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Lactose content of modified enzyme-treated 'dadih'

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Abstract

The preparation of 'dadih', a sweet 'gel-like' fresh milk product which is a popular dessert in the northern part of Peninsular Malaysia is described. The lactose content of modified 'dadih' and enzyme-treated 'dadih' was monitored throughout 7 days, a period over which 'dadih' is normally stored and kept at 4°C. Modified 'dadih', made from fresh milk with 4.49% lactose, yields 3.63% lactose upon formulation, a value still high for lactose-intolerant consumers. A suitable volume of commercially-prepared enzyme, Lactozym 3000L to hydrolyse lactose in 'dadih' was found to be 1 U ml^{-1}, yielding >70% hydrolysis after a 48 h incubation period at 4°C. The colour of enzyme-treated 'dadih' was significantly different ($p < 0.05$) from the untreated samples. Other characteristics, such as texture, aroma and overall acceptability, showed no significant differences ($p > 0.05$).

Keywords: Dadih; Lactose intolerant; β-Galactosidase

1. Introduction

'Dadih' is a traditional food normally consumed as a dessert in the northern region of Peninsular Malaysia (Hamzah, 1983). Physically, it is a sweet soft solid with a fine texture resembling custard and does not break easily when scooped. Despite the fact that 'dadih' has been consumed for decades, information on its chemistry and development technology is rather scarce. Chemical analysis of 'dadih' reveals that it contains fat, protein and total sugars (7.05, 4.79 and 17.2%, respectively) (Hamzah, 1983) if it is made from buffalo's milk, as traditionally prepared in the early days where buffalo was easily available. Today, cow's milk replaces the milk, giving a lower fat content of around 2.8% (Hamzah, 1983).

The traditional way of preparing 'dadih' is as an inoculum method. Inoculum is prepared by adding a dried slice of *Garcinia antoviridis* in the raw milk and leaving overnight at room temperature. The curd is then stirred and filtered and the filtrate is used as inoculum. The inoculum is added to the raw milk (which gives a final pH of 5.85) followed by the addition of sugar and salt, filtering and then dispensing into clay cups for 3–4 h before steaming for 10 min. The curd formed upon steaming (Parry, 1994) is cooled and kept at 4°C before being consumed. However, the traditional method is no longer practised due to inconsistency of texture of the final product and development of syneresis after 24 h. An attempt at commercialising this product using the traditional method was not successful.

Modified methods include the acid method, by addition of citric acid, enzyme method, involving the application of Rennilase, and agar method (Hamzah, 1983) involving addition of agar to give the desirable soft gel texture without causing milk coagulation phenomena (Parry, 1994). The former method is preferred and popular as it is quick and easy to prepare the product by medium-scale vendors and it is acceptable by local consumers as it is very mild compared to the sour taste and 'stale' smell of yoghurt. As with other modified 'dadih', the agar method lacks adequate fermentation and yields relatively high lactose content, sufficient to cause a problem for the lactose-intolerant consumers (Holsinger & Kligerman, 1991).

Lactose intolerance is a term used to describe inability to digest lactose due to the deficiency in lactose in the digestion system (Houts, 1988). Lactose, a disaccharide, originates from mammalian milk, accounting for 5% of fresh milk (Campbell & Marshall, 1975). Lactose is hydrolysed by β-galactosidase, a membrane-bound enzyme located in the small intestine, to D-glucose and D-galactose by a mechanism suggested by Whitaker

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(1972) and absorbed by the intestine (Holsinger & Kligerman, 1991) or fermented by bacteria (Zadow, 1984). Non-Caucasians especially suffer lactose indigestibility, and this causes discomfort and diarrhoea (Holsinger & Kligerman, 1991). However, lactose acts as a promoter in absorption and retention of calcium in the intestine and absorption of magnesium and manganese. The use of β-galactosidase to hydrolyse lactose enzymatically in dairy products, however, provides an alternative for lactose-intolerant consumers (Richmond, Gray, & Stine, 1981). There is a considerable growing interest in the search for more potential β-galactosidases for their use in hydrolysing lactose in dairy foods (Ismail, Mabrouk, & Mahoney, 1997).

