Revista **U.D.C.A** Actualidad & Divulgación Científica
January-June 2021-Volumen 24 No. 1:e1949
ISSN: 2019-2551 en línea
ISSN: 0123-4226 impreso

Check for updates

Scientific article

http://doi.org/10.31910/rudca.v24.n1.2021.1949

Physical and compositional characteristics of cheese and yogurt made from partially demineralized milk protein concentrate

Características físicas y composicionales del queso y yogurt elaborados a partir de un concentrado de proteínas de leche parcialmente desmineralizado

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How to cite: Bejarano-Toro, E.; Sepúlveda-Valencia, J.U.; Rodríguez-Sandoval, E. 2021. Physical and compositional characteristics of cheese and yogurt made from partially demineralized milk protein concentrate. Rev. U.D.C.A Act. & Div. Cient. 24(1):e1949. http://doi.org/10.31910/rudca.v24.n1.2021.1949

Official publication of the Universidad de Ciencias Aplicadas y Ambientales U.D.C.A, University, Accredited as a High-Quality Institution by the Colombian Ministry of Education.

Received: March 3, 2019 **Accepted:** May 7, 2021 **Edited by:** Ingeborg Zenner de Polanía

ABSTRACT

The milk protein concentrate (MPC) has been extensively studied; however, the MPC partial demineralization through the diafiltration (DF) and its effect on MPC ability to produce milk coagulate products has not been fully explored; therefore, it was considered studying the MPC demineralization process with DF and evaluate the effect of this treatment on the compositional and textural characteristics of enzymatically and acid-coagulated products. The MPC of ultrafiltration was diafiltered by two cycles, later this MPC was used to make a fresh cheese, a set yogurt and stirred yogurt. The application of a single DF cycle removed 22.2% of the ashes and 8.12% of the MPC calcium, but no statistically significant differences were present ($P > 0.05$) between the application of two DF cycles. The cheeses with MPC undergone to one cycle and two cycles of DF were less hard and presented less resistance to chewing, and the set yogurt showed lower springiness values due a total solids and calcium content, that was affected by DF. These phenomena increased the coagulation time and the formation of weaker gels. The DF achieved the maximum milk demineralization in a single cycle.

Keywords: Proteins; Ultrafiltration; Diafiltration; Coagulation; Calcium.

RESUMEN

El uso de concentrados de proteína de leche (MPC) ha sido estu-diado ampliamente; sin embargo, su desmineralización parcial por medio de la diafiltración (DF) y el efecto de este tratamiento sobre su aptitud en la elaboración de productos coagulados no está com-pletamente explorada. Se planteó, entonces, estudiar el proceso de desmineralización de un MPC por medio de varios ciclos de DF y evaluar el efecto de este tratamiento sobre las características com-posicionales y texturales de productos coagulados enzimáticamente y por acidez. El MPC, obtenido por ultrafiltración, fue diafiltrado en dos ciclos; luego, el MPC fue usado para elaborar un queso fresco, un yogurt batido y uno cuchareable. La aplicación de un ciclo de DF removió el 22,2% de las cenizas y 8,12% del calcio, pero no hubo diferencias significativas (P>0,05) con respecto a la aplicación de dos ciclos de DF. El queso elaborado con el MPC, con uno y dos ciclos de DF, fue menos duro y presentó menor resistencia a la masticación que el elaborado con MPC sin DF y el yogurt cuchareable presentó menor elasticidad, debido al menor contenido de sólidos totales y calcio, los cuales, fueron afectados por la DF. La desmineralización parcial aumentó el tiempo de coagulación y favoreció la formación de geles más débiles. La DF alcanzó el máximo de desmineralización de la leche en un solo ciclo.

Palabras clave: Proteínas; Ultrafiltración; Diafiltración; Coagulación; Calcio.

