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Influence of the frozen storage on some characteristics of ripened Manchego-type cheese manufactured with a powdered vegetable coagulant and rennet

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Abstract

A study was made of the effect of freezing and nine months' frozen storage on the chemical, microbiological and sensorial characteristics of fully ripened Manchego-type cheese. Chemical components, water activity and nitrogen compounds were not altered by freezing and frozen storage. Soluble and non-protein nitrogen were significantly higher in cheeses produced with the powdered vegetable coagulant (PVC) compared to those made with rennet. Proteolysis continued slowly during frozen storage, with higher amino acid and ammonia nitrogen rates at the end of the storage period. Micrococci (*P*-value <0.05, in cheese made with PVC) and staphylococci counts tended to decrease during frozen storage; however total viable, lactic acid bacteria and mold-yeasts counts were similar throughout the nine months. Changes in sensorial parameters were observed (*P*-value <0.05) throughout freezing and frozen storage, affecting the creaminess of cheeses made with chymosin and the taste intensity and salty taste of cheese obtained with vegetable coagulant. Cheeses obtained with PVC proved to have a more acid taste and greater taste intensity ($P < 0.01$) as well as displaying a more bitter taste, greater creaminess and lower degree of hardness (*P*-value <0.001) than those produced with rennet.

Keywords: Manchego-type cheese; Freezing; Frozen storage; Proteolysis; Microbiology; Organoleptic; Vegetable coagulant

1. Introduction

Due to the largely seasonal nature of a great part of sheep milk production for cheese manufacture, with a marked increase in spring, every year there tends to be a surplus of – mainly traditional – ripened ewes' milk cheese from June to October, causing prices to drop and adversely affecting the marketing and sale of cheeses. To alleviate this general situation and regulate this market, a number of storage-related tests have been performed, hitherto with little success, using a range of procedures such as milk freeze-drying, which led to deficiencies in the taste of the cheeses tested (FIL, 1982); or milk freezing, which prompted the appearance of lactose crystals and casein aggregates. Freezing of concentrated milk induces the incorporation of phosphorus and calcium in micellar casein, prompting drastic alterations in its structure and giving rise to the formation of casein precipitates and protein flocculation (Muir, 1984). It has been reported (Voutsinas, Katsiari, Pappas, & Mallatou, 1995) that Feta cheese prepared from non-filtered frozen ewes' milk gave rise to significant organoleptic deficiencies.

Freezing of cheese curd has traditionally been considered the most suitable alternative for regulating the

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cheese market (Veisseyre, 1980); and numerous studies have been carried out on different cheeses ripened from frozen curds (Alichanidis, Polichroniadou, Tzanetakis, & Vafopoulou, 1981; Bertola, Califano, Bevilacqua, & Zaritzky, 1996; Fontecha, Peláez, Juárez, & Martín-Hernández, 1994). However, few authors have studied the effect of freezing on fully ripened and partially ripened cheeses, even though this preservation method is routinely used in many cheeses factories. The most noteworthy of these studies have focused on partial aspects of the sensory, functional or chemical characteristics of Gorgonzola and Provolone (Ottogalli & Rondinini, 1974), mozzarella (Califano & Bevilacqua, 1999; Chaves, Viotto, & Grosso, 1999; Oberg, Merrill, Brown, & Richardson, 1992) and Cheddar (Kasprzak, Wendorff, & Chen, 1994; Sen & Gupta, 1987). Lück (1977) concluded that frozen storage was suitable for cream cheese, unripened Camembert, and Brick cheese, but not for Gouda or Cheddar cheese. Cervantes, Lund, and Olson (1983) concluded that freezing (one-week storage) and thawing did not affect the quality of mozzarella cheese as assessed by compression, beam bending and sensory evaluation. Recent studies of the effect of freezing rates and frozen storage duration on the sensorial (Tejada et al., 2000), chemical and microbiological characteristics (Tejada, Sánchez, Gómez, Vioque, & Fernández-Salguero, 2002) of ripened Los Pedroches cheese (a homemade Spanish semihard cheese made from raw ewes' milk) concluded that this cheese could be stored at −20 °C for approximately 6 months without any significant alteration of the characteristics studied. However, the frozen storage was not suitable for a small-format, goats' milk cheese (Pino, 2005), producing a crumbly texture. In view of all these results, the old adage that cheese must not be frozen might usefully be re-examined for each variety of cheese.

