PAPER

YOGHURT DRINK FOR LACTOSE AND GALACTOSE INTOLERANT PATIENTS

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ABSTRACT

In this study, yoghurt drink with lactose free and low galactose content for patients with galactose intolerance were produced using a 1:1 mixture of lactose free milk and two different types of infant formula, fortified with strawberry flavor. The results indicated that galactose content of yoghurt drinks produced from lactose free raw materials were declined to a level that is suitable for the diets of patients with galactosemia. Chemical, microbiological and sensory properties of these products were found to match the common quality characteristics of a commercial fermented dairy product.

Keywords: yoghurt drink, Food allergies, galactosemia, lactose intolerance

1. INTRODUCTION

Classic galactosemia is an autosomal recessive disorder of carbohydrate metabolism (OMIM 230400), due to a severe deficiency of the enzyme, galactose-1-phosphate uridyltransferase (GALT, EC 2.7.7.12), which catalyzes the conversion of galactose-1phosphate and uridine diphosphate glucose (UDPglucose) to uridine diphosphate galactose (UDPgalactose) and glucose-1-phosphate. Upon consumption of lactose in the neonatal period, the affected infants develop a potentially lethal disease process with multiorgan involvement. However, since the advent of newborn screening (NBS) for galactosemia, we rarely encounter such overwhelmingly ill newborns. The following case report illustrates the acute neonatal toxicity that may be seen in infants with severe GALT deficiency, marked elevation of galactose-1-phosphate in target tissues and severe hypergalactosemia due to lactose ingestion (RIEDEL et al., 2005; BERRY, 2012). Its estimated incidence is 1/40,000 - 60,000 live births. This form is called the classical galactosemia. Patients with GALT deficiency appear normal at birth but soon develop severe hepatic, renal and gastro-intestinal manifestations that, if not treated, mostly lead to death. Removal of dietary lactose and galactose is essential as this will prevent or decrease the severity of the initial metabolic crisis in the neonate (KERCKHOVE *et al.*, 2015).

Individuals with galactosemia are intolerant of dietary lactose and galactose, primarily found in milk and milk products. If untreated, the disorder can cause liver failure, kidney dysfunction, sepsis, and death. If it is diagnosed soon after birth and treated by removal of lactose and galactose from the diet, the symptoms will resolve and many of the long-term complications, including cataracts and mental retardation, can be prevented (GRANGE, 2004).

Individuals suffering from galactosemia cannot consume products containing galactose, which means that they cannot consume dairy products, which have a critical role in healthy growth and nutrition. A strategy, similar to the introduction of the gluten free products to the diet of individuals with celiac disease, may be adopted for galactosemia suffering individuals by developing galactose free dairy products.

Previous studies have reported the following galactose consumption limit values that were verified by doctors and dieticians based on many years of experience: babies 50 (–200 mg), infants 150 (–200 mg), schoolchildren 200 (–300 mg), youth 250 (–400 mg), adults 300 (-500 mg) galactose/day (VARGA *et al.*, 2006, SCHWEITZER *et al.*, 1998). Depending on these limit values, the galactose content of the yoghurt-like product developed by SZIGETI and KRÁSZ (1992) was found to be higher than the required for children suffering from galactosemia, where VARGA *et al.*, (2006) found the galactose levels of kefir-like products suitable for galactosemia patients from all ages.

Yoghurt is a fermented dairy product commonly produced in the world, produced by the fermentation of milk by *Lb. bulgaricus* and *S. thermophilus* bacteria. Yoghurt drink also carries the health and nutritional value of yoghurt and it is produced by the addition of water to yoghurt. It is called in different ways in different regions such as ayran in Turkey, dough in Iran, tan in Armenia, laban in Syria and Lebanon. As well its many health benefits, yoghurt drink has positive effects on health including the regulation of lactose intolerance and supporting the immune system (ERKAYA *et al.*, 2015).

