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Thermosonicated whey protein concentrate blends on quality attributes of reduced fat Panela cheese

Genaro G. Amador-Espejo [a], Irving I. Ruiz-Lopez [b], Paola J. Gibbens-Bandala [b], Raúl J. Delgado-Macuil [c], Hector Ruiz-Espinosa [b,*]

[a] CONACYT-Centro de Investigación en Biotecnología Aplicada, Instituto Politécnico Nacional, Ex-Hacienda San Juan Molino, Carretera Estatal Tecuexcomac-Tepetitla Km 1.5, Tlaxcala 90700, Mexico
[b] Facultad de Ingeniería Química, Benemérita Universidad Autónoma de Puebla, Av. San Claudio y 18 Sur, Ciudad Universitaria, Puebla 72570, Mexico
[c] Centro de Investigación en Biotecnología Aplicada, Instituto Politécnico Nacional, Ex-Hacienda San Juan Molino, Carretera Estatal Tecuexcomac-Tepetitla Km 1.5, Tlaxcala 9070, Mexico

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ABSTRACT

Aiming at producing a reduced fat cheese (RFC) as an alternative to full-fat Panela cheese, a highly consumed fresh Mexican dairy product, thermosonication (TS) processes (24 kHz, 400 W nominal power, 2, 4 and 6 min; 50, 55 and 60 °C) were evaluated to treat WPC (80% protein) blended with reduced-fat milk (1 and 2% fat), which were later LTLT pasteurized. TS blends were compared in terms of their technological properties (water holding capacity-WPC, gel firmness- GF, color, pH and titratable acidity) with those of a regular full fat (3%) LTLT pasteurized milk used as a control. Afterwards, a regression analysis was carried out with the obtained data in order to select the most appropriate conditions for cheesemaking purposes (similar GF, higher WHC with respect to the control), minimize both fat content and TS treatment duration to minimize energy expenses. According to these restrictions, the selected conditions were 1.5% fat milk-WPC blend, TS treated at 60 °C for 120 s; 1% fat milk-WPC blend, TS treated at 50 °C for 120 s and 1% fat milk-WPC blend, 50 °C for 144 s, which allowed preparing low fat cheeses (LFCs). These TS treatments were applied in a larger scale to elaborate Panela-type LFCs comparing different technological properties (cheese yield, syneresis, water content, texture profile analysis, color and titratable acidity) with those of a full fat variety, at day 1 and during 14 days of refrigerated storage. Results showed similar texture profiles of LFC cheeses and full fat milk cheeses throughout their storage period with significant changes in composition parameters (higher moisture, protein and salt contents, with low fat percentages), syneresis, selected color parameters (hue, b*), with no observed changes in cheese yield, TA and pH during cheese storage. These promising results are encouraging to develop LFCs with no physicochemical or technological defects using novel processing techniques that may help reducing calorie consumption without compromising sensory acceptability.

1. Introduction

A worldwide pandemic linked with several human health complications, overweight and obesity are a major concern for health officials, medical experts, scientists, and food developers alike [1]. Recent evidence indicates that 20% and 40% of world's population could respectively be obese and overweight by 2030 if proper measures are not taken [2]. From the nutritional standpoint, these conditions have been partially associated with a high-fat diet; thus, aimed at health-conscious consumers, the food industry has been developing products with low and reduced fat contents as alternatives to several vastly consumed products such as cheese. Cheese is one of the staples of the human diet and a concentrated source of protein, fat, calcium and other key nutrients; however, as certain consumers are seeking for what they perceive as healthier options, reduced fat cheeses (RFC) have become popular, with a global market size valued at 93.9 billion USD in 2018 and expected to growth 3.8% annually from 2019 to 2025 [3].

Any cheese with less fat than their full-fat counterpart is considered a reduced-fat product although no global criteria has been established to grade them as either RFC or low-fat cheeses (LFC) and a classification

* Corresponding author.
E-mail address: hector.ruiz@correo.buap.mx (H. Ruiz-Espinosa).

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needs to be made for each individual variety [4]. According to Khanal & Bansal [5] fat in RFC is reduced at least in 25% compared to the regular cheese while LFC possess 5–6% fat. Cheese structure and functionality and their related sensory features could be compromised in RFC and LFC as fat plays a key role in the development of cheese quality characteristics. Texture-wise, fat acts as a plasticizer, providing a smooth, creamy mouthfeel while preventing the formation of an overly tight casein network when uniformly distributed within the cheese matrix; because of the lack of fat and relatively high protein-to-fat ratio and colloidal calcium phosphate content, texture of RFC and LFC could be described as firm, rubbery, stiff, crumbly and grainy; besides, their modified microstructure also alters technological features such as melting and stretching. As fat is a source of fatty acids that provide flavor to cheese in themselves or could be transformed to new flavor compounds, RFC are expected to exhibit deficient sensory characteristics including less intense flavors and even off-flavors [6].

Generally aimed at increasing cheese moisture content, numerous strategies have been applied to palliate the defects of RFC and LFC including formulation-based or process-based approaches, which can be used individually or in combination [5]. In the former category, the most common is the addition of fat replacers such as inulin [7,8]; corn dextrin, polydextrose, maltodextrin [9] and other starch-based compounds [10,11], gums such as sodium alginate [12], konjac glucomannan [13] and pectin [14]; emulsions stabilized by gelatin and gum Arabic [15] or rice and pumpkin seed proteins [16], among others. However, as most of these ingredients are not allowed in cheese formulations, dairy ingredients such as rennet casein, microparticulated whey protein [17,18], whey protein isolate [16] and whey protein concentrate (WPC) [19] are preferred. On the other hand, the process-based approaches include modifications of the standard cheese make procedures (including the use of adjunct cultures, accelerated acidification, reduced curd cooking temperature, increased stirring time, reduced salt concentration) or the application of selected nonthermal technologies to cheesemilk or to some cheese ingredients to modify RFC and LFC characteristics [4]. As such, Mayta-Hancco et al [20] evaluated the addition of cream treated by high-pressure homogenization to skim milk with or without sodium caseinate to produce RFC. Cheeses with pressure-treated cream and sodium caseinate exhibited an increased yield and improved textural characteristics and sensory acceptability compared to their untreated reduced fat alternatives. Recently, Gamlath et al. [21] used power ultrasound (US) and/or heat treatment to produce protein aggregates from WPCs, evaluating their effects on selected cheesemaking properties of a nonfat model cheese system; the more hydrophobic aggregates formed through the combined US/heat treatments showed enhanced protein retention in cheese while avoiding an exceedingly compact microstructure, thus, showing promise as alternatives to avoid some of the common problems found in LFC, texture wise.

Panela cheese (PC) is a soft, unripened, pasteurized cow-milk cheese, widely consumed in Mexico and in some parts of the United States. Usually a starter-free product, PC is rennet coagulated, yielding 13–14 kg per 100 L of milk [22]. According to the Mexican regulations [23], PC could be prepared with whole, partially skimmed or skimmed milk; the whole milk-type needs to have a maximum moisture content of 59% and minimum protein and fat contents of 17 and 20%, respectively; PC usually contains 1.3–1.8% salt and pH values of 5.6 to 6.4 [24]. Meanwhile, reduced-fat PCs exhibit both a higher moisture (up to 64%) and lower fat (11–17%) contents. Although quality problems associated with fat reduction in PC have been previously tackled [14] and, recently, PC elaborated with ultrasound-treated milk [25] or US-treated dairy ingredients [26] have been explored, no reports on thermosonicated (TS) partially skimmed milk- WPC blends for manufacturing reduced fat PC have been found. Thus, the aim of this study was to evaluate the use of WPC-added cheesemilks submitted to pre-selected US treatments as means to alleviate potential quality defects of reduced fat fresh cheeses such as Panela.

2. Materials and methods

2.1. Materials

Raw bovine milk was obtained from a local producer (Chipilo, Puebla), stored at 4 °C until used and processed within 24 h. WPC-80 (76% protein, 9% carbohydrates, 10% fat) (ARLA Foods, Denmark), double strength rennet (Cuamix, Chr. Hansen, Mexico) and food-grade calcium were used for cheesemaking purposes.

2.2. Milk sample preparation

Milk was skimmed at 40 °C in a benchtop Elecrem™ separator (Vanves, France) and then standardized at either 1, 2 or 3 g fat/100 g milk. WPC was gradually added to these standardized milks at levels of 1.33, 0.67 or 0 g WPC/100 mL milk, respectively, then mixed at low speed for 30 min with a magnetic stirring plate and left at 4 °C for 24 h to allow complete rehydration. All milk samples and milk blends were analyzed for fat, total protein, solids-non-fat (SNF) and water contents by MilkoScan (S-54B, FOSS Electric A/S, Hillerød, Denmark).

