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Development and storage stability of conjugated linoleic acid fortified yogurt

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Abstract

The core objective of this research work was to fortify milk with conjugated linoleic acid (CLA) and to develop yogurt. Cow milk was analyzed for proximate analysis, minerals content, fatty acid profile and CLA content. After chemical analysis, milk was fortified with 1, 2 and 3% of CLA and yogurts were made. CLA fortified yogurts of cow milk was analyzed for proximate analysis, titratable acidity, synersis, pH, viscosity, CLA and sensory evaluation. Results showed that the proximate composition significantly changed particular fat. CLA contents also changed significantly. The value of titratable acidity was changed significantly in all treatments whereas the entire samples showed lower value of pH after 14 days storage. All treatments showed higher values of viscosity at 0 day and decreased significantly at 14th day. Yogurt samples showed the lowest syneresis value after 14 days of storage. All the treatments were showed overall acceptability. The sensory evaluation indicated good sensory and high textural quality with the maximum consumer satisfactoriness. The results showed that CLA fortification can meet recommended CLA dietary intake for health benefits.

Keywords: CLA, fatty acids, yogurt, fortification, health.

Practical Application: Dairy products are not fulfilling daily CLA requirement. Cow milk was fortified with different percentages of CLA and used to make a common and highly recommended product (yogurt) to meet recommended CLA dietary intake.

1 Introduction

The intake of dietary fat and fatty acids remains noticeable for researchers in the field of human nutrition. This persistent development in research not only leads to categorize fat in different types such as saturated, unsaturated, monounsaturated, polyunsaturated as well as omega fatty acids. However, the crucial role of relatively tiny as well as defined fatty acid named, Conjugated Linoleic Acid (CLA) is naturally occurring fatty acid abundant in such foods that comes from ruminants (Carafa et al., 2020; Hartigh, 2019). CLA has a variety of isomers primarily *cis-9*, *trans-11* CLA and *trans-10, cis-12* CLA, described as positional and geometrical isomers with conjugated dienes at different locations present in food mainly ruminant products such as milk and meat (Kim et al., 2016; Chin et al., 1992). CLA biosynthesis in ruminants is influenced by a lot of elements like type of diet as well as bacterial and enzymatic action in ruminants. However, CLA isomers are formed at two locations in ruminants such as intestine and rumen respectively. Main CLA isomers *cis-9*, *trans-11* CLA made from polysaccharide called linoleic acid comes from diet, whereas through microbial bio-hydrogenation it is produced by linolenic acids (also a polysaccharide) (Kim et al., 2016; Kepler et al., 1966; Parodi, 1999). In cow's milk, *cis-9, trans-11* CLA is produced through bio-hydrogenation and desaturation pathway. Milk and meat contains natural CLA in different concentrations. For example, CLA present in different ranges in meat and milk such as 0.12 – 0.68 g/100g fat and 0.34 – 1.07 g/100 g fat respectively, (Fritsche et al., 1999, 2000). The ranges of CLA present in milk and meat is not enough to meet human daily requirements (Mir et al., 2004; Zlatanos et al., 2008). In order to fulfill the recommended dietary allowance of CLA of human being, manufacturing and selling of milk and meat products are supplemented, fortified or improvement with essential fatty acids, predominantly CLA has been enhanced considerably since late-1990s owing to its bio functionalities. In this perspective, feeding practices of dairy animals has important role in altering the nutrients concentrations, predominantly the fatty acids arrangement in ruminant's milk, meat as well as their products. There are comparatively very little researches on the bioavailability of CLA from dairy products and more specifically, the bioavailability of CLA from these naturally enriched dairy products requires to be discovered. According to researches, the dairy products and animal meats contains 90% and 75% *c-9, t-11* CLA, respectively, whereas plant oils account for < 50% *c-9, t-11* CLA isomer (Chin et al., 1992). Thus, milk is considered an important nutritious diet for all ages particularly for children. The objectives of the study are to increase the CLA content in dairy, to fortify CLA at least 1% to 3% to produce a fortified yogurt and to evaluate physiological and sensory parameters and CLA content of yogurts. Yogurt is considered a healthy, nutritious and favorable functional food from many centuries which delivers essential nutrients including some important vitamins and minerals. Additionally, yogurt is popular among all ages and recommended in many conditions such as diarrhea, constipation, lactose intolerance, irritable bowel syndrome and all GI tract disorders because of its medicinal properties and