The aim of this study was to develop yoghurt drink for individuals with galactosemia and/or lactose intolerance by full hydrolization of lactose content and lowering the galactose levels suitable for the safe consumption of these products by galactosemic individuals. Accordingly, yoghurt drink with galactose levels lower than 200 mg/100 c m³ for galactosemic patients from all ages by using by using a 1:1 mixture of lactose free milk and two different types of infant formula, fortified with strawberry flavor.

2. MATERIALS AND METHODS

2.1. Milk samples and fermented dairy production, experimental design

UHT cow's milk and lactose hydrolyzed UHT cow's milk used in the studies were obtained from Pinar Sut Co. (Izmir, TURKEY). In order to lower the galactose content before the fermentation, lactose hydrolyzed UHT cow's milk was mixed with galactose free infant formulas. The ratios in the mixtures were one part of lactose hydrolyzed milk and one part of galactose free infant formula (1:1). Two different galactose free infant formulas were used as supplements of lactose hydrolyzed milk: Neocate, a maltose based, galactose free infant formula (Milupa/Numico, Netherlands) and Galactomin 19, fructose based, galactose free complete infant formula (SHS, UK). The sensory properties of the two formulas were different, possibly influencing the sensory properties of both raw material mixtures and fermented products. UHT cow's milk as the control group, lactose hydrolyzed milk and the two types of mixtures were inoculated with yoghurt drink cultures respectively (Table 1).

Table 1. Raw material properties of ferme	ented dairy drinks for the	e individuals with galactosemia.
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Raw Material	Dry Matter (%)	Fat (%)	Protein (%)	рН	Lactose (mg/100cm ³)	Galactose (mg/100cm ³)
С	10.31±0.50	1.50±0.06	3.10±0.00	6.7±0.03	4208.35±23.35	0.00±0.00
L	10.19±0.18	1.45±0.05	3.10±0.04	6.6±0.11	0.00±0.00	2160.40±34.21
LN	10.54±0.24	2.50±0.07	2.97±0.02	6.4±0.14	0.00±0.00	1068.11±12.30
LG19	10.42±0.08	2.80±0.01	2.98±0.06	6.5±0.08	0.00±0.00	1080.07±14.10

C: Conventional UHT milk, L: Lactose-free UHT milk,

LN: Lactose-free UHT milk + Neocate, LG19: Lactose-free UHT milk + Galactomin 19.

Commercial freeze-dried yoghurt drink starter culture LB340, containing *Lactobacillus delbrueckii spp. bulgaricus* and *Streptococcus thermophilus*, were obtained from Ezal (Texel, France). The strawberry sauce and flavor used for enhancing the sensory properties of the products were obtained from Aromsa Co. (Kocaeli, Turkey). Skim milk powder used for the preparation of starter cultures were obtained from Pinar Sut Co. (Pinarbasi, Izmir).

500 mL of reconstituted skim milk with 12 % non-fat dry matter were inoculated with freeze-dried yoghurt drink (2 %, in 42° C) cultures. The inoculations ended when the pH levels of the inocula dropped to 4.6. Raw materials prepared for the production of fermented drinks were inoculated with 3.25 % culture in all cases. Incubation parameters for the products were, 3 hours in 42° C. Fermentation were run in duplicate and repeated twice in bottles containing 500 mL of raw materials and 3.25 % inoculum. In order to enhance the sensory properties of products, fermented drinks were fortified with galactose free strawberry sauce (1.8 %) and strawberry aroma (0.1 %). Manufacture of the products were run in duplicate and repeated twice in all cases. 4 different raw materials were coded as follows;

CAY: Control Yoghurt Drink, LAY: Lactose free milk Yoghurt Drink, LNAY: Lactose free milk + Neocate Yoghurt Drink, LG19AY: Lactose free milk + Galactomin 19 Yoghurt Drink.

