Gelation upon Long Storage of Milk Drinks with Carrageenan

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ABSTRACT

The carrageenan-induced stabilization and gelation of ultra-high-temperature-treated milk was studied during long storage. Severe heating (causing increased protein denaturation), lowering of the pH, or the use of κ -carrageenan (instead of ι -carrageenan) led to excessive gelation. It is suggested that the balance between carrageenan-carrageenan interactions and carrageenan-protein interactions determines the gel strength. If the interactions between carrageenan and proteins are decreased, more carrageenan is available for carrageenan-carrageenan interactions, leading to a stronger gel. This is the case if κ -carrageenan is used instead of ι -carrageenan because the former forms weaker interactions with proteins than the latter. Also, heating and pH influence the attachment of whey proteins to the casein micelle surface, hindering the attachment of carrageenan to the casein proteins. Upon storage, gel strength increased. Particle size and rheology measurements indicated that upon storage, tenuous carrageenan-protein aggregates are formed. The firming of the gel was probably related to slow structural arrangements of the gel and not related to slowly changing calcium equilibria or age gelation.

Key words: carrageenan, gelation, milk, storage

INTRODUCTION

In liquid milk products like sterilized milk or cream, carrageenan is added to induce the formation of a weak gel network not perceivable by the consumer. The purpose of this is to trap protein aggregates or fat droplets in a network. As such, instabilities like sedimentation and creaming are reduced, and shelf life is increased. For instance, in UHT treated (i.e., sterilized) milks, carrageenan helps to reduce sedimentation resulting from heat coagulation (Walstra, 1999; Singh, 2004).

Application of carrageenan is particularly important if milk is enriched with vegetable oily material lacking

the surface active material naturally present on the milk fat globule (Deeth, 1997; Walstra, 1999). The interest in these so-called filled milks is rising because of the current consumer heart health trend. Filled milks for instance contain elevated levels of unsaturated fatty acids by addition of vegetable or marine oils.

Unfortunately, the application of carrageenan in UHT milk at typical concentrations of 0.01 to 0.05% (all percentages in article given in wt/wt) can also lead to unwanted gelation, like most gelation processes ending up with phase separation (Mellema et al., 2002). In this study we show that gelation occurs upon long storage of UHT or sterilized filled milk drinks with 0.015% carrageenan.

Schorsch et al. (2000) reported on the phase behavior of systems containing casein and κ -carrageenan at these concentrations. They found instability of systems containing carrageenan at levels higher than 0.015% if the casein concentration was similar to milk (2.8%; Walstra, 1999).

This study aims at understanding the mechanism of stabilization by carrageenan in sterilized milk filled with sterol esters, and the mechanism of the gelation defect thereof. Carrageenan is a polysaccharide obtained by extraction with water or alkaline water from red seaweeds. Three basic types of carrageenan exist: κ -, ι -, and λ -carrageenan, of which κ -carrageenan is the most often referred to as suitable for stabilizing milk products. Note that most commercially applied carrageenans are mixtures of all 3 types, even if they carry the name of either one. A κ -carrageenan mixture is typically added at a concentration of 0.01 to 0.05% in milk drinks (Towle, 1973; Syrbe et al., 1998).

According to literature, the mechanism of carrageenan stabilization basically consists of 2 elements: gelation and complexation. Gelation occurs due to intermolecular bridges between carrageenans. Both κ and ι -carrageenan can gel via double helix formation and alignment of helices into junction zones. Gelation is dependent on the presence of cations like K^+ (κ -carrageenan) and Ca^{2+} (ι -carrageenan; Towle, 1973; Syrbe et al., 1998; Chen et al., 2002; Sedlmeyer et al., 2003). Many commercial carrageenans already contain mixtures of cations to improve gelation properties. The λ -carra-

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geenan is unable to gel due to a high amount of sulfate groups.