INTRODUCTION

The membrane technology, as a filtration and selective concentration technique applied to the dairy industry, has benefited the develop-ment of technological processes, such as water removal and solid-liquid or liquid-liquid separations (Pouliot, 2008; Lauzin et al. 2020). The ultrafiltration (UF) process is crucial in the dairy industry, in which milk proteins are concentrated by removing lactose, minerals, peptides, water and other solutes with low molecular weight (Ken-neth *et al.* 2017). As a result of this process, MPC contains both casein and whey proteins in a similar ratio of whole milk (80:20). These products are generally spray-dried, contain protein levels from 42% to 85% and are used as protein source in the production of other dairy products such as fermented beverages, cheeses and ice creams (Francolino et al. 2010; Patel & Patel, 2014; Eshpari et al. 2014; Bruzantin et al. 2016; Lu *et al.* 2017).

The MPCs in liquid phase can also be applied in the cheese industry since they can improve the nutritional quality of the final product by a greater retention of components, keep a standard composition of the raw material and increase the cheese yield. Furthermore, increasing the total solids in the milk will turn the cheese industry more efficient and a profitable enterprise (Kumar *et al.* 2013).

The first process developed and patented was the MMV methodology, named after its inventors Maubois, Mocquot & Vassal, where the industrial production of a cheese based on milk concentrated between 5 to 7 times by UF was obtained, yielding high quality curd (Maubois *et al.* 1969). In general, the following types of retentate or concentrate can be obtained by concentrating milk through UF: low concentration retentate (VCF: 1.2-2X) (LCR); medium concen-tration retentate (MCR) (VCF: 2-6X) and liquid pre-cheeses (VCF: 6-8X) (Mistry & Maubois, 2004).

The concentration and buffer capacity of the minerals associated with the caseins (Ca, P and Mg) increase in an MPC obtained by UF, generating non-standard textures and flavours in the products with this MPC (Mistry & Maubois, 2004). The membrane permeable solutes in the DF are diluted in the concentrate by the addition of water and can be re-concentrated in successive stages. Therefore, DF is used to increase the protein content in the UF concentrate, removing lactose, and soluble and insoluble minerals (Brans *et al.* 2004; Singh, 2007; Gavazzi-April *et al.* 2018).

The application of DF to milk can lead to the modification of the casein micelles due to the alteration of the minerals associated with their structure and can therefore impact the characteristics of the dairy products (Sandra & Correding, 2013). DF has also been used to produce whey protein concentrates (WPC) from buttermilk and skimmed milk in order to evaluate their functional properties (Svanborg *et al.* 2015). These concentrated powder products have been assessed in different processing conditions to improve their functional characteristics (Mao *et al.* 2012; Banach *et al.* 2013; Cao *et al.* 2015; Chenchaiah et al. 2015). Despite the literature in this field, the use of liquid and demineralized MPC in coagulated products has not been fully explored, and the MPC uses are important to dairy industry. Thus, the aim of this work was to study the effect of partial demineralization and protein increase with the application of two DF cycles on a MPC in dairy products like Burgos cheese and set and stirred yogurts by assessing their compositional and textural characteristics.

MATERIALS AND METHODS

Process of obtaining MPC. The skimmed milk was characterised by Fourier Transform Infrared Spectrometry-FTIR (Milkoscan Foss Instruments, Hillerød, Denmark) for its protein, ash, fat and lactose content. 320L of skimmed milk were used for each experiment. A pilot APV filtration plant (Silkeborg, Denmark) was used for the milk concentration. This plant was equipped with two polyether-sulfone ultrafiltration (UF) membranes (UF-pHt Series GR81PP, Alfa Laval, Lund, Sweden) in parallel with a molecular weight cut-off of 10kDa and an effective filtration area of 13.6m2. The UF process was executed in concentration mode at a transmembrane pressure (TMP) of 4 bar, a temperature of 20°C and a volumetric concentration factor (VCF) of 4, in order to obtain 80L of MPC and 240L of permeate. The ash, calcium and protein contents were determined for the MPC and treatments by the incineration method at 550ºC (IDF 27, 1964); by inductively coupled plasma mass spectrometry (ICP-MS) (ISO 17294-2, 2003) and by the Kjeldahl method (IDF 3 20B, 1993), respectively. The analyses were performed in triplicate.

