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Full Length Research Paper

Nutritional evaluation of yoghurt prepared by different starter cultures and their physiochemical analysis during storage

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Yoghurt was prepared with two different types of starter cultures that are *Lactobacillus bulgaricus* and *L. acidophilus*. In this study 3, 4 and 5% starter cultures were used and stored at 4 °C for 12 days. To analyze the effect of two different cultures and their concentrations on the properties of yoghurt, different physio -chemical tests (protein, lactose, ash, fat, acidity, total solid, pH and moisture) were performed. The results showed that the protein, lactose, ash, fat, acidity and total solid mass were slightly increased while pH and moisture values gradually decreased during storage period of 12 days. The comparative study of starter cultures showed that *L. acidophilus* produced good quality yoghurt as compared to *L. bulgaricus.*

Key words: Yoghurt, starter cultures, physio-chemical, comparative study of starter cultures.

INTRODUCTION

Fermented products are the mainly used in human diet in Pakistan, which have been derived from plant or animal materials. It is an acceptable and essential part of diet in most parts of the world for several centuries. Yoghurt is one of the oldest fermented milk products known. Fermentation of milk involves the action of microorganisms, principally the lactic acid bacteria. These microorganisms convert milk lactose into lactic acid and make milk sour (Kagan, 1985). The popularity of yoghurt is due to its characteristics; the pleasant aromatic flavor, thick creamy consistency and its reputation as food associated with good health (Kleyn et al., 1979; Domagla, 2005).

Present days, most yoghurt is prepared by either using special lactic acid producing organism or by direct acidification of milk by acidulant (Nobuo, 2002). Although the flash-freezing technique used in the production of frozen yoghurt, unlike slow freezing in a freezer, should not kill the live cultures (Meydani, 2006). Yoghurt made from milk (10% fat) with sugar and homogenized at 200 bars was found to be good quality (Balasubramanyan et al., 1991). High quality yoghurt with a pleasant taste depends very much on the ratio of two bacterial species: *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Fuller, 1989). The effect of culture concentration and inoculation temperature (25 °C and 45 °C) on physiochemical, microbial and organoleptic properties of yoghurt produced from three base materials was conducted in a nested experimental design. It was concluded that yoghurt with an acceptable quality could be produced with the three inoculation concentrations (3, 4 and 5%) at 25 °C (Abubakar et al., 2005).

The dairy protein composition is known to influence the structure and the texture character of yoghurt (Saint et al., 2006) . Bitterness in yoghurt is produced during storage due to the function of peptides caused by the proteolytic activity of *L. bulgaricus* (Renz and Puhan, 1975). The acidity of yoghurt varies from 0.7 to 1.1% lactic acid with pH approximately 4.0 to 4.2 (Wanda and Salauen, 2005). Yoghurt is more nutritive than milk in vitamin contents for its digestibility. It is also used as source of calcium and phosphorous (Foissy, 1983). It is believed that yoghurt has valuable "therapeutic properties" and helps curing gastrointestinal disorders (Adolfsson, 2004). Yoghurt may aid digestion, ease diarrhea, boost immunity, protect against cancer (Gibson et al., 1997; Fernandez, 1988; Ripudaman, 2003; Shahani et al., 1976; Perdigon,

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2005; Deeth and Tamine, 1981).

The specific health benefits depend on the strain and viability of the culture in yoghurt (Miller et al., 2008). Probiotic bacteria are completely non-toxic. Probiotics have been consumed as part of cultured food such as yoghurt (Troller, 1973). Probiotics can be suggested for patients in the form of yoghurt with irritable bowel syndrome (Sauby, 2008). *L. acidophilus* inhibits the growth of C*andida albicans*, fungus that cause Vulvoviginal candidiasis (Hilton, 1992; Erika et al., 2000). *L. bulgaricus* produces acetaldehyde that perfumes yoghurt and also produces lactic acid, which helps to preserve the milk (Balows et al., 1991; Zourari et al., 1992). In the present study, it is investigated that the preparation of yoghurt with two starter cultures that are *L. acidophilus* and *L. bulgaricus.* The effects of starter cultures on physiochemical quality of yoghurt have been determined.

