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Physicochemical, microbiological and sensory characteristics of strawberry yoghurt fortified with *Red beet* extract

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ABSTRACT

This research conducted to optimize the formul of fruit yoghurt with different levels of red beet extract (1.25%, 2.5% and 4%) and strawberry flavor (1%, 2% and 4%). Physico-chemical and microbiological characteristics were analyzed to assay the quality of the yoghurt samples. Results showed higher syneresis and lower viscosities in red beet-flavored yogurts compared to the control sample and this effect was found to be concentration-dependent. The titratable acidity of red beet-flavored yogurt containing 2.5% red beet extract was found lower than the samples containing 1.25 and 4% extract and control, while the acidity of red beet-flavored yogurt containing 1.25 and 4% red beet extract was higher than the control. The pH of all the samples and control continued to decrease during storage period. Higher concentrations of red beet extract resulted in more decrease in pH. During 21 days of refrigeration storage, pH and LAB count decreased and titratable acidity increased. Panelists gave higher scores to the yogurt with 2.5% red beet extract, and no significant difference was observed between scores of yogurts produced by adding 1.25 and 4% red beet extract. The overall acceptability values of the yogurts containing 2.5% red beet extract and 2% strawberry flavor was found to be higher than the other types of red beet-flavored yogurts.

KEYWORDS

Flavored yogurt;
Red beet extract;
Physicochemical properties;
Viscosity;
Sensory properties.

INTRODUCTION

Betalians are natural water-soluble nitrogen-containing pigments, which comprise the red-violet betacyanins (Beta vulgaris), whose main pigment is betalain-5-0-B-glycosidase, or betanin, which is the most common betacyanin[8,11]. Recent findings rank beet root among the ten most potent antioxidant vegetables[7]. Betalains respond at least in part for these beneficial properties[21]. These reports have increased the interest in using red beetroot extracts to color food. Recently, there has been an increasing interest in the use of natural food additives and incorporation of health-promoting substances into the diet[18]. Yogurt consumption has doubled in the past 17 y and as of 2003 is a 2.3 billion dollar market[26]. In 2004, there was an 8% increase in the 2.7 billion pounds of yogurt consumed annually in the United States, attributed to increased advertisement and public perception of various health benefits of the product[26]. The improvement of aroma and flavor of yogurt can result in an increase in the consumption of final product. Fruit yogurt found to be more attractive organoleptically due to masking partially the excessive acetaldehyde flavor in plain yogurt[10,25]. The production and consumption of fruit yogurt is low in Iran compared to plain yogurt, therefore, in this study we would like to evaluate the physicochemical, microbial and sensory quality during storage of red beet-flavored yogurt.

MATERIAL AND METHODS

The experiment was conducted in the laboratory of department of food technology & rural industries, Islamic Azad University, branch of Shahrekord. Fresh milk was collected from dairy farm of Shimbar factory. Red beet for extract preparation, strawberry flavor (farmand Co, iran), and starter culture collected from local market.

Preparation of red beet extract

Collected red beets, were washed with clean water and the black spots were removed. Red beets were blended and the juice extracted by juicer. The extract was filtered with clean cloth (hot water washed) and kept in plastic containers at freezing temperature (-20°C) until preparation of red beet yogurt[23].

Yogurt production

Raw milk pasteurized at 85 °C for 10 minutes and subsequently cooled to 39±1 °C. Inoculation was done with desirable proportion of starter culture (2.5%). Once the starter, was completely mixed, the red beet juice incorporated into yoghurt at 1.25%, 2.5% and 4% level and was flavored with strawberry flavor at 1, 2 and 4% levels except in control sample[3]. The plastic cups were pre-washed with boiled water before using. The samples were incubated at 41-43 °C until formation/ coagulation of yoghurt (8-12 hrs). The yoghurt samples were stored at about 4 °C at refrigeration until used.

Chemical analysis of fresh milk

Moisture, total solid (TS) and ash content were determined according to AOAC (2002). Fat content determined by Babcock method using the procedure described by Aggarwala and Sharma (1961). Acidity determined by titration with 0.1 N sodium hydroxide solution using the procedure by Aggarwala and Sharma (1961). Crude protein determined by Kjeldahl described by Ranganna (1976) procedure. Total carbohydrate content of the sample determined by subtracting the measured protein, fat, ash and moisture from 100. pH measured with the help of a pH meter (D-82362، Germany).

