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Study on simultaneous lactose hydrolysis and fermentation in the preparation of lactose hydrolyzed yoghurt and its effect on physico-chemical properties

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Abstract
The persons with the deficiency of lactase enzyme in the epithelial cells of brush boarder of small intestine is cause for lactose intolerance and is a very much prevailing problem in the world (68%) as well as in India (65%). Low lactose and fermented dairy products are the answer for this problem. Yoghurt is one among the most popular fermented milk products. In this study efforts are made to reduce the lactose content in the yoghurt by hydrolyzing it using exogenous source of lactase. Different levels of lactose hydrolysis *viz*., 50, 60, 70 and 80% were tried in the simultaneous lactose hydrolysis and fermentation in the yoghurt. It was observed that as the level of lactose hydrolysis increased, the sensory scores, penetration value and acidity of the lactose hydrolyzed yoghurt (LHY) also increased. Whereas, setting time decreased correspondingly with increased degree of lactose hydrolysis. Based on the sensory scores, 70% LHY was adjudged as very much comparable product with the control.

Keywords: Yoghurt, lactase enzyme, fermentation, lactose hydrolysis

Introduction
Yogurt is a universally well-known unique fermented dairy product, manufactured using a special symbiotic yoghurt starter which had got characteristic flavor, body and texture. Fermented dairy products have good health benefits. Since yogurt mix is often supplemented with milk solids nonfat (MSNF) to increase total solids in the mix, such mix may contain as much as 5.7% of un-hydrolyzed lactose. Lactose is a unique disaccharide in milk which is hydrolyzed by enzyme lactase present in the epithelial cells of brush board of small intestine. It is estimated that approximately 68% of world and 65% of Indian population [3] are suffering lactose intolerance due to deficiency of lactase. The lactose intolerant consumer can't utilize lactose causing disorders such as gastro-intestinal discomfort like bloating, diarrhea, flatulence, abdominal pain, loss of appetite, nausea etc. [9]. Lactose in the yoghurt mix can be hydrolyzed using an external source of lactase from either bacteria or fungus. Advantages of lactose hydrolyzed products are improved functional and nutritional properties with reduced lactose content and make yogurt more digestible by lactose sensitive and lactase deficient individuals. In the present study it was aimed to study the method of simultaneous lactose hydrolysis and fermentation and its effect on sensory and physico-chemical properties of yoghurt.

Materials and methods
Fresh cow's milk was obtained from Students experimental Dairy Plant, DSC Bangalore, Karnataka Veterinary, Animal and Fisheries Science University, Bidar, standardized to 3.0% fat and 11.0% SNF using 'Sagar' brand spray dried SMP (GCMMF Ltd Anand). Yogurt mix was prepared by using 0.3% stabilizer- emulsifier powder. The mix was then preheated to 60°C and homogenized at 2500 + 500 PSI (1st + 2nd stage) using two stage homogenizer. Then pasteurized by heating to 90 °C/5 min in H_2O bath, then cooled 42±1 °C using chilled water bath. Inoculated with yogurt culture *Streptococcus selverious spp. thermophilus* and *Lactobacillus delbrueckii spp. bulgaricus* (1:1 ratio) added at rate of 1.5% of mix and incubated at 42 °C ±1 °C until acidity reaches to 0.8% LA. Control was prepared as above without enzyme. The yogurt was cooled immediately to 5±1 °C with the help of chilled water bath and stored in a refrigerator (5±1 °C) for further analysis.
For the preparation of 50, 60, 70 ad 80% lactose hydrolyzed yoghurt (LHY), enzyme lactase commercially available as 'BIOVEN', manufactured by Biovencer Healthcare Private Limited,

UP, India, having 3000 ALU/ml activity was added at different levels *viz*., 0.16, 0.24, 0.32 32 and 0.40 ml/L of mix to obtain 50, 60, 70 and 80% lactose hydrolysis along with starter culture as mentioned above to the preheated homogenized pasteurized yoghurt mix and incubated at 40±1 °C for 3-3.5 hour or until acidity reaches to 0.8% LA. It was cooled and stored in the refrigerator maintained at 5±1 °C for further analysis.

Fat, SNF and acidity of milk, yoghurt mix and yoghurt were estimated as per the procedures mentioned in FSSAI Manual [5]. Lactose content in the mix was analyzed by using Lane-Eynon Method [7].

$$\% \text{ Lactose} = 5\times(V_1/V_2)$$

Where V_1=Volume in ml, of standard lactose solution taken to reduce 10 ml of Fehling's solution; and

V_2= Volume in ml, of prepared milk filtrate taken to reduce 10 ml of Fehling's solution

% Lactose Hydrolysis =100- (lactose% in the yoghurt/ lactose% in the mix) x 100

Curd tension of yoghurt was measured using Cone Penetrometer (cone and probe weighing 30 g) and is measured as mm penetration in 5 Seconds. This test was conducted in yoghurt sample which was tempered to 10 °C prior to measurement and the curd strength was expressed as mm of penetration in 5 seconds [2]. Whey separation was estimated by measuring the amount of whey (in ml) separated in 60 minutes when 100 ml cup yoghurt was stored at 25±1 °C. Sensory evaluation was carried out by using 9 point hedonic scale score card [10].