2.2. Chemical, microbiological and sensory analyses

The pH was determined using a pH meter (Hanna pH 211 Microprocessor, Portugal). Dry matter, protein and fat contents were determined according to A.O.A.C (2006). Tyrosine levels were measured according to HULL (1947). Acetaldehyde contents of the samples were determined using spectrophotometric method according to ROBINSON *et al.*, (1977). For the determination of lactose and galactose levels, Megazyme K-LACGAR 12/05 enzymatic kit obtained from Megazyme International Ireland Limited (Co.Wicklow, Ireland) was used.

Bacterial enumerations were carried out at the 1st, 10th, 20th, 30th days of the storage period. Samples (1 mL) were diluted with ringer solution (9 mL). Serial dilutions were carried out, and bacteria were counted, applying the pour plate method. *L. bulgaricus* counts in yoghurt drink samples were enumerated in MRS agar (pH 5.8) (Merck / 1.10660, Darmstadt, Germany) anaerobically at 42°C for 48 h, whereas *S. thermophiles* in yoghurt drink samples were counted in M17 agar (pH 6.9) aerobically at 37°C for 48 h (BRACQUART, 1981).

Samples were evaluated for their sensory properties (taste-aroma, consistency, overall). The evaluation cards were prepared according to BODYFELT *et al.*, (1998). The evaluation was performed by the academicians from the Dairy Technology Department of Ege University.

2.3. Statistical analyses

The experiments were performed in two repetitons with three parallels. The mean value of the six values for each sample was calculated (n=6). The obtained data was statistically analyzed by one-way ANOVA using the general linear model. The constant effects (different production process and storage period and the effects of the interactions between these effects were analyzed by analysis of variance (ANOVA) using SPSS© v.15.00 (SPSS Inc., Chicago, Illinois USA). The significant data as a result of ANOVA were tested according to the Duncan multiple comparison test at p <0.05 level.

3. RESULTS AND DISCUSSIONS

3.1. Chemical properties

In terms of obtaining the desired structural and sensory properties of yoghurt drink dry matter content and its properties are the important basic parameters. Many studies have reported that dry matter contents had a direct effect on the structural, microbiological and sensory properties of the products. In the production of fermented dairy products, it is required to comply with the legally prescribed minimum dry matter levels. Dry matter, fat and protein contents of yoghurt drink samples were analyzed on the 1st day of the storage (Table 2). The results showed that dry matter, fat and protein contents of all yoghurt drink and kefir samples were in accordance with the nutrient contents specified in Fermented Dairy Products Communiqué (Communiqué No: 2009/25) in Turkish Food Codex (2009). In accordance with the aim of our study, lactose-free milk and milk-formula mixtures were used in productions. Additionally, no lactose hydrolization process was done in control group that is conventional semi-skimmed UHT drinking milk. Therefore, lactose was not detected in the study, except the control samples coded as CAY (Table 2).

	Dry Matter (%)	Fat (%)	Protein (%)	Acetaldehyde (ppm)	Lactose (mg / 100 cm ³)	Galactose (mg / 100 cm ³)
CAY	10.94±0.08 ^b	1.50±0.00 ^a	2.71±0.19 ^b	6,97±0,01 [°]	2129.20±23.81	120.67±.072 ^b
LAY	10.82±0.47 ^b	1.55±0.00 ^b	3.01±0.36 ^c	7,04±0,01 ^c	≤0.01±0.00	225.51±2.78 ^d
LNAY	11.05±0.50 ^c	2.56±0.00 ^c	2.69±0.10 ^a	6,00 ±0,01 ^a	≤0.01±0.00	153.79±2.05 ^c
LG19AY	10.62±0.21 ^ª	2.76±0.02 ^d	2.66±0.84 ^a	6,30±0,01 ^b	≤0.01±0.00	63.70±6.39 ^a

Table 2. The results for the compositional analysis of yoghurt drinks for the individuals with galactosemia.

^{a, b, c, d}: Values with different lower-case letters in the same column differ significantly (P < 0.05).

