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THE EFFECT OF LACTOSE HYDROLYSIS ON RHEOLOGICAL, PHYSICAL AND SENSORY CHARACTERISTICS OF FROZEN YOGHURT

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Abstract: Objectives: The effect of lactose hydrolysis on the rheological and sensory characteristics of frozen yoghurt was investigated during this study.

Methods: Yoghurt mix was hydrolyzed by commercial lactase enzyme before fermentation step. Results: The study revealed that the hydrolyzed samples had slightly shorter fermentation time and higher acidity. Moreover, lactose hydrolysis affected the rheological properties of the frozen yoghurt mix considerably. At shear rate range (2.6- 50 s^{-1}), the maximum apparent viscosity value of hydrolyzed frozen yoghurt mix was 50% less than the regular samples. Similarly, shear stress value of the hydrolyzed samples was 30% less than the regular samples. Additionally, the hydrolyzed samples showed higher melting rate and significantly lower overrun (39.33±0.732%) compared to (44.63±0.645%) for regular samples. Furthermore, sensory evaluation result showed that creaminess and texture attributes were ranked significantly different ($P \leq 0.05$). It can be concluded that lactose hydrolysis had a negative impact on some quality attributes and profitability of frozen

Keywords- lactose hydrolysis, frozen yoghurt, viscosity, texture

1. INTRODUTION

Frozen yoghurt popularity has considerably increased over the last years among different age groups. It is a frozen dessert that identified by characteristics which are closer to an ice cream but has a distinct sour taste and flavor resulted from the fermentation caused by lactic acid bacterial culture [1]. It is known that high intake of fat increases the risk of heart diseases and obesity [2]. The derive of the increasing demand of frozen yoghurt that, it is considered healthier than ice cream due to low fat content which contribute to 3-6% in comparison with 10-12% in standard ice cream [1]. Additionally, it can be considered as a functional food because, at low storage temperature cultures remained at adequate levels to enhance gastrointestinal health associated with culture survival in the digestive system [3, 4].

Despite the health benefits and nutrients that frozen yoghurt can provide, many consumers who suffer from lactose intolerance cannot risk eating frozen yoghurt. Lactose intolerance is a result of lactase deficiency or lactose malabsorption, it is a situation in which people have digestive symptoms such as abdominal pain, bloating, diarrhea and gas after eating milk or milk product [5, 6]. Lactose is a reducing sugar consist of unit of glucose and unit of galactose connected by glycosidic linkage in β-configuration, described as β-D-galactopyranosyl-(1 4)-D-glucopyranose and found in milk from all mammals [7]. For lactose intolerant people, it is vital to breakdown lactose into glucose and galactose so can be easily absorbed into the bloodstream.

The production of frozen yoghurt consists of mixing and pasteurization of all ingredients to make natural stirred yoghurt with stabilizers, emulsifiers and sugar, then freezing the mix in a standard ice cream machine [8, 9]. In general, there is no standard specifications for frozen yoghurt nevertheless, some references specify that the final product should have a minimum of 0.15% titratable acidity (expressed as lactic acid), 3.25% milk-fat, a pH<5, and a minimum yoghurt content 70% [10, 11, 12]. The production of lactose free frozen yoghurt involves similar processing steps in addition to lactose reduction step. In manufacturing, the reduction of lactose is achieved by ultrafiltration or inoculation of lactase enzyme simultaneously or prior to fermentation step [12, 13]. The rheological properties of frozen yoghurt mix are very critical because they directly affect the physical and sensory attributes of the finish product [11]. Previous studies on lactose hydrolysis showed a negative effect while others showed a positive effect on the quality attributes of yoghurt and frozen yoghurt [12, 13, 14]. These contradicted findings demonstrate that further investigation is necessary due to increasing demand on lactose free products and its economic value. The aim of the current work is to investigate the impact of lactose hydrolysis on the rheological, physical and sensory characteristics of frozen yoghurt.