Manchego cheese is named after the La Mancha region (Spain), where the original product has been traditionally made from sheep's milk. Since 1984, Manchego cheese has been protected by a Spanish Denomination of Origin and its manufacture and final features are controlled by a Regulator Council (Marcos & Esteban, 1983). Cheeses similar to Manchego, produced from ewes' milk but also using binary and ternary milk mixtures of cow, sheep and/or goat milk, do no enjoy the legal protection afforded by Denomination of Origin status, and are traditionally marketed as so-called "Manchego-type cheeses". This type of cheese is mainly produced in the Castilla-La Mancha and Castilla-León regions of Spain and, to a lesser extent, in other Spanish regions. Pasteurised, whole ewes' milk from once- or twice-daily milking is generally used, but raw milk may also be used. Starter cultures may be added and liquid or powdered calf rennet is normally used but vegetable coagulant from dried flowers of the cardoon *Cynara cardunculus* L. has been used in the past. This vegetable coagulant is still used in other Spanish and Portuguese varieties of sheep's cheese enjoying *Appellation d'Origine Controllée* status (Fernández-Salguero & Sanjuán, 1999). The curds are dry-salted or submerged in brine (22° Brix) for several days. Ripening (for 2, 6 or 12 months, depending on the target market outlet, to produce semi-hard to hard consistency) is carried out in a curing chamber at around 10 °C with a varying degree of relative humidity. The cheeses are cylindrical in shape, 10–14 cm high, 18–22 cm in diameter, and weigh 2–3.5 kg. The rind is brown to pale yellow, and the interior is compact with small eyes. The flavour is pronounced, and cheeses ripened for longer than nine months tend to be moderately piquant.

The aim of this paper was to provide much-needed data on the freezing of ripened cheese, and to complement the studies mentioned earlier on the Los Pedroches variety by determining the effect of freezing and frozen storage for 9 months on the chemical, microbiological and sensorial characteristics of ripened Manchego-type cheese (180 days' ripening) made with animal rennet or a powdered vegetable coagulant developed by the authors (Fernández-Salguero, Tejada, & Gómez, 2002) from aqueous extract of the dried cardoon flowers (*C. cardunculus* L.).

2. Materials and methods

2.1. Cheese-making procedure and sampling

Batches of Manchego-type cheese were made on different days using raw ewes' milk and commercial starter culture. The amount obtained during each milking day was split into two batches, one of which was coagulated with animal rennet (AR) by adding about 2.5 g/100 l milk (commercial calf rennet powder with 80% chymosin and 20% pepsin, from Chr. Hansen) and the other using a powdered vegetable coagulant (PVC) obtained as previously described elsewhere (Fernández-Salguero et al., 2002). The milk obtained on another day was split identically into two batches and coagulated with the same types of coagulant. Therefore, two experimental batches were coagulated with calf rennet and another two with vegetable coagulant. A commercial starter culture EZAL® from Larbus S.A. (Madrid, Spain) containing a mixture of *Lactococcus lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis* var. *diacetylactis* and *Streptococcus thermophilus* was added to the milk. Each of the four experimental batches consisted of 4 cheeses (using about 5.6 l of milk/kg of cheese). The clotting temperature for milk was 29 ± 1 °C and the clotting took 45–55 min. After pressing, the cheeses were salted on brine, ripened during 180 days under controlled conditions at 11 °C and 85% relative humidity. Before freezing of cheeses, a sample of each batch was taken for analysis (hereafter referred to as control cheese); the remainder were frozen at −20 °C and kept under frozen storage for 3, 6 and 9 months in a freezer.