Complexation occurs due to protein-carrageenan interactions. A specific interaction is formed between κ -carrageenan and κ -CN at the surface of the casein micelles (Hood and Allen, 1977; Langendorff et al., 2000; Spagnuolo et al., 2005). This interaction is of an electrostatic nature. Even at neutral pH, the κ -CN contains a positively charged region between the residues 20 and 115, which binds to the sulfated groups on the κ -carrageenan (Snoeren et al., 1975). Interactions may also be indirect via cations such as calcium, which can act as a bridge between the carboxyl groups on the proteins and sulfate groups on the carrageenan (Glicksman, 1983; Dickinson, 1998). The latter mechanism can occur with whey proteins and with casein micelles, particularly at neutral pH (Dickinson, 1998).

Even though complexation as well as gelation is necessary for the stabilizing effect of carrageenan in milk, under some conditions the complexation can also interfere with the gelation. Especially at low carrageenan concentration (<0.018%), milk proteins can interfere with carrageenan gel formation, due to the domination of protein-carrageenan interactions over carrageenan-carrageenan interactions. This is most pronounced with the micellar casein, but whey proteins can have this effect also (Drohan et al., 1997; Tziboula and Horne, 1999).

Because of the above, carrageenan-protein interactions also depend on the interactions of the proteins with each other. For instance during heat treatment whey proteins denature and bind to the casein micelles. According to Anema and Li (2003a) the main whey protein β -LG binds specifically to the κ -CN to form a β -LG- κ -CN complex. However, the whey proteins can also interact with each other to form whey protein aggregates. The amount of denaturation and subsequent aggregation of the whey proteins is dependent on heating temperature and method (Odfield et al., 1998).

The type of interaction between proteins during heating is also dependent on the pH of the milk. If the pH of milk is increased before heating, interactions between whey proteins increase, giving more whey protein aggregates. If the milk is acidified before heating, the β -LG- κ -CN interactions increase, leading to more attachment of whey proteins to the casein micelle surface (Corredig and Dalgleish, 1996a; Anema and Li, 2003b; Vasbinder and de Kruif, 2003).

Note that the pH also influences the charge on the molecules, leading to changed interactions between proteins and carrageenan. Galazka et al. (1999) and Dickinson and Pawlowsky (1997) have shown that the interaction of carrageenan with BSA becomes stronger if the pH is decreased (<7).

It has been suggested that the β -LG- κ -CN complex can slowly release itself from the micelle upon long-term storage (months). As a result the micelles lose their stabilizing hairy layer and they will aggregate, eventually leading to gelation of the milk. Gelation resulting from this mechanism is also called age gelation (McMahon, 1996; Datta and Deeth, 2001). According to Walstra (1999), age gelation is preceded by a shape deformation of the casein micelles. This is assumed to be due to ongoing proteolytic enzymatic activity resulting from insufficient heating, which could (in turn) facilitate the release of the β -LG- κ -CN complex.

Most of the above-mentioned gelation and complexation mechanisms have been related to the presence of calcium. Although some research was done on the changes in calcium balance in milk (De La Fuente, 1998; May and Smith, 1998), little is known about calcium balances in milk upon long (weeks/months) storage. Youssef et al. (1992) found a progressive increase in calcium content in UHT milk during 180 d of storage. De La Fuente et al. (2001) have suggested that the increase in calcium content in the soluble phase during prolonged storage of milk may be due to breakdown of casein by residual or reactivated heat-stable proteolytic enzymes. In contrast, Aoki and Imamura (1974) measured a decrease in soluble calcium in concentrated skimmed milk during prolonged storage. Note that no correlation between the changes in soluble calcium content and the onset of age gelation was found (Kocak and Zadow, 1986; Cano-Ruiz and Richter, 1998).

Although much is known about age gelation (i.e., gelation related to proteolytic activity) in UHT milk (McMahon, 1996; Datta and Deeth, 2001), little is known about carrageenan gelation in UHT milk drinks. In this study we investigate the slow gelation of milk under conditions where carrageenan gelation is more important than age gelation (sufficient heating, relatively high concentrations of carrageenan). Gelation of sterol ester-filled milk drinks with carrageenan was monitored over a time period of 16 wk. Because the gels formed are not reversible and easily disturbed, creaming was investigated as an indicator for stabilization and gelation. The influence of variations in composition (such as carrageenan concentration and type or the presence of cations) and processing (such as heating and pH changes) were investigated.