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presence of probiotics (Khaledabad et al., 2020). Probiotics are known as beneficial living organisms that are non-pathogenic in nature and improve the GI tract functioning and activity of gut microbiota as well as it reduces the immune responses. Therefore, probiotics present in yogurt also play an important role in minimizing the cholesterol level in blood and enhance the absorption of calcium (Akpinar et al., 2020). Yogurt is rich in essential vitamins (Vitamin D, A, B_1, B_2, B_6 and B_{12}) and amino acids (methionine, lysine, isoleucine, histidine, valine, tryptophan, phenylalanine, leucine and threonine) which perform their roles in body. The yogurt is an excellent source of energy and fat (97 kcal/100 g and 5.0 g/100 g), respectively (Banerjee et al., 2017).

2 Materials and methods

2.1 Procurement of raw material

Fresh cow's milk and starter culture of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophiles* were collected from the local market of Faisalabad, Pakistan.

2.2 Proximate analysis

Cow milk was evaluated for moisture, crude protein, crude fat, lactose, and total ash as described in American Association of Cereal Chemists (2000).

2.3 Minerals content

Minerals like calcium, magnesium, potassium, iron, sodium, zinc and phosphorus were determined according to Fick et al. (1976) method. Elemental concentrations in the extractants were analyzed using flame photometer.

2.4 Physiochemical characterization of milk

Fatty acid profile

Fatty acid profile of cow milk was examined by following method of Nadeem et al. (2017).

CLA content

Cow milk's CLA content was examined using the procedure of Castro-Gómez et al. (2014).

CLA fortification

Cow milk samples were fortified by different percentages of CLA to total fat using as described in Table 1.

Table 1. Cow milk fortification with different CLA %.

Treatments	Cow milk
T_o (Control)	Whole milk
T_1	Whole milk + 1% CLA
T_2	Whole milk + 2% CLA
T_3	Whole milk + 3% CLA

2.5 Preparation of yogurt

Yogurt samples were prepared using the method as described by Walstra et al. using CLA fortified cow milk (Walstra et al., 2005). All the samples were warmed at 85 °C for about 30 min for pasteurization and then allowed to cool at 42 °C temperature. A mixture of starter cultures of Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophiles (1:1) were added to CLA fortified cow milk samples for yogurt production. Each 450 mL milk samples contain 0.2 U/L starter culture. Nalgene containers of 500 mL were labeled and filled with 450 mL yogurt samples and incubated at temperature 42 °C for final pH of 4.6. Then all the samples were stored in a laboratory refrigerator at 4 °C for further analysis.

2.6 Physiochemical analysis of yogurt

Yogurt samples were stored at temperature 4-6 °C, then analyzed at day 0 and 14 days for titratable acidity, susceptibility to syneresis, pH and viscosity.

Titratable acidity

Titratable acidity was calculated by following Association of Official Analytical Chemists (AOAC) method 947.05. Samples were titrated along with 0.1 N Sodium hydroxide (NaOH) solutions and revealed as percent lactic acid.

Syneresis

Syneresis of yogurt was observed via placing 100 mL of yogurt samples on a funnel having a filter paper. Afterward, the volume of the whey assembled in a tumbler was calculated after drainage of six hours and utilized as an indicator of syneresis.

Syneresis was calculated as following (Equation 1):

$$\text{Syneresis}\left(\%\right)=\frac{Volume\,of\;whey\,collected\,after\,drainage}{Volume\,of\;\;yogurt\,sample}X100 \quad (1)$$

pH

pH of CLA fortified cow milk yogurt was analyzed using electric pH meter (Hanna-pH, 209, Germany).

Viscosity

Viscosity was measured through a Brookfield (LVDVE 230) viscometer followed by Gassem & Frank (1991).

