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Changes in the chemical and *in-vitro* antihypertensive properties of sweet whey obtained from miniature fresh, Chanco and Gouda-style model cheeses

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Research Article

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Abstract

This Technical Research Communication evaluated the influence of various cheese manufacture methods on the composition and *in vitro* antihypertensive activity of sweet whey samples obtained from miniature models for fresh, Chanco and Gouda-style cheese processing using bulk-tank milks throughout a year. Raw milks from morning milking were standardized, pasteurized and used to obtain sweet whey using cheesemaking protocols for each variety on 200 g scale, as well the use of whey dilution at levels of 0, 15, 30 and 45% in Chanco and Gouda-style making. The composition of sweet whey obtained within each cheesemaking variety was similar among different timepoints of the year ($P > 0.05$), which was attributed to similar composition of milks and the use standardized cheesemaking protocols used for this study. As expected, the use of whey dilution led to sweet whey samples with reduced levels of total solids ($P < 0.05$), but they exhibited an improvement of the *in vitro* antihypertensive properties, which may be attributed to the formation of low-molecular weight bioactive peptides due to increased cheese making times. The results of this study suggest that modifying cheese manufacture protocols may have a direct impact on the bioactive properties of sweet whey. Future work will be required to identify and evaluate the feasibility to purify bioactive peptides obtained from sweet whey.

Worldwide estimations indicate that cheese production has experienced an increase of more than 22% during the period 2005–2014, reaching production levels of more than 21 million tonnes per annum (FAO, 2020). A similar increase in cheese production rate has also been observed in Chile, which is focused for both internal and export markets within Latin America, mainly based on the manufacture of three varieties: fresh, Chanco and Gouda-style (Oliveira and Brito, 2006). Fresh cheese consists of a rennet coagulated variety made from pasteurized milk, with no starter cultures added and high moisture content that is usually consumed within 10 d after manufacture (Guzman and Ilabaca, 2007). Chanco cheese has a semi-soft texture and buttery flavours and is made from rennet coagulation of pasteurized milk, with starter cultures added and ripened for 2 to 6 weeks (Vyhmeister et al., 2019). Latin American countries and the United States produce a block Gouda-style cheese made from pasteurized milk, starter cultures, direct or brined salting and ripening times that ranges from 2 weeks up to 6 months (Oliveira and Brito, 2006; Ibáñez et al., 2020). The application of whey dilution (WD; partial removal of whey during manufacture, along with replacement of that volume with water generally ranging from 15 to 45%) is extensively used in the manufacture of Chanco and Gouda cheeses to control acid development (Vyhmeister et al., 2019; Ibáñez et al., 2020). However, one of the disadvantages of this technique is associated with a considerable increase in the volume of sweet whey (i.e., liquid by-product obtained from cheese manufacture; SW) that will have to be further processed. The generation of SW obtained from the cheese industry in the Chilean market (considering ~4 kg/kg cheese in fresh cheese, and ~9 kg/kg cheese in Chanco and Gouda varieties with no WD; Oliveira and Brito, 2006) exceeds 1.5 million metric tonnes per annum and it is estimated that less than 50% of this volume is processed into dried commodity products for feed and food industry, therefore, potential alternative uses have to be explored. In recent years SW components have shown bioactive properties that are potentially beneficial for human health (Yadav et al., 2015). Different peptide fractions obtained from fresh cheese SW have shown antihypertensive properties when studied under *in vitro* conditions (i.e., inhibitory angiotensin converting enzyme activity; ACE-I; Tarango-Hernández et al., 2015) and may potentially be

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Table 1. Composition and pH values of sweet whey obtained from the manufacture of fresh cheese with no WD, and Chanco and Gouda cheeses with different levels of whey dilution