The processing conditions, temperature and transmembrane pres-sure for the application of DF to the MPC were determined by previous tests. The MPC was undergone to one

(M1DF) and two cycles (M2DF) of DF. Osmotized water (240L) with a dilution factor of 4 was added to the MPC for one DF cycle. This mixture was concentrated by UF with an VCF of 4. For two cycles of DF, the previous process was repeated in a successive way. 80L of each diafiltered product were recovered and the membrane was chemi-cally cleaned at the end of each experiment.

Burgos cheese preparation. The cheese was prepared using the MMV methodology (Maubois et al. 1969). The protein content in each MPC was standardized to 12% (w/v). The MPC was pasteur-ized at 75°C for 5 min and cooled to 35°C. Then, 0.02% of CaCl2 was added and the coagulant enzyme was applied (Chy-Max, Chr. Hansen, Denmark). After 30 min, the product was stored at 5°C by 24 h until the analyses were performed.

Set and stirred yogurt preparation. The protein content in each MPC was standardized to 4% (w/v). The MPC samples were heated to 75°C, homogenized at 200 bar and pasteurized at 95°C for 5min. They were then cooled to 43°C, inoculated with Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus and incubated at 42.5°C until they reached a pH of 4.6. Subsequently, some yogurt was remained without any further stirring (set yogurt), whereas some others were stored at 5°C for 12h and their curd was broken manually to transform the stirred yogurt. Both set and stirred yogurts were stored at 5°C by 24h until their analysis.

Textural properties of the yogurts and cheese. The firmness (N), springiness (mm) and cohesiveness of the set yogurt were assessed using a texture analyzer (TA-XTplus, Stable Micro Systems, Godalm-ing, UK) with a 0.5mm probe (SMSP/0.5) and equipped with a 25kg load cell. The samples were introduced into a container where their fermentation took place. The parameters for the test were: 1mm/s pre-test speed, 0,5mm/s test speed, 0,5mm/s post-test speed, a compression distance of 15mm and a test time of 5s (Ferragut *et al.* 2009; Serra *et al.* 2009).

Regarding the stirred yogurt, a back-extrusion test was performed using the same texture analyzer and the same load cell to determine its firmness (N), consistency (N.s), cohesiveness and viscosity index (N.s). The probe was the back-extrusion ring with a 35mm disc and calibrated at a height of 80mm. For this test, the cylinder was filled with the sample and refrigerated for 1h. The test conditions were: 1mm/s pre-test speed, 1mm/s test speed, 10mm/s post-test speed and a compression distance of 30mm (Serra *et al.* 2009; Najgebauer-Lejko *et al.* 2014). For the yogurts, six samples were used for each textural test.

The maximum deformation supported by the cheese samples before fracture was evaluated in previous tests. The cheese was portioned into 2cm^3 cubes to perform the texture profile analysis (TPA) using the texture analyzer previously described, equipped with the 100mm probe (SMSP/100) and the same load cell. The parameters for the test were: 1mm/s pre-test speed, 1mm/s test speed, 1mm/s post-test speed, and 35% strain with a 5s waiting time between the two cycles (Gutiérrez *et al.* 2013). The TPA characteristics were firmness (N), springiness, cohesiveness and chewiness (N). Six replicates of each textural test were performed on the cheese samples.

Composition analysis of products. The ash content was calculated by the method of incineration at 550°C (IDF 27, 1964), the calcium content by ICP-MS (ISO 17294-2, 2003), the protein content by the Kjeldahl method (IDF 20B, 1993) and the total solids by gravimetric method at 100ºC (IDF 4A, 1982). The fat content for the cheese was measured by the Soxhlet method (IDF 5B, 1986) and for the yogurt by the Rose-Gottlieb method (IDF 1D, 1996).