11. MATERIALS AND METHODS

Source of materials

Fresh milk, skim milk powder and cream were obtained from DAL Dairy Factory, commercial Ha-Lactase (5200 NLU/g) and freeze dried culture (*Streptococcus thermophilus and Lactobacillus delbrueckii Subsp. Bulgaricus*) were produced by Chr-Hansen Company (Denmark), raspberry flavor (Symrise, Germany), Natural color Ch-Hansen (Denmark) and sugar was purchased from the local market.

Preparation of yoghurt base mix

The base mix consisted of fat (3.5±0.09%), solid non-fat (10.6 ±0.054%) and TS (14.17±0.019%), was preheated at 66 °C using Thermomix device and homogenized at 180 bar by GEA Panda Plus 2000 homogenizer. Then the mix was heated to 95 °C for 5 min and cooled to 5 °C. Next, the sample was divided into two equal portions control and treated.

Lactose hydrolysis of treated sample

The hydrolysis of the treated sample has taken place at 5±1 °C for 12 hours before fermentation step. The minimum effective dose of lactase enzyme was determined using four different doses (0.04, 0.08, 0.12 and 0.16 %) to avoid changes in the chemical composition that might be caused by enzyme volume. After 9 hours incubation time at 5±1 °C, there was no difference in hydrolysis degree between 0.12% and 0.16 % dose of the enzyme, thus 0.12 % was used.

Fermentation step

Temperature of both samples was increased from 5 to 43 °C using Thermomix devise then, freeze dried culture (*Streptococcus thermophilus and Lactobacillus delbrueckii Subsp. Bulgaricus*) was added. Fermentation of the control and the hydrolyzed samples was carried out simultaneously at the respective fermentation temperature (43±1°C) in the same incubator (memmert UN 55, B213.2549, Germany) device. Fermentation step was stopped at pH=4.60 and samples were stirred during cooling to 25 °C in Thermo Circulator, Lab Tech LCB-R20, Korea. Then, samples were refrigerated at (5 °C) for the next step.

Preparation of frozen yoghurt mix

Each mix sample was prepared by addition of 15% sugar, color 0.03% and 0.03% flavor to 20% of the total quantity of stirred yoghurt and heated to 72 °C for seconds, then the mix was cooled to 5 °C and added to the 80% remaining stirred yoghurt of each sample. This step was done to avoid second pasteurization for the total mix in order to maintain active culture in the frozen yoghurt. Next, the both mixes was aged overnight and processed in an ice cream batch freezing machine (MARK, Italy). The finished product was packed into plastic cups and kept in freezer at -18 °C. All formulations were replicated at least three times.

Physiochemical analysis

The pH was determined according to AOAC [15] using electronic pH meter (JENWAY 3510 pH Meter, designed and manufactured in the UK by Bibby Scientific Stone LTd, model 3510, serial no. 51030). Titratable acidity, fat content, protein content and total solids content were measured according to the methods described previously [15].
The water holding capacity (WHC) of stirred yoghurt was determined by centrifugation of a five grams yoghurt sample at 4500 rpm for 30 min at 10°C (Hettich ROTOFIX 32A, Germany). The WHC was calculated as followed [16]:

$$WHC\ [\%] = \left(1 - \frac{Weight\ of\ whey\ after\ centerifugation}{Yoghurt\ sample\ weight}\right) * 100$$

Lactose was determined by two methods; for the mix by infrared spectrometry using (Milkoscan FT2, FOSS Analytical A/S.69, Slangeruggade, and DK3400 Hillerod Denmark) and in yoghurt and frozen yoghurt mix by High Performance Liquid Chromatography (HPLC) SHIMADZU, Japan.

