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Standardization of different levels of lactose hydrolysis in the preparation of lactose hydrolyzed yoghurt

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Summary

Yoghurt is a unique fermented dairy product, consumed throughout the world and the trend of consumption is increasing due to its unique health benefits. Yoghurt mix is often supplemented with MSNF (milk solids-non-fat), it may contain as much as 5.7% unhydrolysed lactose. Lactose can be hydrolysed with an enzyme lactase. Deficiency of lactase in lactose-intolerant persons leads to gastrointestinal disorders; such consumers may find it difficult to consume lactose unhydrolysed yoghurt. The production of lactose hydrolysed yoghurt by pre-hydrolysing of lactose in the yoghurt mix by enzymatic hydrolysis of lactose was studied using standard materials and methods. Among the different levels of enzyme examined (0.12 to 0.56% of yoghurt mix with increment of 0.04% at each level), 0.16, 0.32 and 0.52% were found suitable to hydrolyse 50, 70 and 90% lactose in the yoghurt mix, respectively. Lactose hydrolysed yoghurt (LHY) prepared from 70% followed by 50% lactose hydrolysed mix (LHM) had significantly higher scores for body and texture, flavour and overall acceptability than control ($P<0.05$). However, 90% LHY secured significantly lower scores for sensory scores ($P<0.05$) and lactose hydrolysis in mix had no effect on colour and appearance of yoghurt at all levels of hydrolysis. Lactose hydrolysis reduced the setting time by 30-45 min over control (210 min) ($P<0.05$). The curd strength was checked by measuring penetration (mm/5sec) using a cone penetrometer. The yoghurt from 50, 70 and 90% LHM had shown significantly increased penetration of 280, 325 and 395 mm/5sec as compared to control (195 mm/5sec) and the amount of whey separation increased as the degree of lactose hydrolysis increased ($P<0.05$).

Key words: Lactose hydrolysed yoghurt, Lactose hydrolysed mix, Lactose unhydrolysed mix, Lactose hydrolysis

Introduction

Yoghurt is a unique fermented dairy product, consumed throughout the world and the trend of consumption is increasing due to its unique health benefits. The uniqueness of yoghurt is attributed to its taste, consistency and health benefits.

Lactose is a unique disaccharide in milk, which is about 4.5 to 4.8% in cow's milk and is hydrolysed by lactase enzyme present in the brush border of small intestine of human beings. Deficiency of these enzymes leads to lactose intolerance. About 53% of Indian (Balasubramanyam, 1988) and 70% of world population (Vonk *et al.*, 2003) are suffering from lactose intolerance, which in turn leads to gastro-intestinal disorders such as bloating, diarrhoea, flatulence, abdominal pain, loss of appetite, nausea, etc (Hourigan, 1984). The body should properly utilize lactose, for that it must be hydrolysed by this enzyme to its component monosaccharides.

Lactose in the milk and milk products

can be hydrolysed by using an external source of lactase from either bacterial or fungal source. The advantages of lactose hydrolysed products are improved functional and nutritional properties. Since yoghurt mix is often supplemented with MSNF (milk solids-non-fat), it may contain as much as 5.7% unhydrolysed lactose. Thus, consumers suffering from lactose intolerance may find it difficult to consume lactose unhydrolysed yoghurt (Hilgendorf, 1981). Several investigations proved that consumption of lactose hydrolysed yoghurt (LHY) resulted in increased digestibility, sweeter taste, faster fermentation, better body, consistency and mouth feel. Considering the above facts the present study was conducted to standardize the levels of lactose hydrolysis (LH) in the yoghurt mix for the preparation of LHY and to evaluate its effect on the physico-chemical properties.