Sedlmeyer and Kulozik (2006) have already suggested that the complexation of carrageenan and milk proteins and resulting gel strength and texture is influenced by the UHT process conditions. They have found an increased particle size in milk protein-carrageenan gels if the UHT temperature was increased. The possible use of particle size measurements using light scattering methods to measure carrageenan-protein in-

teractions has been demonstrated by Langendorff et al. (2000) and Spagnuolo et al. (2005). This is why we will also perform light scattering measurements in addition to viscosity measurements on sterol ester-filled milk with and without carrageenan during storage. It will show any early indications of gelation or aggregation due to interactions of carrageenan. The light scattering measurements will include ultrasonic treatments to distinguish between particle size increases due to droplet coalescence and aggregation.

MATERIALS AND METHODS

Sample Preparation

Commercial milk (skim pasteurized milk, Campina, Zaltbommel, the Netherlands) was used to prepare the milk drinks. Sterol esters (0.5%, ex Unilever, Purfleet, UK) were added to induce creaming in the samples. Other ingredients were: κ -carrageenan (κ -carrageenan deltagel P379, Quest, Naarden, the Netherlands), ι -carrageenan (ι -carrageenan deltagel P388, Quest), and κ/ι -carrageenan hybrid (κ -carrageenan k100, CP Kelco, Atlanta, GA). Calcium chloride (Merck, Darmstadt, Germany) and potassium chloride (Merck) were added to favor carrageenan-protein interactions. Also, EDTA (Akzo Nobel, Arnhem, the Netherlands) and sodium hexametaphosphate (Fluka Chemika, Buchs, Switzerland) were added as calcium complexing agents. Lactic acid (Purac, Gorinchem, the Netherlands) and NaOH (Merck) were added to alter the pH of the milk drinks.

The milk was preheated to 60°C in a tank. The sterol esters were heated overnight at 70°C before addition to the milk. The milk, sterol esters, and other ingredients were mixed using an UltraTurrax blender. The whole mixture was then UHT treated for 8 s at 140°C in a direct heating system (Unilever, Vlaardingen, the Netherlands). The homogenization pressure (APV-Gaulin, Albertslund, Denmark) in the UHT line was 100 bar. The samples were cold filled in polyethylene terephthalate bottles in a flow cabinet. The samples were stored chilled (5°C) in the dark for 16 wk. Variations were made in the sample composition and processing. The variations are summarized in Table 1.

Determination of Gel Strength

Over a period of 16 wk, gel strength was monitored. For this purpose, sterol esters were added to the milk drinks. Any occurrence of weak gelation would reduce creaming of the sterol esters. The amount of creaming is used as a measure of gel strength. Every 4 wk, the samples were analyzed visually for creaming. For quantification, cream categories were made, which are shown in Figure 1.

Particle Size Measurement

Particle size was measured using Helos light scattering (Sympatec, GmbH, Clausthal-Zellerfeld, Germany). Particle size ($D_{3,2}$) was measured with an R1-lens, both before and after an ultrasound treatment of 120 s.

Viscosity Measurement

Viscosity was measured using the advanced rheometer AR1000 (TA Instruments, Etten-Leur, the Netherlands), using a 40-mm diameter, 2% angle cone measuring system. A steady-state shear process was used, increasing shear rate from 0.01 to 250/s. The measuring temperature was 7°C.

Calcium Concentration

Soluble calcium concentration of 2 samples (milk + sterol esters and milk + 0.015% κ -carrageenan k100) was measured after 3, 7, and 14 wk storage at 5°C. The samples were filtered with 3000 Dalton Centriprep filters (centrifugation at $1,500 \times g$); the clear solution with no protein present was measured by plasma emission spectrometry at a wavelength of 317.933 nm.

Protein Denaturation Degree

The protein composition of some specific samples (samples in which the ingredient or milk end product underwent various heat treatment) was determined using HPLC in combination with an UV/visible detector. The denaturation degree (DG) of selected samples was calculated using the following equation:

$$DG = \left(1 - \frac{\text{conc. whey protein sample}}{\text{conc. whey protein "blank"}} \right) \cdot 100\%.$$

Pasteurized skim milk was used as control sample.