Examining the galactose levels of other three lactose free samples, it was found that galactose level in LAS sample was higher than the level reported by VARGA *et al.*, (2006), with 212.46 mg/100 cm³. Different raw material contents had a significant effect on the galactose contents in all kefir samples (p<0,05). It was found that LKF, LNKF and LG19KF samples contained lower galactose than the threshold reported by VARGA *et al.*, (2006), with 161.95, 132.74 and 106.54 mg/100 cm³, respectively. VARGA *et al.*, (2006), in their study, determined the galactose level of kefir sample pre-determined as the control sample produced from lactose free milk as 270 mg/100 cm³, the galactose level of kefir produced from milk-formula mixture containing Pregomin as 169 mg/100 cm³, and the galactose level of kefir produced from their study, the researchers determined the milk-formula ratio as 2 parts milk and 1 part formula (2:1). Comparing those results with our study, the galactose levels obtained in our study appear to be lower than those by VARGA *et al.*, (2006). The most likely reason for this difference is the 1:1 milk-formula ratio we adopted for our method. In all samples pH decreased during storage (Fig. 1).

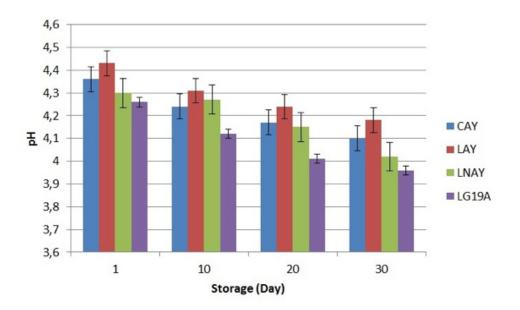


Figure 1. pH values of yoghurt drinks for the individuals with galactosemia.

The differences between the pH values in acidophilus milk samples at the 10th, 20th and 30th day of the storage were found to be statistically not significant (p>0.05). In yoghurt

drink samples, different raw material content had no effect on the pH values at the 1st and the 20th days of the storage (p<0.05). pH values of yoghurt drink samples in our study were similar to those in similar studies by OZER *et al.*, (2005), MARTINI *et al.*, (1991), TONGUC *et al.*, (2013), YERLIKAYA *et al.*, (2013) and ERKAYA *et al.*, (2015). In fermented dairy products, as a result of the hydrolization of lactose by culture bacteria and the formation of lactic acid during incubation, pH reaches to a certain level and coagulates and maintains the gel formation. During ripening and storage, acidity increases and the decrease in pH value continues. The type of the bacteria used in the incubation is largely responsible for the speed of the decrease in pH.

It was reported that the amount of acetaldehyde required for the formation of characteristic aroma in fermented dairy products varied between 13 and 48 ppm (SAHAN *et al.,* 2008; CHENG, 2010). Different raw material compositions had a statistically significant effect on the acetaldehyde contents in all samples (p<0.05). This result was supported by the panelists' comments in taste-aroma evaluations in sensory analyses reporting that they perceived acetaldehyde aroma in products.

Tyrosine values of yoghurt drink samples and the changes in these values during storage are given in Fig. 2. The differences between tyrosine values of yoghurt drink samples were statistically significant (p<0.05). Also, storage had a statistically significant effect on the tyrosine values of the samples (p<0.05). Tyrosine levels obtained in our study was compatible with those reported in studies on various fermented dairy products (OZER *et al.*, 2005, YERLIKAYA *et al.*, 2013). However, tyrosine levels of sample LN were higher than those values. In this perspective, tyrosine values of all the samples and the scores obtained in sample LNAY supports the claim by De MANN (2013) that bitter taste forms as tyrosine content increases. Sample LNAY received the lowest taste-flavor scores in sensory evaluations throughout the whole storage. Panelists reported "bitter taste-flavor formations" in the sensory evaluation sheets many times throughout the sensory evaluation process.