Materials and Methods

Fresh cow's milk was obtained from dairy farm, University of Agricultural Sciences, Bangalore was tested for fat (IS: 1224, Part-I, 1977), solids-non-fat (SNF) and total solids (IS: 1479, Part-II, 1961) and acidity (IS: 1479, Part-I, 1960); and standardized to 3.5% fat and 12.5% MSNF using cream and Anik brand spray dried skim milk powder (SMP) (Brookbond Lipton Ltd.). Yoghurt mix was prepared by using 0.3% stabilizer and 0.2% emulsifier, preheated to 60°C and homogenized at 3000 psi using two stage Renni homogenizer. Then heated to 85°C for 30 min to pasteurize, cooled to 42°C using chilled water bath, and inoculated with 2.0% yoghurt starter culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (obtained from dairy microbiology lab.). The inoculated mix was transferred to 100 ml cups and incubated at 42 ± 1°C for 3.0-3.5 h and product showed the final titratable acidity (TA) of 0.80% LA (IS: 7035, 1973). The duplicate samples were randomly selected for examination at 20 or 30 min intervals. The samples were immediately cooled in a chilled water bath to 5°C to minimize the activity of starter, then transferred to refrigerator and maintained at 5 ± 1°C for further analysis and sensory evaluation.

LHY was prepared by adding lactase enzyme (Lactozyme 3000 L supplied by NOVO Nordisk A/S, Denmark) at different levels *viz*., 0.12 to 0.56% of mix (with increment of 0.04% at each level), after homogenization, pasteurization and cooling to 40°C, it was incubated in incubator for one h at the same temperature. After one hour the samples were heated to 75°C for 2 min to inactivate enzyme, then cooled to 42 ± 1°C and inoculated with 2% starter cultures, incubation and setting of yoghurt was done as in case of control. Samples were stored in refrigerator (5 ± 1°C) for further analysis.

The yoghurt samples were analysed for titratable acidity, (as per IS: 1479, Part-I, 1960). It was estimated by taking 10 g of sample diluting with 20 ml distilled water, which were stirred well and titrated against 0.1N NaOH using phenolphthalein as an indicator until a light pink colour end point of titration appears and was expressed as a percent of lactic acid (LA). The pH of the samples was checked using Elico pH meter by diluting yoghurt with distilled water at 1:1 ratio and mixing well to get homogenous solution. Lactose content in the yoghurt mix before hydrolysis was estimated according to Lawrence (1968). The whey separation in the yoghurt was estimated by measuring the amount of whey expelled from 100 ml yoghurt in a cup when sample kept at 25 ± 1°C for one h. Curd strength of such prepared product was checked by using a cone penetrometer by measuring the penetration cone (weighing 30 g) into 100 ml yoghurt in a cup for 5 sec, which was tempered to 10°C prior to measurement and average reading was expressed in mm/5sec (milli meters). To judge the quality of product based on sensory tests, the product was evaluated by serving the sample to panel of five experienced judges using 9-point hedonic score card (Nelson and Trout, 1964). To check the percentage of lactose hydrolysis in lactose hydrolysed yoghurt mix, the freezing point depression method was used, and it was estimated according to Davood *et al*. (1982) using a Cryoscope. The results were statistically analysed and tested for significance using CRD (Completely

Randomized Design) and the critical difference was computed and the significance of the difference in the treatments was tested at 5% level using Graph Pad Prism Computer Programme, Version 4.03 (Graph pad prism computer programme, USA, 2005).

Results

The lactase enzyme (Lactozyme®) was used at different concentrations to hydrolyse lactose in yoghurt mix and to find out at what level of lactose hydrolysis a good quality yoghurt can be produced. It was noticed from Table 1 that as the concentration of enzyme increased from 0.12 up to 0.56%, the degree of lactose hydrolysis (LH) also increased from 46.3 to 90.06%; it was indicated by the constant decrease in freezing point which was statistically significant (P<0.05). However, beyond 0.52% enzyme concentration, the increase in freezing point depression was non-significant (P<0.05). So, for further studies only 0.16, 0.32 and 0.52% Lactozyme was used to hydrolyse 50, 70 and 90% lactose in the yoghurt mix.