MATERIALS AND METHODS

The raw materials such as Olper milk, gelatin and sugar were purchased from local market, Lahore. *L. acidophilus* and *L. bulgaricus* were purchased from a multinational Company situated in Lahore and was used as starter culture for the preparation of yoghurt samples . The yogurt was prepared by heating the milk up to about 90 C for a period of 30 min so as to kill the bacteria. Subsequently, milk was cooled at 42 C and yoghurt starter cultures that is; *L. acidophilus* and *L. bulgaricus* were mixed into the heated milk separately. Two different freshly prepared yoghurts were incubated at 38 - 42 C for 4 h. Further these were stored at 4 C in a refrigerator and were subjected to physiochemical evaluations.

Physio-chemical analysis

Different physio-chemical parameters such as moisture, ash, fat, protein and lactose in all prepared yoghurt samples were estimated by the methods as described in A.O.A.C. (2005). Acidity was determined by using phenolphthalein as indicator by titration of 0.1 N NaOH. pH was determined by using pH meter.

RESULTS AND DISCUSSION

Different physio-chemical characteristics of two types of yoghurts were analyzed during 12 days of storage period. All experiments were set up in two experimental groups. Physiochemical changes occurring in the yoghurt samples during storage are shown in the Tables 1 - 4.

The results indicated that the coagulation time of different yoghurt samples decreased with the increase of percentage of starter culture. The coagulation of fermented milk was due to casein protein contents. The results are in line with the observation of Machida et al. (2002).

It was found that there was a gradual decrease in moisture contents in all yoghurt samples with the passage of time. *L. bulgaricus* yoghurt showed rapid decline in moisture percentage than *L. acidophilus* yoghurt. The average moisture value of *L. bulgaricus* was 86.05 with a standard deviation of 0.87. The average moisture value of *L. acidophilus* was 85.22 with a standard deviation of 0.64. Differences in moisture percentage were not significant and therefore did not influence the yoghurt quality. Haq (1974) and Rashid et al. (1978) reported the decrease in moisture content in yoghurt during storage as 86.03 - 83.34%, which is similar to our findings.

The ash contents in all yoghurt samples within 12 days of storage. The insignificant increase in ash contents was because of loss of CO_2 and water during charring of yoghurt samples. The average ash value of *L. bulgaricus* yoghurt was 0.81 with a standard deviation of 0.12.The average ash value *L. acidophilus* was 0.96 with a standard deviation of 0.08. The results are in agreement with the findings of Akin and Guler (2005) who reported the ash value of probiotic yoghurt as 0.95%.

The analysis of fat values showed the maximum increase in fat, which was 4.32% in the treatment containing *L. bulgaricus* with a standard deviation of 0.61. there was increase in fat contents due to acidic pH. These findings are in accordance with the results of Mutlu et al. (2005) who observed that fat contents of bio-yoghurt ranged from 3.1 - 4.5% during storage.

The increase in protein content in yoghurt depends on the proteolytic activity of lactic acid bacteria, which hydrolyses proteins (caseins) into peptides and amino acids (Thomas and Mills, 1981). The protein values in experimental treatments were higher as compared to control sample that was found to enhance the quality of yoghurt. The average protein value of *L. bulgaricus* yoghurt was 4.67 with a standard deviation of 0.58. The average protein value of *L. acidophilus* was 4.55 with a standard deviation of 0.56. Janhoj et al. (2006) showed that the protein contents of low fat stirred yoghurt ranged from 3.4 - 5.6%, which are similar to our findings.

With the passage of time total solid mass could be increased. The increase in total solid contents could be due to loss of moisture. The average value of solid mass of *L. bulgaricus* yoghurt was from 15.26% with a standard deviation of 0.91. The average solid value of *L. acidophilus* yoghurt was 15.60% with a standard deviation of 0.56. Abubakar et al. (2005) conducted a study on physiochemical properties of yoghurt prepared from three base materials cow milk, whole milk and powder milk. They estimated that the total solids were increased in three samples. These results were parallel to our findings.

The reduction in pH can be due to breakdown of lactose into lactic acid. Starter culture yielded a different pH profile with the passage of time. The lag time for the pH decrease during storage reflected the acidification rate of the culture involved. Yoghurt quality is therefore affected due to microbial growth. The average pH value of *L. bulgaricus* yoghurt was 4.18 with standard deviation of 0.68 where as with *L. acidophilus* yoghurt pH was 4.29 with a standard deviation of 0.61. These results are similar with the findings of Nighswonger et al. (1996). The average acidity of *L. bulgaricus* yoghurt was 0.97 with a

Table 1. Physio-chemical changes (Mean ± SD) in moisture, fat and coagulation time of Yoghurt samples during storage.

