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Physicochemical and sensory properties of Manchego-type cheese fortified with nanoemulsified curcumin

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Abstract

The effect of incorporating nanoemulsified curcumin in Manchego-type cheese on its physicochemical and sensory properties was investigated. Nanoemulsified curcumin was prepared by the thin-film hydration-emulsification and ultrasonication methods, and added to Pelibuey sheep milk at 5, 7.5, and 10 ppm of curcumin to make enriched Manchego-type cheeses. The curcumin content of the enriched cheeses was determined by high-performance liquid chromatography. The average curcumin retention coefficient was 0.83 ± 0.03. The total phenolic content and antioxidant activity according to the FRAP and DPPH assays increased in the curcumin-enriched cheeses as compared to the control cheese. No significant differences were observed in the moisture, protein, fat, and ash contents, nor in water activity and yield; however, significant differences were observed in the soluble nitrogen and free fatty acid contents and colour. According to surveyed panellists, the addition of nanoemulsified curcumin to cheese modified its colour, odour, and appearance, but not its flavour, texture, and overall acceptability. The present work highlights the potential of incorporating nanoemulsified curcumin in Manchego-type cheese to enhance its beneficial health effects.

Introduction

Consumers are increasingly aware of the importance of diet for good health, and often prefer food products that can improve their quality of life. This has led to the development of functional foods and increasing market for their consumption (Domínguez-Díaz *et al.,* 2020). In addition to their nutritional value, functional foods may reduce the risk of diseases such as cancer, type-2 diabetes, dyslipidaemia, and cardiovascular diseases (CVD) (Granato *et al.,* 2020).

One functional compound with protective health effects against CVD, the principal cause of death worldwide, is curcumin (Li *et al.,* 2020). It has a remarkable antioxidant capacity that helps to reduce the progression of CVD (Benzer *et al.,* 2018). Its protective effect has been demonstrated *in vitro* and *in vivo*, and even confirmed in clinical assays (Salehi *et al.,* 2020).

Curcumin is a yellow polyphenolic compound extracted from the rhizome of *Curcuma longa* L., a herbaceous plant in the family Zingiberaceae, whose use as spice dates back to 1900 BC. Over the centuries, it has also been used as a traditional medicine to treat various ailments (Amalraj *et al.,* 2017). Curcumin is the main bioactive compound of the turmeric rhizome, and responsible for its potent antioxidant, anti-inflammatory, antimicrobial, antimutagenic, and anticancer properties (Patel *et al.,* 2020).

Curcumin has been characterised as safe by the Food and Drug Administration (FDA) of the United States for use as spice, colouring, and flavouring agent (Amalraj *et al.,* 2017). It is often used in curries, mustard, sauces, beverages, cheeses, butters, and French fries, as well as in rice, pasta, meat, vegetable, and salad dishes (Gupta *et al.,* 2013). However, one of the main problems in obtaining the benefits of curcumin is its low bioavailability, mainly due to its poor solubility in water, chemical instability under physiological conditions, rapid intestinal metabolism, malabsorption, and rapid elimination from the body (Kunnumakkara *et al.,* 2019).

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Several strategies have been tested to improve the bioavailability of curcumin including its incorporation in nanoemulsions (NEs), solid dispersions, inclusion complexes with cyclodextrins and copolymeric micelles, or its encapsulation in polymeric nanoparticles, lipid-based nanoparticles, and liposomes (Kharat and McClements, 2019). NEs have the advantage of incorporating hydrophilic, amphiphilic, or lipophilic compounds in addition to being non-toxic and having high physical stability, optical clarity, digestibility, and bioaccessibility (Jiang *et al.,* 2020). Curcumin has in fact been demonstrated to have greater bioavailability in NEs (Ochoa-Flores *et al.,* 2017). Thus, curcumin NEs could be incorporated into a wide range of food products to help prevent or treat different diseases, including CVD.

Cheese is a part of diet of a large segment of the world's population; and in Mexico, Manchego-type cheese is one of the most consumed (Salazar-Montoya *et al.,* 2018). It is made from pasteurised cow's milk added with mesophilic starter cultures and beef rennet extract. It is traditionally coloured with bixin to an orange-yellow colour. Therefore, curcumin could potentially be used as a potential and alternative colorant with bioactivity and health benefits. However, it is necessary to obtain information on the effects of the addition of nanoemulsified curcumin on the physicochemical and sensory properties of cheese. Given this background, the objective of the present work was to evaluate the effect of the incorporation of nanoemulsified curcumin on the physicochemical and sensory characteristics of Manchego-type cheese.

Materials and methods

Materials and reagents

Raw Pelibuey sheep milk was obtained from the ranch "El Rodeo" located at 17° 84' N, 92° 81' W, at 10 masl (Tabasco, Mexico). Curcumin (purity ≥ 98%) was purchased from LKT Laboratories (St. Paul, USA), and soybean lecithin (95% phosphatidylcholine) from Avanti Polar Lipids (Alabaster, USA). Medium-chain oil was purchased from Sound Nutrition (Dover, USA), and glycerol (99.5% USP grade, vegetable-based) from KIC Chemicals Inc. (New Paltz, USA). High-performance liquid chromatography (HPLC)-grade solvents were purchased from Tecsiquim (CDMX, Mexico), and all other reagents from SIGMA (Mexico City, Mexico).