In the present study, no significant differences were observed among the control and treated yoghurt samples for colour and appearance (Table 2). Yoghurt obtained from 50 and 70% LHM secured significantly (P<0.05) higher scores for body and texture, flavour and overall acceptability, but yoghurt from 90% LHM secured lower scores for all attributes (P<0.05) than control.

The prepared samples were then subjected to study different physico-chemical properties like acid production,

Table 1: Effect of lactase on the rate of lactose hydrolysis in yoghurt mix

Enzyme concentration (%)	Freezing point depression (°C)	Degree of lactose hydrolysis (%)
0.12	0.17830^{a}	46.31
0.16	0.19760^{b}	51.16
0.20	0.2713^{c}	56.06
0.24	0.2357^{d}	60.64
0.28	0.2524^{e}	64.80
0.32	0.2708^{f}	69.38
0.36	0.2893^{g}	73.93
0.40	0.3063^{h}	78.21
0.44	0.3256^{i}	83.02
0.48	0.3439^{j}	87.57
0.52	0.3528^{k}	89.84
0.56	0.3539^{k}	90.06
CD	0.0127	ND

Note: Figures in the Table indicates the average of three trails. Superscripts with similar alphabets are not significant from each other. ND: Not determined

Table 2: Effect of lactose hydrolysis on the sensory characteristics of yoghurt prepared from lactose hydrolysed mix

Level of lactose hydrolysis (%)	Colour and appearance	Body and texture	Flavour	Overall acceptability
Sensory scores on 9-point hedonic scale				
0	7.94^{a}	8.00^{a}	7.83^{a}	8.05^{a}
50	7.96^{a}	8.15^{a}	8.25^{a}	8.20^{a}
70	7.93^{a}	8.10^{a}	8.50^{b}	8.50^{b}
90	7.83^{a}	7.30^{b}	6.52^{c}	6.64^{c}
CD	0.23	0.62	0.59	0.51

Note: Figures in the Table indicates the average of three trails. Superscripts with similar alphabets are not significant from each other when read column wise. Samples were incubated at 42°C till setting. CD: Critical difference

Table 3: Effect of lactose hydrolysis on the rate of acidity development during setting of yoghurt

Level of lactose hydrolysis (%)	Incubation period in minutes						
	0	60	90	120	150	180	210
0	0.17^{a}	0.20^{a}	0.28^{a}	0.38^{a}	0.52^{a}	0.68^{a}	0.81
50	0.17^{a}	0.32^{b}	0.42^{b}	0.61^{b}	0.76^{b}	0.90^{b}	ND
70	0.17^{a}	0.34^{b}	0.43^{b}	0.61^{b}	0.77^{b}	0.90^{b}	ND
90	0.17^{a}	0.34^{b}	0.45^{bc}	0.63^{bc}	0.79^{b}	0.93^{b}	ND
CD	0.01	0.03	0.02	0.03	0.03	0.03	ND

Note: Figures in the Table indicates the average of three trails. Superscripts with similar alphabets are not significant from each other when read column wise. CD: Critical difference. ND: Not determined whey separation, setting time and curd strength. The rate of lactic acid production was significantly ($P<0.05$) more in LHY than control, but no significant ($P<0.05$) increase in the rate of acid production was observed within different levels of LH (50, 70 and 90%). LHY took 30 to 40 min less time to reach ≈ 0.7% of acidity (Table 3).

The common defect observed in yoghurt is whey separation. Results in the present study indicated that whey separation increased significantly ($P<0.05$) from 1.0 to 1.8 ml with increase in the degree of LH (Table 4). However, between 50 and 70% LHY, increase in whey separation was non-significant; whereas it was found significant ($P<0.05$) in 90% LHY. It was also noticed that there was a significant ($P<0.05$) decrease in coagulation time (by 30-40 min) and curd strength (from 0-102.6%) in LHY when compared to control. The decrease in curd strength increased from 195 to 395 mm as the level of LH increased from 0 to 90%.