Item	WD (%)	Period						SEM
		January *Summer*	March *Summer*	May *Autumn*	July *Winter*	September *Spring*	November *Spring*	
Fresh cheese								
TS (g/100 g)	–	6.04^{a}	5.76^{a}	5.93^{a}	6.18^{a}	5.89^{a}	5.80^{a}	0.378
Protein (g/100 g)	–	1.06^{a}	0.88^{ab}	0.73^{b}	0.83^{ab}	0.76^{ab}	0.80^{ab}	0.065
Ash (g/100 g)	–	0.48^{a}	0.47^{a}	0.42^{a}	0.42^{a}	0.47^{a}	0.43^{a}	0.030
Lactic acid (g/100 g)	–	0.03^{a}	0.03^{a}	0.03^{a}	0.03^{a}	0.03^{a}	0.03^{a}	<0.001
pH	–	6.68^{a}	6.67^{a}	6.68^{a}	6.68^{a}	6.71^{a}	6.72^{a}	0.030
Chanco								
TS (g/100 g)	0	6.20^{a,A}	6.22^{a,A}	6.25^{a,A}	6.30^{a,A}	6.20^{a,A}	6.13^{a,A}	0.052
	15	5.51^{a,A}	5.50^{a,B}	5.49^{a,B}	5.47^{a,B}	5.47^{a,B}	5.54^{a,AB}	0.048
	30	4.61^{a,B}	4.64^{a,C}	4.76^{a,C}	4.80^{a,BC}	4.80^{a,BC}	4.92^{a,B}	0.084
	45	4.44^{a,B}	4.23^{a,C}	4.33^{a,C}	4.17^{a,C}	4.14^{a,C}	4.16^{a,C}	0.073
Protein (g/100 g)	0	1.01^{a,A}	0.87^{a,A}	0.85^{a,A}	0.95^{a,A}	0.89^{a,A}	0.87^{a,A}	0.044
	15	0.76^{a,A}	0.80^{a,A}	0.73^{a,A}	0.86^{a,A}	0.74^{a,A}	0.76^{a,A}	0.034
	30	0.63^{a,A}	0.71^{a,A}	0.68^{a,A}	0.70^{a,A}	0.66^{a,A}	0.67^{a,A}	0.042
	45	0.69^{a,A}	0.62^{a,A}	0.61^{a,A}	0.60^{a,A}	0.59^{a,A}	0.70^{a,A}	0.036
Ash (g/100 g)	0	0.57^{a,A}	0.55^{a,A}	0.60^{a,A}	0.57^{a,A}	0.51^{a,A}	0.49^{a,A}	0.016
	15	0.44^{a,AB}	0.50^{a,AB}	0.54^{a,AB}	0.41^{a,B}	0.45^{a,A}	0.44^{a,A}	0.018
	30	0.43^{a,AB}	0.43^{a,AB}	0.41^{a,BC}	0.39^{a,B}	0.43^{a,A}	0.38^{a,A}	0.014
	45	0.41^{a,B}	0.40^{a,B}	0.37^{a,C}	0.41^{a,B}	0.43^{a,A}	0.35^{a,A}	0.014
Lactic acid (g/100 g)	0	0.23^{ab,A}	0.24^{ab,A}	0.20^{b,A}	0.23^{ab,A}	0.28^{a,A}	0.24^{ab,A}	0.008
	15	0.18^{ab,AB}	0.18^{ab,B}	0.17^{ab,A}	0.23^{ab,A}	0.26^{a,AB}	0.19^{ab,AB}	0.009
	30	0.16^{a,B}	0.16^{a,B}	0.17^{a,A}	0.19^{a,A}	0.21^{a,BC}	0.16^{a,B}	0.006
	45	0.16^{a,B}	0.17^{a,B}	0.15^{a,A}	0.19^{a,A}	0.19^{a,C}	0.19^{a,AB}	0.007
pH	0	6.21^{a,B}	6.22^{a,A}	6.23^{a,B}	6.22^{a,B}	6.20^{a,A}	6.22^{a,B}	0.006
	15	6.27^{a,AB}	6.27^{a,A}	6.29^{a,AB}	6.27^{a,AB}	6.22^{a,A}	6.26^{a,B}	0.006
	30	6.33^{a,A}	6.32^{a,A}	6.32^{a,AB}	6.30^{a,AB}	6.23^{a,A}	6.28^{a,B}	0.012
	45	6.34^{ab,A}	6.27^{bc,A}	6.34^{ab,A}	6.34^{ab,A}	6.21^{c,A}	6.40^{a,A}	0.020
Block Gouda								
TS (g/100 g)	0	5.78^{a,A}	5.82^{a,A}	6.32^{a,A}	5.65^{a,A}	6.33^{a,A}	6.02^{a,A}	0.129
	15	5.06^{a,AB}	5.26^{a,AB}	5.40^{a,AB}	5.15^{a,AB}	5.18^{a,B}	5.08^{a,AB}	0.078
	30	4.87^{a,AB}	4.46^{a,BC}	4.89^{a,BC}	4.81^{a,AB}	4.66^{a,B}	4.72^{a,B}	0.118
	45	4.21^{a,B}	3.57^{a,C}	4.27^{a,C}	4.30^{a,B}	4.50^{a,B}	4.33^{a,B}	0.096
Protein (g/100 g)	0	0.88^{a,A}	0.73^{a,A}	0.84^{a,A}	0.67^{a,A}	0.85^{a,A}	0.90^{a,A}	0.047
	15	0.85^{a,A}	0.67^{a,A}	0.80^{a,A}	0.68^{a,A}	0.76^{a,A}	0.70^{a,A}	0.035
	30	0.68^{a,A}	0.55^{a,A}	0.62^{a,A}	0.76^{a,A}	0.63^{a,A}	0.65^{a,A}	0.036
	45	0.54^{a,A}	0.59^{a,A}	0.65^{a,A}	0.65^{a,A}	0.50^{a,A}	0.60^{a,A}	0.043
Ash (g/100 g)	0	0.48^{a,A}	0.47^{a,A}	0.52^{a,A}	0.45^{a,A}	0.48^{a,A}	0.46^{a,A}	0.012
	15	0.41^{a,AB}	0.41^{a,AB}	0.40^{a,AB}	0.39^{a,AB}	0.42^{a,AB}	0.38^{a,AB}	0.011
	30	0.39^{a,AB}	0.33^{a,BC}	0.41^{a,AB}	0.37^{a,AB}	0.34^{a,BC}	0.39^{a,AB}	0.014
	45	0.33^{a,B}	0.27$^{a.B}$	0.36^{a,B}	0.29^{a,B}	0.29^{a,C}	0.33^{a,B}	0.013