Treatment	Starter culture	Coagulation Time	Moisture % Days				Fat % Days			
		H	0	4	8	12	0	4	8	12
T_1 (control)	Unknown	6:25	86.40 ± 0.75	86.26 ± 0.86	86.17 ± 0.93	86.02 ± 1.10	3.61 ± 0.56	3.62 ± 0.56	3.63 ± 0.56	3.64 ± 0.56
T_2	3%	5:50	86.29 ± 0.83	86.20 ± 0.83	86.12 ± 0.83	86.05 ± 0.87	3.99 ± 0.14	4.00 ± 0.14	4.01 ± 0.14	4.02 ± 0.14
T_3	4%	5:35	85.87 ± 1.03	85.80 ± 1.07	85.70 ± 1.07	85.89 ± 1.04	3.76 ± 0.69	3.77 ± 0.69	3.78 ± 0.69	3.79 ± 0.69
T_4	5%	4:50	85.29 ± 0.85	85.16 ± 0.88	85.06 ± 0.95	85.00 ± 0.96	4.29 ± 0.61	4.30 ± 0.61	4.31 ± 0.61	4.32 ± 0.61
T_5	3%	6:15	86.26 ± 0.87	86.22 ± 0.87	86.18 ± 0.87	86.14 ± 0.87	3.85 ± 0.89	3.86 ± 0.89	3.87 ± 0.89	3.88 ± 0.89
T_6	4%	5:55	84.71 ± 0.74	84.66 ± 0.74	84.62 ± 0.74	84.58 ± 0.74	3.58 ± 0.53	3.58 ± 0.53	3.59 ± 0.53	3.60 ± 0.53
T_7	5%	5:10	85.35 ± 0.66	85.32 ± 0.66	85.27 ± 0.65	85.22 ± 0.64	4.36 ± 0.54	4.37 ± 0.54	4.38 ± 0.54	4.39 ± 0.54

Table 2. Physio chemical changes (Mean ± SD) in Ash and Protein of Yoghurt samples during storage.

Treatment	Starter culture	Ash % Days				Protein % Days			
		0	4	8	12	0	4	8	12
T_1 (control)	Unknown	0.70 ± 0.10	0.70 ± 0.10	0.71 ± 0.10	0.72 ± 0.10	4.89 ± 0.88	4.90± 0.88	4.91 ± 0.88	4.92 ± 0.88
T_2	3%	0.81 ± 0.10	0.81 ± 0.09	0.82 ± 0.10	0.83 ± 0.10	4.61 ± 0.55	4.62± 0.55	4.63 ± 0.54	4.65 ± 0.54
T_3	4%	0.87 ± 0.10	0.88 ± 0.10	0.89 ± 0.10	0.89 ± 0.10	4.45 ± 0.59	4.46± 0.60	4.48 ± 0.60	4.50 ± 0.60
T_4	5%	0.79 ± 0.12	0.80 ± 0.12	0.80 ± 0.12	0.81 ± 0.12	4.62 ± 0.58	4.64± 0.59	4.65 ± 0.58	4.67 ± 0.58
T_5	3%	0.70 ± 0.17	0.70 ± 0.17	0.71 ± 0.17	0.72 ± 0.17	4.50 ± 0.52	4.51± 0.52	4.53 ± 0.52	4.54 ± 0.53
T_6	4%	0.94 ± 0.09	0.94 ± 0.08	0.95 ± 0.08	0.96 ± 0.08	4.50 ± 0.56	4.52± 0.56	4.54 ± 0.56	4.55 ± 0.56
T_7	5%	0.87 ± 0.10	0.87 ± 0.10	0.88 ± 0.10	0.89 ± 0.10	4.60 ± 0.62	4.61± 0.62	4.63 ± 0.61	4.65 ± 0.61

Table 3. Physio chemical changes (Mean ± SD) in Total solids and pH of yoghurt samples during storage.

