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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SUPPLEMENTARY FILE

Table S1 Dates of milking, composition of herd and climate conditions of location[1] at different sampling periods

Item	January *Summer*	March *Summer*	May *Autumn*	July *Winter*	September *Spring*	November *Spring*
Cattle details[2]						
Days of sampling	22, 25, 30	12, 15, 19	7, 10, 14	2, 6, 11	24, 27, 30	21, 26, 29
Number of lactating cows	224	233	227	223	215	236
Proportion of cows from spring-calving[3] (%)	37.9	36.9	37.9	37.2	28.4	34.0
Proportion of cows from summer-calving[4] (%)	13.1	15.0	15.4	15.7	16.3	13.8
Proportion of cows from autumn-calving[5] (%)	25.1	24.9	28.1	30.9	32.1	29.3
Proportion of cows from winter-calving[6] (%)	23.9	23.2	18.5	16.3	23.1	22.9
Climate conditions[7]						
High temperature (ºC)	29.1	26.9	18.5	15.3	18.6	25.2
Low temperature (ºC)	10.4	8.2	3.3	1.4	4.1	7.7
Precipitation (mm)	1	6	71	95	37	8
Relative humidity (%)	60	65	74	75	73	62

[1] Pirque, Chile (33º38'28" S, 70º34'27" W)
[2] Animals were fed with design diets (67% forage and 33% concentrate) to meet nutritional requirements at their appropriate lactation time (NRC 2001).
[3] Months of September, October and November.
[4] Months of December, January and February.
[5] Months of March, April and May.
[6] Months of June, July and August.
[7] Data from Santibanez (2017).

Table S2 Cheese manufacture protocol applied to obtain sweet whey samples from fresh, Chanco and Gouda cheeses on a miniature model scale[1].

Step	Fresh cheese[2]	Chanco cheese[3]	Gouda-style cheese[4]
Initial milk temperature	31 ºC in all treatments.		
Addition of starter	None.	CHN-22 (Chr. Hansen, Milwaukee, WI, USA) at a rate of 0.25 g/kg. Ripening time of 30 min.	CHN-19 (Chr. Hansen, Milwaukee, WI, USA) at a rate of 0.26 g/kg and adjunct of Lh-B02 (Chr. Hansen, Milwaukee, WI, USA) at a rate of 0.024 g/kg. Ripening time of 30 min.
Addition of $CaCl_2$	0.26 g/kg milk (77 g/100 g purity; Dilaco Ltda., Santiago, Chile), based on milks containing 3.2 g/100 g protein. 3 min of equilibration under continuous agitation.		
Addition of coagulant	0.1 ml/kg milk (Chy-Max® Ultra, 1000 International Milk Clotting Units [IMCU]/ml, Chr. Hansen, Milwaukee, WI, USA), based on milks containing 3.2 g/100 g protein.		
Cutting the gel	45 min after coagulant addition. Horizontal and vertical cuts of 0.5 cm. No healing time.	45 min after coagulant addition. Horizontal and vertical cuts of 0.5 cm. Healing time 3 min.	50 min after coagulant addition. Horizontal and vertical cuts of 0.5 cm. Healing time 15 min.

(Continued)

Table S2 (Continued)

Step	Fresh cheese[2]	Chanco cheese[3]	Gouda-style cheese[4]
Agitation	10 min of gentle agitation, followed by 15 min of regular agitation.	10 min of gentle agitation.	10 min of gentle agitation.
Partial draining whey (1)	None.	At levels of 0, 15, 30 or 45% of curd/whey mixture. Removed whey fractions were cooled in ice bath.	At levels of 0, 15, 30 or 45% of curd/whey mixture. Removed whey fractions were cooled in ice bath.
Water addition (1)	None.	Addition of deionized water at 31 ºC at same level of removed whey.	Addition of deionized water at 31 ºC at same level of removed whey.
Cooking (1)	None.	From 31 to 38 ºC at a heating rate of 1 ºC/3 min. Temperature was maintained at cooking temperature until pH decreased to 6.2.	From 31 to 38 ºC at a heating rate of 1 ºC/3 min. Temperature was maintained at cooking temperature for 20 min.
Partial draining whey (2)	None.	None.	At a level of 30% of curd/whey mixture. Removed whey fractions were cooled in ice bath.