2.3. Thermosonication and LTLT pasteurization treatments

For US treatments, a UP 400S ultrasonic processor (400 W, 24 kHz, 120 μm amplitude) (Hielscher, Teltow, Germany) equipped with a 22-mm diameter titanium probe was employed. As determined by calorimetry [27] the power delivered was 101.5, 99.1 and 96.5 W for milk blends with 1, 2 and 3% fat, respectively.

Standardized milk samples (2 L) were placed in a 3-L double-walled vessel and probe was immersed 3 cm into the liquid. Ultrasonication was carried out at 24 kHz, 100% amplitude according to a complete randomized design with milk fat content (1, 2 or 3 g fat/100 g milk), treatment time (2, 4 or 6 min) and temperature (50, 55, 60) as factors and three replicates. Temperature was controlled by an external water circulation system (AD07R-20, PolyScience, llinois, USA), to avoid US-related temperature increases; besides, as treatment times were kept short, temperature of milks samples did not rise>1 °C of that of the expected temperature at any time. Right after US treatment, milk samples were LTLT pasteurized (63 °C 30 min) in a bench-top device (FJ15 Milky, F. Janschitz Co., Austria) for food safety assessment and upon treatment completion, it was cool down to 4 °C and kept under refrigeration until used.

2.4. Technological properties of cheesemilk

The feasibility of using US-treated milk-WPC blends for cheesemaking purposes was assessed by determining water holding capacity (WHC) and gel firmness (GF) in gel model systems as described below.

WHC was calculated as the ability of a rennet-coagulated milk sample to retain water when submitted to centrifugation [28]. A 30-g milk sample (32 °C) was placed in a 50 mL tube; 45 μL of a 1:10 diluted rennet (Cuamix, Chr Hansen Mexico) solution was added to each sample and milk was allowed to coagulate at 32 °C for 45 min. Curds were centrifuged (4500 rpm, 40 min, 10 °C) in a 320R device (Hettich, Massachussets, USA) The whey expelled (mL) and the curd weight at the bottom of the tube were registered and WHC was calculated as (Eq. (1)).

$$WHC = \left(\frac{M \times m_0}{100} - E\right)\left(\frac{100}{M - E}\right) \quad (1)$$

Where, M = sample weight before centrifuge; E = exuded mass; m_0 = initial water content percentage in the curd (wet basis), gravimetrically determined. Results were expressed as g of water content per 100-g curd.

GF was assessed as described by Gutiérrez-Méndez et al [29] with some modifications. 28 μL of a calcium chloride solution (6.6 M) were added to a 280-mL sonicated milk sample. The mixture was LTLT

pasteurized (63 ± 1 °C, 30 min), cooled down (32 °C) and its pH was adjusted to 6.15 with 0.1 N HCl. Later, 420 μL of a 1:10 rennet solution (Cuamix, Chr Hansen Mexico) was added. Milk was thoroughly homogenized, and 70-g samples were poured into 110 mL wide mouth bottles which were placed into a water bath and allowed to curd at 32 °C for 45 min. After samples were tempered in ice for 5 min, GF was evaluated in a TA-XT Plus texturometer (Stable Microsystems, UK), with a load cell of 30 kg using a 2.5 cm- diameter cylindrical probe with a penetration distance and speed of 10 mm and 1 mm/s, respectively. GF was expressed as g force.

Additionally, milk color, pH and titratable acidity were determined, comparing these attributes with those of 3% milkfat LTLT milk. Color evaluations were carried out in a calibrated HunterLab Colorflex colorimeter (Reston, VA, USA), in CIELAB scale (L*, a*, b*); chroma and hue angles were calculated from these parameters, as well as net color change (ΔE*), which was evaluated as described in Eq. (2)

$$\Delta E^{*} = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (2)$$

Meanwhile, pH was determined in milk mixtures, using a UB-10 pH meter (Denver Instruments, Denver, CO, USA). The evaluation was carried out by submerging a previously calibrated electrode into each milk sample, until a stable lecture was attained (about 2 min). Titratable acidity (TA) (as % lactic acid) was determined according to AOAC method 947.05 [30].

2.5. Statistical analysis of cheesemilk data and TS treatment selection.

General linear model (GLM) was used to determine the significance of main factors and their interactions on technological properties of cheesemilk, followed by Tukey's or Dunnett's tests (to compare results of blends with those of 1, 2 or 3% fat LTLT milks) (Minitab 16, State College, PA, USA). Meanwhile, to select the most appropriate TS treatment for cheesemaking purposes, a regression analysis ($P < 0.05$) was performed with Matlab R2012a software (MathWorks Inc., Natick, MA, USA) employing treatment time (2, 4 and 6 min) temperature (50, 55 and 60 °C) and milkfat content (1, 2 or 3 g fat/100 g milk) as processing parameters and milk WHC, GF, color, pH and TA as dependent variables. A 3% fat LTLT processed milk was used as a control. The values obtained for each of the proposed responses were analyzed using the regression model shown in Eq. (3), to identify the significance of the factors for each of the responses evaluated:

$$y = b_0 + b_1T + b_2G + b_3t + b_{12}TG + b_{13}Tt + b_{23}Gt + b_{123}TGt + b_{11}T^2 + b_{33}t^2 \quad (3)$$

Where:

T = Treatment temperature, G =% fat, t = ultrasonic treatment time.

The quality of the model fit was evaluated using the generalized coefficient of determination (R^2). The effects of the variables were shown in 2D contour plots.

2.6. Panela cheese elaboration

PCs were elaborated according to a make procedure proposed by Lobato-Calleros et al [14] with some modifications. Before cheesemaking, TS and control milks were thermisized at 32 °C for 30 min; later, diluted rennet (150 μL/L rennet diluted in 10 mL water) was added, and milk was curdled for 30 ± 2 min. Curd was separated in cheese cloth, pressed overnight in hoops, vacuum-packed in polypropylene bags and stored at 4 °C.

2.7. Cheese analyses

Except for compositional analyses, cheeses were sampled at day 1, 7 and 14. After elaboration, cheese fat, water, protein and salt contents were determined by the Gerber, gravimetric, Kjeldhal and Volhard methods [30,31]. Titratable acidity was determined according to the Mexican regulation [32] and expressed as % lactic acid. Cheese pH was determined with a calibrated portable pH meter with a cheese probe [31]. Simple cheese yield was expressed as kg of cheese per kg of milk employed [31]. Cheese syneresis was reported as the amount of expelled whey (mL) on each cheese during their refrigerated storage. Cheese texture characteristics (hardness, cohesiveness, springiness and chewiness) were determined by a Texture Profile Analysis (TPA); TPA was performed in a TA-XT Plus texturometer (Stable Microsystems, UK), with a load cell of 30 kg; 20-mm diameter × 20.mm high cheese cylinders were compressed twice to 50% of their original size with a 25-mm cylindrical probe 1 mm/s. Force-time curves were obtained at 1 mm/s and used to calculate the parameters above [33]. Cheese color evaluations were carried out as previously described for milk.

2.8. Statistical analysis of cheese data.

The analysis of variance (one way or two-way ANOVA) of results followed by Tukey's test pairwise comparisons ($P < 0.05$) was performed using Minitab 16 (Minitab Inc., State College, PA, USA) software. All experiments were carried out in duplicate, and results were expressed as mean ± standard deviation. Cheeses prepared with 3% milkfat pasteurized-only milk was used as control.

3. Results and discussion

3.1. Preparation of milk blends

Average composition values (determined at least in duplicates) of standardized milk samples, milk-WPC blends and their corresponding protein/fat ratios are presented in Table 1. As expected, SNF, protein and protein/fat ratio significantly increased ($P < 0.05$) after the addition of WPC to milk at all fat contents; besides, the same compositional parameters significantly augmented with the amount of WPC added. However, as the protein source is not casein-rich, no immediate conclusions on the effect of such increments on a possible improved cheese yield and dry matter content can be made, as it has been previously reported when either milk protein concentrates or isolates are used [33,34]. The inclusion of whey proteins in cheesemilk is a common practice in some industries, especially in denatured form [35] and as protein aggregates [21].

Table 1
Composition of standardized milks and milk-WPC blends.