Sensory evaluation

The samples of yogurt prepared were evaluated organoleptically on 7 point hedonic scale by a 10 judges panel (10 laboratory staff of Shimbar dairy factory). The samples were evaluated for body, texture, flavor and overall acceptability as described by Land and Shepher (1988). The judges were briefed as to the method of scoring for different quality characteristics. Descriptions about the criteria evaluated were given prior to evaluation[27].

pH measurement

Yogurt pH was measured in duplicate using a pH model (D-82362، Germany). Titratable acidity of milk and the yogurts, expressed as % lactic acid, was determined on triplicate samples following method 947.05 of the AOAC (1999). Yogurt pH and titratable acidity were measured at days 1, 7, 14, and 21.

Total tritatable acidity measurement

Yogurt sample (1 ml) was mixed thoroughly with 9 ml of distilled water phenolphthalein solution (0.1%, 3 drops) was added and the yogurt suspension was titrated using 0.1 M NaoH. The mixture was stirred continuously and titrated was continued until the indicator changed to a definite pink color lasting for 30 seconds. The volume of NaOH required to neutralize the yogurt acid was recorded and used to calculate the content of titratable acids (lactic acid percentage equivalent) using the following formula:

$$LA\% = \frac{10 \times VNaOH \times 0.009 \times 0.1}{W} \times 100\%$$

Where, 10 = Dilution factor; W = weight of sample for titration; VNaOH = Volume of NaOH used to neutralize the lactic acid; 0.1 = Normality of NaOH.

Syneresis measurement

One hundred grams of yoghurt sample was placed on a filter paper resting on a top of a funnel. After 2 h of drainage at 70C, the quantity of whey collected in a 50 ml graduated cylinder was used as an index of syneresis[13]. Syneresis (%) was based on the volume of clear supernatant per 100-mL yogurt.

Viscosity measurement

Apparent viscosity was determined by using a RV brookfield viscometer (Stoughton, USA) on 100 mL yogurt samples at room temperature. Samples were stirred for 40 sec before measurement. Readings were converted to centipoises units. All viscosity values were measured at 10 rpm with spindle #5[19].

Lactic acid bacteria (LAB) and yeast and mold counts

Total LAB counts were determined for the starter culture and yogurt samples. Aliquots of 10 g were diluted with 90-mL sterile peptone water (0.1% w/v) and serial dilutions were prepared. Mann, rogosa, sharpe agar (Oxoid, Basingstoke, U.K.) was used for assaying total lactic acid bacteria by the double layer plating technique. Plates were incubated at 32C for 48–72 h[34]. Yeast and mold counts were also conducted on the yogurts. The serial dilutions were placed on potato dextrose agar (Oxoid) acidified with 1% lactic acid and the plates were incubated at 30C for 5 days[17]. All yogurt samples were duplicate plated at days 1, 7, 14 and 21.

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using the general linear model (GLM) procedure of the statistical analysis system[33]. The means were separated by use of the least significant difference (LSD) test. Significant differences were determined at α=0.05[29].

RESULTS

Chemical analysis of milk

Quality of milk used for yoghurt production was analyzed before using. Moisture, total solid, fat, protein, ash, lactose, acidity, pH, and solid non-fat (SNF) were determined. Results of chemical analysis of milk are shown in (TABLE 1). The results are more or less similar to other researcher's. Protein percentage of raw milk samples was 3.32, which is within the normal range of 2.3 to 4.4[35]. Mean acidity of the experimental samples was 0.17 percent which is within the normal range.

Organoleptic properties of red beet-flavor yogurts

The organoleptic evaluation of the red beet-flavored yogurt was carried out at weekly intervals for 21 days and the mean scores obtained summarized in TABLE 2. Results of organoleptic evaluation showed that significant differences were observed among red beet-flavored yogurts (P< 0.05) except texture, while differences were obtained significant statistically between the scores of all organoleptic properties of the yogurt flavor made by adding 1.25 and 2.5 and 4% of red beet extracts. By adding higher dosages of red beet extract, the flavor of yogurt was scored lower by panelists (P<0.05). However, statistically significant differences were not found between the scores of samples with 1 and 2% strawberry flavor addition. In addition, the panelists preferred yogurt produced with 2% of Red beet extract (TABLE 2).