(Continued)

Table S2 (Continued)

Step	Fresh cheese[2]	Chanco cheese[3]	Gouda-style cheese[4]
Water addition (2)	None.	None.	Addition of deionized water at 38 ºC at a level of 20% of initial curd/whey mixture.
Cooking (2)	None.	None.	Temperature was maintained at 38 ºC until pH decreased to 6.1.
Total draining whey	Centrifugation of samples at 1700 *g* × 20 min at 25 ºC. Removed whey fractions (i.e., supernatants) were cooled in ice bath for 30 min.		
Mixing of sweet whey fractions	None.	Mixing of partial drained whey (1) + total drained whey.	Mixing of partial drained whey (1) + partial drained whey (2) + total drained whey.
Removal of fat and suspended particles	Sweet whey samples were centrifuged at 3000 g × 30 min at 4 ºC and then filtered through Whatman® 1 filter paper.		
Pasteurisation and storage of samples	65 ºC × 30 min, cooled to 4 ºC and stored overnight for further analyses.		

[1] Shakeel-Ur-Rehman *et al*. (1998).
[2] Adapted from Guzman & Ilabaca (2007).
[3] Adapted from Vyhmeister *et al.* (2019).
[4] Adapted from Ibáñez *et al.* (2020).

Table S3 Composition of standardised milks[1] obtained from different periods and used for the manufacture of miniature cheese models.

Item	Period						SEM
	January	**March**	**May**	**July**	**September**	**November**	
	Summer	*Summer*	*Autumn*	*Winter*	*Spring*	*Spring*	
TS (g/100 g)	11.84[a]	11.83[a]	11.80[a]	11.87[a]	11.85[a]	11.76[a]	0.017
Fat (g/100 g)	3.53[a]	3.50[a]	3.48[a]	3.47[a]	3.43[a]	3.55[a]	0.018
Protein (g/100 g)	3.09[a]	3.17[a]	3.16[a]	3.16[a]	3.40[a]	3.15[a]	0.032
P:F[2]	0.88[a]	0.91[a]	0.90[a]	0.90[a]	0.97[a]	0.90[a]	0.009
Lactose (g/100 g)	4.59[a]	4.56[a]	4.48[a]	4.54[a]	4.34[a]	4.53[a]	0.039
L:P[3]	1.49[a]	1.44[a]	1.43[a]	1.45[a]	1.28[a]	1.45[a]	0.024
Ash (g/100 g)	0.67[a]	0.61[a]	0.67[a]	0.67[a]	0.61[a]	0.58[a]	0.025

[abc] Means within the same row not sharing a common superscript differ ($P < 0.05$), as compared by Tukey multiple comparison test.

[1] Levels of total solids (gravimetric method), fat (Gerber method), total protein (N × 6.38) and ash (gravimetric method) were measured as described by Ibáñez et al. (2019); whereas lactose content was estimated by the difference obtained between levels of total solids minus protein, fat and ash.

[2] Protein-to-fat ratio.

[3] Lactose-to-protein ratio.

Values represent mean and standard error of the mean (SEM; n = 3).

Table S4 Concentration of major whey proteins[1] from sweet whey obtained from the manufacture of fresh, Chanco and block Gouda cheeses with varying levels of whey dilution and obtained at different seasons.