	%Milk fat				
	3%	2%*		1%*	
Component	Milk	Milk	Blend	Milk	Blend
Fat (%)	2.96 ± 0.06^{A}	1.99 ± 0.05	2.04 ± 0.04^{B}	1.00 ± 0.06	1.12 ± 0.04^{C}
SNF (%)	7.71 ± 0.11 ^{A}	7.86 ± 0.10^{a}	8.30 ± 0.26 ^{Bb}	8.15 ± 0.23^{a}	9.16 ± 0.23 ^{Cb}
Protein (%)	2.84 ± 0.04 ^{A}	2.90 ± 0.04^{a}	3.32 ± 0.04 ^{Bb}	3.01 ± 0.08^{a}	3.97 ± 0.08 ^{Cb}
Water (%)	89.33 ± 0.10	90.15 ± 0.19	89.66 ± 0.29	90.85 ± 0.33^{a}	89.71 ± 0.32^{b}
Protein/Fat ratio	0.96 ± 0.01 ^{A}	1.46 ± 0.02^{a}	1.63 ± 0.02 ^{Bb}	3.02 ± 0.14^{a}	3.54 ± 0.12 ^{Cb}

*WPC added to standardized milk: 3% fat: no WPC added (control); 2% fat: 0.67 g/100 mL milk; 1% fat: 1.33 g/100 mL milk.

[a] Different superscripts in the same row within the same milkfat percentage indicates a significant difference ($P < 0.05$).

[A] Different superscripts in the same row between blends indicates a significant difference ($P < 0.05$).

3.2. Technological properties of cheesemilks

3.2.1. Water holding capacity and gel firmness

Both WHC and GF of gels prepared with TS milk-WPC blends are shown in Table 2. WHC is the ability of proteins and other hydrocolloids to retain free water after going through a series of processing stages, without exudation or syneresis [36]. WHC results for cheesemilk blends were compared with those of their pasteurized-only counterparts. It is evident that most of WPC-containing thermosonicated samples exhibited a higher WHC than that of 3% fat LTLT milk (44.93 ± 1.02 g H_20/100 g curd), regardless of the amount of WPC added to milk. According to GLM results, the three main factors (milkfat content, temperature and US treatment time) and their triple interaction had a significant effect ($P < 0.05$) on the WHC. Meanwhile, Dunnett comparisons indicated that two TS treatments resembled the WHC exhibited by 3% milkfat control sample, being 1% milkfat, US-treated for 2 min at 50 and 60 °C. The remaining treatments exhibited higher WHC values with no significant differences to that of 1% (52.87 ± 2.31 g H_20/100 g curd) and 2% milkfat (55.24 ± 2.57 g H_20/100 g curd) control samples. Finally, the highest WHC values (>61 g H_20/100 g curd and different to any control sample) were obtained with 2% milkfat blends sonicated at $T \geq 55$ °C and for at least 4 min.

An increase in cheese yield have been observed when WPC-fortified cheesemilk is used, frequently because of a superior water retention in the cheese matrix [37]. The incorporation of whey proteins to casein-containing dairy products such as milk could avert water expulsion from the gel network by forming casein-whey protein complexes that immobilize water, avoiding syneresis [38]. The increase in WHC obtained by incorporating WPC may resemble the behavior observed when adding fat replacers to cheese of both protein (e.g. microparticulated whey proteins) or polysaccharide nature, which causes the protein matrix to unfold, allowing a higher water retention while improving texture and increasing yield in reduced and low-fat cheeses [39]. Previous studies suggest that WHC increases because water directly binds to fat mimetics such as WPC and these in turn may interfere with casein matrix contraction, thus reducing the driving force involved in water expulsion from the curd [40]. Another possible explanation for the increase in WHC in the US process is related to cavitation and its mechanical effects, that causes the milk fat globule membrane to break down, causing caseins and denatured whey proteins to integrate into the newly formed membrane at exposed sites, thus improving the interaction between fat globules and the protein network; as a result, WHC increases due to greater amount of whey protein incorporated and its well-known ability to retain water [41]. Such increase in the binding of water molecules is due to a greater exposure of the hydrophilic sections of the amino acids to the surrounding aqueous phase [42]. Recently, Cheng et al [43] reported an increase in WHC up to 15.5% in a thermoformed gel prepared from a WPC solution: soybean oil emulsion when the concentrate was pretreated for 10 min with a dual frequency (20/28 kHz) probe ultrasound device. investigated the rheological and thermophysical properties of 10% WPC solutions, treated by US (20 kHz, 15 min) which was attributed to changes in gel porosity and pore size caused by US.

The GF values of TS milk-WPC blends are also shown in Table 2. According to the corresponding GLM analysis, both double (%fat-temperature and %fat- TS time) and the triple interaction of main factors had a significant effect ($P < 0.05$) on GF. Again, Dunnett's grouping allowed to distinguish the treatments whose behavior resemble the most to control sample (3% fat LTLT milk) or to the other partially skimmed milks. Most TS treatments exhibit GF values not significantly ($P < 0.05$) different from that of the control, which is promising from the technological standpoint since it points to a TS-induced softening of the casein network, considering that one of the major drawbacks of reducing fat in cheese is an increase in gel strength [44]. On the other hand, only 6-min TS, 1% milk-WPC blends treated at 50 and 55 °C showed no significant difference with 1% fat milk gels

An adequate curd firmness at cutting is paramount for cheesemaking to maximize fat retention while obtaining an appropriate moisture content and preventing fine losses. Thus, considering its role as a predictor of milk suitability for cheese manufacture, it was of key importance to determine the firmness of gels prepared with TS milk blends. A firmer gel structure at increasing protein concentrations have been reported with whey protein gels due to hydrophobic interactions and intermolecular disulfide bonds [45]; besides, Zisu et al [46] reported changes in GF of WPC dispersions submitted to heating (80 °C, 20 min) reporting that selected US pretreatments (20 kHz, 31–50 W of effective power) increased gel strength with sonication time (1–60 min), exhibiting less syneresis and significant differences in gel microstructure when compared to non– sonicated WPC gels. However, in casein-whey protein mixes, a reduction in GF is usually expected due a to the formation of a more open structure in comparison to the tight one found in RFCs, as whey proteins work as fat mimetics; for example, Li et al [47] recently reported the formation of weaker gels in blended model systems composed of casein, WPC and microparticulated WP. Similarly, the inclusion of WPC into the cheese matrix have been proven effective for reducing GF. Using microparticulated WPC in low-fat kashar cheese (a *pasta* filata-type cheese) Koca & Metin [48] reported a decrease in hardness attributed to higher moisture content in non-fat substance, moisture-to-protein ratio and total filler volume. However, excessive amounts of whey protein could interfere with curd firmness due to the formation of βLg - κCN complexes through thiol disulfide interchange when subjected to heat treatment [49] and other processes. Masotti et al [50] described a number of milk pre-treatments aimed at enriching cheese with whey proteins, such as heat treatment, membrane technology, high hydrostatic pressure, high pressure homogenization, use of transglutaminase and hybrid treatments. However, selected US

Table 2
Thermosonication effect on water holding capacity and gel strength of milk-WPC mixtures.

US t (min)	°C	Water Holding Capacity [1] (g H_20/100 g curd)		Gel Strength [1] (g)	
		% Fat		% Fat [1]	
		1	2	1	2
2	50	46.46 ± 3.64^{AB}	58.45 ± 2.53^{BC}	39.23 ± 1.73^{AB}	33.48 ± 2.12^{A}
	55	57.66 ± 5.13^{BC}	58.07 ± 1.49^{C}	35.58 ± 0.81^{A}	34.11 ± 3.84^{A}
	60	51.78 ± 3.60^{ABC}	59.40 ± 2.88^{C}	33.05 ± 0.87^{A}	39.38 ± 2.51^{AB}
4	50	56.79 ± 0.58^{BC}	57.70 ± 3.29^{BC}	38.42 ± 0.26^{AB}	35.98 ± 2.57^{AB}
	55	55.33 ± 0.69^{BC}	61.67 ± 0.01^{D}	35.20 ± 0.35^{A}	38.83 ± 0.39^{AB}
	60	54.06 ± 1.69^{BC}	62.65 ± 4.52^{D}	34.92 ± 1.77^{A}	38.20 ± 0.57^{AB}
6	50	55.31 ± 2.19^{BC}	54.39 ± 0.74^{BC}	41.27 ± 3.30^{ABC}	37.03 ± 1.38^{AB}
	55	59.04 ± 1.92^{C}	65.32 ± 2.55^{D}	40.58 ± 1.03^{BC}	33.78 ± 1.45^{A}
	60	56.53 ± 5.02^{BC}	67.25 ± 1.45^{D}	38.78 ± 3.50^{AB}	34.00 ± 1.98^{A}

(A) With 3% milkfat milk; (B) With 2% milkfat milk; (C) With 1% milkfat milk; (D) Significantly different to all control samples.
WHC of LTLT milks: 3% Milkfat: 44.93 ± 1.02; 2% Milkfat: 52.87 ± 2.3; 1% Milkfat: 55.24 ± 2.57.
GF of LTLT milks: 3% Milkfat: 36.08 ± 2.51; 2% Milkfat: 40.18 ± 3.25; 1% MilkFat: 47.05 ± 3.00.
[1] Mean ± standard deviation (n = 2).