Table 4: Effect of lactose hydrolysis on the physico-chemical characteristics of yoghurt

Level of lactose hydrolysis (%)	Whey expelled (ml/100 g)	Coagulation time (min)	Curd strength (mm/5sec)
0	1.00^{a}	210^{a}	195^{a}
50	1.50^{b}	180^{b}	280^{b}
70	1.60^{b}	180^{b}	325^{b}
90	1.80^{bc}	165^{c}	395^{b}
CD	0.21	10.87	2.45

Note: Figures in the table indicates the average of three trails. Superscripts with similar alphabets are not significant from each other when read column wise

Discussion

Many people in the world are suffering from lactose intolerance due to deficiency of enzyme lactase (Vonk *et al.*, 2003). The products of lactose hydrolysed milk are the best solution for their problem. Lactase can be used for the production of fermented dairy products like yoghurt. The results of the present investigation indicated that as the concentration of enzyme increased in the yoghurt mix, the degree of lactose hydrolysis to its component monosaccharides (glucose + galactose) increased. Monosaccharides are more soluble in solution which, in turn, indicated by the depression in the freezing point of lactose hydrolysed yoghurt. However, beyond 0.52% (corresponds to 89.84% LH) enzyme concentration, the increase in freezing point depression was not significant due to attained saturation point of degree of hydrolysis (Hilgendorf, 1981). Hence, it is not advisable to hydrolyse lactose beyond 90%.

Lactose hydrolysis in yoghurt mix showed an improvement in body and texture of 50 and 70% LHY than unhydrolysed yoghurt which may be due to increased content of monosaccharides that are more soluble and imparted soft body and a creamier texture. However, weaker body and texture of 90% LHY may be due to higher amount of monosaccharides which reduced the viscosity (Hilgendorf, 1981; Davood *et al.*, 1982; Wilson *et al.*, 2003).

Characteristic flavour of yoghurt is due to production of lactic acid, acetaldehyde and other carbonyl compounds during fermentation by starter organisms. Fifty percent and 70% LHY had better flavour than control, may be due to availability of more amount of glucose for the production of flavour compounds and also due to faster production of more lactic acid than control; poor flavour of 90% LHY may be due to the intense sweet taste, almost bitter after-taste (Hilgendorf, 1981), which attributed to the production of bitter peptides (Antila *et al.*, 1977; Davood *et al.*, 1982; Smith and Bradley, 1984).

Development of acidity in fermented milks mainly depends on type and quantity of carbohydrates. The rate and amount of lactic acid production was more in LHY than control, may be attributed to the availability of more quantity of easily fermentable sugar (glucose) which, in turn, required for the faster growth of starters (Knanagaeva *et al.*, 1980; Baeve, 1981; Whalen *et al.*, 1988).

Whey separation, a defect in yoghurt may be due to low MSNF, low preheat treatment of yoghurt mix or elevated temperature of incubation (Tamine and Robinson, 1988). This defect leads to slow rate of acid production in yoghurt and adversely affects the sensory quality of the product. The present study indicated that increase in the degree of LH resulted in increase in whey separation which may be due to more soluble sugar content and destabilized protein (casein) in LHY that lead to the weaker curd strength (Hilgendorf, 1981; Davood *et al*., 1982; Technical Review, NOVO Industries, A/S, 1987; Mahipal Reddy, 1989; Uma, 1991). Decreased coagulation time in LHY, attributed to the faster fermentation of glucose than lactose in control (Hilgendorf, 1981; Mahipal Reddy, 1989).

The results of the present study indicated that good quality LHY can be produced from 50 and 70% lactose hydrolysed yoghurt mix, which provides better technology to produce pleasant yoghurt, relatively lower lactose content and wider range of consumer acceptability than conventional "unflavoured" or "plain" yoghurt. Each manufacturer must determine if added cost of lactase enzyme is offset by savings in time, added sweetener (if any) and the greater palatability of the finished product. Reducing the cost of production through utilization of immobilized lactase will be the subject of future research.