2.7 Sensory analysis

CLA fortified yogurt samples were analyzed for color, smell, appearance, taste, texture and body, thickness (by spoon and mouth), sourness and overall acceptability through a trained board of seven adjudicators. The panel is nominated in accordance with their engrossment and expertise in dairy products organoleptic assessment. It was done individually by judges using nine-point hedonic scale (Costa et al., 2020). All score from 1-9 shows disliking to liking and 5 point was used for neutral. Yogurts were removed from fridge-freezer (4 °C) 1 hour earlier to sensory analysis, placed at room temperature

Stone & Sidel (2004) Three random yogurt samples were offered to panel in transparent and ordor less plastic cups along with a glass of water aiming to rinse mouth between the samples. Cups were coded with three random numbers (Souza et al., 2021).

2.8 Statistical analysis

The findings were articulated as mean ± SEM. Statistical analysis was accomplished with the help of Analysis of Variance test (ANOVA). Statistical variations among different samples were examined through Duncan's Multiple Range Test and level of significance (5%) was used (Steel et al., 1997).

3 Results and discussion

3.1 Proximate analysis

Results showed that cow milk has following moisture, protein, fat, lactose and total ash content (87.01 ± 0.12, 3.19 ± 0.09, 4.03 ± 0.03, 0.47 ± 6.45, 4.25 ± 0.13 and 0.70 ± 0.01 respectively. The findings are similar to Ahmad et al. (2013) and Medhammar et al. (2012). They reported that cow milk is highly nutritive regarding proximate composition (Ahmad et al., 2013; Medhammar et al., 2012).

3.2 Mineral elements

Mineral elements of cow milk such as Potassium (K), Calcium (Ca), Phosphorus (P), Sodium (Na), Magnesium Mg), Zinc (Zn), Iron (Fe) were presented in Figure 1. The following mean were recorded: CA (118.90 ± 3.70 mg/100 g), P (95.05 ± 5.71 mg/100 g), Mg (12.42 ± 2.23mg/100 g), K (146.01 ± 4.50mg/100 g), Na (48.65 ± 3.69 mg/100 g), Fe (0.07 ± 0.01 mg/100 g), and Zn (0.37 ± 0.00 mg/100 g) respectively. According to Rodriguez et al. (2001) the concentrations of some minerals such as Zn and Mg change due to climate and season while the amount of other minerals remain same, there is no effect of season and climate on it.

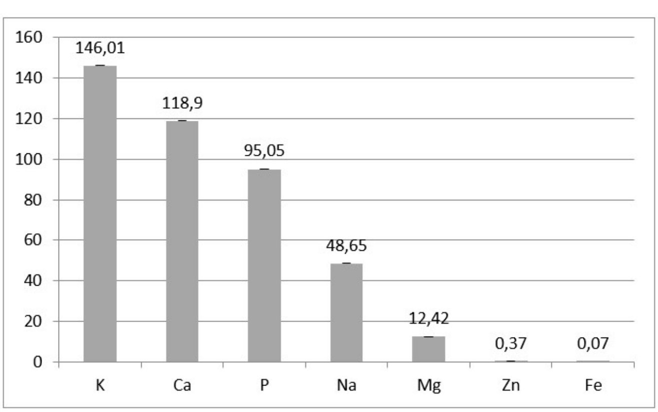

Figure 1. Major mineral elements of cow milk (mg/100g). Results are expressed as Mean ± SD. Potassium (K), Calcium (Ca), Phosphorus (P), Sodium (Na), Magnesium Mg), Zinc (Zn), Iron (Fe). 43. science and technology, (2nd ed). (pp. 808). Boca Raton, FL: CRC Press. 44. Fermented milks. Dairy.