With respect to flavor value, the red beet-flavored yogurt manufactured with 2.5% of red beet extracts and 2% strawberry flavor was more acceptable by panelists. As a result of panelists' evaluation, with respect to flavor, color, and perceived texture, significant differences were not observed between examined yogurts by adding different amounts of strawberry flavors. The perceived flavor value of 1% strawberry flavor added yogurt was found to be the lowest, while the values of the yogurts made by adding 2% strawberry flavor was highest (P < 0.05). In addition, panelists also stated that the amount of the red beet yogurt containing 1 and 4% strawberry flavor was less desirable. Yogurts produced with adding 2.5% red beet extract and 2% strawberry flavor were preferred by panelists with higher value of others red beet yogurt. Although, the result was statistically significant, the overall acceptability value of red beet-flavored yogurt with 1.25 and 4% red beet extract was slightly lower than the yogurt added 2.5% red beet extract.

TABLE 1 : Composition of cow milk, used for yogurt making

Composition	pH	Acidity (%)	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Carbohydrate/Lactose (%)	SNF (%)
Raw Milk	6.6	0.18	87.52	4.15	3.31	0.72	4.28	8.37

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TABLE 2 : Effect of red beet extract and strawberry flavor concentrations on sensory properties of yogurt.

Properties			Flavor	Texture	Color	Overall Acceptability
Different types of yogurts	0% Red beet extract	CY	5.60±0.65	5.88±0.52	5.82±0.49	5.02±1.14
		S0	5.36±0.72	5.88±0.53	5.82±0.45	5.02±1.23
		S1	5.49±0.75	5.87±0.72	5.84±0.48	5.01±1.76
		S2	5.64±0.54	5.90±0.48	5.86±0.49	5.02±1.19
		S3	6.02±0.61	5.90±0.49	5.83±0.65	5.00±1.43
		$ST\bar{X}$	5.60 NS	5.89 NS	5.83 NS	5.01 NS
	1.25% Red beet extract	CY	5.60±0.64	5.88±0.59	5.82±0.65	5.02±1.27
		S0	5.52±0.63	5.91±0.56	5.96±0.63	5.03±1.20
		S1	5.63±0.64	5.88±0.50	5.99±0.63	5.02±1.12
		S2	5.60±0.73	5.93±0.65	5.92±0.72	5.04±1.59
		S3	5.71±0.65	5.92±0.63	6.00±0.74	5.02±1.19
		$ST\bar{X}$	5.61 NS	5.90 NS	5.96 *	5.03 NS
	2.5% Red beet extract	CY	5.60±0.65	5.88±0.48	5.82±0.56	5.02±1.83
		S0	5.43±0.71	5.89±0.62	6.02±0.59	5.04±2.04
		S1	5.77±0.73	5.92±0.54	6.05±0.54	5.04±1.98
		S2	5.80±0.64	5.94±0.59	6.09±0.53	5.05±2.01
		S3	5.86±0.69	5.93±0.63	6.14±0.66	5.06±1.88
		$ST\bar{X}$	5.69 *	5.91 NS	6.03 *	5.04 *
	4% Red beet extract	CY	5.60±0.52	5.88±0.61	5.82±0.53	5.02±1.45
		S0	5.60±0.64	5.92±0.65	5.86±0.59	5.02±1.53
		S1	5.65±0.76	5.89±0.64	5.90±0.69	5.03±1.34
		S2	5.57±0.68	5.91±0.57	5.91±0.65	5.04±1.49
		S3	5.69±0.65	5.94±0.71	5.84±0.71	5.05±1.57
		$ST\bar{X}$	5.62 NS	5.91 NS	5.86 *	5.03 NS

CY = Control yogurt, K0 = 0% of strawberry flavor, K1 = 1% of strawberry flavor, K2 = 2% of strawberry flavor, K3 = 4% of strawberry flavor, * = significant at 5% level, NS = Non significant

Physical and chemical properties of experimental yogurt

Plain yoghurt (no flavor and extract added) was compared with yoghurts incorporating different concentrations (1.25%, 2.5% & 4%) of extract of red beet (R1, R2 & R3), and (1%, 2% & 4%) of Strawberry flavor (S1, S2 & S3).