Characterisation of milk

Density, titratable acidity, fat, protein, lactose, ash, total solids, and non-fat solids of milk were determined by the methods described by the Association of Official Analytical Chemists (AOAC, 2005). An extraction of the milk (1 mL) was carried out with 10 mL of a solution of 1 N hydrochloric acid:95% ethanol (15:85, v/v), shaken at 300 rpm for 1 h at 30°C, followed by centrifugation at 7,000 rpm for 15 min at 5°C. The supernatant was recovered and used to determine total phenolic content (TPC) and antioxidant activity (AA). TPC was determined by the Folin-Ciocalteu method according to Singleton *et al.* (1999), and expressed as mg gallic acid equivalents (GAE)/L of milk. AA was determined by the DPPH method according to Brand-Williams *et al.* (1995), and expressed as mmol Trolox Equivalents (TE)/mL of milk. In addition, AA was evaluated through a FRAP assay according to Benzie and Strain (1996), and expressed as mmol TE/mL of milk. The fatty acid composition of the milk was determined by gas chromatography (GC) after the extraction with chloroform:methanol (1:2, v/v). The extracted fat was derivatised with 0.25 M sodium methoxide in methanol:diethyl ether (1:1, v/v) to obtain the fatty acid methyl esters, and glyceryltriundecanoate was used as the internal standard. Methyl esters were quantified in a Perkin Elmer model AutoSystem XL gas chromatograph fitted with a split-splitless injector, flame ionisation detector, and Perkin Elmer Elite Series PE-225 (30 m × 0.25 mm × 0.25 μm) capillary column. All analytical determinations were performed in triplicate (n = 3). Data were expressed as means ± standard deviations.

Preparation and characterisation of nanoemulsified curcumin

Nanoemulsified curcumin (NEC) was prepared by the thin-film hydration-emulsification and ultrasonication methods as reported by Ochoa-Flores *et al.* (2017). Briefly, soybean lecithin was dissolved in absolute ethanol and added to medium-chain oil. Curcumin was then added, and the mixture was rotary evaporated to generate a dry and thin film, which was hydrated with deionised distilled water (DDW) and glycerol, warmed to 45°C, and homogenised to produce a coarse emulsion. The coarse emulsion was then ultrasonicated to obtain the NEC, which was stored at 4°C until use. The NEC formulation consisted of soybean lecithin 10% (w/w), medium-chain oil 5% (w/w), DDW 42.5% (w/w), glycerol 42.5% (w/w), and 2.5 mg of curcumin per g of nanoemulsion. The droplet size, polydispersity index, and zeta potential of NEC were measured with a Nano-ZS90 dynamic light scattering device at a 90° fixed angle at 25°C. The curcumin concentration and entrapment efficiency

were determined by HPLC according to the method reported by Wichitnithad *et al.* (2009). All analytical determinations were performed in triplicate (n = 3). Data were expressed as means ± standard deviations.

Preparation of Manchego-type cheese

The milk was pasteurised at 63°C for 30 min, and distributed in four stainless steel tubs. After cooling to 37°C, NEC was added to the milk of three tubs (at 5, 7.5, and 10 ppm of curcumin, respectively) for the preparation of curcumin-enriched cheeses. Ten ppm of bixin in solution were added to the control treatment. Calcium chloride (0.02%) and lyophilised lactic culture M127 from Bioprox (2 g/100 L) were added; the mixture was stirred for 10 min, and allowed to stand for 30 min. Rennet of microbial origin from Cuamex (15 mL/100 L) was added. The curdling process lasted for 45 min. The curd was cut into 1 cm^3 cubes, stirred for 30 s, and allowed to stand for 15 min. The temperature was then raised to 40°C for 30 min for scalding and contraction of the curds. The whey was removed, and the curds were moulded and pressed for 18 h. Salting was carried out by rubbing salt (25 g/kg) on the exterior, and the cheeses were maturated for 60 days at a temperature of 10°C.

Characterisation of Manchego-type cheese

Moisture, total protein, fat, and ash contents of the curcumin-enriched cheeses and control cheese were determined by the methods described by the AOAC (2005). Water activity was measured using an Aqualab instrument at 25.5°C. Fractions of soluble nitrogen in water (SN-W), sodium citrate (SN-pH 4.6, adjusted to a pH of 4.6), 12% trichloroacetic acid (SN-TCA), and 2.5% phosphotungstic acid (SN-PTA) were prepared according to Desmazeaud *et al.* (1975). The nitrogen content of each fraction was determined by the macro-Kjeldhal method (AOAC, 2005). The SN-W content of cheeses, expressed as a percent of total nitrogen (TN) content, was reported as the ripening index (RI). Free fatty acid content (FFAC) was quantified by the methodology of Deeth and Fitz-Gerald (1976), and expressed as potassium hydroxide (KOH) mE/100 g of fat. An extraction of the cheeses was carried out according to the methodology described by Rashidinejad *et al.* (2013) to determine the TPC and AA. The TPC was determined by the Folin-Ciocalteu method according to Singleton *et al.* (1999), and expressed as mg GAE/100 g of cheese. The DPPH and FRAP assays to evaluate the AA of the cheeses were performed as described earlier for milk, and the results were expressed as mmol TE/g of cheese.