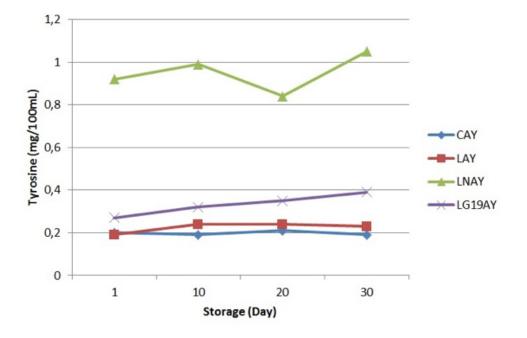


Figure 2. Tyrosine values of yoghurt drinks.

3.2. Microbiological properties

Examining the microbiological properties of yoghurt drink samples, it was found that different raw material contents had a significant effect on the *L. bulgaricus* counts in yoghurt drink samples at the 10th and 20th day of the storage (Table 3). The effect of storage period on the *L. bulgaricus* counts in all yoghurt drink samples was significant (p<0.05). Using different raw materials had a significant effect on the *S. thermophilus* counts of yoghurt drink samples (p<0.05). In addition, the effect of storage on *S. thermophilus* counts of yoghurt drink samples was statistically significant (p<0.05). *S. thermophilus* counts of yoghurt drink samples were above log 7 cfu/ml and the lowest value was determined in LAY with log 6.97 cfu/ml on the 10th day of the storage (Table 4). *Lb. bulgaricus* and *S. thermophilus* counts in LNAY and LG19AY samples maintained their viability during 30-day storage period. *Lb. bulgaricus* and *S. thermophilus* counts in our samples were higher than the results found in previous studies by AKALIN and UNAL (2010) and were similar to those obtained by VARGA *et al.*, (2003), YERLIKAYA *et al.*, (2012), TONGUC *et al.*, (2013) and ERKAYA *et al.*, (2015).

Table 3. Microbiological contents of yoghurt drinks for the individuals with galactosemia.

Helv	Storage (Day)					
	1 st	10 th	20 th	30 th		
Lb. bulgaricus (log cfu/ml)						
CAY	8.07±0.26Y ^b	8.12±0.10Y ^b	7.42±0.04X ^a	7.57±0.08X		
LAY	7.85±0.34XY ^{ab}	8.02±0.01Y ^b	7.58±0.07XY ^b	7.40±0.01X		
LNAY	7.50±0.07X ^a	$8.03 \pm 0.06 W^{b}$	7.61±0.01XY ^b	7.71±0.02Y		
LG19AY	7.46 ±0.03XY ^a	7.79±0.01Y ^a	7.32±0.07X ^a	7.54±0.25XY		
S. thermophilus (log cfu/ml)						
CAY	7.37±0.04Y ^a	7.12±0.10X ^b	7.63±0.03W ^b	7.16±0.01X ^a		
LAY	7.48±0.01W ^{ab}	6.97±0.04X ^a	7.85±0.04Z ^b	7.22±0.02Y ^a		
LNAY	7.41±0.00Y ^{ab}	7.30±0.01X ^c	7.39±0.00Y ^a	7.53±0.03W ^b		
LG19AY	7.54±0.08Y ^b	7.27±0.02X ^{bc}	7.66±0.07Y ^b	7.66±0.08Y ^b		

^{a.b.}: Values with different lower-case letters in the same column differ significantly (p<0.05). X, Y, W, Z: Values with different capital letters in the same row for each analysis differ significantly (P <0.05).

3.3. Sensory properties

In yoghurt drink samples, using different raw material formulations had a significant effect on the taste-aroma properties on the 1st, 10th and the 20th day of the storage (p<0,05). LNAY received considerably lower points compared to the other samples (Table 4). The difference between taste-aroma scores of the samples was found to be statistically not significant on the 30th day of the storage (p>0.05). Also, it was found that storage had no significant effect on the taste-aroma properties of the samples (p>0.05). The panelists reported that taste-aroma characteristics of yoghurt drink samples, including LNAY sample, which received the lowest points, were very stable throughout the storage, and no negative developments occurred such as increase in acidity or souring. As a result of ANOVA and Duncan tests, it was found that different raw material composition of the yoghurt drink samples had a significant effect on their consistency properties (p<0.05).