Titratable acidity properties of the experimental yogurts

The lowest mean value of titratable acidity was found at the 1st day of storage, as the highest value was found at the 21st day of storage. In the range of 0.86-0.88%, for the 1st day of storage, sample S3 had the lowest mean value, for the 21st day of storage and the sample CY (control yogurt) had the highest mean value of titratable acidity. Some authors reported similar results [35]. This might be due to the acid production in the experimental yogurts during storage as a result of the fermentation of lactose by the action of the starter cultures [14]. Laye et al., (1993) reported lower titratable acidity values than ours and similar results were reported by Isleten and Karagul-Yuceer (2008) for non-fat yogurt. Titratable acidity of the control and red beet-flavored yogurts increased significantly during the storage period at 4ºC ($P < 0.05$).

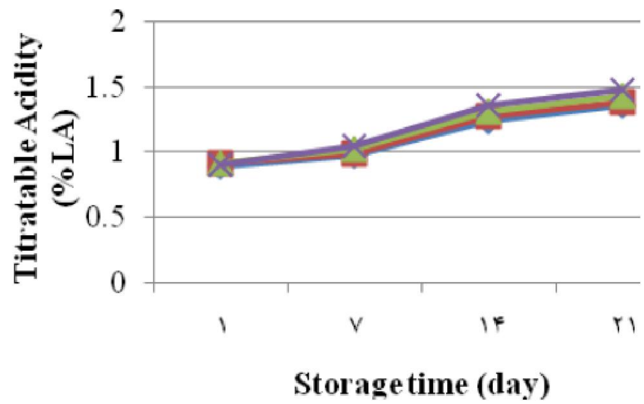

Figure 1 : Effect of red beet extracts on titratable acidity of yogurts during storage at 4ºC for 21 days; ◆: CY control, ■ S1: 1.25%, ▲ S2: 2.5%, × S3: 4% Red beet extract respectively

Some researchers reported that the titratable acidity of fruit-flavored yogurts increased along with storage[24,31]. In the red beet-flavored yogurts containing 4% red beet extract, the rapid increase in titratable acidity continued up to the end of storage (Figure 1). The titratable acidity of red beet-flavored yogurts contained 4 % red beet extract was found more than the yogurt with 1.25% red beet extract and control, while the acidity of the yogurt with 1.25% red beet extract was lower than the control (Figure 1).

pH properties of the experimental yogurts

pH measures free H+ ion whereas the total titratable acidity measure total organic acid that present in yogurt. Both measurements are important because acidification is the key mechanism during yogurt fermentation[1]. The declining of pH during fermentation was due to the protocooperative action of two strain of bacteria *Str.thermophilus* and *Lb.bulgaricus*[1]. The presence milk sugar (carbon source) and milk protein (nitrogen source) in the rich medium of milk and optimum incubation environment (pH 7 and 41ºC) encourage the bacterial strain (*Str.thermophilus*) to grow rapidly. They transform lactose acid into lactic acid, acetaldehyde, diacetyl, and formic acid. The accumulation of all these fermentation products corresponds to the increasing of acid production during fermentation. The liberation of lactic acids reflects the high metabolic activity of the lactic acid bacteria[2]. In general, the pH values of all samples decreased during storage and these differences were found to be significant (P<0.05). This can be explained by further metabolic activities of starter cultures during storage[12].

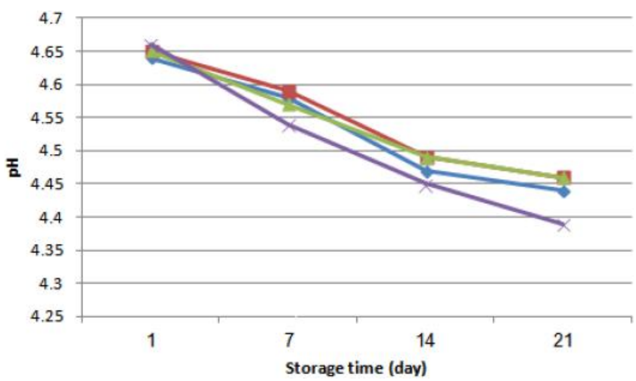

Figure 2 : Effect of red beet extracts on pH of control and red beet yogurts during storage at 4ºC for 21 days: ◆CY: control and, ■S1: 1.25%, ▲S2: 2.5%, and × S3: 4% Red beet extract respectively.