Statistical analysis

Experiments in the present study were designed based on CRD with replications to study the effect lactose hydrolysis on the quality of lactose hydrolyzed yoghurt. The test of the significance was carried out as per the procedure laid down by [12]. The critical difference was computed and the significance of the difference in the treatments was tested at 5% level.

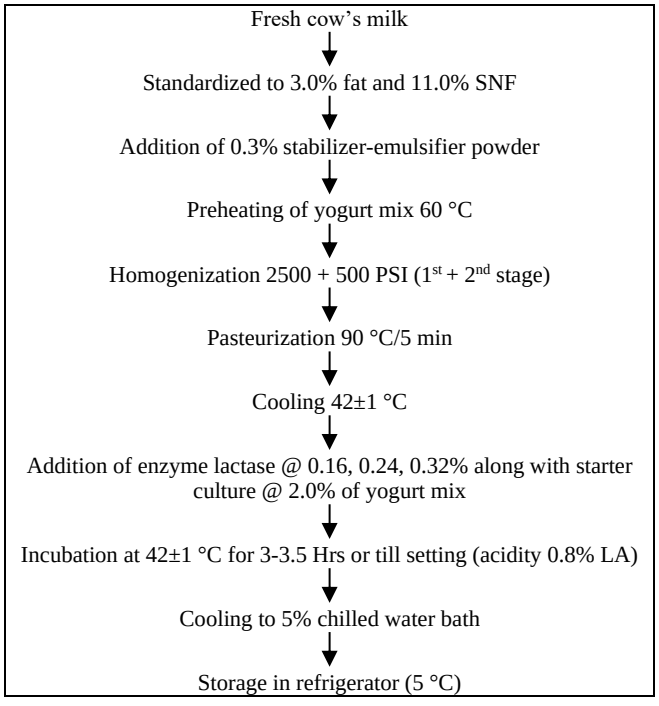

Fig 1: Flow diagram for the production of lactose hydrolyzed yoghurt

Results and Discussion

In present investigation the enzyme lactase was tried at different concentration from initial concentration of 0.16% to 0.40% maintaining 0.08% difference between two successive levels to study its effect on rate of hydrolysis which was determined by estimating percent UN hydrolyzed lactose present in the product. It is observed from Table1 that as the level of enzyme addition increased the degree of lactose hydrolysis also increased. The results of the present investigation are in agreement with the findings of [1].

The lactic acid content in yoghurt plays a key role in determining the sensory quality of the yoghurt. The acidity content of the samples treated with different levels of enzymes is significantly higher than that of control samples at all the stages of fermentation. Further, there was increase in the acidity content with increased enzyme concentration (Table 2). Present findings are in accordance with the findings of [9]. The purpose of this study was to show that a low lactose yoghurt with acceptable quality can be produced by adding lactase simultaneously with the starter culture. This manufacturing method would be more convienent and time saving than using pre lactose Hydrolysis yoghurt mix for for production of the lactose hydrolyzed yoghurt.

From Table 3 it was noticed that at all the four levels of enzyme additions *viz*; 0.16, 0.24, 0.32 and 0.40% tried in the preparation of lactose hydrolyzed yoghurt secured statistically non-significant ($p\leq0.05$) scores for color and appearance. However the yoghurt prepared using 0.16 and 0.24% enzyme secured negligibly higher score of 8.15 compared to control for body and texture. Yoghurt with 0.24% enzyme secured the higher sensory score of 8.50 and the yoghurt prepared using 0.40% scored the lowest of 6.25 when compared to the control. All enzyme concentrations secured statistically non-significant ($p\leq0.05$) higher sensory scores for all attributes than control except 0.40% concentration whose scores were statistically significantly lower than all treatments ($p\leq0.05$). [11] Also reported a decrease in sensory scores for lactose hydrolyzed yoghurt with more than 80%. The increased sensory scores for up to 70.85% lactose hydrolyzed yoghurt may be due to increased sweetness and flavor in the product. Whereas at more than 70% lactose hydrolysis product had week body & texture as well as harsh fruity flavor [11] hence, 0.32% enzyme concentration was found to be optimum in the preparation of yoghurt from simultaneous lactose hydrolysis fermentation method.