The curcumin content of the enriched cheeses was determined by reverse-phase HPLC according to Wichitnithad *et al.* (2009). Briefly, 2.5 g of finely grated cheese was mixed with 10 mL of 95% ethanol to facilitate the curcumin extraction. The mixture was homogenised for 3 min at 10,000 rpm, sonicated for 15 min in an ultrasonic bath at room temperature, and centrifuged for 15 min at 4,000 rpm. The supernatant was recovered and filtered through Whatman #1 filter paper. The extraction was repeated one more time with fresh solvent, and the extracts were pooled to a volume of 25 mL with 95% alcohol. The extracts were then filtered through Whatman #42 filter paper, and kept at 10°C until analysis. Samples were injected into a Waters HPLC System fitted with a Econosphere C_{18} 5-µm column (250 × 4.6 mm). Curcumin was detected at 425 nm with a UV-visible detector (Waters model 2487). The mobile phase consisted of acetonitrile:2% acetic acid (40:60, v/v) run isocratically at a flow rate of 2.0 mL/min. The retention time for curcumin was 2.7 min. The curcumin retention coefficient (CRC) in the cheeses was calculated using the equation CRC = C_c/C_m, where Cc and Cm are the concentration of curcumin in cheese and milk, respectively.

The fat in the cheeses was extracted with chloroform:methanol (1:2, v/v). Briefly, 10 g of finely grated sample was homogenised with 30 mL of extraction solvent and 2 mL of DDW; then, 10 mL of chloroform was added to homogenise again for 30 s. Another 10 mL of DDW was added, and the mixture was homogenised once more for 30 s. The homogenate was filtered and transferred to a separatory funnel; the lower phase was drained and rotary evaporated. The extracted fat was derivatised, and the fatty acid methyl esters was quantified by GC under the conditions described earlier for the characterisation of milk fat.

The colour of the cheeses was measured in L^*, a^*, and b^* units on the CIE Lab colour scale at three different sites on the surface using the CM-5 Konica Minolta colorimeter. All analytical determinations were performed in triplicate (n = 3). Data were expressed as means ± standard deviations.

A sensory analysis of the curcumin-enriched Manchego-type cheeses was performed through a consumer acceptability test (Liggett *et al.,* 2008; Braghieri *et al.,* 2015). A total of 100 untrained panellists (54 males and 46 females, aged 18 to 62) were recruited from the lobby of the Industrial Process Division Building on the campus of The Technological University of Tabasco (Tabasco, Mexico). They were selected based on their desire to evaluate Manchego-type cheese, and because they ate Manchego-type cheese at least once a month. Each participant evaluated four 2.0- to 2.5-g cubes

(three of the curcumin-enriched cheeses and one of the control cheese) in a controlled sensory analysis laboratory.

Cheese cubes were served in random order at approximately 10°C in a polypropylene container, and labelled with a neutral 3-digit number. Room-temperature spring water was provided for rinsing. For each cheese, panellists were asked to assess the overall acceptability and the following 5 sensory properties: appearance, colour, odour, flavour, and texture. Consumers ranked these features on a 9-point hedonic scale labelled at the left end as "extremely unpleasant" (score = 1), at the right end as "extremely pleasant" (score = 9), and at the central point (score = 5) as "neither pleasant nor unpleasant" (Braghieri *et al.,* 2015).

Experimental design and data analysis

The experiment was carried out according to a completely randomised design with a total of four treatments, one corresponding with the control cheese (10 ppm of bixin) and three with the curcumin-enriched cheeses (5, 7.5, and 10 ppm of curcumin, respectively), and three repetitions each. The results were analysed using a repeated measures ANOVA and Tukey's honestly significant difference *post-hoc* comparison to determine whether there were significant differences between the samples. Analyses were conducted in Statistica (version 6.0, StatSoft Inc., Tulsa, OK).

Results and discussion

Characterisation of milk

The physicochemical characteristics of the Pelibuey sheep milk used to make Manchego-type cheeses added with NEC are shown in Table 1. The milk was rich in total solids (17.14% ± 0.19%), particularly in protein (5.66% ± 0.07%) and fat (5.41% ± 0.25%), which is common for sheep milk. These results are consistent with those reported by Gómez-Cortés *et al.* (2009) and Bucevic-Popovic *et al.* (2014), who reported values from 4.91 to 5.80% for protein content, 5.60 to 6.51% for fat content, and 16.53 to 17.97% for total solids content.

The milk had a TPC of 139.34 ± 26.37 mg GAE/L, and AA of 15.99 ± 3.58 and 5.06 ± 0.97 mmol TE/mL according to the DPPH and FRAP assays, respectively. This AA can be attributed to the presence of some vitamins, enzymes, proteins, and amino acids, as well as some peptides resulting from the hydrolysis of proteins (Bucevic-Popovic *et al.,* 2014). In accordance with our results, Yilmaz-Ersan *et al.* (2018) evaluated the AA of raw sheep milk using the DPPH and FRAP methods, and found a higher AA of 8.7 mg TE/100 mL with the DPPH assay in comparison to the FRAP assay (5.82 mg TE/100 mL). Several other authors have used these assays to evaluate the AA of milk (Bucevic-Popovic *et al.* 2014; Yilmaz-Ersan *et al.,* 2018). However, according to Zarban *et al.* (2009), the DPPH method is the most appropriate for the evaluation of the AA of milk.

Table 1. Physicochemical characteristics of Pelibuey sheep milk used to make curcumin-enriched Manchego-type cheeses.