When pH decreased, aroma and acidic taste increased as a result of decreased flavoring characteristics. Similarly, the pH of the control and red beet-flavored yogurts continued to decrease during the storage. Lower concentration of the red beet extract in the yogurt resulted in a faster decrease in the pH, while higher red beet extract concentration caused to increase in the pH. This was probably due to the nature pH of the added ingredients to the yogurts.

Syneresis properties of the yogurts

The syneresis values of yogurts were affected significantly (P<0.05) by both red beet extracts concentration and storage time and the changes were shown in (Figure 3). The highest mean value (46.06 mL/100 g) of syneresis was recorded in sample S3 and the lowest mean value (42.62 mL/100 g) in sample CY (control). As seen in Figure 3, the addition of red beet extract caused a increase of syneresis values in all samples of red beet-flavored yogurts and the differences between the control and these samples were statistically significant (P<0.05). All yogurts with red beet extracts showed a higher syneresis percentage compared to plain yogurt. Yogurt with 4 % (w/w) red beet extract showed the highest syneresis (52.8%). This increasing in syneresis is probably due to decreasing in water holding capacity that led to more releases of whey.

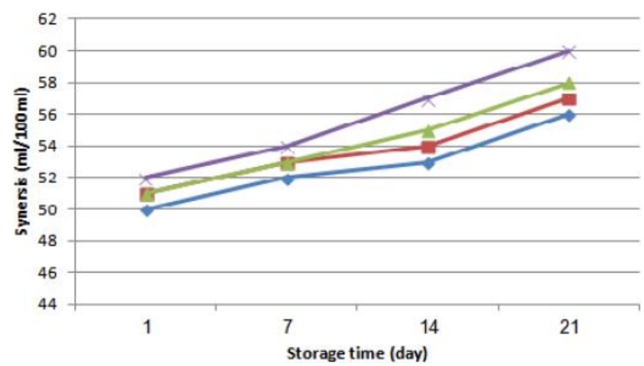

Figure 3 : Syneresis of control and red beet yogurts during storage at 4ºC for 21 days : ◆ CY: control, ■ S1: 1.25%, ▲ S2: 2.5% × S3: 4% Red beet extract respectively.

The introduction of red beet extracts did not increase the fiber contents in yogurt, which otherwise would hold the water and thus increase the syneresis. The watery structure of the extracts themselves may lead to more releases of whey in the red beet-flavor yogurts. The higher syneresis shown in red beet-flavor yogurt was most probably caused by higher active water content contributed by the added extracts.

Viscosity properties of the yogurts

The viscosities of the control and red beet-flavored yogurt increased rapidly up to day 7, and continued to increase slowly up to day 14 of storage and afterwards decreased slowly. Similar viscosity pattern of yogurt

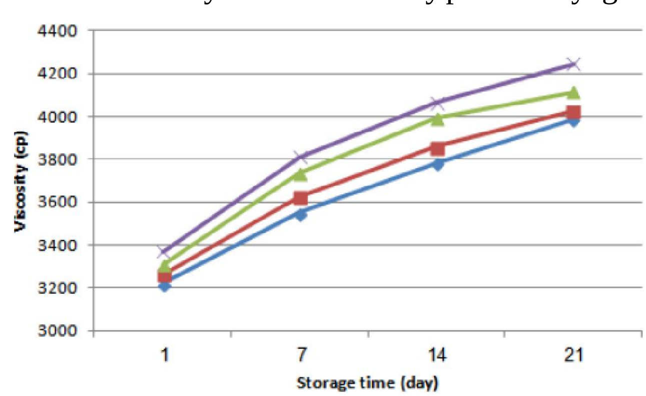

Figure 4 : Viscosity of control and red beet yogurts during storage at 4°C for 21 days: ◆ CY: control and, ■ S1: 1.25%, ▲S2: 2.5% and × S3: 4% Red beet extract respectively.