3.3 Fatty acids profile and CLA concentration of cow milk

Cow milk was evaluated for fatty acids composition and has shown in Table 2. Fatty acid content of cow milk fat varied with other ruminant's fat. Following Fatty acid content was recorded in cow milk: C4:0 (3.22% wt), C6:0 (2.05% wt), C8:0 (1.40% wt), C10:0 (3.02% wt), C12:0 (3.66% wt), C14:0 (12.94% wt), C16:0 (32.31% wt), C18:0 (9.10% wt), C18:1(23.03% wt), C18:2 (0.78% wt), C18:3 (0.76% wt), and C20:4 (0.24% wt) respectively as presented in Table 3. The findings are similar to Castro-Gómez et al. (2014), Mulbry et al. (2009), Cescut et al. (2011). They described significant difference in FAME composition. Saturated fatty acids, unsaturated fatty acids, and polyunsaturated fatty acids in cow milk 2.3 ± 0.02% wt, 1.1 ± 0.01% wt and 0.2 ± 0.02% wt were recorded respectively. Ahmad et al. (2013) stated that the difference is because of ruminant's diet, breed and season etc. Cow milk contains 2.4 ± 0.01% wt CLA content as showed in Table 4. CLA has different isomers but most abundant isomer is *cis-9, trans-11* CLA (2.66 ± 0.12 mg/g fat) present in cow milk. Another famous isomer is present in trace amounts (0.07 ± 0.02 mg/g fat) named *trans-10, cis-12* CLA. Kraft et al. (2003) found varying concentrations of CLA with its isomers in cow's milk whose gazing patterns are varied with each other.

Table 2. Proximate composition of cow milk.

Nutrients	g/100 gm
Protein	3.19 ± 0.09
Fat	4.03 ± 0.03
Lactose	4.25 ± 0.13
Ash	0.70 ± 0.01
Moisture	87.01 ± 0.12

Table 3. Fatty acids profile of cow milk (g/100g fat).

Fatty acids in cow's milk		g/100g fat
C4:0 butyric acid		3.22 ± 0.03
C6:0 caproic acid		2.05 ± 0.21
C8:0 caprylic acid		1.40 ± 0.44
C10:0 capric acid		3.02 ± 0.02
C12:0 lauric acid		3.66 ± 0.01
C14:0 myristic acid		12.94 ± 0.03
C16:0 palmitic acid		32.31 ± 0.05
C18:0 stearic acid		9.10 ± 0.43
C18:1 oleic acid		23.03 ± 0.76
C18:2 linoleic acid		0.78 ± 0.02
C18:3 linolenic acid		0.76 ± 0.01
C 18:2 CLA		0.44 ± 0.01
C 18:2 CLA	*cis-9, trans-11* CLA	0.41 ± 0.12
	trans-10, cis-12 CLA	0.03 ± 0.02
C20:4 arachidonic acid		0.24 ± 0.04
SFA		2.3 ± 0.02
MUFA		1.1 ± 0.01
PUFA		0.2 ± 0.02

Saturated fatty acids (SFA), Monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), Conjugated Linoleic Acid (CLA).

3.4 Proximate composition of yogurt

The results of proximate composition of yogurt samples showed in Table 4. Moisture content of all yogurt samples revealed an increase during storage. From the results, the mean values of the crude protein were significantly different between 0 day and 14 days of storage but there is non-significant difference between all the treatments. The decrease in crude protein and lactose content may be due to the presence of starter cultures. The fat content found to be higher in all treatments after 14 days of storage. The higher value in fat may be due to the starter cultures present in yogurts. Starter cultures can make CLA and its isomers (Paszczyk et al., 2020). All the treatments showed non-significant difference regarding proximate composition.

3.5 CLA content of yogurt

CLA content and two CLA isomers (*cis-9, trans-11* CLA and *trans-10, cis-12* CLA) of CLA fortified cow milk yogurt samples are presented in Table 5. Significant increase was noticed in CLA and its isomers of all treatments during storage. Treatment T_3 showed highest value of CLA (2.89 ± 0.06 g/100 g fat), *cis-9, trans-11* CLA (2.81 ± 0.01 g/100 g fat) and *trans-10, cis-12* CLA (0.08 ± 0.02 g/100 g fat) after storage. The increase in CLA content and its isomers is due the presence of starter cultures (bacteria). According to Paszczyk et al. (2020) the content of CLA in cow milk yogurts was higher at 7^{th} day of storage compared to 1 day. They reported that changes in CLA and its isomers in yogurts were produced when it was stored at temperature 5 °C for 14 days.

3.6 Physio-chemical examination of yogurt

Titratable acidity, pH, viscosity and syneresis

Titratable acidity, pH, viscosity and syneresis characteristics of yogurt are presented in Table 6. The significant change in

Table 4. Proximate composition of yogurt.