Rheological Analysis

Rheological characteristics were determined using rotational rheometer Rheolab QC Anton Paar (SN 81811601), Austria with temperature control device (C-PTD 180/AIR/QC) (SN 81834495). About 30 g of the sample was placed carefully in a concentric measuring cup and vein stirrer A ST22-4V-40/113 was used. Flow behavior was analyzed using controlled shares rate factor (CSR) at shear rate range (0-50 s^{-1}) at 5±1 °C, this range was selected for the measurement of both the stirred yoghurt and the frozen yoghurt because beyond this value the changes were marginal. The flow curve data were fitted to Power Law model.

$\sigma = K\,(\dot{Y})^n$

Where σ is the shear stress, k is a consistency index and n is behavior index.

Overrun

Overrun of the frozen yogurts was calculated based on the following formula [11].

$$Overrun\ [\%] = \left(\frac{Weight\ of\ mix - Weight\ of\ frozen\ yoghurt}{Weight\ of\ frozen\ yoghurt}\right) * 100$$

Melting rate (w/w) was determined for 50 min at room temperature 23 °C [17]. The weight of collected melted sample was recorded at ten minutes intervals.

Sensory evaluation

Samples were presented in comparative or simultaneous design [18] and the evaluation was based on seven points hedonic scale, the result is presented as a mean value of the assessment of thirty un-trained panelists for taste, sourness, sweetness, creaminess and texture attributes.

Statistical analysis

The results in the current study are presented as a mean value of analysis of three replicates with standard deviation.
The statistical significance difference between the control and hydrolyzed sample was evaluated by T-test at $P \leq 0.05$ significant level using IBM SPSS Statistics for Windows program (Version 22.0. Armonk, New York, USA).

111. RESULTS AND DISCUSSION

Lactose hydrolysis and chemical composition

The degree of hydrolysis data is presented in Figure 1. As can be seen the degree of hydrolysis was increasing as enzyme dose increases. The degree of hydrolysis was largely affected by the dose of the enzyme in the initial 6 hours then leveled off. After 9 hours the difference between 0.12 and 0.16% was neglected. As a result 0.12% dose for 12 hours was capable of reducing lactose content from 5.54% to 0.03%. The chemical composition of the samples before and after fermentation step was shown in Table 1. Fermentation time to reach strike (pH= 4.6) for the control and hydrolysis samples was 270 min. and 280 min., respectively. Hydrolyzed sample fermentation time was 10 min. shorter than the control samples and the acidity was slightly higher than the control samples (0.90% and 0.87%, respectively). A similar difference in acidity was also reported for hydrolyzed samples 0.89% compared to 0.87% for control samples [12]. The increase in acidity could be correlated with enhanced fermentation due to availability of glucose and galactose for lactic acid bacteria caused by lactase enzyme. Shorter fermentation time of hydrolyzed milk was also observed [13, 19, 20].

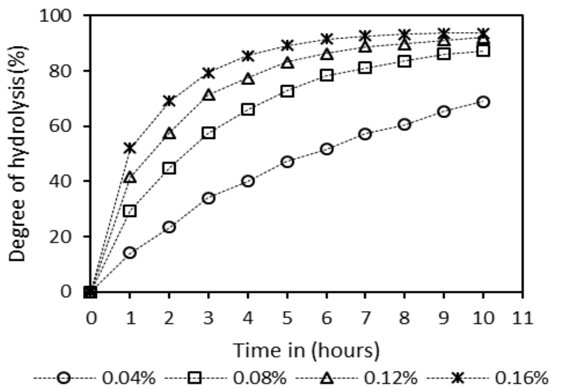

Figure 1: Degree of hydrolysis of lactose sugar for yoghurt mix at different lactase enzyme dose (0.04, 0.08, 0.12 and 0.16 %) at temperature at 5 ± 1 °C

Table 1: Chemical composition of yoghurt base mix before fermentation, control frozen yoghurt mix and hydrolyzed frozen yoghurt mix