Item	WD	Period						SEM
		January	March	May	July	September	November	
	(%)	*Summer*	*Summer*	*Autumn*	*Winter*	*Spring*	*Spring*	
Fresh								
β-lac (mg/mL)	-	2.60^{a}	3.08^{a}	3.16^{a}	3.04^{a}	2.84^{a}	2.96^{a}	0.106
α-lac (mg/mL)	-	1.04^{a}	0.89^{a}	0.98^{a}	1.02^{a}	0.96^{a}	0.89^{a}	0.054
BSA (mg/mL)	-	0.34^{a}	0.30^{a}	0.27^{a}	0.33^{a}	0.33^{a}	0.32^{a}	0.017
Chanco								
β-lac (mg/mL)	0	$2.65^{a,A}$	$2.70^{a,A}$	$3.41^{a,A}$	$2.97^{a,A}$	$2.84^{a,A}$	$2.81^{a,A}$	0.087
	15	$2.80^{a,A}$	$2.21^{a,A}$	$2.55^{a,AB}$	$2.50^{a,A}$	$2.74^{a,A}$	$2.68^{a,A}$	0.113
	30	$2.33^{a,A}$	$2.67^{a,A}$	$2.53^{a,AB}$	$2.27^{a,A}$	$2.93^{a,A}$	$2.49^{a,A}$	0.084
	45	$2.10^{a,A}$	$2.58^{a,A}$	$2.28^{a,B}$	$2.55^{a,A}$	$2.32^{a,A}$	$2.50^{a,A}$	0.114
α-lac (mg/mL)	0	$0.85^{a,A}$	$0.99^{a,A}$	$0.98^{a,A}$	$0.90^{a,A}$	$0.99^{a,A}$	$0.95^{a,A}$	0.038
	15	$0.89^{a,A}$	$0.88^{a,A}$	$0.71^{a,A}$	$0.82^{a,A}$	$0.98^{a,A}$	$0.93^{a,A}$	0.046
	30	$0.78^{a,A}$	$0.63^{a,A}$	$0.73^{a,A}$	$0.70^{a,A}$	$0.81^{a,A}$	$0.77^{a,A}$	0.027
	45	$0.91^{a,A}$	$0.73^{a,A}$	$0.68^{a,A}$	$0.75^{a,A}$	$0.70^{a,A}$	$0.73^{a,A}$	0.048
BSA (mg/mL)	0	$0.30^{a,A}$	$0.25^{a,A}$	$0.31^{a,A}$	$0.34^{a,A}$	$0.31^{a,A}$	$0.31^{a,A}$	0.019
	15	$0.27^{a,A}$	$0.24^{a,A}$	$0.31^{a,A}$	$0.32^{a,A}$	$0.24^{a,A}$	$0.32^{a,A}$	0.013
	30	$0.27^{a,A}$	$0.21^{a,A}$	$0.25^{a,A}$	$0.32^{a,A}$	$0.23^{a,A}$	$0.23^{a,AB}$	0.019
	45	$0.23^{ab,A}$	$0.27^{ab,A}$	$0.28^{ab,A}$	$0.32^{a,A}$	$0.15^{ab,A}$	$0.13^{b,B}$	0.022
Block Gouda								
β-lac (mg/mL)	0	$2.80^{a,A}$	$2.81^{a,A}$	$2.73^{a,A}$	$2.87^{a,A}$	$2.75^{a,A}$	$2.69^{a,A}$	0.041
	15	$2.57^{a,AB}$	$2.60^{a,A}$	$2.60^{a,A}$	$2.67^{a,A}$	$2.59^{a,A}$	$2.68^{a,A}$	0.033
	30	$2.24^{a,AB}$	$2.23^{a,AB}$	$2.44^{a,A}$	$2.28^{a,A}$	$2.36^{a,A}$	$2.29^{a,A}$	0.039
	45	$1.77^{a,B}$	$1.66^{a,B}$	$2.03^{a,A}$	$2.14^{a,A}$	$2.42^{a,A}$	$2.04^{a,A}$	0.152
α-lac (mg/mL)	0	$0.87^{a,A}$	$0.82^{a,A}$	$0.75^{a,A}$	$0.87^{a,A}$	$0.83^{a,A}$	$0.84^{a,A}$	0.042
	15	$0.66^{a,A}$	$0.73^{a,A}$	$0.64^{a,A}$	$0.86^{a,A}$	$0.69^{a,A}$	$0.74^{a,A}$	0.039
	30	$0.54^{a,A}$	$0.73^{a,A}$	$0.68^{a,A}$	$0.74^{a,A}$	$0.58^{a,A}$	$0.65^{a,A}$	0.036
	45	$0.59^{a,A}$	$0.69^{a,A}$	$0.79^{a,A}$	$0.69^{a,A}$	$0.61^{a,A}$	$0.64^{a,A}$	0.035
BSA (mg/mL)	0	$0.27^{a,A}$	$0.31^{a,A}$	$0.30^{a,A}$	$0.34^{a,A}$	$0.30^{a,A}$	$0.32^{a,A}$	0.019
	15	$0.27^{a,A}$	$0.25^{a,A}$	$0.25^{a,A}$	$0.28^{a,A}$	$0.26^{a,A}$	$0.26^{a,A}$	0.013
	30	$0.23^{a,A}$	$0.20^{a,A}$	$0.21^{a,A}$	$0.32^{a,A}$	$0.23^{a,A}$	$0.17^{a,A}$	0.017
	45	$0.23^{a,A}$	$0.20^{a,A}$	$0.22^{a,A}$	$0.25^{a,A}$	$0.22^{a,A}$	$0.22^{a,A}$	0.013

[1] Quantification was performed by reversed-phase high performance liquid chromatography (RP-HPLC), using standards of β-lactoglobulin (β-lac), α-lactoalbumin (α-lac) and bovine serum albumin (BSA), according to the method 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 sampling periods.