Treatments		T_o	T_1	T_2	T_3
Moisture	0 day	84.01 ± 0.12^b	85.86 ± 0.175^b	86.65 ± 0.178^b	87.67 ± 0.173^b
	14 day	86.66 ± 0.176^a	88.96 ± 0.135^a	89.75 ± 0.143^a	90.77 ± 0.178^a
Protein	0 day	4.237 ± 0.882^a	4.245 ± 0.881^a	4.196 ± 0.883^a	4.239 ± 0.880^a
	14 day	3.937 ± 0.12^b	3.945 ± 0.14^b	3.896 ± 0.13^b	3.939 ± 0.15^b
Fat	0 day	4.03 ± 0.12^b	4.57 ± 0.14^b	5.57 ± 0.13^b	6.57 ± 0.15^b
	14 day	4.51 ± 0.06^a	5.16 ± 0.05^a	7.24 ± 0.06^a	9.24 ± 0.04^a
Lactose	0 day	3.89 ± 0.53^a	3.88 ± 0.57^a	3.90 ± 0.55^a	3.86 ± 0.56^a
	14 day	3.69 ± 0.01^b	3.68 ± 0.03^b	3.80 ± 0.01^b	3.66 ± 0.02^b
Ash	0 day	0.87 ± 0.01^a	0.84 ± 0.03^a	0.89 ± 0.02^a	0.86 ± 0.03^a
	14 day	0.77 ± 0.12^b	0.74 ± 0.11^b	0.79 ± 0.14^b	0.76 ± 0.13^b

Table 5. CLA content of yogurt (mg/100g).

Treatments		T_o	T_1	T_2	T_3
CLA g/100gfat	0 day	0.44 ± 0.06^b	0.54 ± 0.05^b	1.54 ± 0.02^b	2.54 ± 0.02^b
	14 day	0.48 ± 0.02^a	0.59 ± 0.02^a	1.67 ± 0.04^a	2.89 ± 0.06^a
cis-9, trans-11 CLA g/100gfat	0 day	0.41 ± 0.01^b	0.52 ± 0.02^b	1.49 ± 0.06^b	2.48 ± 0.03^b
	14 day	0.43 ± 0.01^a	0.56 ± 0.02^a	1.60 ± 0.03^a	2.81 ± 0.01^a
trans-10, cis-12 CLA g/100gfat	0 day	0.03 ± 0.03^b	0.02 ± 0.01^b	0.05 ± 0.02^b	0.06 ± 0.02^b
	14 day	0.05 ± 0.12^a	0.03 ± 0.00^a	0.07 ± 0.01^a	0.08 ± 0.02^a

Table 6. Titratable acidity, pH, viscosity and syneresis of yogurt.

Parameter	Storage	**Treatments**			
		T_o	T_1	T_2	T_3
Acidity	Day 0	0.70 ± 0.008^b	0.73 ± 0.005^b	0.75 ± 0.007^b	0.78 ± 0.006^b
	Day 14	0.81 ± 0.007^a	0.84 ± 0.006^a	0.86 ± 0.005^a	0.87 ± 0.008^a
pH	Day 0	5.83 ± 0.03^a	5.81 ± 0.02^a	5.79 ± 0.032^a	5.84 ± 0.02^a
	Day 14	4.81 ± 0.03^b	4.83 ± 0.02^b	4.86 ± 0.032^b	4.89 ± 0.02^b
Viscosity	Day 0	2842 ± 0.03^a	2860 ± 0.02^a	2808 ± 0.02^c	2801 ± 0.01^a
	Day 14	1850 ± 0.03^b	1890 ± 0.02^b	1933 ± 0.02^b	1908 ± 0.01^b
Syneresis	Day 0	9.62 ± 0.02^a	9.59 ± 0.04^a	9.57 ± 0.01^a	9.68 ± 0.03^a
	Day 14	18.22 ± 0.11^b	17.85 ± 0.08^b	18.03 ± 0.21^b	19.01 ± 0.07^b

T_o: control, T_1: cow milk yogurt fortified with 1% CLA, T_2: cow milk yogurt fortified with 2% CLA, T_3: cow milk yogurt fortified with 3% CLA.

Table 7. Sensory evaluation of yogurt.