This study attempts to investigate the effect of adding β-galactosidase (E.C. 3.2.1.23), or lactase, on the level of lactose present in the modified 'dadih' upon storage using the agar method.

2. Materials and methods

2.1. Determination of lactose content in fresh milk

Fresh milk obtained from local farms was immediately used for lactose determination. Lactose content was determined using HPLC as described by Bakken, Hill, and Amundson (1992). A volume of 0.5 ml was transferred to a test-tube and diluted to 10 ml with a mixture of acetone:water, 75:25% ratio. The mixture was then centrifuged at 200 rpm for 30 min. The supernatant was filtered through Sep-Pak and Millipore 0.45 μm. The filtrate, 20 μl, was injected into the column, 'Sugar-Pak', operated at 90°C using filtered deionised and degassed water as a mobile phase with a flow rate of 0.5 ml min^{-1}.

2.2. Determination of lactose and glucose in 'dadih'

A sample of 'dadih' was removed from the cup and blended using a commercial laboratory Waring blender for 15 s. Lactose and glucose content were determined as described earlier using fresh milk except that the sample was treated with boiling water for 5 min to deactivate the enzyme before addition of 75% acetone.

2.3. Determination of β-galactosidase activity (E.C. 3.2.1.23)

Commercial enzyme, Lactozym 3000L (Novo Nordisk Ltd.), was used. Enzyme activity, β-galactosidase was determined by incubating 0.1 ml of enzyme aliquot with 4 ml lactose solution (5%, w/v) in 25 mM phosphate buffer, pH 6.5, at 37°C for 30 min. The activity was stopped by boiling for 5 min. The mixture was cooled to room temperature and filtered through Sep-Pak and Millipore, 0.45 μm. The glucose produced was determined using HPLC.

2.4. Preparation of 'dadih'

Fresh milk was initially pasteurised at 80°C for 10 min using a steam-jacketed cooker (Green TDB-G10). A volume of 160 ml solution containing molten agar (3%), sugar (44%) and sodium chloride (1.25%) was added to 834 ml of the pasteurised milk and left to cool at 45°C before adding β-galactosidase. A volume of either 0.5, 1, 2, 3, 4, 5 and 6 ml Lactozym 3000L was added to the mixture, adjusting the final volume to 1 l with distilled water. A control was also prepared with the addition of 6 ml of distilled water only. The mixture was then stirred and dispensed immediately into plastic cups of 230 ml capacity with a diameter of 6.5 cm and depth of 7.5 cm. The set mixture was immediately cooled and sealed, and thereafter referred to as 'dadih' and was kept at 4°C until further analysis.

2.5. Effect of enzyme concentration on lactose content in 'dadih'

The effect of β-galactosidase on hydrolysis of lactose in 'dadih' was determined by the amount of lactose remaining after incubation for 7 days at 4°C.

2.6. Microbial count

A sample (5 g) was homogenised with 495 ml 0.1% peptone water using a stomacher (Lab Blender 400) for 20 s and further dilution was done using 0.1% peptone water. Counts were conducted by inoculating the sample on Petrifilm (Petrifilm™ 3M). The Petrifilm was incubated at 32°C for 48 h for aerobic counts whereas, for the yeast and mould counts, the plates were incubated at 21°C for 3–7 days.

2.7. Sensory evaluation of 'dadih'

'Dadih' and enzyme-treated 'dadih', at a concentration of 1 U ml^{-1} stored for 48 h at 4°C were used by 20 untrained panels for sensory analysis using a hedonic scale test (9 point scale). Characteristics such as aroma, colour, taste, texture and overall acceptance were determined.

2.8. Determination of colour

The colour of 'dadih' was determined using a Hunter Lab model D25M Optical Sensor (Hunter Associates Laboratory, Inc. Restron, VA), calibrated using a white ceramic tile with reflectance values of $X = 83.24$, $Y = 85.32$ and $Z = 100.92$. A representative sample of 'dadih' was placed in a 6 cm diameter Petri dish to a

depth of 1 cm. The sample dish was covered to avoid stray light. Hunter L (lightness), +a (red) to −a (green), and +b (yellow) to −b (blue) were then determined for each sample. Each value represented a mean value of five replicate determinations.