[A] Superscripts indicate no significant difference of WHC with that of a control sample ($P < 0.05$).

conditions could be as effective a tool as the above. Gamlath et al. [21] reported that sufficiently large, less surface hydrophobic heat/US-produced whey protein aggregates incorporated in model non-fat cheeses did not greatly affect rennet gelation of skim milk compared to untreated microfiltered whey protein, which significantly impaired gelation.

3.2.2. Other physicochemical properties of WPC-milk blends

Color modifications in milk-WPC blends after US treatment are presented in Table 3. The GLM analysis shows that a significant ($P < 0.05$) effect of all main factors and their double and triple interactions (except for temperature × fat) on L*, while for a* only fat and time were significant; and for b* main factors and their double and triple interactions were significant. Table 4 present the results obtained for hue (h*), chroma (C) and net color change (ΔE) and similar results were obtained. For hue and ΔE, the GLM analysis showed that all main factors and their interactions were significant ($P < 0.05$), the same as for chroma (except for 10.68 to temperature × fat content interaction). These results indicate that changes in milkfat and the intensity of TS conditions cause important modifications in milk color. By increasing fat content, milk displays significantly ($P < 0.05$) higher luminosity, a* (less green) and b* (yellower) values which agrees with previous results [43]. US and TS could increase milk luminosity by decreasing milkfat globule size [51] and, to a lesser degree, whey protein particle size [52] that creates a more homogenous sample with smaller particles that increase visible light scattering. 1% milkfat samples did not differ significantly in L with the control sample, but all 2% fat milk samples exhibit a significantly ($P < 0.05$) higher luminosity than the 3% fat LTLT milk. Meanwhile, hue angles ranged from 4.10 to 4.50 in 1% fat milk-WPC blends and from 3.94 to 5.04 in 2% fat samples, with most 2% fat milk blends having a significantly higher value than its 1% fat counterpart treated at the same TS conditions and no trend in terms of TS time and temperature. Chroma values significantly ($P < 0.05$) increased with fat content at all TS conditions, ranging from 10.68 to 22.83 in 1% fat samples and from 33.03 to 47.26 in 2% fat blends. Higher chroma values indicate a more vivid color in 2% fat samples due to a greater carotenoid concentration. Finally, the net color changes show that 1% milk fat samples resemble the most to the control (3% fat LTLT pasteurized milk) rather than the 2% fat blends, which could be due to the higher WPC concentration in the former.

According to GLM analysis, pH values were only significantly affected by temperature and the % Fat × TS time interaction. Although in average all TS conditions caused milk pH to drop after treatment at both fat concentrations (from 6.60 to 6.64 to 6.51–6.57 in 1% fat milk blend and from 6.58 to 6.64 to 6.51–6.58 in 2% fat milk blend), only a few of them exhibit a significant difference ($p < 0.05$) (*Data not show*). These results are in accordance with previous reports. Marchesini et al [53] observed a decrease in pH in US (24 kHz, 400 W nominal power) milk from 6.77 to 6.30 with increasing wave amplitude (70–100%) and US time (50–300 s). Meanwhile, Chandrapala et al [54] reported small (-0.1), fully reversible, US time-dependent changes in pH of ultrasonicated (20 kHz, 50% amplitude, 31 W real ultrasonic power) skim milk; according to these authors, pH reductions in US-treated milk may come from the concomitant temperature increase or from the cavitation-induced formation of nitric acid when nitrogen and oxygen reacts. However, this pH change is short-lived and thus, cannot truly affect mineral balance in milk

Regarding titratable acidity, no significant ($P < 0.05$) differences

Table 3
Thermosonication effect on milk-WPC mixtures color.

Parameter	US t(min)	T (°C)	% Fat	
			1	2
L*	2	50	78.91 ± 0.05^{Ba}	82.28 ± 0.36^{ABb}
		55	79.58 ± 0.59^{Aa}	82.59 ± 0.09^{Ab}
		60	78.92 ± 0.04^{Ba}	81.39 ± 0.23^{Bb}
	4	50	79.52 ± 0.47^{Aa}	83.33 ± 0.35^{Ab}
		55	79.54 ± 0.3^{Aa}	82.95 ± 0.02^{Ab}
		60	79.48 ± 0.12^{Aa}	82.93 ± 0.23^{Ab}
	6	50	79.87 ± 0.25^{Aa}	82.54 ± 0.05^{Ab}
		55	80.03 ± 0.09^{Aa}	82.80 ± 0.29^{Ab}
		60	79.73 ± 0.19^{Aa}	83.18 ± 0.17^{Ab}
a*	2	50	-4.03 ± 0.02^{Aa}	-3.32 ± 0.11^{Ab}
		55	-4.06 ± 0.01^{Aa}	-3.32 ± 0.04^{Ab}
		60	-4.00 ± 0.00^{Aa}	-3.30 ± 0.05^{Ab}
	4	50	-4.05 ± 0.01^{Aa}	-3.42 ± 0.01^{Ab}
		55	-4.08 ± 0.07^{Aa}	-3.58 ± 0.21^{Ab}
		60	-4.08 ± 0.14^{Aa}	-3.49 ± 0.01^{Ab}
	6	50	-4.15 ± 0.05^{Aa}	-3.26 ± 0.11^{Ab}
		55	-4.14 ± 0.08^{Aa}	-3.52 ± 0.08^{Ab}
		60	-4.13 ± 0.10^{Aa}	-3.55 ± 0.13^{Ab}
b*	2	50	0.76 ± 0.04^{Ba}	3.10 ± 0.03^{Ab}
		55	1.69 ± 0.1^{Aa}	3.56 ± 0.14^{Ab}
		60	1.37 ± 0.25^{Aa}	2.15 ± 0.05^{Cb}
	4	50	1.12 ± 0.13^{Ba}	3.71 ± 0.37^{Ab}
		55	1.60 ± 0.23^{Aa}	3.12 ± 0.18^{Ab}
		60	1.38 ± 0.11^{Aa}	3.64 ± 0.33^{Ab}
	6	50	1.64 ± 0.25^{Aa}	2.91 ± 0.22^{ABb}
		55	1.75 ± 0.10^{Aa}	2.94 ± 0.12^{ABb}
		60	1.58 ± 0.25^{Aa}	3.18 ± 0.36^{Ab}

Control 3% fat LTLT pasteurized milk. L * 80.06 ± 0.40; a * −3.38 ± 0.12; b * 2.12 ± 0.07.
Results are media ± standard deviation (n = 3).
[a] Different superscript in the same row within the same parameter indicates a significant difference ($P < 0.05$).
[A] Different superscript in the same column within the same parameter indicates a significant difference ($P < 0.05$).

Table 4
Thermosonication effect on hue (*h**) parameter of milk-WPC mixtures.

t US (min)	°C	Hue (h*)		Chroma (C)		Net color change (ΔE)	
		% Fat		% Fat		% Fat	
		1	2	1	2	1	2
2	50	4.10 ± 0.02^{Ca}	4.54 ± 0.06^{ABb}	10.68 ± 0.44^{Ba}	43.03 ± 1.17^{Ab}	1.90 ± 0.01^{ABa}	2.43 ± 0.34^{Bb}
	55	4.39 ± 0.03^{Aa}	4.87 ± 0.13^{ABb}	22.62 ± 1.23^{Aa}	46.99 ± 0.77^{Ab}	1.02 ± 0.23^{Ca}	2.91 ± 0.15^{ABb}
	60	4.27 ± 0.08^{Ba}	3.94 ± 0.07^{Cb}	18.90 ± 3.17^{Aa}	33.03 ± 0.28^{Bb}	1.51 ± 0.15^{BCa}	1.32 ± 0.13^{Ca}
4	50	4.20 ± 0.05^{Ca}	5.04 ± 0.27^{Ab}	15.40 ± 1.62^{Aa}	47.26 ± 2.78^{Ab}	1.35 ± 0.25^{BCa}	3.63 ± 0.47^{Ab}
	55	4.38 ± 0.15^{ABa}	4.75 ± 0.04^{ABb}	21.39 ± 2.51^{Aa}	41.08 ± 3.32^{ABb}	1.04 ± 0.22^{Ca}	3.06 ± 0.06^{ABb}
	60	4.31 ± 0.17^{Ba}	5.04 ± 0.24^{Ab}	18.63 ± 0.77^{Aa}	46.18 ± 2.18^{Ab}	1.19 ± 0.04^{BCa}	3.26 ± 0.05^{ABb}
6	50	4.47 ± 0.14^{Aa}	4.37 ± 0.06^{BCa}	21.55 ± 2.74^{Aa}	41.73 ± 3.13^{ABb}	0.96 ± 0.14^{Ca}	2.60 ± 0.12^{Bb}
	55	4.50 ± 0.11^{Aa}	4.58 ± 0.02^{Aa}	22.83 ± 0.82^{Aa}	39.87 ± 1.75^{ABb}	0.86 ± 0.03^{Ca}	2.86 ± 0.30^{ABb}
	60	4.42 ± 0.18^{ABa}	4.77 ± 0.15^{ABa}	20.83 ± 2.58^{Aa}	41.79 ± 4.31^{ABb}	1.02 ± 0.00^{Ca}	3.30 ± 0.27^{ABb}

Control 3% fat, LTLT pasteurized milk. h * 32.15 ± 1.78, C * 3.99 ± 0.06.
Results are media ± standard deviation (n = 3).
[a] Different superscript in the same row within the same parameter indicates a significant difference ($P < 0.05$).
[A] Different superscript in the same column within the same parameter indicates a significant difference ($P < 0.05$).

were found in the treated samples after US treatment (from 0.17 ± 0.01 to 0.18 ± 0.01) (data not shown). Contrary to these results, Bermúdez-Aguirre et al. [55] reported an increase in TA (from 0.11 to 0.14% lactic acid) in US-treated (24 kHz, 63 °C, 30 min) milk, hypothesizing that it may come from US-enhanced lipolytic free fatty acid release or from the production of nitrite, nitrate and hydrogen peroxide during cavitation.