Parameter	Value
Density at 15°C (g/mL)	1.0304 ± 0.0003
Acidity (% of lactic acid)	0.22 ± 0.001
Fat (%)	5.41 ± 0.25
Protein (%)	5.66 ± 0.07
Lactose (%)	4.50 ± 0.07
Ash (%)	1.06 ± 0.05
Total solid (%)	17.14 ± 0.19
Non-fat solid (%)	11.73 ± 0.22
TPC (mg GAE/L)	139.34 ± 26.37
AA by DPPH (mmol TE/mL)	15.99 ± 3.58
AA by FRAP (mmol TE/mL)	5.06 ± 0.97

TPC = total phenolic content; AA = antioxidant activity; GAE/L = Gallic Acid Equivalents per L of milk; and TE/mL = Trolox Equivalents per mL of milk.

Table 2 shows the concentration of fatty acids (FAs) in the milk. The most abundant FAs were palmitic (16:0), oleic (18:1 *n*-9), myristic (14:0), and stearic (18:0) acids, with a concentration of 22.554 ± 0.651, 22.082 ± 0.783, 9.253 ± 0.324, and 8.738 ± 0.263 g/100 g of fat, respectively. The milk included high proportions of short-chain FAs (SCFAs) (13.629 ± 0.395%) and medium-chain FAs (MCFAs) (13.992 ± 0.602%). Saturated FAs (SFAs) constituted about 60% of the total FAs, whereas the proportion of polyunsaturated FAs (PUFAs) was very low (2.384 ± 0.113%). Oleic acid was the second most abundant FA in the milk, and also the most abundant of the monounsaturated FAs (MUFAs). Four odd-chain FAs were detected: 15:0, 17:0, 15:1 *n*-8, and 17:1 *n*-7.

The FA concentrations of the milk analysed herein are similar to those reported by Payandeh *et al.* (2017), who found the SFAs palmitic (16:0), stearic (18:0), myristic (14:0), capric (10:0), and butyric (4:0) acids at concentrations of 23.437, 11.847, 8.540, 5.880, and 3.667 g/100 g of fat, respectively. They also found oleic acid (18:1 *n*-9) to be the most abundant MUFA (23.232 g/100 g). Overall, the

Table 2. Concentration of fatty acids in the fat of Pelibuey sheep milk used to make curcumin-enriched Manchego-type cheeses.

Fatty acid		g/100 g of fat
Butyric acid	4:0	3.106 ± 0.113
Caproic acid	6:0	2.318 ± 0.055
Caprylic acid	8:0	2.174 ± 0.093
Capric acid	10:0	6.031 ± 0.360
Lauric acid	12:0	4.070 ± 0.259
Myristic acid	14:0	9.253 ± 0.324
5-Tetradecenoic acid	14:1 *n*-9	0.200 ± 0.006
7-Tetradecenoic acid	14:1 *n*-7	0.196 ± 0.005
Myristoleic acid	14:1 *n*-5	0.359 ± 0.012
Pentadecanoic acid	15:0	0.668 ± 0.019
7-Pentadecenoic acid	15:1 *n*-8	0.208 ± 0.007
Palmitic acid	16:0	22.554 ± 0.651
7-Hexadecenoic acid	16:1 *n*-9	0.397 ± 0.011
Palmitoleic acid	16:1 *n*-7	0.868 ± 0.027
11-Hexadecenoic acid	16:1 *n*-5	0.304 ± 0.016
Heptadecanoic acid	17:0	0.436 ± 0.010
10-Heptadecenoic acid	17:1 *n*-7	0.205 ± 0.016
Stearic acid	18:0	8.738 ± 0.263
Oleic acid	18:1 *n*-9	22.082 ± 0.783
Linoleic acid	18:2 *n*-6	2.063 ± 0.157
Linolenic acid	18:3 *n*-3	0.321 ± 0.040
Short chain	(SCFAs)	13.629 ± 0.395
Medium chain	(MCFAs)	13.992 ± 0.602
Long chain	(LCFAs)	31.729 ± 0.924
Saturated	(SFAs)	59.350 ± 1.920
Monounsaturated	(MUFAs)	24.818 ± 0.883
Polyunsaturated	(PUFAs)	2.384 ± 0.113
Atherogenicity index	(AI)	2.340 ± 0.005

SCFAs = 4:0 + 6:0 + 8:0 + 10:0; MCFAs = 12:0 + 14:0 + 15:0; LCFAs = 16:0 + 17:0 + 18:0; SFAs = SCFAs + MCFAs + LCFAs; MUFAs = 14:1 *n*-9 + 14:1 *n*-7 + 14:1 *n*-5 + 15:1 *n*-7 + 16:1 *n*-9 + 16:1 *n*-7 + 16:1 *n*-3 + 17:1 *n*-8 + 18:1 *n*-9; PUFAs = 18:2 *n*-6 + 18:3 *n*-3; and AI = (12:0 + (4 × 14:0) + 16:0)/(PUFAs + MUFAs).

concentrations of the SFAs, MUFAs, and PUFAs as a whole were 65.923, 29.372, and 4.513 g/100 g, respectively. In relation to the odd-chain FAs, Gómez-Cortés *et al.* (2009) reported the presence of 15:0, 17:0, 15:1, and 17:1 FAs at concentrations of 0.94, 0.54, 0.09, and 0.19%, respectively, in accordance with our results.