Statistical analysis. The results were presented as means + SD. For the response variables (physical, chemical and compositional charac-teristics) of each product, a one-way analysis of variance (ANOVA) was used at a 5% significance level, considering the factor as the number of diafiltrations: without DF (MPC) (control), one cycle (M1DF) and two cycles (M2DF). The least significant difference (LSD) was used to compare the treatments, when significant dif-ferences were found. All analyses were performed using Statgraphic Centurion 16.1. (Statpoint Technologies, INC).

RESULTS AND DISCUSSION

Process for obtaining MPC, M1DF and M2DF. The skimmed milk used in the study was composed of 33,3g/L of protein, 6,6g/L of ash, 0,7g/L of fat, 49,3g/L of lactose, 92,9g/L of total solids and 125,83mg/100mL of calcium, the milk used for the concentration process is within the parameters required by Colombian legislation and by other authors, to categorize it as skimmed milk (Ministerio de la Protección Social, 2006; Fox et al. 2015). The composition of MPC, M1DF and M2DF is presented in the table 1. The ash contents were 9,8% and 9,6% and the calcium contents were 369,2mg/100g and 357,5mg/100g for M1DF and M2DF, respec-tively, and were significantly lower ($P<0.05$) than those presented by the MPC which were 12,6% and 401,8 mg/100g, respectively. Banach et al. (2013) obtained similar results when applying DF to an MPC, removing part of the ash content, mainly from the soluble fraction of minerals.

Likewise, a removal of soluble minerals with DF has been reported, mainly K, Na and Cl (Chenchaiah et al. 2015; Kenneth *et al.* 2018). Based on this, it could be indicated that a large part of the soluble fraction of minerals was removed in the first DF cycle and, for the second cycle, the colloidal minerals (Ca, Mg and P) remained, which are associated with the structure of the milk and solubilized slowly (Mistry & Mauboius, 2004; Gaucheron, 2005). Such behavior was observed in this investigation because ashes were reduced in M1DF and M2DF with respect to MPC, going from 12.6% to 9.8% and 9.6% in MPC, M1DF and M2DF, respectively. Thus, a second DF cycle is not necessary because the removal of minerals from the samples was not significant between M1DF and M2DF (Table 1).

Composition of processed products. No significant differences ($P>0.05$) in protein content were found between the cheeses and yogurts made with MPC, M1DF and M2DF, with 122.5g/kg, 121.2g/kg and 122.9g/kg, respectively, confirming the correct milk standardization. On the other hand, significant differences (P<0.05) were found in the total solids, ash and calcium contents among the products made with MPC compared to those prepared with M1DF and M2DF. These results indicated that the DF process was effec-tive in UF-permeable solutes removal, such as lactose and ash, in which calcium was the most representative. However, it is important to note that these components are soluble fractions removed with the water used in the DF. Gaucheron (2011) showed that the milk calcium had a soluble and micellar fraction that represents the 30% and 70% of the total calcium, respectively. These data agree with the calcium fraction removed between MPC and M1DF (Table 2).

The DF using the UF membrane is a technology aimed at obtaining MPC and WPC with high protein content, since the amount of protein within the total solids of the product increases by decreasing lactose and soluble ions in the concentrate (Ferrer *et al.* 2014). The calcium content was reduced from 401.8mg/100g to 369. mg/100g in a M1DF and 357.5mg/100g in M2DF, and the total solids from 179.3g/kg on MPC to 127.0g/kg on M2DF. These results agree with Eshpari et al. (2014) who found significant differences in the lactose content between an MPC (UF) and an MPC (UF) with a DF cycle and Ferrer et al. (2014) found a significant reduction in the soluble and insoluble calcium content in an MPC when different volumes of DF were applied. In contrast with these reductions, on the concentrate samples used for Burgos cheese, the protein accounted for 69% of total solids on MPC and on M1DF and M2DF the 87.1% and and 95.9% of total solids, respectively, these data indicate that de DF by UF is effective in milk proteins concentration. On the concentrate samples used to make yogurt the trend of protein concentration was similar because on the MPC the protein represented a 38.3% of the total solids and on the M1DF and M2DF the 75.3% and 61.9%, respectively.