References

Antila, P; Lehto, M and Antila, V (1977). The use of lactase treated milk in the manufacture of yoghurt. 20th International Congress on Production of Low Lactose Milk and Milk Products for Lactose Intolerant People. Paris. P: 48.

Baeve, VS (1981). Possibility of producing low lactose milk products for infant feeding using ß-gal. Vopeory Itaniya. 2: 63-65.

Balasubramanyam, BV (1988). Advantages of low lactose milk. Dairyman. 40: 189-191.

Davood, N; Dariani, DN; Frank, JF and Lowenstein, M (1982). Manufacture of low lactose yoghurt by simultaneous lactose hydrolysis and bacterial fermentation. Cult. Dairy Prod. J., 17: 18-19, 22.

Graph pad prism computer programme (2005). Version 4.03, Graph Pad Software Inc., San Diego, USA.

Hilgendorf, MJ (1981). Optimization of fungal lactase levels in yoghurt manufacturing. Cult. Dairy Prod. J., 16: 5-7.

Hourigan, JA (1984). Nutritional implications of lactose. Aust. J. Dairy Technol., 39: 114-120.

IS: 1224, Part-I (1977). Specifications for operators for determination of milk fat by Gerber method. Bureau of Indian Standards, New Delhi.

IS: 1479, Part-I (1960). Methods of test for dairy industry rapid examination of milk. Bureau of Indian Standards, New Delhi.

IS: 1479, Part-II (1961). Methods of test for dairy industry rapid examination of milk. Bureau of Indian Standards, New Delhi.

IS: 7035 (1973). Specifications for fermented milk products. Bureau of Indian Standards, New Delhi.

Knanagaeva, IS; Tikhomirvo, AS and Kulikova, AK (1980). Effect of lactose hydrolysis on activity of lactic acid bacteria. Pischevaya Technologiya. 6: 133-134 (cited in FSTA 14: 275).

Lawrence, AJ (1968). The determination of lactose in milk products. Aust. J. Dairy Tech., 23: 103.

Mahipal Reddy, K (1989). Use of yeast cell bound lactase for the preparation of lactose hydrolysed milk and low lactose yoghurt. MSc Thesis, University of Agriculture Sciences, Bangalore, India.

Nelson, JA and Trout, GM (1964). *Judging dairy products*. 4th Edn., IV EA. Olsen Publishing Co., Wilwaukee. P: 357.

Smith, FE and Bradley, RLJr (1984). Acceptance of yoghurt made with and without lactose hydrolysis. Cult. Dairy Prod. J., 19: 31-33, 35-37.

Tamine, AY and Robinson, RK (1988). Fermented milks and their future trends: technological aspects. Part II. J. Dairy Res., 56: 281-304.

Technical Review, NOVO Industries, A/S (1987). A technical review of Lactozyme-R "NOVO enzymes product brochure". NOVO Industries AS 2880, Bagsvaerd, Denmark.

Uma, MV (1991). Preparation and use of permeabilized yeast cells (*Kluyveromyces fragilis*) for production of low lactose milk and milk products. MSc Thesis, University of Agriculture Sciences, Bangalore, India.

Vonk, RJ; Priebe, MG; Koetse, HA; Stellaard, F; Lenoir-Wijnkoop, I; Antoine, JM; Zhong, Y and Huang, CY (2003). Lactose intolerance: analysis of underlying factors. Eur. J. Clin. Invest., 33: 70-75.

Whalen, AC; Gilmore, TM; Spurgeon, KR and Parson, JG (1988). Yoghurt manufactured from whey-caseinate blends and hydrolyzed lactose. J. Dairy Sci., 71: 299-305.

Wilson, JH; Wilson, EJ; Matak, KE; Duncan, SE and Sumner Susan, HCR (2003). *The influence of lactose hydrolysis on the strength and sensory characteristics of vanilla ice cream*. American Society of Agricultural and Biological Engineers, St. Joseph, Michigan. PP: 036-054.