3.3. US treatment selection

The quadratic effect of the fat percentage was not considered, since it is only found in two levels. The minimum and maximum levels selected for each of the variables were Treatment temperature (minimum 50, medium 55, maximum 60 °C); % Fat (minimum 1%; maximum 2%); US treatment time (minimum 2, medium 4, maximum 6 min), The response variables were WHC, GF, ΔpH, TA and ΔE (see Supplementary material). Subsequently, the regression analysis was performed for each of the responses. The significance of the parameters was evaluated at a 95% confidence level. The coefficients of determination (R^2) for each response were: WHC 0.727; GF 0.588; pH 0.582; ΔE 0.871; TA 0.362. This indicates that the model adequately fits WHC and ΔE data and, to a minor extent, GF and pH data, while the proposed model did not adjust properly for TA responses. Contour plots of WHC and GF at 2, 4, and 6 min of US time are shown in Fig. 1, as well as their overlaid plots. Based on these results and the possible technological impact of response variables on the final product, the optimization process was carried out considering the predictive properties WHC and GF. For a quantitative analysis, the regression model was programmed in Excel software through the Solver add-in function (Microsoft Inc, USA). With this, the coded values for temperature, fat and sonication time that met the following restrictions were obtained according to the scenarios described below:

- Scenario 1. Minimize the fat content, reducing the US time treatment (to minimize energy expenses) while keeping WHC and GF similar to those of control sample (3% fat LTLT milk), in order to emulate the responses of the control, but reducing its caloric contribution.
- Scenario 2. Minimize the fat content, maintaining a GF similar to that of the control but increasing WHC from 15% up to about 20%. This would allow us to obtain RFCs with a GF similar to their full fat counterparts, but with a significant increase in performance.

The treatments that met the established restrictions were 1.5% fat milk-WPC blend, TS treated at 60 °C for 120 s; 1% fat milk-WPC blend, TS treated at 50 °C for 120 s; and 1% fat milk-WPC blend, TS treated at 50 °C for 144 s; these will be hereafter referred to as Treatment 1 (T1), Treatment 2 (T2) and Treatment 3 (T3), respectively. These treatments were proposed for the cheesemaking experiments, as they are considered the most promising ones to obtain RFCs with the proposed characteristics, based on their predicted WHC (T1: 55.28, T2: 49.50, T3: 53.00) and GF (T1: 36.09, T2: 38.42, T3: 37.26). These were respectively higher than and similar to the experimental WHC (44.93 ± 1.02) and GF (36.08 ± 2.5) of control milk.

3.4. Panela cheese elaboration and characterization

WPC-milk blends were prepared according to the optimized conditions described above; again, 3% fat LTLT pasteurized cheesemilk was used as control. Average composition values of standardized milk and milk-WPC blends are shown in Table 5. Protein-to-fat ratio of control, T1 (the same as T2) and T3 were 0.93, 2.33 and 3.77, respectively. These cheese milks were used to prepare PCs.

3.4.1. Cheese composition

Composition of PCs elaborated with control and TS milks is presented in Table 6. Significant increments ($P < 0.05$) in moisture, protein

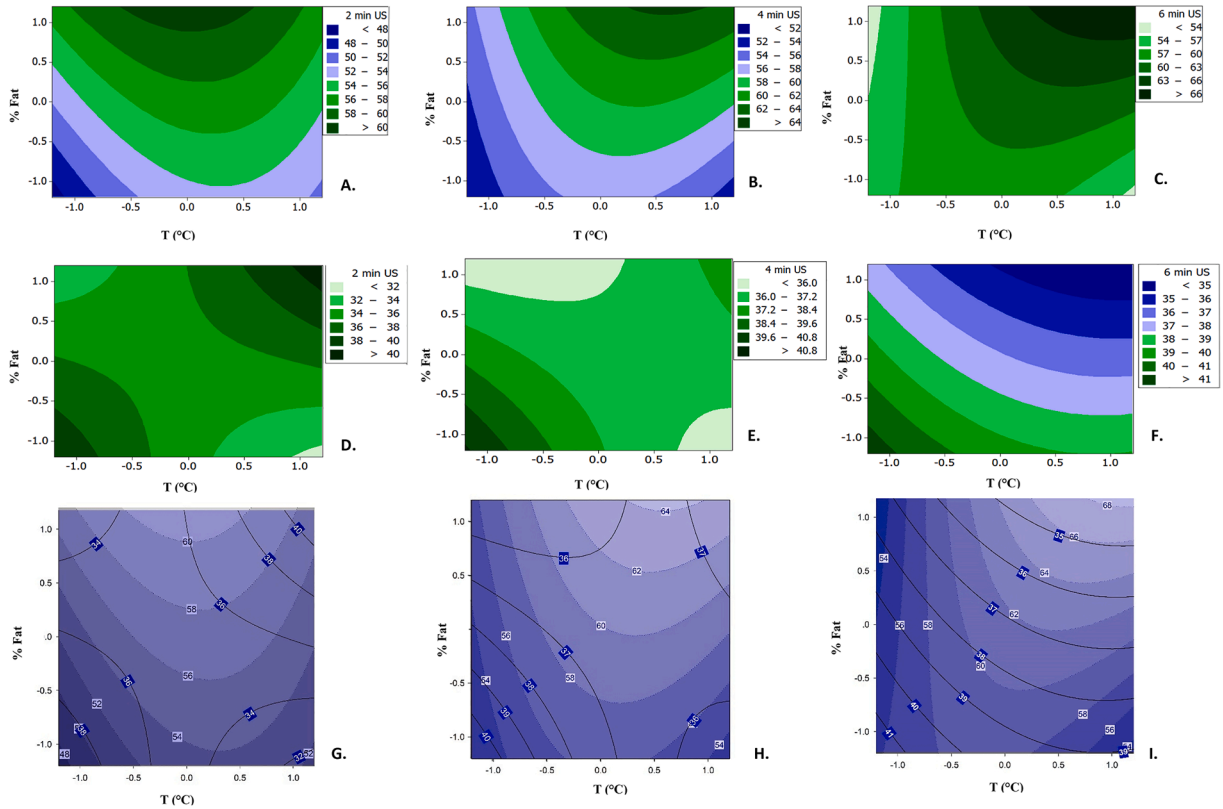

Fig. 1. Contour plots of water holding capacity (WHC) and gel firmness (GF) of thermosonicated milk-WPC blends A-C: WHC; D-F: GF; G-I: Overlaid contour plots of WHC + GF. A, D, G: TS = 2 min; B, E, H: TS: 4 min; C, F, I: TS = 6 min Coded variables: Temperature, °C: 50(-1), 55(0), 60(1); % Fat; 1(-1), 2(0), 3(1). In overlaid contour plots, WHC is in dotted lines, GF is in continuous lines.

Table 5
Average milk composition before and after WPC addition.

Treatment	Cheese milk composition before WPC addition					Cheese milk composition after WPC addition[2]					
	Fat (%)	SNF[1] (%)	Lactose (%)	Protein (%)	Water (%)	Fat (%)	SNF[1] (%)	Lactose (%)	Protein (%)	Water (%)	P/F Ratio
Control	2.98 ± 0.03	7.60 ± 0.24	4.15 ± 0.13	2.78 ± 0.09	89.48 ± 0.28	2.98 ± 0.03	7.55 ± 0.24	4.15 ± 0.13	2.78 ± 0.09	89.48 ± 0.27	0.93 ± 0.03
T1	1.55 ± 0.02	7.90 ± 0.23	4.34 ± 0.13	2.92 ± 0.08	90.60 ± 0.25	1.56 ± 0.06	8.67 ± 0.23	4.39 ± 0.12	3.64 ± 0.08	89.71 ± 0.25	2.33 ± 0.05
T2/T3	1.00 ± 0.05	8.10 ± 0.64	4.45 ± 0.35	3.00 ± 0.24	90.86 ± 0.68	1.05 ± 0.05	9.12 ± 0.63	4.51 ± 0.35	3.96 ± 0.23	89.73 ± 0.67	3.77 ± 0.16

Results are media ± standard deviation (n = 3).
[1] SNF: Solids-non-fat.