2.2. Compositional analyses

Determinations included measurements of moisture, protein, lactic acid, ash and sodium chloride (AOAC, 1980) and fat (Gerber-Van Gulik), pH and water activity (a_w) as described previously (Fernández-Salguero, Sanjuán, & Montero, 1991).

2.3. Nitrogen fractions

Soluble nitrogen (SN) at pH 4.6, non-protein nitrogen [NPN, soluble in 12% trichloroacetic acid, (TCA)], amino acid nitrogen (AAN), and ammonia nitrogen (NH_3-N) were determined as described elsewhere (Carmona, Sanjuán, Gómez, & Fernández-Salguero, 1999). All samples were analyzed in duplicate.

2.4. Microbiological analyses

All microbial groups, except staphylococci, were analysed according to APHA (1984) as follows: aerobic bacteria were determined on plate count agar (PCA, Oxoid Limited, Basingstoke, UK) and incubated at 30 °C for 72 h; total enterobacteria (Gram negative and citocromo-oxidase negative) on violet red bile glucose agar (VRBG) and incubated at 37 °C for 24–48 h; coliforms on violet red bile agar (VRBA) and incubated at 37 °C for 24 h; micrococci on mannitol salt agar (MSA; Oxoid) incubated at 37 °C for 72 h; lactic acid bacteria on MRS agar in anaerobiosis and incubated a 37 °C for 24 h and moulds and yeasts on potato dextrose agar (PDA; Oxoid) and incubated at 26 °C for 96 h. Staphylococci on Baid Parker agar (BP; Oxoid) incubated at 37 °C for 24–48 h as described elsewhere (Buttiaux, 1974). All determinations were made in duplicate and expressed as log colony-forming units (cfu) per gram of sample.

2.5. Sensory evaluation

Sensorial analysis was carried out for each cheese by 12 trained panelists; selected and trained in accordance with the ISO 8586-1 standard (1993) in a sensorial panel, equipped with individual cabins which complied with the ISO 8589 standard (1988). Sensorial evaluation sessions were carried out as previously described elsewhere (Tejada et al., 2000).

2.6. Statistical analysis

The results obtained at the different frozen-storage stages were subjected to an analysis of variance (ANOVA) using the SAS 6.09 software package SAS (1989). Once the existence of significant differences had been determined between the different specimens, Tukey's multiple means comparison test was performed.

3. Results and discussion

Overall mean values and standard deviations for moisture, fat, protein, lactic acid and NaCl (in g/100 g of cheese), various nitrogen fractions (SN, NPN, AAN and NH_3-N, in g/100 g TN), as well as pH and a_w of cheeses obtained with animal rennet (AR) and powdered vegetable coagulant (PVC) from *C. cardunculus* are shown in Table 1.

No significant differences (P-value >0.05) were observed between controls and cheeses kept under frozen storage for up to nine months, using both types of coagulant, with regard to the chemical components analysed, including nitrogen fractions as well as pH and a_w (Table 1).

Table 1
Average values and standard deviations of chemical components (g/100 g of cheese), pH , a_w and different nitrogen fractions (g/100 g TN) of ripened cheeses obtained with animal rennet (AR) and powdered vegetable coagulant (PVC) and frozen storage for 3, 6, and 9 months