2.9. Statistical analysis

Data were analysed using the analysis of variance (ANOVA) procedure of the MINITAB version 10 for Windows (MINITAB Inc.). When significance was indicated, means were separated using Fisher's least square difference test ($\alpha = 0.05$).

3. Results and discussion

Local fresh milk was found to contain 4.49 % lactose, a level which could cause discomfort due to lactose intolerance after normal consumption of 220 ml (Holsinger & Kligerman, 1991). Lactose content of fresh milk has been reported elsewhere to be between 4.4–5.2% (Nikerson, 1974). However, milk composition is also influenced by the breed, lactation period, feed, age and several other factors (Johnson, 1974).

Upon formulation, 'dadih' contained 3.63% lactose, a reduction of 19%. However, the level is still likely to cause discomfort if the consumer is lactose-intolerant (Holsinger & Kligerman, 1991). The modified 'dadih' method includes the use of commercial β-galactosidase enzyme (Lactozym 3000L) extracted from *Kluyveromyces fragilis*, involving the addition of agar to give a smooth gel-like product. The effect of treatment of 'dadih', using Lactozyme 3000L, at different incubation times at 4°C on the lactose content, is shown in Fig. 1. Greater amounts of enzyme employed in 'dadih' hydrolysed lactose at a faster rate. Activities of 2, 3, 4, 5 and 6 U ml^{-1} 'dadih' achieved >70% hydrolysis, giving values of 73.8, 91.2, 93.9, 93.9 and 94.7%, respectively, at 24 h incubation. Intolerance symptoms can be eliminated if the level of lactose is reduced by 70% (Holsinger & Kligerman, 1991), giving a calculated value of 1.23% lactose when around 220 ml 'dadih' is consumed. 'Dadih', with added enzyme of concentration 1 U ml^{-1} achieved >70% hydrolysis on 48 h incubation while 0.5 U ml^{-1} Lactozym 3000L achieved the hydrolysis target at day 4. Hydrolysis of lactose in the untreated 'dadih' was also detected, probably due to the fermentation of microorganisms present in the 'dadih' (Muir, 1990). However, the hydrolysis of lactose by the untreated sample was only slight >20% at day 7, with the lowering of lactose from 3.63 to 2.9% only.

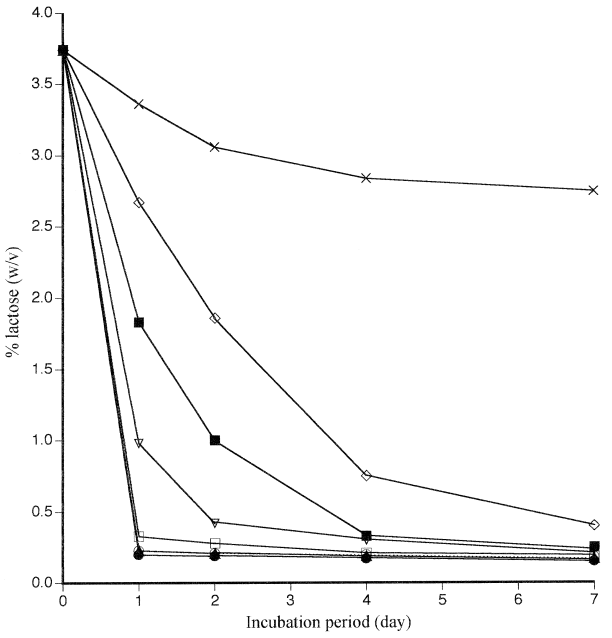

Fig. 1. Effect of enzyme concentration on the hydrolysis of lactose during stoage at 4°C. × 0.00 U ml^{-1}; ◇ 0.50 U ml^{-1}; ■ 1.00 U ml^{-1}; ▽ 2.00 U ml^{-1}; □ 3.00 U ml^{-1}; ○ 4.00 U ml^{-1}; △ 5.00 U ml^{-1}; ● 6.00 U ml^{-1}.