Analysis	*Yoghurt base mix*	*Control frozen yoghurt mix*	*Lactose free frozen yoghurt mix*
Fat (%)	3.50±0.00[a]	3.23±0.06[b]	3.20±0.00[b]
Protein (%)	3.72±0.02[a]	3.35±0.07[b]	3.3±0.08[b]
pH	6.54±0.00[a]	4.25±0.005[b]	4.20±0.00[c]
TA (%)	0.23±0.0045[a]	0.87±0.002[b]	0.90±0.003[c]
Lactose (%)	5.54±0.004[a]	4.33±0.047[b]	0.03±0.000[c]
TS (%)	14.20±0.055[a]	26.34±0.096[b]	26.25 ±0.098[b]

Data is presented as mean ±SD. Different superscript letters in the same raw indicate statistically significant differences (P≤0.05)

Water holding capacity

The water holding capacity (WHC) or forced syneresis of stirred yoghurt is expressed as the amount of serig.um that is expelled from a gel during centrifugation (Isanga and Zhang, 2009). The result revealed 49.70±0.003% for control stirred yoghurt and 50.22±0.004% for hydrolyzed stirred yoghurt with no significant difference was observed. Similarly, the forced syneresis of stirred yoghurt ranged from 46.2 to 54.1%. However, there was no evidence that lactose hydrolysis had positive or negative influence [13].

Rheological properties of stirred yoghurt and frozen yoghurt mix

Viscosity is a critical aspect of frozen dessert that directly affects the quality and sensory attributes such as texture and mouth feel. As can be seen, in Figure 2 control yoghurt sample and hydrolyzed yoghurt sample apparent viscosity measurements values at 5±1 °C were very close. The maximum values obtained were 42.47 and 41.72 Pa.s respectively. Likewise, shear stress values of the hydrolyzed yoghurt were slightly lower than the control yoghurt sample, the maximum values was obtained at 50 s^{-1} shear rate were 157.86 and 149.15 respectively, however there was no significant difference. On the other hand, Figure 3 shows that hydrolyzed frozen yoghurt mix viscosity value at 5±1°C and shear rate 2.6 s^{-1} was 50% less than the control mix (5.64 Pa.s and 10.59 Ps.s, respectively). Similarly, shear stress value of hydrolysis samples was 30% less than the control samples. The maximum values obtained were 29.58 Pa and 43.56 Pa, both results were significantly different (P≤0.5). The flow curve data was fitted by the Power Law model as summarized in Table 2. The result showed that the behavior index *(n)* is less than one and this confirming that all samples exhibit pseudo-plastic behavior. However, hydrolyzed samples seem to be more sensitive to shear rate increase. The values of consistency coefficient *(K)*, flow behavior index *(n)* were significantly different for frozen yoghurt mix and control sample. This significant change was thought to be caused by structural change of breakdown of disaccharide to monosaccharide. The hydrolyzed fermented products from similar starter culture exhibited significant lower apparent viscosity (0.16 and 0.24 Pa.s) compared with control untreated samples (0.29 and 0.35 Pa.s) [13]. In contrast, the hydrolyzed frozen yoghurt had higher viscosity than control frozen yogurt however, the total solids of hydrolyzed samples was slightly higher than the control (19.46% and 18.76%, respectively)
[12].

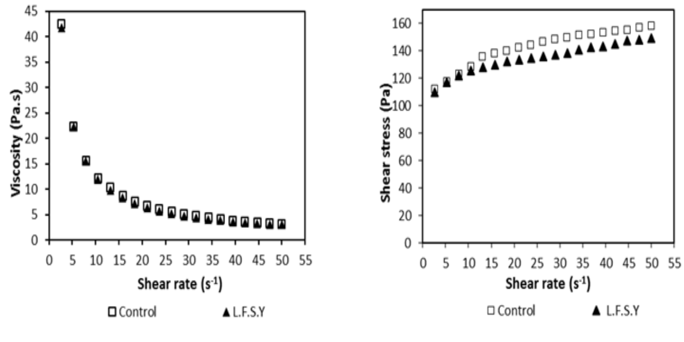

Figure 2: Controlled share rate (CSR) measurements of apparent viscosity and shear flow curve for control stirred

yoghurt and lactose free stirred yoghurt (L.F.S.Y) samples at 5 ± 0.5 °C and shear rate range (0-50 s-1)