	AR				PVC			
	Control	3	6	9	Control	3	6	9
Moisture	35.73 ± 1.99^a	35.38 ± 2.52^a	33.29 ± 1.72^a	36.35 ± 2.05^a	34.85 ± 0.78^a	35.72 ± 1.53^a	34.69 ± 0.51^a	34.63 ± 1.09^a
Fat	37.50 ± 0.71^a	37.25 ± 1.06^a	37.00 ± 1.41^a	37.15 ± 0.92^a	38.00 ± 0.00^a	37.00 ± 0.00^a	36.75 ± 1.06^a	35.18 ± 1.87^a
Protein	25.22 ± 0.31^a	23.43 ± 0.04^a	23.87 ± 0.04^a	23.08 ± 1.08^a	25.16 ± 0.22^a	23.27 ± 0.35^a	23.51 ± 0.08^a	23.67 ± 1.51^a
Lactic acid	1.16 ± 0.45^a	0.98 ± 0.08^a	0.95 ± 0.01^a	0.93 ± 0.05^a	1.19 ± 0.11^a	1.23 ± 0.04^a	1.24 ± 0.04^a	1.18 ± 0.16^a
NaCl	1.70 ± 0.45^a	1.64 ± 0.45^a	1.55 ± 0.45^a	1.69 ± 0.34^a	1.54 ± 0.01^a	1.49 ± 0.03^a	1.51 ± 0.04^a	1.51 ± 0.03^a
pH	5.46 ± 0.11^a	5.52 ± 0.04^a	5.44 ± 0.03^a	5.71 ± 0.11^a	5.57 ± 0.23^a	5.63 ± 0.11^a	5.49 ± 0.07^a	5.77 ± 0.28^a
a_w	0.933 ± 0.00^a	0.951 ± 0.01^a	0.931 ± 0.01^a	0.937 ± 0.00^a	0.937 ± 0.00^a	0.959 ± 0.02^a	0.939 ± 0.00^a	0.930 ± 0.00^a
SN	33.58 ± 0.74^a	32.60 ± 3.26^a	31.97 ± 3.03^a	33.65 ± 0.89^a	49.05 ± 0.64^a	50.32 ± 3.24^a	49.06 ± 1.81^a	49.07 ± 0.62^a
NPN	25.90 ± 2.55^a	25.52 ± 2.23^a	25.96 ± 2.55^a	25.85 ± 1.26^a	32.25 ± 2.90^a	32.50 ± 4.04^a	31.60 ± 3.76^a	32.28 ± 2.94^a
AAN	6.29 ± 0.57^a	6.39 ± 0.99^a	7.29 ± 1.38^a	7.45 ± 1.97^a	5.70 ± 0.47^a	6.02 ± 1.16^a	5.99 ± 0.54^a	6.66 ± 0.01^a
NH_3-N	3.06 ± 0.13^a	3.42 ± 0.10^a	3.49 ± 0.28^a	3.64 ± 0.53^a	3.57 ± 1.00^a	4.06 ± 1.17^a	4.07 ± 1.39^a	4.55 ± 1.33^a

[a] Means of the same parameter in the same row for each coagulant without a common superscript are different (P-value < 0.05).

SN values differed markedly between the cheeses coagulated with the two types of rennet. Mean SN values for the samples analyzed (control and 3, 6, and 9 months' freezing) showed that cheese made with vegetable coagulant contained over 33% more soluble nitrogen than that made with animal rennet (P-value <0.001). This difference in soluble nitrogen formation, also reported by other authors in different varieties of ewe cheeses made with vegetable coagulant (Fernández-Salguero & Sanjuán, 1999; Núñez, Fernández del Pozo, Rodríguez Marín, Gaya, & Medina, 1991; Vioque et al., 2000), is due to the strong proteolytic activity shown by enzymes (cyprosins or cardosins) in *C. cardunculus* L. flowers, as compared to chymosin; the plant coagulant is reported to exhibit virtually maximum enzyme activity at the pH studied (Heimgartner et al., 1990). This comparatively intense proteolytic activity could be used as a proteinase system in the accelerated ripening of some ewes' milk cheese varieties (Law & Wigmore, 1982).