Characterisation of NEC

The NEC formulated with curcumin, soy lecithin, glycerol, DDW, and medium-chain oil had a small droplet size of 59.98 ± 1.15 nm, a polydispersity index of 0.35 ± 0.02, and a zeta potential of -6.88 ± 1.03 mV. The NEC had a curcumin concentration of 2.50 ± 0.09 mg/g of NE as determined by HPLC with a high entrapment efficiency (100.43 ± 0.54%). Often, the use of co-surfactants helps to optimise NE formation. So, the addition of glycerol in the proper amount can help to enhance the quality of the NE, which can become optically translucent if the droplet size, refractive index, and turbidity are reduced (Saberi *et al.,* 2013). In the present work, the use of glycerol in the NE allowed a droplet size of only 60 nm to be obtained

along with a more or less low polydispersity index value. All of these features make NEC suitable for use in food processing (Silva *et al.,* 2012).

Characterisation of Manchego-type cheese

Cheese is a good source of proteins, minerals, and functional compounds such as bioactive peptides, conjugated linoleic acid, and a small amount of phenols (Henning *et al.,* 2006). In addition, herbs, extracts, essential oils, or other bioactive compounds of natural origin have been added as a source of beneficial compounds that promote consumer health (Khalifa and Wahdan, 2015; Rashidinejad *et al.,* 2016; Del Olmo *et al.,* 2019). In the present work, the fortification of Manchego-type cheese with curcumin (at 5, 7.5, and 10 ppm) did not significantly affect ($p > 0.05$) the moisture, protein, fat, ash, total solid, and non-fatty solid contents, nor the water activity and yield as compared to the control cheese (Table 3).

These results are consistent with those reported by Rashidinejad *et al.* (2013; 2016) for full-fat cheese added with green tea catechins, and low-fat cheese added with catechin and epigallocatechin. However, Giroux *et al.* (2013) found that the addition of green tea extract to Cheddar cheese at a high concentration (2 g/kg of milk) had a significant effect ($p < 0.05$) on the moisture content as compared to the control cheese. Therefore, it is possible that a higher concentration of NEC could have a greater effect on the composition of cheese than that observed herein. Khalifa and Wahdan (2015) also found that the addition of 500, 750, and 1,000 ppm cranberry extract in a soft white cheese increased its moisture content and yield.

Proteolysis and lipolysis are two of the most

Table 3. Physicochemical characteristics of curcumin-enriched Manchego-type cheeses.

Parameter	Control	NEC		
		5 ppm	7.5 ppm	10 ppm
Moisture (%)	50.87 ± 0.94^{a}	49.42 ± 0.77^{a}	49.34 ± 2.36^{a}	44.17 ± 4.67^{a}
Protein (%)	22.43 ± 0.43^{a}	22.85 ± 0.41^{a}	23.31 ± 0.11^{a}	23.54 ± 0.63^{a}
Fat (%)	21.82 ± 1.22^{a}	22.50 ± 1.47^{a}	23.57 ± 0.61^{a}	24.46 ± 2.00^{a}
Ash (%)	3.64 ± 0.03^{a}	3.66 ± 0.04^{a}	3.71 ± 0.18^{a}	3.75 ± 0.01^{a}
Total solid (%)	49.13 ± 0.94^{a}	50.58 ± 0.77^{a}	50.66 ± 2.36^{a}	55.83 ± 4.67^{a}
Non-fat solid (%)	27.31 ± 2.01^{a}	28.08 ± 2.24^{a}	27.09 ± 2.00^{a}	31.37 ± 4.53^{a}
Water activity at 25.5°C	0.969 ± 0.001^{a}	0.972 ± 0.001^{a}	0.972 ± 0.003^{a}	0.973 ± 0.004^{a}
Yield (%)	17.27 ± 0.67^{a}	16.05 ± 0.50^{a}	16.94 ± 0.10^{a}	16.29 ± 0.56^{a}
TN (%)	3.52 ± 0.07^{a}	3.58 ± 0.07^{a}	3.65 ± 0.03^{a}	3.69 ± 0.09^{a}
SN-W (%)	1.01 ± 0.06^{a}	1.15 ± 0.19^{ab}	1.17 ± 0.05^{ab}	1.36 ± 0.15^{b}
SN-pH 4.6 (%)	0.60 ± 0.06^{a}	0.65 ± 0.01^{ab}	0.69 ± 0.04^{ab}	0.74 ± 0.07^{b}
SN-TCA (%)	0.51 ± 0.03^{a}	0.51 ± 0.02^{a}	0.55 ± 0.03^{a}	0.55 ± 0.02^{a}
SN-PTA (%)	0.15 ± 0.01^{a}	0.19 ± 0.02^{ab}	0.22 ± 0.02^{b}	0.30 ± 0.01^{c}
RI (%)	28.84 ± 2.01^{a}	32.26 ± 5.64^{ab}	31.89 ± 1.41^{ab}	37.01 ± 5.13^{b}
FFAC (KOH mE/100 g)	3.13 ± 0.25^{a}	2.14 ± 0.14^{b}	1.97 ± 0.13^{b}	1.99 ± 0.23^{b}
TPC (mg GAE/100 g)	213.49 ± 3.56^{a}	245.65 ± 8.81^{b}	280.90 ± 3.13^{c}	307.91 ± 4.41^{d}
AA by DPPH (mmol TE/g)	11.66 ± 0.81^{a}	11.90 ± 0.39^{a}	12.66 ± 0.47^{ab}	13.73 ± 0.64^{b}
AA by FRAP (mmol TE/g)	3.45 ± 0.03^{a}	3.74 ± 0.30^{a}	4.27 ± 0.09^{b}	4.73 ± 0.10^{c}
C content (ppm)	-----	4.30 ± 0.09^{a}	6.14 ± 0.19 ^{b}	8.04 ± 0.44^{c}
CRC	-----	0.86 ± 0.02^{a}	0.82 ± 0.03^{a}	0.80 ± 0.04^{a}