Table 2: Calculated flow model parameters consistency index (*k*) and behavior index (*n*) and the coefficient of determination *(R^2)*

Samples	*Power Law Model (Shear stress vs shear rate)*		
	k	*n*	*R^2*
Control	97.250	0.124	0.990
L.F.S.Y	98.810	0.102	0.989
Control	24.803	0.144	0.991
L.F.M	12.070	0.231	0.989

L.F.S.T: lactose free stirred yoghurt; L.F.M: lactose free frozen yoghurt mix.

Overrun

The results revealed that the hydrolyzed sample has about 39.31±0.741% overrun and 44.35±0.674% for the control sample, the result was significantly different (P≤0.05). This means lactose hydrolysis resulted in 5% loss in the yield compared to control frozen yoghurt sample. Overrun or air incorporated during the dynamic freezing process is the most important property of frozen dessert [11, 21]. Tight control of over overrun is essential since it is directly tied to yield [22]. Moreover the frozen dessert containing yoghurt instead of milk has lower overrun in comparison with traditional ice cream [23]. In contracts, the lactose hydrolysis has no influence on the overrun capacity and the percentage were approximately 32.7±1.1% and 32.3±1.4% for control and hydrolyzed samples, respectively [12].

Meltdown rate

Frozen yoghurt melting property (removal of latent heat) is an important physical property because it affects eating experience. It is highly dependent on the microstructure and significant for determination of the optimum storage conditions. Melting rate over time data are presented graphically in Figure 4. Over the selected observation period, both samples displayed linear melting rate with different slope. The hydrolyzed samples have higher melting rate than the control samples. The maximum difference was about 12% after 50 min. storage at elevated room temperature (23 °C). Hence, total solids, fat content, protein and homogenization of both samples were not different. The difference in melting rate was thought to be caused by freezing point depression caused by lactose hydrolysis. Glucose and galactose as monosaccharide can cause greater freezing point depression than the lactose [11]. Additionally, the different overrun percentage as illustrated in Figure 4 can also influence melting rate. Higher overrun is known to decrease thermal diffusivity and cause isolation effect therefore delays meting rate [24]. Moreover, this result can be correlated to the difference in viscosity between the two samples as shown in Figure 3. Higher viscosity of the mix results in higher melting resistance due to the more stable emulsion was also reported previously [25, 26].

Sensory evaluation

Sensory evaluation results in Table 3 shows small differences in the sourness and sweetness perception between the samples. Initially, hydrolyzed samples were perceived as sweeter and less acidic even though the acidity measurement and pH was slightly higher than the control samples.

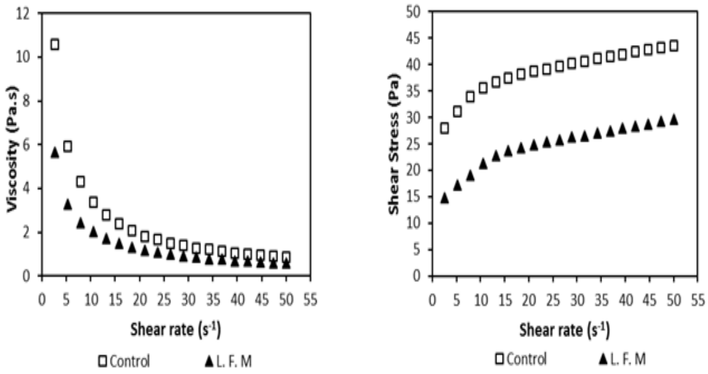

Figure 3: Controlled share rate (CSR) measurements of apparent viscosity and shear flow curve for control frozen yoghurt mix and lactose free frozen yoghurt mix (L.F.M) samples at 5± 0.5 °C and shear rate range (0-50 s-1)