	Quality Attributes							
Treatments	Color and Appearance	Smell	Taste	Texture and Body	Thickness (spoon)	Thickness (mouth)	Sourness	Overall Acceptability
T_o	8.59 ± 0.35^{d}	6.07 ± 1.80^{b}	7.09 ± 0.09^{d}	7.96 ± 1.69^{a}	8.81 ± 0.42^{c}	8.30 ± 0.16^{c}	5.80 ± 1.81^{a}	7.71 ± 0.44^{c}
T_1	8.71 ± 0.41^{c}	6.10 ± 1.45^{a}	7.11 ± 0.05^{c}	7.81 ± 1.72^{d}	8.79 ± 0.45^{d}	8.26 ± 0.18^{d}	5.79 ± 1.80^{c}	7.75 ± 0.43^{c}
T_2	8.99 ± 0.50^{a}	6.05 ± 1.51^{b}	7.89 ± 0.04^{a}	7.88 ± .81^{c}	8.86 ± 0.51^{b}	8.33 ± 0.17^{b}	5.88 ± 1.79^{b}	7.89 ± 0.49^{b}
T_3	8.80 ± 0.45^{b}	6.11 ± 1.95^{a}	7.66 ± 0.06^{b}	7.90 ± 1.45^{b}	8.83 ± 0.35^{b}	8.40 ± 0.19^{a}	5.65 ± 1.52^{d}	7.90 ± 0.51^{a}

T_o: control, T_1: cow milk yogurt fortified with 1% CLA, T_2: cow milk yogurt fortified with 2% CLA, T_3: cow milk yogurt fortified with 3% CLA.

titratable acidity, pH, viscosity and syneresis was observed during storage of yogurt. The high value of titratable acidity was noticed after 14 days storage in all treatments (0.81 ± 0.007, 0.84 ± 0.006, 0.86 ± 0.005 and 0.87 ± 0.008 respectively,), while all the samples showed the lower value of pH. In a recent research the acidity was lower at 0 day and higher at day 14th which is same to that recounted by (Bhagiel et al., 2015). The difference may be due to storage time. Significant changes were detected for viscosity during storage while treatments mean showed non-significant difference. Results revealed the reduction in viscosity of yogurt at 14 day of storage. It was observed a reduction in yogurt viscosity by increasing the time of storage (Hanif et al., 2012). Aryana et al. (2006) also reported a decrease in yogurt after storage. The decrease in syneresis of yogurt samples (9.62 ± 0.02, 9.59 ± 0.02, 9.57 ± 0.02, 9.68 ± 0.03) was observed during the period of storage in all the treatments (T_o, T_1, T_2 and T_3 respectively,) but between the samples difference is non-significant. Rima et al. (2017) are also observed same trend (Rima et al., 2017). They reported a decrease in syneresis of cow milk yogurt.

3.7 Sensory analysis

Table 7 shows the average scores of different sensory attributes of CLA fortified cow milk yogurt samples. The sensory evaluation showed good sensory and high textural quality along with the highest consumer acceptability of yogurt samples. The results are in accordance with earlier study conducted by Shori & Baba (2012), who reported no difference in sourness, bitterness and overall acceptability between two groups of yogurts. According to them, cow milk yogurt showed greater score in aroma (Shori & Baba, 2012). Yilmaz-Ersan et al. (2017) also notice the same trend (Yilmaz-Ersan et al., 2017).

4 Conclusion

An essential fatty acid such as linoleic acid converted into conjugated linoleic acid which has a variety of isomers primarily *cis-9*, *trans-11* CLA and *trans-10, cis-12* CLA, present in food mainly ruminant products such as milk and meat. Milk and meat are abundant with natural CLA in different concentrations. For example, milk contains 0.34 – 1.07 g/100 g fat CLA and in meat its range is 0.12 – 0.68 g/100 g fat. CLA ranges of milk and meat is not sufficient to meet human daily requirements. In order to meet the recommended dietary allowance of CLA of human being, manufacturing and selling of milk and meat products are supplemented, fortified or improved with CLA has been increased from last decade due to CLA bio-functionalities. Results indicate the significant change in proximate composition particularly fat and CLA contents. Cow milk has been fortified with 1, 2 and 3 percentages of CLA and used to make a common and highly recommended product (yogurt) among individuals of all ages aim to meet recommended CLA dietary intake.

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