Values are reported as mean ± S.D. of triplicate determinations ($n = 3$). Means with different letters are significantly different ($p < 0.05$). SN-W = soluble nitrogen in water; SN-pH 4.6 = soluble nitrogen at pH 4.6 adjusted with sodium citrate; SN-TCA = soluble nitrogen in trichloroacetic acid; SN-PTA = soluble nitrogen in phosphotungstic acid; RI = ripening index, reported as percent of SN-W/TN; TN = total nitrogen; FFAC = free fatty acid content; TPC = total phenolic content; AA = antioxidant activity; C = curcumin; and CRC = curcumin retention coefficient.

important processes that occur during cheese ripening. During these processes, some peptides and amino acids are released, which constitute the SN fractions (Giroux *et al.* 2013). Fat in cheese can also be degraded, resulting in the release of FAs that impact sensory characteristics (Kurcubic *et al.,* 2015). In the present work, curcumin-enriched Manchego-type cheese had a higher SN fraction than the control sample. Significant differences ($p < 0.05$) were observed in the SN-W and SN-pH 4.6 fractions of cheese with 10 ppm of curcumin, as well as in the SN-PTA fraction of cheeses with 7.5 and 10 ppm of curcumin as compared to the control cheese. Overall, however, the cheeses made with NEC had a lower FFAC ($p < 0.05$) than the control cheese (Table 3).

Tarakci *et al.* (2006) found that the addition of mendi to Turkish cheese has a significant effect on the RI, although these differences were only significant ($p < 0.05$) for cheeses added with the highest concentrations of mendi tested (2 and 3%) and not for concentrations of 0.5 and 1%, which is in agreement with our results. Khalifa and Wahdan (2015) and Giroux *et al.* (2013) found that the supplementation of cheeses with dried cranberry or green tea extract, respectively, resulted in a significant decrease in proteolysis as compared to control cheeses. These differences can be attributed to variation in the moisture content of cheese, inhibition of proteolytic enzymes, or steric hindrance resulting from the union between polyphenols and casein, which hinders hydrolysis.

On the other hand, Kurcubic *et al.* (2015) evaluated the effect of an extract of *Kitaibelia vitifolia* on the characteristics of a Serbian cheese, and Khalifa and Wahdan (2015) evaluated the effect of a cranberry extract on a soft white cheese. Both observed a significant decrease in the FFAC of the cheeses made with these extracts as compared to the control cheeses. These results are similar to those obtained herein. However, the addition of wild garlic to a Turkish cheese led to a significant increase in the FFAC as compared to the control cheese (Tarakci *et al.* 2011). These differences, as reported by Del Olmo *et al.* (2019), may be due to the nature of the materials used and their lipolytic activity, the characteristics of the extracts, or the cheese-making process.

As expected, higher values of TPC and AA were found for the curcumin-enriched cheeses as compared to the control cheese due to addition of curcumin, a natural polyphenolic compound with powerful antioxidant activity. Higher TPC and AA were found in cheeses with a higher amount of NEC (Table 3), and significant differences ($p < 0.05$) were observed in TPC among the evaluated treatments. The AA determined by the DPPH method showed significant differences ($p < 0.05$) between the control cheese and the cheese added with 10 ppm of curcumin. The AA determined by the FRAP method showed significant differences ($p < 0.05$) between the control cheese and the cheeses added with 7.5 and 10 ppm of curcumin. These results are consistent with those reported by Rashidinejad *et al.* (2016), who reported that the fortification of cheeses with different products rich in polyphenols significantly increased their TPC and AA.

The curcumin content of the cheeses enriched with 5, 7.5, and 10 ppm of curcumin during the cheese-making process was 4.30 ± 0.09, 6.14 ± 0.19, and 8.04 ± 0.44 ppm, respectively, according to HPLC. Table 3 shows the CRC of the enriched cheeses. No significant differences ($p > 0.05$) were found among the cheeses, and the average CRC was 0.83 ± 0.03. The high CRC values indicate that the added phenolic compound was highly retained in cheese curds, and this also implies that its loss was minimal during the cheese-making process. According to Han *et al.* (2011), the RC of polyphenols depends on various factors such as their interaction with binding sites on the protein molecules, their polarity and solubility in water or lipid micelles, and their distribution between the solid matrix and liquid phase. Some studies have shown that curcumin can hydrophobically bind with milk casein and β-lactoglobulin, which may greatly improve its solubility and stability (Wu and Wang, 2017). Helal *et al.* (2015) investigated the retention of different phenolic compounds during curd formation on polyphenol-enriched cheeses and found a positive correlation between the degree of hydrophobicity of the phenolic compound and its RC in curds.