(*Continued*)

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Table 1. (*Continued.*)

Item	WD (%)	Period						SEM
		January *Summer*	March *Summer*	May *Autumn*	July *Winter*	September *Spring*	November *Spring*	
Lactic acid (g/100 g)	0	$0.14^{a,A}$	$0.13^{a,A}$	$0.17^{a,A}$	$0.16^{a,A}$	$0.14^{a,A}$	$0.14^{a,A}$	0.021
	15	$0.11^{a,A}$	$0.14^{a,A}$	$0.16^{a,A}$	$0.15^{a,A}$	$0.12^{a,A}$	$0.12^{a,A}$	0.027
	30	$0.11^{a,A}$	$0.13^{a,A}$	$0.17^{a,A}$	$0.14^{a,A}$	$0.11^{a,A}$	$0.13^{a,A}$	0.036
	45	$0.11^{a,A}$	$0.11^{a,A}$	$0.14^{a,A}$	$0.13^{a,A}$	$0.11^{a,A}$	$0.11^{a,A}$	0.024
pH	0	$6.53^{a,A}$	$6.56^{a,A}$	$6.51^{a,A}$	$6.51^{a,A}$	$6.44^{a,A}$	$6.42^{a,A}$	0.132
	15	$6.59^{a,A}$	$6.55^{a,A}$	$6.54^{a,A}$	$6.55^{a,A}$	$6.53^{a,A}$	$6.56^{a,A}$	0.063
	30	$6.61^{a,A}$	$6.58^{a,A}$	$6.52^{a,A}$	$6.56^{a,A}$	$6.58^{a,A}$	$6.54^{a,A}$	0.082
	45	$6.64^{a,A}$	$6.60^{a,A}$	$6.53^{a,A}$	$6.55^{a,A}$	$6.61^{a,A}$	$6.57^{a,A}$	0.075