Treatment	Starter culture	Total solids % Days				pH Days			
		0	4	8	12	0	4	8	12
T_1 (control)	Unknown	14.32 ± 0.83	14.36 ± 0.83	14.40 ± 0.83	14.44 ± 0.830	3.64 ± 0.65	3.63 ± 0.65	3.62 ± 0.65	3.61 ± 0.650
T_2	3%	13.75 ± 0.91	13.97 ± 0.75	13.90 ± 0.91	13.95 ± 0.92	3.48 ± 0.53	3.46 ± 0.53	3.45 ± 0.54	3.43 ± 0.54
T_3	4%	15.14 ± 0.91	15.18 ± 0.91	15.22 ± 0.91	15.26 ± 0.91	4.24 ± 0.68	4.22 ± 0.68	4.20 ± 0.68	4.18 ± 0.68
T_4	5%	14.64 ± 0.60	14.68 ± 0.61	14.72 ± 0.60	14.76 ± 0.60	3.58 ± 0.53	3.56 ± 0.52	3.54 ± 0.52	3.51 ± 0.52
T_5	3%	14.44 ± 0.88	14.48 ± 0.88	14.52 ± 0.88	14.56 ± 0.88	4.35 ± 0.61	4.33 ± 0.61	4.31 ± 0.61	4.29 ± 0.61
T_6	4%	14.70 ± 0.84	14.74 ± 0.84	14.78 ± 0.84	14.82 ± 0.85	4.04 ± 0.87	4.01 ± 0.87	3.99 ± 0.86	3.96 ± 0.86
T_7	5%	15.49 ± 0.56	15.52 ± 0.55	15.56 ± 0.56	15.60 ± 0.56	4.33 ± 0.65	4.30 ± 0.65	4.27 ± 0.66	4.24 ± 0.66

Table 4. Physio chemical changes (Mean ± SD) in acidity and lactose of yoghurt samples during storage.

Treatment	Starter culture	Acidity Days				Lactose % Days			
		0	4	8	12	0	4	8	12
T_1 (control)	Unknown	0.57 ± 0.11	0.61 ± 0.12	0.65 ± 0.130	0.69 ± 0.13	4.57 ± 0.72	4.59 ± 0.71	4.61 ± 0.71	4.62 ± 0.71
T_2	3%	0.68 ± 0.09	0.71 ± 0.10	0.74 ± 0.10	0.78 ± 0.12	4.60 ± 0.60	4.61 ± 0.590	4.63 ± 0.60	4.65 ± 0.61
T_3	4%	0.73 ± 0.08	0.79 ± 0.08	0.84 ± 0.09	0.89 ± 0.09	4.85 ± 0.94	4.86 ± 0.940	4.87 ± 0.94	4.88 ± 0.94
T_4	5%	0.83 ± 0.10	0.88 ± 0.09	0.93 ± 0.08	0.97 ± 0.07	5.15 ± 0.48	5.17 ± 0.48	5.19 ± 0.47	5.21 ± 0.47
T_5	3%	0.64 ± 0.10	0.71 ± 0.10	0.78 ± 0.10	0.84 ± 0.09	4.63 ± 0.57	4.65 ± 0.57	4.66 ± 0.57	4.67 ± 0.57
T_6	4%	0.71 ± 0.09	0.77 ± 0.09	0.82 ± 0.09	0.80 ± 0.12	4.54 ± 0.66	4.56 ± 0.66	4.57 ± 0.65	4.59 ± 0.64
T_7	5%	0.66 ± 0.12	0.70 ± 0.12	0.75 + 0.12	0.80 ± 0.12	4.57 ± 0.77	4.58 ± 0.77	4.59 ± 0.77	4.61 ± 0.77

*Means of chemical measurements, * S.D stands for Standard deviation.

standard deviation of 0.07 while the average acidity of *L. acidophilus* was 0.80 with a standard deviation of 0.12. The results showed that the acidity tends to increase in all yoghurt treatments within 12 days of storage. The fast increase of acidity in yoghurt prepared by *L. acidophilus* is expressed due to its lower buffering capacity and higher content of non protein nitrogen and vitamins which are needed for fast growing microorganisms (Abrahamsen et al., 1991, Salvador and Fiszman (2004). A significant increase in amount of lactose observed between different groups of yoghurt.

The average lactose value of *L. bulgaricus* yoghurt was 5.21 with a standard deviation of 0.47 while lactose value of *L. acidophilus* was 4.61 with a standard deviation of 0.77. The increase in lactose contents might be due to fermentation action done by bacteria, which improved the quality of yoghurt. These findings are in accordance with the observations of Lopez et al. (1997) and Lerebours et al. (1989). Standard deviations (SD) reveal the uniformity within each sample of yo-ghurt.

Both starter cultures concentrations resulted in minor differences and had no significant affect on physio-chemical quality of yoghurt. Slight but potentially important changes were observed in different yoghurt samples within 12 days of storage period.

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