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during gelation process was reported by Jumah et al (2001). On the other hand, the viscosities of the red beet-flavored yogurts were influenced by the rates of the extracts addition (Figure 4).

The addition of the red beet extracts increased the mean viscosity values of all yogurts, and it was also found to be concentration-dependent ($P<0.05$). All yogurts formulated with red beet extracts showed no significantly higher viscosity values compared to the plain yogurt (TABLE 4). Probably, the addition of red beet extracts reinforced the yogurt micelle matrix and the red beet fiber fragments did not interfere with the fine structure of the yogurt. Yogurt, with the highest red beet extract content (2.5%), showed the highest viscosity values. Lower levels of red beet extracts probably altered the casein micelle matrix structure of the yogurts in such a way as to have contributed to the low viscosity values. Similarly, Otero et al. (2000) observed higher viscosity values when the fiber content was increased in yogurt preparation.

Microbial properties of red beet- flavor yogurts

LAB and yeast and mold counts

The starter culture used in yogurt preparation had a LAB count of $3.2 \pm 0.4 \times 106$ cfu/mL. LAB counts of yogurt containing 1.25, 2.5 and 4% added red beet extract are shown in Figure 5, LAB counts of yogurts, except yogurt containing 4% red beet extract, at day 1 were $3 \pm 0.1 \times 106$ cfu/mL, which was good stated for LAB counts of yogurts. During the 21 days of storage, LAB counts decreased significantly ($P<0.05$). Addition of red beet extract did affect LAB counts. Çon et al. (1996) reported that LAB counts of fruit-flavored yogurts were 8.9 log cfu/g and 8.4 log cfu/g at days 1 and 13, respectively, and Akin and Konar (2001) found

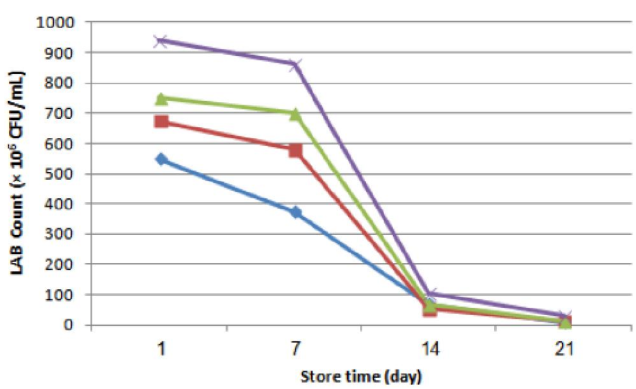

Figure 5

LAB counts for yogurts made from cows' milk varied between 8.3–8.7 log cfu/g at day 1 and 8.5–8.6 log cfu/g at day 15. In comparison to these reports, LAB counts in this study were generally less and decreased significantly more over the 21-day period. The decrease was possibly related to the slightly higher storage temperature and larger pH reduction that was observed (Figure 5).

CONCLUSION

Set-type red beet-flavored yogurt was made by adding red beet extract and flavored with strawberry flavor at different ratios. The effects of the red beet extract addition on the physicochemical, microbiological and organoleptic properties of yogurt were examined. Based on sensory panel evaluations by trained panelists, yogurts with 4% red beet extract and 4% strawberry flavor addition had the highest overall acceptability compared to other yogurts. The titratable acidity, pH, viscosity, syneresis, and organoleptic properties of experimental yogurts were determined at weekly intervals for 21 days. Statistically significant differences were found between the control and red beet-flavored yogurts in terms of viscosity and syneresis. During 21 days of refrigerated storage, however, yogurt pH and LAB counts decreased and titratable acidity increased. The addition of the red beet extract to yogurt resulted in an increase in the syneresis, and a decrease in the viscosity. During the storage, the values of the titratable acidity, viscosity and syneresis of experimental yogurts increased, while pH of the yogurts decreased significantly ($P<0.05$). Yogurt with 2.5% red beet extract and 2% strawberry flavor was more acceptable and high scored with respect to overall acceptability value by panelists. By this research a new type of red beet- flavor yogurt with high iron content was produced, that by use of this vegetable, commercial yogurt could be enriched.

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