Values represent mean and standard error of the mean (SEM; $n = 3$).
Levels of total solids (gravimetric method), total protein ($N \times 6.38$), ash (gravimetric method), lactic acid (titratable acidity method) and pH were measured as described by Ibáñez et al. (2019).
^{abc}Means within the same row not sharing a common uppercase superscript differ ($P < 0.05$), comparing the effect of timepoint period.
^{ABC}Means within the same column (for a particular parameter) not sharing a common uppercase superscript differ ($P < 0.05$), comparing the effect of whey dilution at a single treatment.

used as ingredients. We hypothesize that modifications to cheese making conditions, such as the application of WD, lead to changes in the composition of SW affecting their *in vitro* antihypertensive properties. Therefore, the objective of this study was to characterize the composition and ACE-I properties of SW obtained from model cheese manufacture methods using bulk tank milk from a dairy farm at different timepoints of a year.

Materials and methods

This study was conducted at the experimental dairy unit from the Pontifical Catholic University of Chile (Pirque, Chile) from January to November 2018. The cattle were comprised by approximately 240 Holstein cows in milk, producing in average 35 l/d/cow. Forty litres of raw milk were obtained directly from the bulk-tank at 4°C, 2 h after morning milking (5:00 AM) at six different timepoints: January, March (summer), May (autumn), July (winter), September and November (spring), 2018. Details of cattle in terms of number of lactating cows, lactation and climate conditions of location of study are shown in Supplementary Table S1. Milk samples were transported to the Department of Animal Science (Pontifical Catholic University of Chile) for further analyses and processing. Milks were standardized to a fat content of 3.5 g/100 g, transferred into individual 200 ml containers, pasteurized at 65°C × 30 min, cooled down and stored overnight at 4°C. The following morning, milk samples were heated at 31°C and used to make model cheeses on a miniature scale (200 g), according to the protocol described by Shakeel-Ur-Rehman et al. (1998) with their respective modifications for fresh (Guzman and Ilabaca, 2007), Chanco (Vyhmeister et al., 2019) and Gouda-style varieties (Ibáñez et al., 2020). Details of processing conditions are included in Supplementary Table S2, in which Chanco and Gouda-style varieties were also manufactured using WD at levels 0, 15, 30 or 45%.

Milk and SW obtained from cheesemaking were analysed for chemical composition [total solids (TS; gravimetric method), fat (Gerber method), total protein ($N \times 6.38$; Kjeldahl method), ash (gravimetric method) and lactose (weight difference method)], titratable acidity, pH and protein profile by reversed phased-high performance liquid chromatography (RP-HPLC), as previously described (Ibáñez et al., 2019). In addition, the *in vitro* ACE-I activity of SW samples was analysed according the spectrophotometric method described by Lu et al. (2016). Each cheesemaking process was made in triplicate within 2 weeks for each experimental period. Since SW from fresh cheese was obtained with no WD, one-way analysis of variance (ANOVA) was performed to evaluate the effect of period on the chemical properties ($P < 0.05$). In contrast, SW from Chanco and Gouda-style cheese making were studied based on a 6 × 4 full factorial design using a general linear model to evaluate effects of period (January, March, May, July, September and November), levels of WD (0, 15, 30 and 45%) and their interactions on the composition and ACE-I properties. When significant differences were found ($P < 0.05$), means were analysed by Tukey's multiple comparison test. All analyses were performed using Minitab® 19 (Minitab Inc., State College, PA, USA).

Results

The standardized milks used to make model cheeses had no significant differences ($P > 0.05$) on their composition at different timepoints (Supplementary Table S3), including the protein-to-fat and lactose-to-protein ratios. The composition, pH and titratable acidity of model SW samples obtained from various cheese making protocols is presented in Table 1 and showed a great variability among varieties. Levels of TS in SW obtained from fresh cheese were in the range of 5.8–6.2 g/100 g, whereas those levels in treatments obtained from Chanco and Gouda-style cheese making (with no WD) were among 6.1–6.3 and 5.7–6.3 g/100 g, respectively. An increase in the level of WD during cheese manufacture led to SW with decreased levels of total solids, ash, titratable acidity and, as expected, increased pH values ($P < 0.05$). In contrast, the content of total protein remained constant when level of WD increased. This is also in accordance with the content of major whey proteins analysed by RP-HPLC (Supplementary Table S4), in which β-lactoglobulin (β -lac), α-lactalbumin (α-lac) and bovine serum albumin (BSA) accounted for ~50% of total proteins. Nevertheless, chromatograms from protein analysis of SW (Supplementary Fig. S1) also showed the presence of

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other unidentified peaks. Independently of the cheese variety and/or level of WD, the composition of SW was not greatly affected when obtained from different times of the year ($P > 0.05$).