Although the optimum activity of Lactozym 3000L is known to be around pH 6.8 at 47°C (Kulp, 1975; Mahoney & Witaker, 1978), it was used at the storage temperature, 4°C, due to practicality of application, causing no additional delay in the processing line. The activity of the Lactozyme 3000L was 1029.5 U per ml determined at 37°C for the ease of analysis; this yields 1 U ml^{-1} upon dilution if 1 ml of the enzyme is used in 1000 ml of final volume formulation. Although glucose, an indicator product of hydrolysis, was detected, it could be utilised by the microorganisms in 'dadih' immediately upon release. Hence the level of glucose detected in 'dadih' was a balance between the rate of hydrolysis and the rate of utilisation of the microorganisms present. Highest glucose concentration was found with 6 U ml^{-1} lactase treatment, yielding 3.2% glucose at 24 h incubation (Fig. 2). Rapid production of glucose was accompanied by a drop after 24 h as noted for treatment with >2U ml^{-1} 'dadih', suggesting that the rate of utilisation was greater than their production at the 48 h incubation period. However, the glucose level of 'dadih' with 0.5 U ml^{-1} and 1 U ml^{-1} achieved a maximum at day 2, with values of 1.42 and 1.83%, respectively. The level of glucose continued to drop upon storage. The microbial growth was found to be related to the enzyme activity (Fig. 3). This indicates that the by-product hydrolysis was utilised for growth. Rapid microbial growth was noted for treatment using 4, 5, and 6 U ml^{-1} lactase at the 24 h incubation period, with values of 2.8×10^4, 4.9×10^4 and 6.5×10^4, respectively. However, viable counts dropped upon storage. Lactase treatment with 3.0 U ml^{-1} and lower gave counts lower than 1×10^4 throughout the incubation period.

According to the Malaysian Food Act (1983) and the Food Regulations (1985), milk and dairy products are

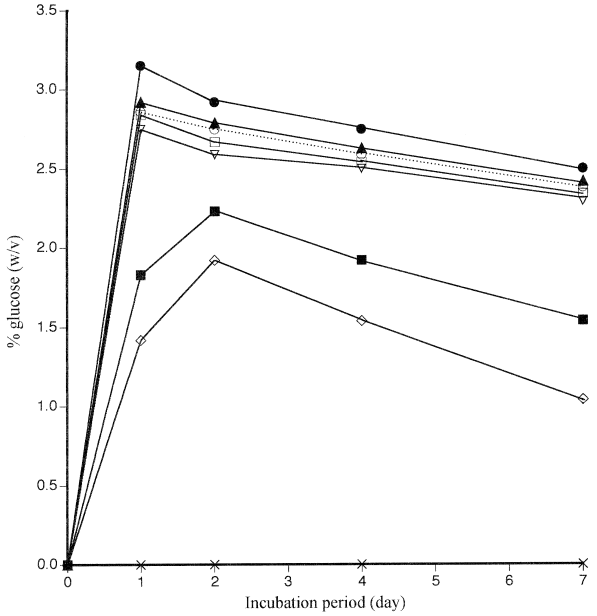

Fig. 2. Glucose concentration of 'dadih' during storage at 4°C. × 0.00 U ml^{-1}; ◇ 0.50 U ml^{-1}; ■ 1.00 U ml^{-1}; ▽ 2.00 U ml^{-1}; □ 3.00 U ml^{-1}; ○ 4.00 U ml^{-1}; ▲ 5.00 U ml^{-1}; ● 6.00 U ml^{-1}.