The physico-chemical properties studied for the simultaneous lactose hydrolyzed yoghurt were whey separation, setting time and curd strength (Table 4). The yoghurt samples obtained by 0.16 and 0.24% lactase treatment had 1.26 ml whey expelled, fairly higher than that of control. Whereas the samples obtained by 0.32 and 0.40% lactase treatment had 2.20 and 2.25 ml whey expelled respectively indicating significant increase over the control ($p\leq0.05$). This may be due to protein destabilization caused by higher content of lactic acid and increased the solubility of monosaccharaides. The observation of this study is in conformation with the findings reported by [8]. It is also observed from the results that increase in the enzyme concentration significantly decreased the setting time over the control from 210 min. in to 170 min. Significant ($p\leq0.05$) differences in setting time between lactase treated yoghurt samples may be due to production of easily fermentable monosaccharides namely glucose and galactose from lactose hydrolysis which might have helped for the faster production of lactic acid [4]. The

curd strength was measured in terms of penetration. The increased concentration of lactase in samples increased the cone penetration. This increase was found statistically significant ($p \leq 0.05$) indicating weaker three dimensional protein network in lactose hydrolyzed yoghurt [6].

Table1: Effect of enzyme conc. on degree of lactose hydrolysis during fermentation of yoghurt in simultaneous lactose hydrolysis fermentation process

Treatment	Enzyme conc. (%)	Lactose hydrolyzed (%)
T_1	0.00	50.64
T_2	0.16	60.48
T_3	0.24	65.69
T_4	0.32	70.85
T_5	0.40	78.21

Note: n=3; Superscripts with similar alphabets are not significant to each other when read column wise

Table2: Effect of enzyme conc. on rate of acidity development during fermentation of yoghurt in simultaneous lactose hydrolysis fermentation process

Enzyme Period in min.							
Conc.(ml/L) 0 60 90 120 150 180 210							
Percentage of lactic acid							
0.00	0.170[a]	0.22 [a]	0.30 [a]	0.39 [a]	0.51 [a]	0.66 [a]	o.79
0.16	0.173 [a]	0.28 [b]	0.40 [b]	0.64 [a]	0.75 [b]	0.90 [b]	--
0.24	0.173 [a]	0.30 [b]	0.42[b]	0.64 [a]	0.76 [c]	0.91 [b]	--
0.32	0.170 [a]	0.30 [b]	0.43 [b]	0.66 [a]	0.80 [c]	0.92 [b]	--
0.40	0.170 [a]	0.32[c]	0.48 [c]	0.66 [a]	0.81 [d]	0.95 [c]	--
CD	0.0109	0.0287	0.0219	0.0348	0.0268	0.0279	--
CV (%)	3.33	5.17	3.94	2.71	2.13	1.97	--

Note: n=3; Superscripts with similar alphabets are not significant to each other when read column wise

Table 3: Effect of level of enzyme in simultaneous lactose hydrolysis fermentation process on sensory quality of yoghurt

Enzyme conc. (%)	Colour and appearance	Body and texture	Flavor	Overall acceptability
Score on 9 point hedonic scale				
0.00	8.10 [a]	8.00 [a]	8.00 [a]	8.05 [a]
0.16	8.05 [a]	8.15 [a]	8.10 [a]	8.15 [a]
0.24	8.00 [a]	8.25 [a]	8.20 [a]	8.25 [a]
0.32	8.00 [a]	8.25 [a]	8.50 [b]	8.50 [b]
0.40	7.95 [a]	7.20 [b]	6.25 [c]	6.50 [c]
CD	0.2378	0.2569	0.3927	0.4329
CV (%)	3.87	6.11	4.96	5.28

Note: n=3; Superscripts with similar alphabet's are not significant to each other when read column wise

Table 4: Effect of enzyme conc. on physico-chemical characteristic of yoghurt during simultaneous hydolysis fermentation process

Enzyme conc. (%)	Coagulation time (min.)	Whey expelled (ml/h)	Curd strength (mm/5 sec)
0.00	210 [a]	1.00 [a]	195 [a]
0.16	175 [b]	1.26 [a]	230 [b]
0.24	175 [b]	1.26 [a]	285 [c]
0.32	170 [b]	2.20 [b]	355 [d]
0.40	170 [b]	2.50 [c]	430 [e]
CD	13.4912	0.2253	19.6488
CV (%)	4.12	7.61	3.60

Note: n=3; Superscripts with similar alphabets are not significant to each other when read column wise

Conclusion

Under the selected processing conditions as the lactase enzyme addition increased from 0.16 to 0.40% of the yoghurt mix resulted in corresponding increase in the level of lactose hydrolysis from 60.48 to 78.21%; acidity from 0.90 to 0.95% LA. Furthermore, setting time was reduced by approximately 19%. The 70% lactose hydrolyzed yoghurt is much better than the control.

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