[2] WPC added: 3% fat: no WPC added (control); 1.5% fat: 1 g/100 mL milk; 1% fat: 1.33 g/100 mL milk.

Table 6
Average composition of cheeses elaborated with full-fat pasteurized and reduced-fat thermosonicated milk with WPC.

Treatment	Control[1]	T1	T2	T3
Moisture (%)	60.41 ± 1.35^{A}	66.23 ± 1.03^{B}	66.53 ± 1.12^{B}	67.08 ± 1.00^{B}
Protein (%)	15.91 ± 1.14^{A}	22.04 ± 0.35^{C}	20.31 ± 1.35^{BC}	19.12 ± 0.06^{B}
Fat (%)	15.22 ± 0.73^{A}	7.61 ± 1.08^{B}	5.56 ± 0.77^{C}	5.22 ± 0.25^{C}
Ash (%)	3.67 ± 0.35^{A}	3.56 ± 0.13^{A}	3.88 ± 0.36^{A}	3.83 ± 0.20 ^{A}
Salt (%)	1.37 ± 0.06^{A}	1.89 ± 0.20^{B}	2.37 ± 0.03^{C}	2.68 ± 0.03^{C}
pH (day 1)	6.38 ± 0.02^{A}	6.37 ± 0.01^{A}	6.40 ± 0.01^{A}	6.35 ± 0.11^{A}
pH (day 14)	6.36 ± 0.08^{A}	6.33 ± 0.20^{A}	6.49 ± 0.01^{A}	6.29 ± 0.19^{A}
Curd yield (kg/100 kg milk)[2]	20.75 ± 2.04^{A}	22.25 ± 1.91^{A}	20.96 ± 1.31^{A}	20.21 ± 0.61^{A}
Cheese yield (kg/ 100 kg milk)[2]	13.10 ± 0.82^{A}	14.34 ± 0.58^{A}	13.10 ± 0.64^{A}	13.46 ± 1.19^{A}

Results are means ± standard deviation (n = 3).
[A] Different superscript in the same row indicates a significant difference ($P < 0.05$).
[1]Control: cheese prepared with 3% fat LTLT milk, with no WPC addition.
[2]Curd yield was calculated before salting and pressing the curd; cheese yield on day 1 after pressing (15 h).
[2]After 14 d of refrigerated storage.

and salt contents were found in all TS cheeses, as well as a significant ($P < 0.05$) reduction in fat content. Moisture content of TS-milk cheeses were in range of 66.23 to 67.08% which represents a 9.63 to 11.04% increment from that of LTLT-milk cheeses (60.41%), although no significant differences were found among TS cheeses. Similar moisture contents (about 68.7%) in PCs prepared with US treated milk (400 W nominal power, 24 kHz, 5–10 min, 50–100% amplitude, 16 °C) were recently reported [25] which represented about a 6% increase compared with that of a raw-milk PC (64.96%). Bermúdez-Aguirre et al [41] also reported an increase in moisture content from 62.3 to 64.8% in pasteurized milk cheeses to 67–71% in *Queso fresco*, another typical Latin-American fresh cheese, made with TS milk (129 mW/mL at 63 °C for 10–30 min or at 72 °C for 15–60 s). A higher water content in cheese hints at a probable restructuring in the cheese matrix, allowing a greater water uptake by incorporating the very hygroscopic whey proteins into the surface of the casein micelles [56]; the presence of whey proteins during cheese manufacture could also delay whey expulsion by blocking curd pores [57]. In this sense, Almanza-Rubio et al [58] reported a significant increase in moisture content (55 to 60%) of cream cheeses made with TS milk (20 W, 51 °C, 30 min), with respect to those elaborated with LTLT pasteurized milk. When milk is heated and/or sonicated, the denatured whey proteins can form a complex with the hairy layer of κ-CN on the outer part of the casein micelle. Binding β-LG and α-LA to casein micelles increases WHC of the protein matrix [59], and hence the moisture content in cheese.

In terms of protein content, all TS treatments significantly ($P < 0.05$) increased this parameter in comparison to that of control PC (15.91%) from 19.12% (T3) to 22.04% (T1), although no significant differences were found between the latter and that of T2 (20.31%) (Table 6). It could have been expected that cheeses prepared with milk with the higher P/F ratio (T2, T3) presented the highest protein concentration in cheese, because of the higher WPC added (1 g/100 mL milk in T1 vs 1.33 g/100 mL in T2, T3); however, it was the one with the lower P/F ratio (T1) that exhibited the highest protein concentration. It is hypothesized that the slightly higher fat content in T1 milk played a pivotal role in increasing protein content in PC. As ultrasound is frequently used as a homogenization technique for reducing milk fat globule size [60], the smaller fat globules produced by TS treatment (especially when carried out at about 60 °C) have more membrane sites for protein binding, thus promoting the incorporation of both caseins (or casein fragments) and denatured whey proteins [51]. Thus, the additional whey proteins retained in the cheese matrix could be bound to the more abundant fat globules in T1 milk. Mixed results of the utilization of US to increase protein content in PCs were reported by Carrillo-López et al [25], where cheeses made with sonicated (5 min, 24 kHz 50–100% amplitude, 400 W,) slightly increased protein content of PCs (from 21.46% to 22.69%) whereas identical US treatments carried out for 10 min in fact reduced the average protein content in PC (18.9%).

The salt content of PCs (Table 6) increased with moisture content; as such, control PCs exhibited the lowest salt content (1.37%) with a moisture content of 60.41%; meanwhile, the salt content of TS-milk cheeses ranged from 1.89 to 2.68% (the latter with T3 cheese, whose moisture content is 67.08%). This result is the opposite of what was expected; salt is usually inversely related to moisture content, although salt content in cheeses might be modulated by adjusting selected cheese make procedure parameters [61]. As commercial PCs regularly contains 1.5–2.2% salt [22], the appropriate changes in the cheesemaking steps needs to be made to guarantee a salt content within the desired range. The significantly higher salt content in PCs elaborated with TS milks could be related to the milk fat globule size. Cheeses manufactured with microfiltered small milk fat globule milks have been observed to retain more salt and more moisture than its regular-sized counterparts [62].

As expected, fat content in TS-milk cheeses was significantly ($P < 0.05$) lower than those elaborated with full-fat LTLT-milk (15.22%); T1 milk cheeses had 50% less fat while fat contents of T2 and T3 cheeses ranged from 5.22 to 5.56%, a 63.5–65.7% reduction with respect to the control (Table 6). As commercial reduced-fat PCs and Panela-like cheeses have a fat content ranging from 11 to 18%, and regular Panela type cheeses may contain 15 to 28% fat [63], the fat percentage in PCs elaborated with TS-milks (well below 11%) places them in the LFC

category [5], without most of the quality issues related to those products, as it will be shown later.

Regarding the pH in the control and TS cheeses, no significant ($p > 0.05$) changes were detected in the pH of experimental and control cheeses neither at day 1 nor after 14 days of refrigerated storage (Table 6); in overall, these values are within the range of those found in previous reports [22,24]. It is well known that pH is one of the parameters that affects the textural properties of cheese the most, due to its effect on the protein network. A pH close to the isoelectric point promotes strong ionic and hydrophobic forces, which result in the formation of a compact casein network, typical of hard cheeses; meanwhile, at a higher pH the caseins acquire more negative charges, which generates a certain repulsion between protein aggregates, generating a cheese with higher moisture, more elastic and less compact [64]. Meanwhile, Bermúdez-Aguirre et al. [55] reported minor changes in pH values in TS-treated milk (24 kHz, 400 W) with respect to that of heat-treated milk. As mentioned earlier, a slight, temporary reduction in milk pH was expected because of the cavitation-induced nitric acid formation and the processing temperature used [54] or an increase in lipolytic activity caused by the enlarged surface area of US-homogenized fat globules [25]. In a similar fashion, titratable acidity in the evaluated cheeses did not significantly ($P > 0.05$) change because of the treatment applied or during refrigerated storage; TA was in the range of 0.07–0.10% lactic acid (*Data not shown*). Ramírez-López & Vélez-Ruiz [24] reported similar TA values for Panela cheese at day 1 (0.07%) and throughout refrigerated storage (0.13% at day 8; 0.22% at day 15). Titratable acidity increases in high moisture, fresh cheeses during refrigerated storage could be caused by lactic acid fermentation or residual enzymatic activity.