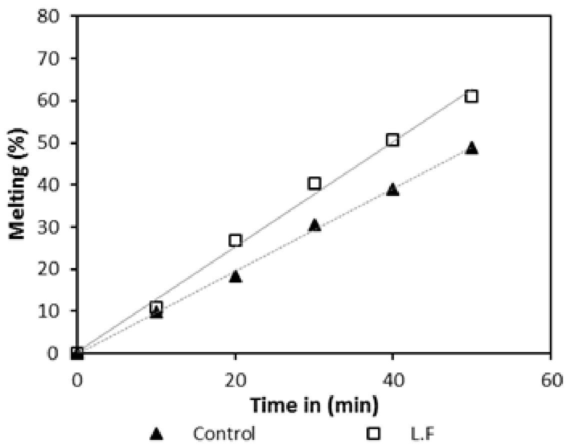

Figure 4: Average meltdown rate of control frozen yoghurt (R^2= 0.9945) and lactose free frozen yoghurt L.F (R^2=0.9983) at room temperature (23 °C)

This was thought to be caused by the overlap of sweet taste resulting from the breakdown of lactose sugar. However, the results were not significantly different. Frozen yoghurt is expected to have certain sensory characteristics during eating experience such as sweet taste, rich flavor, smooth creamy texture and sour taste as a result of fermentation. The present result supported the previous work [13, 27, 28, 29], which stated that hydrolyzed products taste sweeter because of the higher sweetness of the individual monosaccharaides. On the other hand, hydrolyzed sample was perceived as less creamy and has weaker body even though both samples have similar fat content and total solids. The result was significantly different (P≤0.05). This could be related to the differences in the mix viscosity because it has direct effect on the texture. This finding was in line to the previous reports stated that higher mix viscosity leads to better consistency and mouth feel of the frozen dessert [22, 25, 30]. Lastly, taste was ranked equally for both samples. Decline in sensory quality of hydrolyzed sample was compatible to rheological and physical results. Similarly it was reported that the sensory quality of yoghurt decreases with increasing lactose hydrolysis degree [14]. In contrary some findings reported that lactose hydrolysis promotes a smooth and creamy consistency for frozen yoghurt [12].

Table 3: Sensory results based on seven point hedonic scale for creaminess, sweetness, sourness, texture and taste attributes

Sample	*Creaminess*	*Sweetness*	*Sourness*	*Texture*	*Taste*
Control	6.5±0.618[A]	6.3±0.690[A]	6.2±0.0600[A]	6.6±0.0611[A]	6.4±0.715[A]
Lactose free frozen yoghurt	6.1±0.427[B]	6.6±0.495[A]	6.4±0.835[A]	6.2±0.495[B]	6.4±0.558[A]

Data is presented as mean ±SD. Different letters in superscript indicate statistically significant differences (P≤0.05)

V1. CONCLUSION

In the current work, the effect of lactose hydrolysis on frozen yoghurt physical and sensory characteristics has been investigated. Lactose hydrolysis has slightly reduced fermentation time and increased the acidity. On the other hand, rheological properties of were remarkably affected. In general, hydrolyzed sample had lower viscosity and shear stress values, while both samples had shear thinning property following Power Law model. Additionally, overrun was 5% lower in the hydrolyzed sample which means significant economic loss on large scale production, while melting rate was higher. The sensory attributes were also affected by lactose hydrolysis. The hydrolyzed sample was perceived significantly less creamy and has weaker body than the control sample by the panelists. It can be concluded that, lactose hydrolysis can significantly reduce some of frozen yoghurt quality attributes as well as, the overrun. Therefore, these negative impacts shall be addressed through product and process optimization.

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CONFLICTS OF INTERESTS

The authors declared that no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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