NPN (containing mainly small peptides of 2 and 20 residues and free amino acids) in cheeses produced with vegetable coagulant was significantly higher than in those obtained with animal rennet (P-value <0.01). Although lactic bacteria and other enzymes (McSweeney & Fox, 1993) are the principal agents for NPN production, greater proteolytic activity in the breakdown of caseins and their first breakdown products in cheeses obtained with *C. cardunculus* enzymes suggests that cheeses obtained with the powdered vegetable coagulant contain more substrates (casein polypeptides) for producing higher amounts of low molecular weight nitrogen than those obtained with animal rennet (Table 1).

No significant differences were detected in AAN and NH_3-N between the cheeses kept in frozen storage for 9 months and the other cheeses. Nevertheless, the slight increase in AAN and NPN contents in stored cheeses probably prompts an increase in pH values as frozen storage advances, and suggests that bacterial peptidases and amino peptidases continue to act, albeit slowly, during frozen storage, with a gradual increase in the concentrations of oligo-peptides, peptides, amino acids, amines and ammonia. This increase is also brought about by the fact that during freezing there is no increase in the number of microorganisms and, consequently, these nitrogenous substances are not used as metabolites.

Table 2 shows mean values and standard deviations for microbial counts in controls and in cheeses frozen and kept under frozen storage for 3, 6 and 9 months. Total viable, lactic acid bacteria and mould-yeast counts remained virtually unchanged during frozen storage, and were very similar to those recorded in controls for both types of coagulant assayed. There was generally a slight decrease in staphylococci and micrococci counts throughout frozen storage. This decrease was significant (P-value <0.05) for micrococci in cheeses made with PVC from 6 months' frozen storage onwards. Enterobacteriaceae or coliforms were not found either in controls or frozen-stored cheeses after 180 days' ripening, even though cheeses were made with raw milk.

Table 3 shows the mean scores for sensorial attributes for the batches analysed. Changes observed (P-value <0.05) throughout freezing and frozen storage for up to 9 months affected the creaminess of cheeses made with chymosin and the taste intensity and salty taste for cheese obtained with vegetable coagulant. In general, changes taking place over several months' frozen storage were of minor importance. Cheeses obtained with PVC proved to have a more acid taste and greater taste intensity (P-value <0.01) as well as displaying a more bitter taste, greater creaminess and lower degree of hardness (P-value <0.001) than those produced with AR. Finally, general acceptance of the frozen cheeses obtained with both coagulant assayed decreased slightly (P-value >0.05) in relation to the control cheeses.

Given that all chemical components, most microbiological characteristics and most organoleptic test scores were very similar in non-frozen controls and cheeses kept in frozen storage, it may be concluded that freezing is a suitable method for regulating the market and that

Table 2
Log counts of different microbial groups (mean values and standard deviation) in ripened cheeses obtained with animal rennet (AR) and powdered vegetable coagulant (PVC) and frozen storage for 3, 6, and 9 months

	AR				PVC			
	Control	3	6	9	Control	3	6	9
Total viable	7.62 ± 0.06^a	7.84 ± 0.26^a	7.56 ± 0.02^a	7.76 ± 0.14^a	7.56 ± 0.08^a	6.68 ± 2.34^a	7.77 ± 0.37^a	7.99 ± 0.13^a
Enterobacteriaceae	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
Coliforms	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
Staphylococci	4.36 ± 0.06^a	4.33 ± 0.001^a	3.45 ± 0.35^a	3.76 ± 0.39^a	4.15 ± 0.13^a	4.41 ± 0.33^a	3.49 ± 0.33^a	3.37 ± 0.23^a
Micrococci	5.95 ± 0.12^a	5.71 ± 0.62^a	5.70 ± 0.62^a	5.64 ± 0.16^a	4.55 ± 0.13^a	4.41 ± 0.28^a	3.49 ± 0.33^b	3.67 ± 0.23^b
Lactic acid bacteria	7.88 ± 0.06^a	7.79 ± 0.16^a	7.85 ± 0.20^a	7.88 ± 0.23^a	7.90 ± 0.07^a	8.21 ± 0.11^b	7.95 ± 0.26^b	7.93 ± 0.19^a
Moulds-yeasts	2.51 ± 0.13^a	3.80 ± 0.48^b	2.29 ± 1.10^a	2.52 ± 0.33^a	2.70 ± 0.001^a	2.97 ± 0.33^a	2.24 ± 0.00^a	2.23 ± 0.04^a

[a,b] Means of the same microbial group for each coagulant in the same row without a common superscript are different (P-value <0.05).