Among the yoghurt drink samples, LG19AY received the highest consistency points and much better and more tasteful product compared to the control sample. The difference between general scores of the yoghurt drink samples was found to be statistically not significant at the 30° day of the storage (p>0.05). CAY, LAY and LG19AY samples had no significant differences in terms of general sensory analysis scores at the 1st, 10th and 20° days of the storage (p>0.05), and LNAY was statistically different than the other three yoghurt drink samples during the storage (p<0.05).

	Storage (Day)				
	1 st	10 th	20 th	30 th	
Taste- Aroma					
CAY	6.87±0.17 ^b	6.75±1.06 ^{ab}	6.80±0.28 ^{ab}	7.40±0.84 ^b	
LAY	6.65±0.21 ^b	7.55±0.07 ^b	6.65±0.49 ^{ab}	7.01±0.55 ^b	
LNAY	4.10±0.14 ^a	5.15±0.49 ^a	4.57±1.80 ^a	5.80±0.28 ^a	
LG19AY	7.10±0.14 ^b	8.00±0.00 ^b	7.51±0.12 ^b	7.30±0.98 ^b	
Consistency					
CAY	7.02±1.10 ^{ab}	7.50±0.70 ^{ab}	7.37±0.32 ^b	7.50±0.14 ^b	
LAY	7.35±0.77 ^b	7.95±0.77 ^b	7.64±0.50 ^b	7.60±0.00 ^b	
LNAY	4.72±0.67 ^a	6.10±0.14 ^a	5.22±0.88 ^a	5.47±0.38 ^a	
LG19AY	7.87±0.17 ^b	8.35±0.21 ^b	8.00±0.56 ^b	8.10±0.14 ^b	
General					
CAY	6.75±0.35 ^b	7.05±0.63 ^b	6.80±0.28 ^b	7.50±0.70 ^b	
LAY	6.87±0.17 ^b	7.45±0.07 ^b	6.94±0.48 ^b	7.01±0.55 ^b	
LNAY	4.52±0.38 ^a	5.60±0.56 ^a	4.71±1.00 ^a	5.88±0.68 ^a	
LG19AY	7.20±0.28 ^b	8.25±0.35 ^b	7.80±0.28 ^b	7.76±0.90 ^b	

Table 4. Sensory evaluation of yoghurt drinks for the individuals with galactosemia.

^{a,b,c}: Values with different lower-case letters in the same column differ significantly (p < 0.05).

4. CONCLUSIONS

Consumption of dairy products by patients with galactosemia in their daily diet leads to consequences physiologically far more different and serious than those in lactose intolerance cases. Therefore, galactosemia patients have to eliminate dairy products from their daily diet completely in order not to experience these serious adverse effects and physiological damages. In our study, it was found that galactose levels in yoghurt drink produced from lactose free milk and infant formula mixtures for patients with galactosemia were lower than the galactose threshold values reported in the referred studies (VARGA *et al.*, 2006) Yoghurt drink samples exhibited good acidity development, microbiological content, and the stability of these contents. In our study, LG19AY sample was possibly the most successful sample among the yoghurt drinks fulfilling the aim and purpose of our study. Strawberry flavor fortification, comparing with the previous studies, improved the sensory properties and positive results were obtained in the sensory analysis. However, it is necessary to confirm these results with further studies and subsequently these products can be presented to the consumption of the patients.

ACKNOWLEDGEMENTS

The authors thank the Ege University Scientific Research Fund (Project No: 2009-ZRF-018) Council for financial support to this study. The authors also would like to thank Pinar Sut Inc. and Aromsa Inc. for their lactose free milk and flavor procurements.

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Paper Received January 9, 2017 Accepted July 10, 2017