Textural properties of processed products. The cheeses made with MPC showed more firmness and resistance to chewing than those made with M1DF and M2DF, which can be explained by the lower calcium content in diafiltered milk that went from 389.6mg/100g of MPC to 356.2 and 358,6mg/100g of M1DF and M2DF, respectively (Tables 2 and 3).

This mineral is important for the enzymatic coagulation of milk, especially for its soluble fraction, which is involved in the secondary phase of the coagulation process when the casein micelles interact with the rennet (Ferrer *et al.* 2014; Eshpari *et al.* 2015), then the milk with lower soluble calcium resulted in weaker enzymatic curds.

The firmness of dairy gels obtained enzymatically from MPC56 and MPC85 standardized to equivalent levels of protein was compared, and the gel made with MPC56 was harder due to the higher content of soluble calcium (Sandra & Corredig, 2013). The soluble calcium in milk decreases with DF; therefore, gels with lower firmness are obtained when this milk is enzymatically

Table 1. Characterisation of the MPC*.

	Treatments		
Compounds	**MPC**	**M1DF**	**M2DF**
Protein (g/kg)	122.5±0.9a	121.2±1.0a	122.9±1.2a
Ash (%)	12.6±1.1a	9.8±0.9b	9.6±1.0b
Calcium (mg/100g)	401.8±13.3a	369.2±7.6b	357.5±12.7b

* The results are the mean ± standard deviation. Values of the same row with different letters present a statistically significant difference (P<0.05).

Table 2. Composition of Burgos type cheese and set or stirred yoghurts*.

Characteristicas	**Treatments**					
	Burgos type cheese			**Yoghurt***		
	MPC	**M1DF**	**M2DF**	**MPC**	**M1DF**	**M2DF**
Total solids (g/kg)	17.3±5.7a	138.9±2,3b	127.0±7.9c	108.8±10.8a	58.3±5.1b	66.9±0.9b
Protein (g/kg)	119.4±5.9a	121.1±2.4a	121.9±2.2a	41.7±2.2a	43.9±1.4a	41.2±0.6a
Ash (g/kg)	14.2±0.9a	10.8±0.5b	11.5±0.3b	7.9±0.8a	4.3±0.4b	3.5±0.2b
Calcium (mg/100g)	389.6±7.6a	356.2±7.0b	358.6±3.0b	158.8±2.4a	116.8±9.4b	109.1±3.5b

*The results are the mean ± standard deviation. Values on the same row with different letters present a statistically significant difference for each product (P<0.05).

coagulated (Ferrer *et al.* 2014; Liu *et al.* 2017). This behavior was similar to that observed in this investigation where the firmness was lower in the Burgos cheese made from M1DF and M2DF with respect to the firmness of the cheese obtained from the MPC (Table 3) with values of 349.7N, 290N and 261.5N for the cheese made from MPC, M1DF and M2DF, respectively. This is attributed to the fact that the calcium was not significantly reduced between M1DF and M2DF but there were differences with respect to MPC. The lesser firmness in the cheeses with M1DF and M2DF can also be generated because the DF increases the presence of the K, α-s and β caseins in the soluble phase, which increases the rennet coagulation time and reduces the clots firmness (Sandra & Corredig, 2013; Ferrer *et al.* 2014).

The yogurt is a coagulated product obtained through the fermentation of milk by lactic acid bacteria, the yogurt coagulation is caused by the hydrophobic and electrostatic interactions between proteins when the milk reaches the isoelectric point of the caseins (pH = 4.6) (Lourens-Hattingh & Vijoen, 2001; Sandoval-Castilla *et al.* 2004). Even at this point, the calcium phosphate is partially separated from the casein micelles (Fox, 2001; Chandan *et al.* 2006; Ferrer *et al.* 2014; Lauzin *et al.* 2020).

