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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; *Lactobacillus bulgaricus* and *Lactobacillus acidophilus*. The preparation was made by 3, 4 and 5% concentrations. It was stored at 4°C for 12 days. To analyze the effect of the two different cultures and their concentrations on the properties of yoghurt, different physio-chemical tests were performed. These two starter culture slightly enhanced the quality of yoghurt. 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 the 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: Starter culture, yogurt quality, physicochemical tests.

INTRODUCTION

The major portion of human diet consists of fermented products, which are derived from plant or animal materials. Fermented products have been an acceptable and essential part of diets 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 sour the milk by converting the milk sugar lactose to lactic acid (Kagan, 1985). The popularity of yoghurt stems from its number of characteristics such as the pleasant aromatic flavor, thick creamy consistency and its reputation as a food associated with good health (Kleyn et al., 1979; Domagla, 2005).

Presently, most yoghurt is prepared by either using special lactic acid producing organism or by direct acidification of milk by an acidulant (Nobuo, 2002). Although the flash-freezing technique used in the production of frozen yoghurt, unlike slow freezing in a freezer does not kill the live cultures (Meydani, 2006), yoghurt made from milk (10% fat) with sugar and homogenized at 200 bars was found to be of 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 and 45°C) on physiochemical, microbial and organoleptic properties of yoghurt produced from three based 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 at low incubation (Abubakar et al., 2005).

The dairy protein composition is known to influence the structure and 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 *Lactobacillus bulgaricus* (Renz and Puhan, 1975). The acidity of yoghurt varies from 0.7 to1.1% 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 sources of calcium and phosphorous (Foissy, 1983). It is believed that yoghurt has valuable "therapeutic properties" and helps in curing gastrointestinal disorders (Adolfsson, 2004). Yoghurt may aid digestion, ease diarrhea, boost

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

Treatment	Starter culture (%)	Coagulation time (h)	Moisture (%)				Fat (%)			
			0 day	4 days	8 days	12 days	0 day	4 days	8 days	12 days
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

immunity and protect against cancer (Gibson et al., 1997; Fernandes, 1988; Ripudaman, 2003; Shahani et al., 1976; Perdigon, 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). *Lactobacillus acidophilus* inhibits the growth of *Candida albicans*, a coli form bacteria that causes *Vulvoviginal candidiasis* (Hilton, 1992; Erika and Ringdahl, 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). The present investigation is concerned with the preparation of yoghurt with two starter cultures; *L. acidophilus* and *L. bulgaricus*. The effects of the starter cultures on the physio-chemical quality of yoghurt have also been determined.

MATERIALS AND METHODS

Raw materials such as Olper milk, gelatin and sugar were purchased from a local market in 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 method employed in preparing the yoghurt consists of heating the milk up to about 90°C for a period of 30 min so as to kill the bacteria. Subsequently, the milk was cooled to 42°C and yoghurt starter cultures (*L. acidophilus* and *L. bulgaricus*) were mixed into the heated milk separately. Two different freshly prepared yoghurts were incubated at 38 to 42°C for 4 h; these were then stored at 4°C in a refrigerator and subjected to physiochemical evaluations.

Physio-chemical analyses

Different physio-chemical parameters such as moisture, ash, fat, protein and lactose in all prepared yoghurt samples were estimated by the method 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 dissolving yoghurt sample in water and using an electrode (pH meter).

RESULTS AND DISCUSSION

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

The results indicated that the coagulation time of different yoghurt samples decreased with an increase in percentage of the 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 content 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 while that 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 yoghurt quality. Haq (1974) and Rashid et al. (1978) reported a decrease in moisture content in yoghurt during storage to be 86.03 to 83.34% which is similar to our findings.

Very minute changes were observed in ash content in all yoghurt samples within the 12 day-storage period. The insignificant increase in ash contents was because of the 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 while *L. acidophilus* was 0.96 with a standard deviation of

Table 2. Physio-chemical changes (mean ± SD) in ash and protein of yoghurt samples during storage.

Treatment	Starter culture (%)	Ash (%)				Protein (%)			
		0 day	4 days	8 days	12 days	0 day	4 days	8 days	12 days
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	0.8 1± 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 (%)				pH			
		0 day	4 days	8 days	12 days	0 day	4 days	8 days	12 days
T_1 (control)	Unknown	14.32 ± 0.83	14.36 ± 0.83	14.40 ± 0.83	14.44 ± 0.83	3.64 ± 0.65	3.63 ± 0.65	3.62 ± 0.65	3.61 ± 0.65
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				Lactose (%)			
		0 day	4 days	8 days	12 days	0 day	4 days	8 days	12 days
T_1 (control)	Unknown	0.57 ± 0.11	0.61 ± 0.12	0.65 ± 0.13	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 ± 0.59	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 ± 0.94	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

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 a maximum increase in fat, which was 4.32% in the treatment containing *L. bulgaricus* with a standard deviation of 0.61. Increase in fat content appeared to be due to acidic pH. These findings are in accordance with the results of Mutlu and Guler (2005) who observed that the fat content of bio-yoghurt ranged from 3.1 to 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 the 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 while the values for *L. acidophilus* were 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 to 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 15.26% with a standard deviation of 0.91 while that 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 powdered milk. They estimated that total solids were increased in the three samples. These results were parallel to our findings.

The reduction in pH can be due to the breakdown of lactose into lactic acid. Starter culture yielded a different pH profile with the passage of time. The lag time for pH decreases during storage and this reflected the acidification rate of the culture involved. Yoghurt quality is therefore affected by microbial growth. The average pH value of *L. bulgaricus* yoghurt was 4.18 with standard deviation of 0.68 whereas 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 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 acidity tends to increase in all yoghurt treatments within the 12 day-storage period. The fast increase in 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 micro-organisms (Abrahamsen and Rystaad, 1991; Salvador and Fiszman, 2004). A significant increase in the amount of lactose was observed between the different groups of yoghurt.

The average lactose value of *L. bulgaricus* yoghurt was 5.21 with a standard deviation of 0.47 while that of *L. acidophilus* was 4.61 with a standard deviation of 0.77. The increase in lactose content might be due to fermentation action by bacteria; this improved the quality of yoghurt. These findings are in accordance with the observations of Lopez et al. (1997) and Lerebours et al. (1989). Standard deviation (SD) reveals uniformity within each sample of yoghurt.

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

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