[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.

Values represent mean and standard error of the mean (SEM; n = 3).

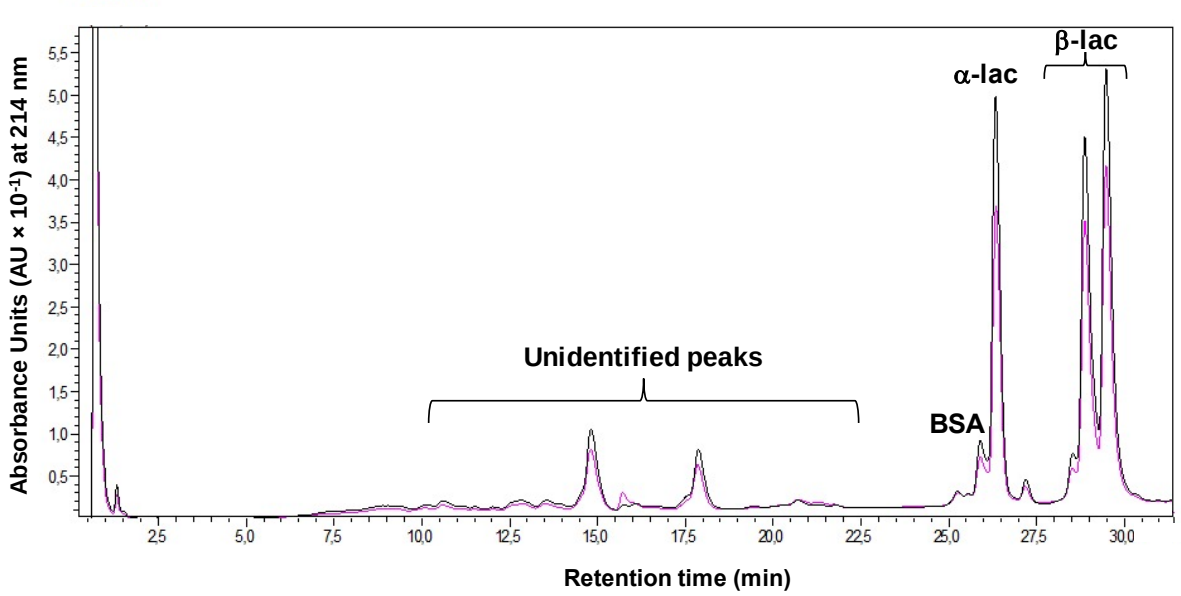

Fig. S1 Reference milk protein profile chromatogram obtained from sweet whey samples made from Chanco cheeses using 0 (black line) and 45% (magenta line) of whey dilution.

References

Guzman V & Ilabaca C (2007). Utilizacion de leche de vaca, cabra y oveja en la pequena empresa [Use of cow, goat and sheep milk in small dairy industry], Santiago, Chile: Fundacion para la Innovacion Agraria, Chile.

Ibáñez RA, Govindasamy-Lucey S, Jaeggi JJ, Johnson ME, McSweeney, PLH, & Lucey JA (2020). Low and reduced fat milled curd, direct-salted Gouda cheese: Comparison of lactose standardization of cheese milk and whey dilution techniques. *Journal of Dairy Science*, **103**, 1175–1192.

Ibáñez RA, Vyhmeister S, Muñoz MF, … Vargas-Bello-Pérez E (2019). Influence of milk pH on the chemical, physical and sensory properties of a milk-based alcoholic beverage. *Journal of Dairy Research*, **86**, 248–251.

NRC (2001). *Nutrient requirements of dairy cattle*, 7th edn, Washington DC, United States: National Academy Press.

Santibanez F (2017). *Atlas agroclimático de Chile - Vol. III* [Agro climatic atlas of Chile], Santiago, Chile: University of Chile - Center for Agriculture and Environment.

Shakeel-Ur-Rehman, McSweeney PLH & Fox PF (1998). Protocol for the manufacture of miniature cheeses. *Lait*, **78**, 607–620.

Vyhmeister S, Geldsetzer-Mendoza C, Medel-Marabolí M, Fellenberg A, Vargas-Bello-Pérez E & Ibáñez RA (2019). Influence of using different proportions of cow and goat milk on the chemical, textural and sensory properties of Chanco–style cheese with equal composition. *LWT*, **112**, 108226.