi. The growth phase was found to be increased with increasing sugar concentrations at every inoculum level. The lactic acid formation was found to be highest at low sugar concentrations. The specific growth rate was optimum at 6% sugar level and found to decrease with an increase of lactic acid in the medium. The pH decreased sharply by the action of lactic acid bacteria towards the metabolite and a rapid decrease in pH was seen in low concentration of sugar. The kinetics data thus obtained can be used to develop a suitable mathematical model of lactic fermentation.

Acknowledgements

The authors appreciate all the faculties and staff of the Central Campus of Technology, Dharan, Nepal for their support and technical assistance.

Funding

This research has not received a particular award or grant from subsidizing organizations in general society, business, or not-for-profit driven sectors.

Compliance with Ethical Standards

Conflict of Interest

The authors declare no conflict of interest.

Ethical approval

The study did not involve any inhumane animal study.

References

AOAC. (2005). *Official Methods of Analysis* (16th ed.). Association of Official Analytical Chemists.

Chandan, R. C. (2004). Other fermented dairy products. In E. (R. C. Chandan and A. Kilara (Ed.), *"Advances in the Microbiology and Biochemistry of Cheese and Fermented Milk"*. (pp. 46-49). Elsevier Applied Science Publishers.

De, S. (2000). *Outlines of Dairy Technology* (1 ed.). Oxford University Press.

Dewan, S., & Tamang, J. P. (2007). Dominant lactic acid bacteria and their technological properties isolated from the Himalayan ethnic fermented milk products. *Antonie van Leeuwenhoek*, *92*(3), 343-352. https://doi.org/https://doi.org/10.1007/s10482-007-9163-5

Gemechu, T. (2015). Review on lactic acid bacteria function in milk fermentation and preservation. *African Journal of Food Science*, *9*(4), 170-175. https://doi.org/https://doi.org/10.5897/AJFS2015.1276

Ginovart, M., López, D., Valls, J., & Silbert, M. (2002). Simulation modelling of bacterial growth in yoghurt. *International Journal of Food Microbiology*, *73*(2-3), 415-425. https://doi.org/https://doi.org/10.1016/S0168-1605(01)00668-7

Joshi, D., & Bahadur, K. T. (2015). An overview of smallholder dairy production and marketing in Nepal. *Smallholder dairy production and marketing—Opportunities and constraints*, 33.

Kharel, G., Acharya, P., & Rai, B. (2010). Traditional foods of Nepal. *Highland. Publication Kathmandu, Nepal*.

Leroy, F., & De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science & Technology*, *15*(2), 67-78. https://doi.org/https://doi.org/10.1016/j.tifs.2003.09.004

Maheshwari, D. (2002). *Practical Microbiology*. S. Chand Publishing.

Mocquot, G., & Hurel, C. (1970). The selection and use of some micro-organisms for the manufacture of fermented and acidified milk products. *Journal of the Society of Dairy Technology*, *23*(3), 130-146.

Narender, R., Behare, P., D., Khetra, Y., Kharui, K., & Hussain, S. A. (2013). *Dairy Products Technology (Dairy Technology)* (1st ed., Vol. 1). The Secretary, Central Board of Secondary Education, Shiksha Kendra, 2, Community Centre, Preet Vihar, Delhi-110092.

NDDB. (2001). *Laboratory Handbook for Dairy Industry* (1 ed.). National Dairy Development Board.

Ranganna, S. (1986). *Handbook of Analysis and Quality Control for Fruits and Vegetables Products* (2nd ed.). Tata McGraw-Hill Publishing Co. Ltd.

Tamang, J. P., Watanabe, K., & Holzapfel, W. H. (2016). Diversity of microorganisms in global fermented foods and beverages. *Frontiers in microbiology*, *7*, 377. https://doi.org/10.3389/fmicb.2016.00377

Tramer, J. (1973). Yogurt cultures. *International Journal of Dairy Technology*, *26*(1), 16-21. https://doi.org/10.1111/j.1471-0307.1973.tb02053.x

Varghese, K. S., & Mishra, H. (2008). Modelling of acidification kinetics and textural properties in dahi (Indian yogurt) made from buffalo milk using response surface methodology. *International Journal of Dairy Technology*, *61*(3), 284-289. https://doi.org/10.1111/j.1471-0307.2008.00411.x

Walia, A., Mishra, H., & Kumar, P. (2013). Effect of fermentation on physico-chemical, textural properties and yoghurt bacteria in mango soy fortified yoghurt. *African Journal of Food Science*, *7*(6), 120-127. https://doi.org/10.5897/AJFS08.049

Younus, S., Masud, T., & Aziz, T. (2002). Quality evaluation of market yoghurt/dahi. *Pakistan Journal of Nutrition*, *1*(5), 226-230.