Table 4 shows the concentration of FAs in cheese per treatment. In both cheese and milk, there was a high content of SFAs including palmitic (16:0), myristic (14:0), and stearic (18:0) acid. In regard to MUFAs, the oleic acid content (18:1 *n*-9) is notable. Significant differences ($p < 0.05$) in the FA concentrations were observed between the cheeses with NEC and the control cheese for 15 of the 21 FAs evaluated. No significant differences ($p > 0.05$) were found in the concentration of caprylic (8:0), capric (10:0), lauric (12:0), myristic (14:0), and stearic (18:0) acids, or in the concentration of the MUFA linolenic acid (18:3 *n*-3). The addition of NEC increased the concentration of palmitic acid (16:0), MUFAs, and one PUFA (linoleic acid, 18:2 *n*-6). However, no significant differences ($p > 0.05$) were

Table 4. Concentration of fatty acids in g/100 g of fat of curcumin-enriched Manchego-type cheeses.

Fatty acid	Control	NEC		
		5 ppm	7.5 ppm	10 ppm
4:0	3.32 ± 0.34[a]	13.02 ± 0.18[c]	6.50 ± 0.46[b]	2.82 ± 0.11[a]
6:0	2.09 ± 0.33[a]	4.58 ± 0.49[b]	3.65 ± 0.01[b]	2.09 ± 0.03[a]
8:0	2.03 ± 0.22[a]	2.69 ± 0.20[a]	2.22 ± 0.21[a]	2.00 ± 0.01[a]
10:0	5.49 ± 0.61[a]	5.77 ± 0.25[a]	5.62 ± 0.13[a]	5.44 ± 0.07[a]
12:0	3.43 ± 0.10[a]	3.43 ± 0.03[a]	3.50 ± 0.40[a]	3.66 ± 0.05[a]
14:0	7.00 ± 0.98[a]	7.40 ± 0.66[a]	7.95 ± 0.91[a]	8.66 ± 0.10[a]
14:1 *n*-9	0.15 ± 0.01[a]	0.17± 0.01[ab]	0.18 ± 0.01[b]	0.19 ± 0.01[b]
14:1 *n*-7	0.14 ± 0.01[a]	0.15 ± 0.01[a]	0.16 ± 0.01[ab]	0.18 ± 0.01[b]
14:1 *n*-5	0.26 ± 0.01[b]	0.22 ± 0.01[a]	0.25 ± 0.01[b]	0.34 ± 0.01[c]
15:0	0.50 ± 0.01[a]	0.55 ± 0.03[ab]	0.61 ± 0.02[bc]	0.65 ± 0.01[c]
15:1 *n*-7	0.15 ± 0.01[a]	0.17 ± 0.02[ab]	0.19 ± 0.01[ab]	0.20 ± 0.01[b]
16:0	16.80 ± 0.74[a]	19.57 ± 0.75[b]	20.31 ± 0.66[b]	20.66 ± 0.17[b]
16:1 *n*-9	0.23 ± 0.01[a]	0.32 ± 0.01[b]	0.36 ± 0.03[b]	0.37 ± 0.01[b]
16:1 *n*-7	0.28 ± 0.01[a]	0.37 ± 0.02[b]	0.38 ± 0.03[b]	0.38 ± 0.01[b]
16:1 *n*-3	0.20 ± 0.01[a]	0.27 ± 0.02[b]	0.27 ± 0.01[b]	0.29 ± 0.01[b]
17:0	0.31 ± 0.01[a]	0.40 ± 0.02[ab]	0.43 ± 0.05[b]	0.42 ± 0.01[b]
17:1 *n*-8	0.16 ± 0.01[a]	0.17 ± 0.01[a]	0.23 ± 0.03[ab]	0.26 ± 0.01[b]
18:0	6.61 ± 0.58[a]	7.34 ± 0.78[a]	7.40 ± 0.05[a]	7.95 ± 0.09[a]
18:1 *n*-9	15.36 ± 0.37[a]	16.37 ± 1.55[a]	18.20 ± 0.89[ab]	20.12 ± 0.17[b]
18:2 *n*-6	1.48 ± 0.06[a]	1.69 ± 0.06[ab]	1.79 ± 0.14[ab]	1.92 ± 0.01[b]
18:3 *n*-3	0.25 ± 0.02[a]	0.24 ± 0.04[a]	0.24 ± 0.05[a]	0.32 ± 0.01[a]
SCFAs	12.93 ± 1.50[a]	26.06 ± 1.12[c]	17.99 ± 0.82[b]	12.35 ± 0.21[a]
MCFAs	10.94 ± 1.09[a]	11.38 ± 0.72[a]	12.05 ± 1.33[ab]	12.97 ± 0.16[b]
LCFAs	23.72 ± 1.32[a]	27.31 ± 1.55[b]	28.13 ± 0.75[b]	29.03 ± 0.27[b]
SFAs	47.59 ± 3.90[a]	64.75 ± 3.39[d]	58.17 ± 2.90[c]	54.35 ± 0.64[b]
MUFAs	16.94 ± 0.42[a]	18.20 ± 1.63[ab]	20.23 ± 1.01[b]	22.35 ± 0.19[c]
PUFAs	1.72 ± 0.08[a]	1.93 ± 0.10[ab]	2.04 ± 0.18[b]	2.24 ± 0.02[b]
AI	0.26 ± 0.02[a]	0.26 ± 0.04[a]	0.25 ± 0.03[a]	0.24 ± 0.01[a]