Experimental SW exhibited an *in-vitro* capacity on inhibiting ACE among 55 and 95% (results not shown). To reduce the variability of ACE-I values in SW samples, these results were expressed as the concentration of protein required to inhibit the ACE to 50% their original activity (i.e., IC_{50} values; Fig. 1). SW from fresh, Chanco and Gouda-style cheeses showed no significant differences ($P > 0.05$) in the IC_{50} values throughout the year. The IC_{50} values found in SW from fresh cheese (Fig. 1a) ranged among 4.8 and 6.3 mg/ml. In contrast, increasing levels of WD during cheese manufacture (from 0 to 45%) led to SW with a significant ($P < 0.05$) reduction of IC_{50} values (i.e., improvement of ACE-I activity) from 5.0–5.8 to 3.1–4.3 mg/ml (Chanco; Fig. 1b) and 4.6–5.2 to 3.1–3.8 mg/ml (Gouda-style; Fig. 1c).

Discussion

Similarities in the composition of model SW samples obtained at different timepoints of the year could be mainly attributed to similar composition of standardized milks (Supplementary Table S3), as well as the application of standard cheesemaking protocols based on milk composition and rate of acidification (Supplementary Table S2). Lactation stage greatly affects bovine milk composition, such as a reduced protein and fat content, along with increased lactose content in mid lactation milks, as compared with milk obtained from late lactation (Hinz et al., 2012). However, the cattle used for our study did not show a marked trend on a particular lactation stage (Supplementary Table S1), maintaining similar lactose-to-protein ratio throughout the year (Supplementary Table S3), along with standardization of fat content. In a standardized cheese making process, the addition of calcium chloride and rennet are based on the concentration of protein, along with controlling acid development (i.e., changes of pH) at critical steps during processing (Vyhmeister et al., 2019; Ibáñez et al., 2020), which reduce variability of cheese and SW composition. The use of WD aims to reduce acid development in cheeses (Ibáñez et al., 2020), including those varieties produced and consumed in Latin America (Chanco and Gouda-style; Oliveira and Brito, 2006). As expected, increasing levels of WD (due to the addition of water to levels of up to 45% of the mixture curd-whey and/or the application of a second dilution performed during the manufacture of Gouda cheeses; Supplementary Table S2) removes more lactose (and/or lactic acid) from the curd, but also leads to SW with reduced levels of TS. However, one of the major disadvantages associated with increased levels of WD, from a sustainability point of view, is the use of more energy required to remove water (Ibáñez et al., 2020). Levels of major whey proteins found in SW samples are in accordance with those reported by the literature, since levels of β-lac, α-lac and BSA account for 45–55% of total protein fractions from SW (Yadav et al., 2015). The presence of other unidentified peak fractions in RP-HPLC chromatograms (Supplementary Fig. S1) could be associated with other minor proteins, as well as peptides released during cheesemaking, such as the glycomacropeptide (GMP) fraction derived from κ-casein that accounts up to 20% of total protein fraction from SW (Yadav et al., 2015).

The ACE-I properties of SW samples obtained from various cheese varieties using different levels of WD are in accordance with Miguel et al. (2009), who found that the IC_{50} values from unhydrolysed milk proteins were ≥ 1 mg/ml and, when hydrolysed,