3.4.2. Cheese yield

Table 6 also shows the curd and cheese yields of PC prepared with control and TS-treated milks. Cheese yield depends on several factors including composition and milk pretreatments [56]. Clearly, to optimize cheese yield, the emphasis must always be on minimizing the losses of the main components (protein and fat) while maintaining an appropriate moisture content. However, with RFC the aim shifts to replace as much fat as possible with a fat mimetic such as WPC; although WPC in itself is capable of binding water, TS also provides an alternative to enhance whey protein incorporation into the cheese matrix, thus further improving cheese yield. According to San Martín-González et al. [65], increases of $\geq 0.5\%$ in cheese yield can be considered sufficient to generate a significant profit for the manufacturer. No significant ($P < 0.05$) difference was found on curd and cheese yields for full-fat LTLT milk and TS-milk cheeses. An increase in yield of cheeses prepared with TS-milk has been previously observed and attributed to an US induced modification of milk structure; milk fat globules and casein micelles could change their shape and size by US-generated cavitation waves in the milk, producing a new casein-fat complex and giving the milk better physicochemical characteristics for cheesemaking [51]. On the other hand, Sfakianakis et al. [66] probed that high intensity US promotes whey proteins denaturation, generating the formation of large aggregates; these and single denatured whey proteins can form complexes with casein micelles which increases WHC of the cheese matrix, increasing yield. The lack of significance in curd and cheese yield differences between control and experimental cheeses could be because of the short length of the TS processes (120–144 s). Other studies presented increases in cheese yield when using TS-milk for cheesemaking purposes, but with considerably longer treatment times. In this sense, Almanza et al. [58] reported an average yield of 19.5% in cream cheese manufactured with TS-milk (20 W, 51 °C, 30 min), which was higher than that of control cheese (10.9%). Bermúdez-Aguirre et al. [41], reported that a TS-pretreatment of milk (24 kHz, 129 mW / mL, 63 °C, 30 min) improved *Queso fresco* cheese yield from 10.7% (control) to 20.6%. However, as flavor issues might arise when US-treating milk for longer times [25], keeping processing times short is paramount from both the economical and sensory standpoints.

3.4.3. Cheese syneresis

Changes in the composition of PCs during their refrigerated storage (4 °C) were observed, which were due to moisture loss by syneresis, a common quality defect in fresh cheeses, like Panela [67]. Syneresis might affect cheese color, appearance and texture; besides, keeping cheese in contact with the expelled whey promotes microbial growth and chemical reactions on the cheese surface that may compromise their stability. Table 7 reports moisture loss in LTLT-milk and TS-milk cheeses at day 1 and after 14 days of refrigerated storage as well as syneresis and fat and protein in the expelled whey. Significant differences ($P < 0.05$) in syneresis and cheese moisture loss of control and experimental cheeses were detected, with TS-milk cheeses exhibiting higher syneresis (19.05–20.83 mL) than their full-fat LTLT-milk counterpart (16.81 mL) and greater cheese moisture losses in all but cheeses made with T2-milk (5.03% in control vs. 4.61% in T2). According to Fernandes García et al. [68], increased cheese syneresis during refrigeration is directly related to TA and inversely related to pH, as acidity affects protein contraction. However, as no significant differences in pH and TA of control and experimental PCs were found, differences in syneresis might be due to the low-fat content in TS- milk cheeses, as the presence of fat lessens protein contraction and reduces syneresis. Similar results regarding the syneresis behavior in TS cheeses compared to heat treated ones was observed by Bermúdez-Aguirre & Barbosa-Cánovas et al. [41] who also observed better results with longer TS treatments (30 min); the authors concluded that the reduction in syneresis in longer US treatments can be attributed to the homogenizing effect that sound waves have on fat globules, casein molecules and other milk components. Syneresis volume of PCs elaborated with US-treated milks has been recently reported [25]; no significant differences in expelled whey of control and experimental cheeses were found when milk was ultrasonicated (24 kHz, 400 W, 50 or 100% amplitude) for 5 min, but syneresis greatly increased (37%) when US-treating milk for 10 min. On the other hand, no significant ($P < 0.05$) differences in fat content of experimental and TS-milk cheeses were found at days 1 and 14 of refrigerated storage; however, their protein content did exhibit significant ($P < 0.05$) differences at day 1 and all cheeses continued losing protein during storage. Protein retention is critical both the nutritional and functional standpoints and the proposed processes need to be further reviewed to try the reduce this parameter. The formation of whey protein aggregates from filtered whey proteins and WPC during US and TS procedures and its incorporation into cheese milk is a promising alternative for improving protein retention in similar systems as ours [21].

3.4.4. Texture profile analysis

The decrease in fat and its substitution in cheese, causes substantial

Table 7
Syneresis, moisture loss in cheese and average whey composition of cheeses elaborated with full-fat pasteurized and reduced-fat thermosonicated milk with WPC.

Treatment	Control[1]	T1	T2	T3
Syneresis, day 14 (mL)	16.81 ± 0.11^{A}	19.05 ± 1.50^{B}	19.20 ± 1.53^{B}	20.83 ± 0.29^{B}
Cheese moisture loss (%) at 14 d	5.03 ± 0.52^{A}	7.70 ± 1.26^{B}	4.61 ± 0.06^{A}	6.43 ± 1.31^{AB}
Fat in whey (%), day 1	0.46 ± 0.13^{A}	0.39 ± 0.05^{A}	0.39 ± 0.07^{A}	0.39 ± 0.08^{A}
Fat in whey (%) day 14	0.03 ± 0.02^{A}	0.03 ± 0.03^{A}	0.04 ± 0.03^{A}	0.02 ± 0.01^{A}
Protein in whey (%), day 1	3.12 ± 0.12^{A}	3.59 ± 0.15^{B}	3.69 ± 0.10^{B}	3.74 ± 0.09^{B}
Protein in whey (%), day 14	9.88 ± 1.19^{A}	8.90 ± 0.58^{A}	9.28 ± 0.81^{A}	9.16 ± 1.00^{A}

Results are means ± standard deviation (n = 3).
[A]Different superscript in the same row indicates a significant difference ($P < 0.05$).
[1]Control: cheese prepared with 3% fat LTLT milk, with no WPC addition.

changes in the final product, affecting both texture and flavor characteristics and compromising their sensory acceptability, as fat heavily contributes to cheese composition, structure, melting behavior and its interaction with both polar and non-polar molecules [6]. In RFC and LFC product development it is critical to determine the possible textural variations that cheese may suffer to identify an appropriate fat mimetic and/or processing approach aimed at reducing their differences with the reference product. Several previous studies indicate that the use of different fat substitutes or milk pretreatments could modulate textural properties of RFCs [14,21,26,48]. TPA results are shown in Table 8. No significant differences ($P < 0.05$) on most of the TPA parameters evaluated (Hardness, springiness, cohesiveness, chewiness) were observed between TS treatments and control cheese at day 1 and during 14 days of refrigerated storage. Only cohesiveness (and chewiness in consequence) at days 7 and 14 did significantly change ($P < 0.05$), with TS-milk cheeses generally exhibiting higher values in these parameters. Higher cohesiveness values have been associated with greater moisture content in regular cheeses and with the presence of fat mimetics in RFCs [69]. Thus, texture-wise, using WPC as fat substitute and pretreating the WPC-milk blend with the selected TS treatments seems promising for RFC and LFC manufacture, as the attributes of full-fat PC can be emulated. Usually, the parameter that is most affected in RFCs is hardness; the strength of the protein network in cheese (casein-casein crosslinks) may be influenced by, among other factors, the content of moisture, fat and minerals. The action of removing half or more of the fat content of cheese milk implies the formation of harder and more rigid cheeses, as fat globules work as structure breakers in the casein matrix [21]. However, the addition of WPC and TS treatments allowed the incorporation of water to the protein network, causing an increase in moisture content with a reduction in the firmness of the structure, which indicates a greater separation between caseins. Hardness values resemble those previously reported for regular PCs [24] although the rest of the parameters have considerably lower values, while other studies report similar springiness and cohesiveness values [22] even if both hardness and chewiness are markedly higher. Bermudez-Aguirre et al [41] observed a significant, US-time dependent reduction in hardness and chewiness of fresh cheeses made with TS-milks

Some textural parameters of both control and experimental PCs changed over time, specifically hardness and chewiness. An increase in cheese hardness (and consequently, in chewiness) was related to the moisture loss by protein contraction, independently of the type of cheese milk pretreatment.