Values are reported as mean ± S.D. of triplicate determinations ($n = 3$). Means with different letters are significantly different ($p < 0.05$). SCFAs = 4:0 + 6:0 + 8:0 + 10:0; MCFAs = 12:0 + 14:0 + 15:0; LCFAs = 16:0 + 17:0 + 18:0; SFAs = SCFAs + MCFAs + LCFAs; MUFAs = 14:1 *n*-9 + 14:1 *n*-7 + 14:1 *n*-5 + 15:1 *n*-7 + 16:1 *n*-9 + 16:1 *n*-7 + 16:1 *n*-3 + 17:1 *n*-8 + 18:1 *n*-9; PUFAs = 18:2 *n*-6 + 18:3 *n*-3; and AI = (12:0 + (4 × 14:0) + 16:0)/(PUFAs + MUFAs).

observed in the atherogenic index (AI) between the curcumin-enriched cheeses and the control cheese.

The colour measurements of the evaluated treatments are shown in Table 5. The values of L^*, a^*, and b^* between the cheeses with NEC and the control cheese were significantly ($p < 0.05$) different. The L^* and a^* values were lower in cheeses with NEC as compared to the control cheese, while the b^* values were higher in the cheeses with NEC, indicating that the addition of NEC decreased the luminosity of cheese and gave it a green-yellow colour. Some authors such as Tarakci *et al.* (2011) and Giroux *et al.* (2013) similarly reported that the luminosity (L^*) of cheese decreased as the concentration of additives increased. Giroux *et al.* (2013) also reported an increase in the b^* values as the concentration of additives increased, which accentuated the yellow colour of cheese. In relation

Table 5. Colour parameters (*L**, *a**, and *b**)(1) and sensory scores(2) of curcumin-enriched Manchego-type cheeses.

Parameters	Control	NEC		
		5 ppm	7.5 ppm	10 ppm
*L**	77.13 ± 1.51[a]	75.03 ± 0.49[ab]	74.03 ± 1.04[ab]	76.50 ± 0.36[b]
*a**	0.57 ± 0.67[a]	-0.90 ± 0.78[b]	-0.73 ± 0.76[b]	-1.90 ± 0.78[c]
*b**	11.57 ± 1.66[a]	18.07 ± 2.12[b]	18.80 ± 1.77[b]	20.30 ± 0.90[b]
Appearance	6.74 ± 0.81[a]	5.67 ± 1.00[b]	5.77 ± 1.08[b]	5.70 ± 1.22[b]
Colour	6.80 ± 0.69[a]	6.06 ± 0.95[b]	6.09 ± 0.87[b]	6.01 ± 1.06[b]
Odour	6.85 ± 0.68[a]	6.16 ± 0.88[b]	6.40 ± 0.60[ab]	6.16 ± 0.92[b]
Flavour	6.08 ± 1.17[a]	6.27 ± 1.22[a]	6.31 ± 1.24[a]	6.19 ± 1.36[a]
Texture	6.97 ± 1.00[a]	6.92 ± 1.06[a]	7.04 ± 1.02[a]	6.00 ± 1.15[a]
Overall acceptability	6.82 ± 1.14[a]	6.46 ± 1.06[a]	6.66 ± 1.05[a]	6.73 ± 1.13[a]

(1)Values are reported as mean ± S.D. of triplicate determinations ($n = 3$). (2)Values are reported as mean ± S.D. of 100 determinations ($n = 100$). Means with different letters are significantly different ($p < 0.05$).

to the *a** value, Tarakci *et al.* (2011) found a significant decrease in this value in a Turkish cheese following the addition of wild garlic. However, Giroux *et al.* (2013) discovered an increase in the *a** value as the concentration of green tea increased, highlighting the impact of this additive and its colour on the colour of processed cheese.

Table 5 shows the results of the liking test with 100 consumers. All sensory properties of the evaluated samples, except appearance, were rated above the neutral point (neither pleasant nor unpleasant). These results show that both Manchego-type cheese made with the colouring agent bixin and NEC were perceived as having good eating quality. The enrichment of cheeses with curcumin did not influence overall acceptability in terms of flavour and texture. However, curcumin-enriched cheeses received significantly lower ratings ($p < 0.05$) in relation to the acceptability of the appearance, colour, and odour as compared to the control cheese. Although the mean acceptability ratings might seem low, these scores reflect findings similar to other studies on cheeses. Liggett *et al.* (2008) conducted preference mapping with Swiss cheeses, and obtained consumer acceptability scores ranging from 4.4 to 6.0 on a 9-point scale. More recently, Braghieri *et al.* (2015) evaluated the consumer acceptability of Scamorza cheeses, and obtained scores ranging from 6.2 to 7.0.

Conclusions

Several studies have evidenced the remarkable antioxidant capacity of curcumin and its protective effect against CVD. The present work describes the enrichment of milk with NEC to develop a novel cheese product. The resulting Manchego-type cheeses enriched with NEC had high CRC values and, consequently, high TPC and AA values. These cheeses also had similar physicochemical properties as the control cheese, and were rated by panellists as having good eating quality. Based on these results, Manchego-type cheese made from Pelibuey sheep milk and enriched with NEC could represent an innovative functional food considering that curcumin is a bioactive compound with proven beneficial health effects.

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