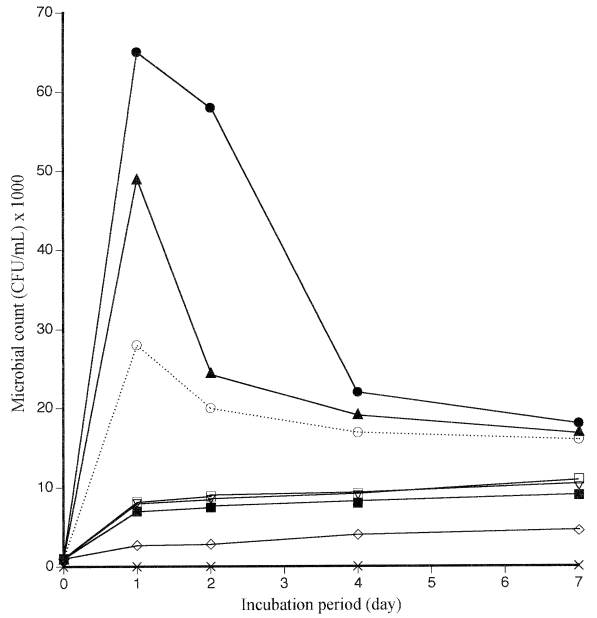

Fig. 3. Microbial counts of 'dadih' during storage at 4°C. × 0.00 U ml^{-1}; ◇ 0.50 U ml^{-1}; ■ 1.00 U ml^{-1}; ▽ 2.00 U ml^{-1}; □ 3.00 U ml^{-1}; ○ 4.00 U ml^{-1}; ▲ 5.00 U ml^{-1}; ● 6.00 U ml^{-1}.

regarded as safe if the total aerobic count is not exceeding 1×10^5 ml^{-1}. Mould and yeast were not detected, probably because they were efficiently destroyed during pasteurisation. Moreover, yeast was not able to ferment lactose (Davis, 1981).

Sensory tests of treated and untreated 'dadih' indicate that both were equally accepted (Table 1). While colour shows a significant difference ($p < 0.05$), other characteristics such as aroma, taste, texture and overall acceptance did not show significant differences ($p > 0.05$). The panels indicate a preference for 'dadih' treated with 1 U ml^{-1} 'dadih'. The colour of 'dadih' treated with lactase with a final activity of 1 U ml^{-1} was slightly yellowish, as indicated by the 'b' values, which could be discerned by the panels (Table 2).

Table 1
Sensory characteristics of 'dadih'

Characteristics	Score mean	
	'Dadih' without enzyme	'Dadih' with enzyme
Aroma	3.67a	3.78a
Colour	3.53a	2.95b
Taste	2.72a	2.72a
Texture	2.89a	2.78a
Overall acceptance	3.00a	3.00a

Score mean of each row having the same letter indicates insignificant difference ($\alpha = 0.05$).

Table 2
Comparison of 'L', 'a' and 'b' values of 'dadih' stored at 4°C

Hunter values	Day	Concentration of enzyme (U ml^{-1})							
		0.00	0.50	1.00	2.00	3.00	4.00	5.00	6.00
L	0	79.4	81.8	83.3	82.2	81.6	83.0	82.4	84.5
	7	79.9	82.3	82.9	82.1	82.5	80.8	81.1	81.5
	14	79.8	80.7	82.4	80.9	81.3	82.1	81.0	84.4
a	0	−4.4	−4.2	−4.0	−4.4	−4.2	−4.2	−4.8	−5.1
	7	−4.2	−4.0	−3.8	−3.8	−3.7	−3.9	−3.7	−3.5
	14	−4.1	−4.1	−4.1	−4.2	−4.1	−4.1	−4.3	−4.1
b	0	9.1a	10.4b	10.6b	10.1b	9.7a	9.8b	10.3b	10.0b
	7	9.7a	10.9b	11.1b	10.9b	10.3b	10.5b	10.5b	9.9a
	14	9.4	10.5	10.8	10.5	9.8	10.2	10.6	N10.3

Values having different letters indicate significant difference at level $p < 0.05$.

4. Conclusion

The preparation of modified 'dadih' using Lactozym 3000L with a final activity of 1 U ml^{-1} (1 ml l^{-1}) was found to be suitable to reduce lactose content of 'dadih' > 70%, a limit considered tolerable for lactose-intolerant consumers, at 48 h storage at 4°C, a period over which 'dadih' is normally stored before distributing to consumers. There is a great potential for commercialising the enzyme-treated modified 'dadih' to fill the wide gap of the market created by lactose-intolerant consumers in the Asian region.

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