3.4.5. Cheese color

Table 9 details the instrumental color characteristics of control and TS cheeses at day 1 and throughout their refrigerated storage; L, a*, b* values are in the range of those reported previously for PC [24], except for lightness whose values are considerably higher in this report. No significant ($P < 0.05$) differences in b* and chroma values were found between experimental and control cheeses at all sampling times, despite of the differences in fat content and the contribution of the latter to yellowness in various dairy foods. Meanwhile, lightness significantly ($P < 0.05$) diminished during storage time (except for T1) but did not exhibit a significant difference between TS-milks and control sample (except for T3 after 14 d of storage); this was unexpected since dairy products elaborated with homogenized milk are usually whiter, more

Table 8
Texture profile analysis during storage of cheeses elaborated with full-fat pasteurized and reduced-fat thermosonicated milk with WPC.

Parameter	Treatment	Storage (days)		
		1	7	14
Hardness (kg-f)	Control	0.70 ± 0.04^{Aa}	1.13 ± 0.06^{Ab}	1.15 ± 0.04^{Ab}
	T1	0.69 ± 0.03^{Aa}	1.25 ± 0.10^{Ab}	1.26 ± 0.07^{Ab}
	T2	0.78 ± 0.07^{Aa}	1.16 ± 0.07^{Ab}	1.19 ± 0.09^{Ab}
	T3	0.71 ± 0.05^{Aa}	1.20 ± 0.09^{Ab}	1.32 ± 0.03^{Ab}
Springiness (Dimensionless)	Control	0.85 ± 0.01^{Aa}	0.86 ± 0.02^{Aa}	0.84 ± 0.01^{Aa}
	T1	0.86 ± 0.00^{Aa}	0.88 ± 0.01^{Aa}	0.87 ± 0.01^{Aa}
	T2	0.86 ± 0.01^{Aa}	0.87 ± 0.01^{Aa}	0.87 ± 0.01^{Aa}
	T3	0.85 ± 0.00^{Aa}	0.88 ± 0.01^{Aa}	0.86 ± 0.01^{Aa}
Cohesiveness (Dimensionless)	Control	0.66 ± 0.01^{Aa}	0.63 ± 0.04^{Aa}	0.69 ± 0.02^{Aa}
	T1	0.70 ± 0.01^{Aa}	0.73 ± 0.03^{Ba}	0.75 ± 0.01^{Ba}
	T2	0.65 ± 0.09^{Aa}	0.77 ± 0.02^{Ba}	0.78 ± 0.01^{Ba}
	T3	0.67 ± 0.02^{Aa}	0.74 ± 0.00^{Bb}	0.65 ± 0.04^{Aa}
Chewiness (kg-f)	Control	0.40 ± 0.01^{Aa}	0.62 ± 0.02^{Ab}	0.67 ± 0.02^{Ab}
	T1	0.41 ± 0.01^{Aa}	0.81 ± 0.08^{Bb}	0.82 ± 0.05^{Bb}
	T2	0.43 ± 0.07^{Aa}	0.78 ± 0.05^{Bb}	0.81 ± 0.08^{Bb}
	T3	0.41 ± 0.02^{Aa}	0.78 ± 0.05^{Bb}	0.75 ± 0.05^{ABb}

Results are means ± standard deviation (n = 3).
[a] Different superscript in the same row within the same parameter indicates a significant difference ($P < 0.05$).
[A] Different superscript in the same column within the same parameter indicates a significant difference ($P < 0.05$).
[1]Control: cheese prepared with 3% fat LTLT milk, with no WPC addition.

Table 9
Color during storage of cheeses elaborated with full-fat pasteurized and reduced-fat thermosonicated milk with WPC.

Parameter	Treatment	Storage (days)		
		1	7	14
L*	Control	92.43 ± 0.33^{Aa}	91.61 ± 0.61^{Aab}	91.38 ± 0.21^{Ab}
	T1	92.35 ± 0.55^{Aa}	92.20 ± 0.24^{Aa}	91.48 ± 0.17^{Aa}
	T2	91.90 ± 0.21^{Aa}	91.36 ± 0.23^{Aa}	90.38 ± 0.27^{Ab}
	T3	91.84 ± 0.50^{Aa}	91.53 ± 0.66^{Aab}	89.53 ± 1.26^{Bb}
a*	Control	0.98 ± 0.33^{Aa}	1.01 ± 0.42^{Aa}	1.28 ± 0.14^{Aa}
	T1	−0.09 ± 0.04^{Ba}	0.17 ± 0.13^{Bb}	0.37 ± 0.02^{Bc}
	T2	0.08 ± 0.06^{Ba}	0.21 ± 0.07^{Ba}	0.21 ± 0.13^{Ba}
	T3	−0.22 ± 0.08^{Ba}	−0.29 ± 0.11^{Ba}	0.20 ± 0.08 ^{Bb}
b*	Control	13.58 ± 0.29^{Aa}	13.91 ± 0.72^{Aa}	14.48 ± 0.34^{Aa}
	T1	12.82 ± 0.80^{Aa}	13.32 ± 0.68^{Aa}	14.28 ± 0.54^{Aa}
	T2	12.58 ± 0.43^{Aa}	13.90 ± 1.05^{Aa}	13.93 ± 0.67^{Aa}
	T3	12.60 ± 0.78^{Aa}	13.52 ± 1.44^{Aa}	14.85 ± 1.34^{Aa}
h*	Control	85.88 ± 1.30^{Aa}	85.92 ± 1.67^{Aa}	84.96 ± 0.54^{Aa}
	T1	89.55 ± 0.19^{Ba}	89.27 ± 0.60^{Bab}	88.50 ± 0.01^{Bb}
	T2	89.64 ± 0.25^{Ba}	89.13 ± 0.30^{Ba}	89.15 ± 0.48^{Ba}
	T3	89.00 ± 0.34^{Ba}	88.35 ± 1.11A^{Ba}	89.26 ± 0.25^{Ba}
C*	Control	13.62 ± 0.31^{Aa}	13.95 ± 0.73^{Aa}	14.54 ± 0.34^{Aa}
	T1	12.82 ± 0.80^{Aa}	13.32 ± 0.68^{Aa}	14.28 ± 0.54^{Aa}
	T2	12.58 ± 0.43^{Aa}	13.90 ± 1.05^{Aa}	13.93 ± 0.68^{Aa}
	T3	12.60 ± 0.78^{Aa}	13.53 ± 1.43^{Aa}	14.85 ± 1.34^{Aa}
ΔE*	T1	1.47 ± 0.37^{Aa}	1.17 ± 0.37^{Aa}	1.65 ± 0.32^{Aa}
	T2	1.79 ± 0.45^{Aa}	1.87 ± 0.25^{Aa}	2.49 ± 1.07^{Aa}
	T3	1.48 ± 0.51^{Aa}	1.31 ± 0.27^{Aa}	1.03 ± 0.16^{Aa}

Results are means ± standard deviation (n = 3).
[a] Different superscript in the same row within the same parameter indicates a significant difference ($P < 0.05$).
[A] Different superscript in the same column within the same parameter indicates a significant difference ($P < 0.05$).
[1] Control: cheese prepared with 3% fat LTLT milk, with no WPC addition.

brilliant than those of non-homogenized sources [70]. Only parameters a* (-green; + red) and hue angle showed significant ($P < 0.05$) differences between TS-milk cheeses and the full-fat cheese at all sampling times; a* was significantly lower (0.08 to −0.22) than the control (0.98) while hue angle was significantly higher (89.0–89.6 vs. 85.9). In overall, it is important to notice that no clear differences in cheese color could be perceived by the naked eye; this was further probed when no significant differences in net color change could be found between control and experimental cheeses at all sampling times (Table 9).

4. Conclusions

Based on the obtained results, it is considered that the selected short TS treatments (120 s at 50 and 60 °C, 144 s at 50 °C) of low-fat milk (1, 1.5%) added with WPC 80 as fat mimetic and later LTLT pasteurized constitute viable procedures to obtain low-fat PCs that exhibit similar physicochemical, technological and textural characteristics to those of a PC prepared with full fat, LTLT pasteurized milk. Although additional sensory, microbiological and microstructural tests are necessary to fully validate these findings, and probably to select the best treatment among these three proposed, this is good evidence of the use of the TS technology to reduce fat in highly-consumed products like fresh cheese.

CRediT authorship contribution statement

Genaro G. Amador-Espejo: Conceptualization, Investigation, Writing - original draft. **Irving I. Ruiz-Lopez:** Methodology, Formal analysis, Conceptualization. **Paola J. Gibbens-Bandala:** Methodology, Investigation, Writing - original draft. **Raúl J. Delgado-Macuil:** Methodology, Formal analysis. **Hector Ruiz-Espinosa:** Conceptualization, Methodology, Resources, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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