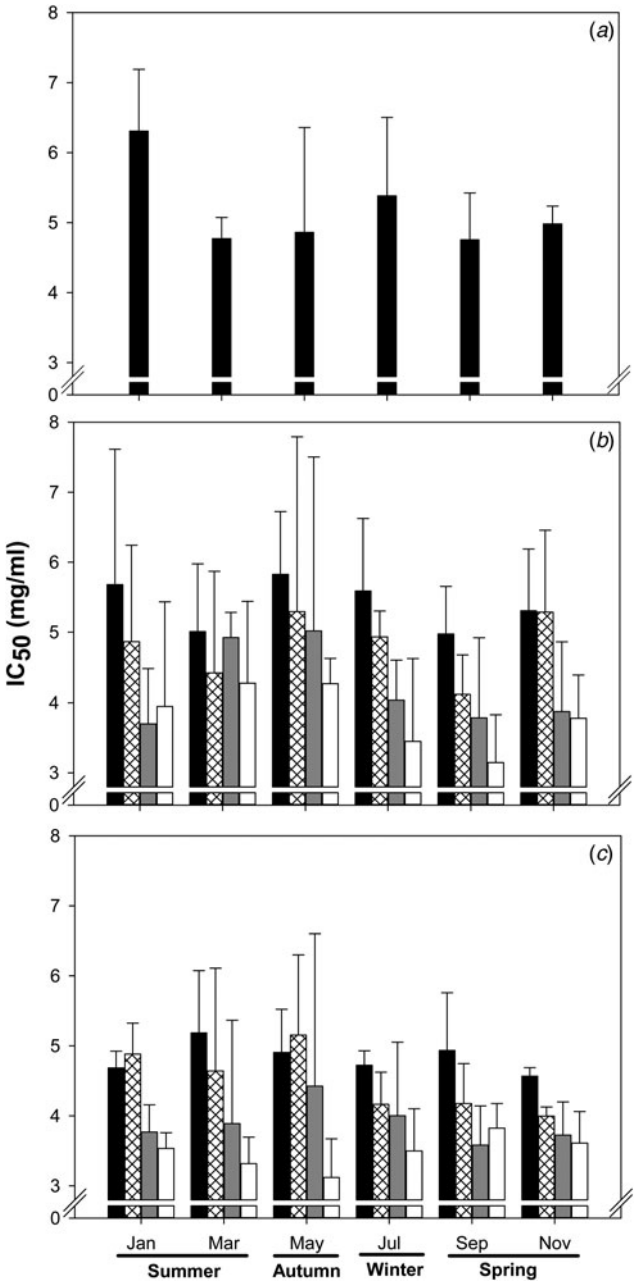

Fig. 1. Experimental IC_{50} values (i.e., concentration required to inhibit ACE to 50% of its original activity) of sweet whey obtained from the manufacture of fresh (a), Chanco (b) and Gouda-style cheeses (c) using 0 (■), 15 (//), 30 (▩) and 45% (□) of whey dilution and obtained at different timepoints. Values represent mean and standard deviation ($n = 3$).

they could be significantly improved. Tarango-Hernández et al. (2015) found that low-molecular fractions (<5 kDa) obtained from different fresh-style cheeses SW exhibited the highest ACE-I activities when compared to other major fractions and were probably attributed to the presence of sequences peptides generated during cheese manufacture. In our study, we believe that an increase of ACE-I activity (i.e., reduced IC_{50} values) in Chanco and Gouda-style SW samples using higher proportions of WD could be associated with an increase in the production of low molecular weight water-soluble ACE-I casein-derived peptides, which is probably explained due to the capacity of chymosin for hydrolysing

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other individual caseins (α_s-, β-), the activity of starter cultures enzymes (Uniacke-Lowe and Fox, 2017) and extended cheese making times required to acidify the whey/curd mixture before whey drainage, due to the addition of water for dilution, which may have allowed more enzymatic hydrolysis.

No major changes in the composition of model SW samples were found when milks supplied at different times of the year had similar composition, in addition with the application of standardized cheese making protocols. However, modification of cheese manufacture protocols (such as the application of various WD levels) may lead to differences in the composition and ACE-I properties of model SW. One of the many applications of SW could be associated with the purification of low-molecular compounds with bioactive properties generated from cheese manufacture that could be potentially used as ingredients. However, future work will be required to evaluate its feasibility.

In conclusion, the use of whey dilution led to sweet whey samples with reduced levels of total solids but improved *in vitro* antihypertensive properties, which may be attributed to the formation of low-molecular weight bioactive peptides due to increased cheese making times. Further work will be required to identify and evaluate the feasibility to purify bioactive peptides obtained from sweet whey.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029920001041.

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