Table 3
Average values and standard deviations of some attributes in ripened cheeses obtained with animal rennet (AR) and powdered vegetable coagulant (PVC) and frozen storage for 3, 6, and 9 months

	AR				PVC			
	Control	3	6	9	Control	3	6	9
Color	5.98 ± 0.02^{a}	5.62 ± 0.71^{a}	5.80 ± 0.06^{a}	6.18 ± 0.46^{a}	5.97 ± 0.19^{a}	5.93 ± 0.69^{a}	6.19 ± 0.02^{a}	6.94 ± 0.08^{a}
Odor	5.97 ± 1.14^{a}	5.43 ± 0.92^{a}	4.98 ± 0.40^{a}	4.22 ± 1.61^{a}	5.82 ± 0.62^{a}	6.15 ± 0.04^{a}	6.09 ± 0.70^{a}	526 ± 0.90^{a}
Intensity taste	6.70 ± 0.72^{a}	6.68 ± 0.26^{a}	6.02 ± 0.09^{a}	6.22 ± 0.72^{a}	6.90 ± 0.28^{a}	7.20 ± 0.06^{a}	7.89 ± 0.27^{b}	6.77 ± 0.26^{a}
Acid taste	5.17 ± 0.08^{a}	5.90 ± 0.34^{a}	5.62 ± 0.21^{a}	4.68 ± 1.27^{a}	6.37 ± 0.11^{a}	6.53 ± 0.03^{a}	6.33 ± 0.12^{a}	5.96 ± 0.40^{a}
Salty taste	5.62 ± 0.09^{a}	5.92 ± 0.24^{a}	5.29 ± 0.49^{a}	4.87 ± 0.63^{a}	4.97 ± 0.40^{b}	5.53 ± 0.12^{ab}	5.62 ± 0.06^{a}	4.34 ± 0.07^{c}
Bitter taste	4.26 ± 0.05^{ab}	4.95 ± 0.37^{a}	4.69 ± 0.36^{a}	3.67 ± 0.02^{b}	5.96 ± 0.97^{a}	6.38 ± 0.50^{a}	6.27 ± 0.14^{a}	5.50 ± 0.55^{a}
Hardness	5.97 ± 0.49^{a}	5.61 ± 0.41^{a}	5.69 ± 0.32^{a}	6.81 ± 0.77^{a}	3.84 ± 0.18^{a}	2.76 ± 0.59^{a}	3.21 ± 0.33^{a}	4.46 ± 0.64^{a}
Creaminess	4.89 ± 0.03^{a}	4.20 ± 0.72^{ab}	3.74 ± 0.12^{bc}	3.09 ± 0.02^{c}	7.03 ± 0.12^{a}	6.86 ± 0.57^{a}	6.28 ± 0.61^{a}	6.40 ± 0.85^{a}
Acceptance	6.62 ± 0.27^{a}	5.74 ± 0.46^{a}	5.37 ± 0.38^{a}	5.62 ± 0.35^{a}	6.19 ± 0.28^{a}	5.66 ± 0.20^{a}	5.72 ± 0.05^{a}	5.01 ± 0.38^{a}

[a,b,c] Means of the same variable for each coagulant in the same row without a common superscript are different (P-value < 0.05).

fully ripened Manchego-type cheese may suitable be stored frozen at −20 °C for up to at least 6 months.

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