Schulz-Collins & Senge (2004) did not find significant differences in the yogurt firmness made from demineralized milk and explained that adding calcium to milk has no significant effect on the acid coagulation of dairy products. In this investigation, no significant differences were found in the firmness with 22.4g, 26.4g, and 24.4g to the stirred yogurt made from MPC, M1DF and M2DF, respectively and 27.3g, 32.1g and 30.3g to the set yogurt made from MPC, M1DF and M2DF, respectively. Sandoval-Castilla *et al.* 2004, Supavititpatana et al. 2008 and Hashim et al. 2009 reported set yogurts with higher firmness, adhesiveness and springiness values because the raw material was skimmed milk standardized to 4% protein and supplemented with starch, gelatin or fiber that helped improve the general characteristics of the product´s texture.

When diafiltered milk is used in the set yogurt preparation a less elastic product is obtained. This effect is attributed to the lower content of total solids in the M1DF and M2DF products (Table 2) since DF in UF membranes removes lactose and milk minerals. When milk solids like minerals, specifically the calcium is removed from the casein micelles, acid gels with higher loss tangents (tan δ) are generated, which explains their lower springiness (Haque & Sharma, 2002; Ozcan *et al.* 2011).

The firmness, consistency, cohesiveness and viscosity index of stirred yogurt showed no significant differences (P>0.05) among the samples (Table 4). Najgebauer-Lejko et al. (2014) prepared a similar product with semi-skimmed milk supplemented with green tea, and found the values of firmness, consistency, cohesiveness and viscosity index lower than those reported in this study for all treatments. The calcium content reduction in the milk did not affect the textural properties of the stirred yogurt, which can be considered as a concentrated dispersion of gel particles in serum (Marle *et al.* 1999).

From the results obtained it can be concluded that the UF application combined with DF is an effective way to achieve a partial MPC demineralization. However, no significant difference in the mineral content was found when more than one DF cycle was ap-plied. The textural differences of the fresh cheese were attributed to the mineral imbalance generated by the DF of the MPC, mainly in the calcium content. When acid coagulation happened in the yogurts, no significant effects of partial MPC demineralization were observed. The filtration effect was significant on protein structures and milk mineral content, this change affects the composition and texture of dairy products. Further studies are needed on the sensory differences and other physicochemical characteristics of the dairy products made from these treated milks in order to establish more accurate effects on their quality.

Acknowledgements. COLCIENCIAS, Productos Lácteos Aura and the Universidad Nacional de Colombia are acknowledged for the financial support to E. Bejarano-Toro provided through contract 575 of 2013. <u>Conflict of interests:</u> The paper was prepared and revised by all authors, who declare that there is no conflict of interest that jeopardizes the validity of the results.

Table 3. Texture profile analysis for cheeses made with MPC, M1DF and M2DF.

Characteristics	**Treatments**		
	MPC	**M1DF**	**M2DF**
Firmness (N)	349.7±13.4a	290.0±10.5b	261.5±21.0b
Cohesiveness	0.87±0.0a	0.87±0.0a	0.86±0.0a
Springiness (mm)	0.97±0.0a	0.97±0.0a	0.97±0.0a
Chewiness (N)	295.1±21.4a	234.7±19.4b	209.9±16.8b

The results are the mean ± standard deviation. Values on the same row with different letters present a statistically significant difference (P<0.05).

Table 4. Textural characteristics of yoghurts made with MPC, M1DF and M2DF.

Product	Characteristic	Treatments		
		MPC	M1DF	M2DF
Stirred yogurt	Firmness (N)	0.219±0.013a	0.259±0.025a	0.239±0.018a
	Consistency (N.s)	5.78±0.504a	6.87±0.62a	6.25±0.56a
	Cohesiveness *	17.5±1.0a	19.1±1.9a	17.8±1.5a
	Viscosity index (N.s)*	0.29±0.021a	0.356±0.037a	0.265±0.058a
Set yogurt	Firmness (N)	0.267±0.025a	0.314±0.031a	0.297±0.029a
	Cohesiveness	0.4±0.0a	0.36±0.0a	0,36±0.0a
	Springiness (mm)	0.97±0.0a	0.93±0.0b	0.94±0.0b

The results are the mean ± standard deviation. Values of the same row with different letters present a statistically significant difference ($P<0.05$).
*Absolute value
0.267 0.314 0.297

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