Proteolytic Activity

The presence and activity of proteases and peptidases in milk was measured using a standard protease substrate kit (Boehringer-Mannheim, Germany).

RESULTS AND DISCUSSION

Effect of Ingredient Parameters on Carrageenan Gel Strength

In this section, we will present the ingredient parameters influencing the (carrageenan) gel strength of the milk drinks.

Three types of carrageenan were tested, namely κ -carrageenan, ι -carrageenan, and a commercial κ/ι -car-

Table 1. Description of the ingredient and processing variables

Variables	Standard	Variations	
Carrageenan types	κ/ι -Carrageenan hybrid	ι -Carrageenan	κ -Carrageenan
Carrageenan concentration (%)	0.01/0.015	0.03	0.05
Salt addition	No	CaCl ₂ (0.05%)	KCl (0.05%)
Sequestering agent addition	No	Sodium hexametaphosphate (0.05%)	EDTA (0.07%)
Heating system	Direct	Indirect	—
Heating temperature (°C)	140	150	—
pH (measured at 60°C)	6.2	6.0	6.5

rageenan hybrid. Carrageenan concentrations varied between 0 to 0.05%. All samples contained skim milk and 0.5% sterol esters (density at 20°C: 0.95 kg/L), which were added to induce creaming. In addition, 2 control samples were measured, one without sterol esters and one without carrageenan.

Figure 2A and B represent the creaming during 16-wk storage in the control samples and the samples containing various carrageenan types and concentration. As expected, no creaming was found in absence of sterol esters, whereas severe creaming was found in the absence of carrageenan.

As we can see in Figure 2B, creaming decreased as the concentration of κ/ι hybrid increased, indicating that a stronger network is formed if the carrageenan concentration is increased. More creaming was found in samples containing ι -carrageenan vs. those containing κ -carrageenan, which indicates that κ -carrageenan forms a stronger network than ι -carrageenan.

Drohan et al. (1997) and Tziboula and Horne (1999) found that the gelation of carrageenan could be hindered by protein-carrageenan interactions. One of the differences between ι - and κ -carrageenan is that ι -carrageenan forms stronger complexes with (whey) proteins than κ -carrageenan does (Galazka et al., 1999). This would result in more protein-carrageenan interac-

tions in the presence of ι -carrageenan, leaving less carrageenan for gelation and thus a weaker gel network. This is in accordance with our findings.

Carrageenan gelation is not only dependent on the amount and type of carrageenan molecules, but also on the presence of cations in solution (Towle, 1973; Dickinson, 1998; Syrbe et al., 1998). Figure 3 shows the effect of addition of CaCl₂ or KCl and the effect of addition of calcium sequestering agents (sodium hexametaphosphate and EDTA) on creaming of sterol esters in milk.

As we can see in Figure 3, creaming decreases (and gel strength thus increases) if (additional) cations are added. Creaming is higher in samples containing sequestering agents (and thus a lower concentration of cations), which confirms that the gel network is weaker in the absence of cations.

Effect of Processing Parameters on Carrageenan Gel Strength

In this section we will discuss the influence of heating and variations in pH on carrageenan gel strength. The heating and pH influence the denaturation of the whey proteins and the subsequent binding of the whey proteins to the casein micelles. The protein denaturation

	Cream category					
Sample structure
Cream structure
Category	0	1	2	3	4	4

Figure 1. Overview of the sample and cream structure and appearance in the cream categories (0 to 4).

Figure 2. The influence of carrageenan concentration on creaming in a milk drink. Creaming is indicated on a scale of 0 to 4, where a value of 0 represents no creaming and a value of 4 represents severe creaming. Scores were given every 4 wk. A) Milk drinks containing skim milk and 0.5% sterol esters; skim milk, 0.015% κ/λ -carrageenan hybrid, and 0.5% sterol esters; and skim milk and 0.015% κ/λ -carrageenan hybrid. B) Milk drinks containing skim milk, 0.5% sterol esters, and various concentrations (0.01, 0.03, or 0.05%) of κ -carrageenan, λ -carrageenan, or κ/λ -carrageenan hybrid.

degree of the samples that were heated at 140 and 150°C with a direct and indirect UHT system are shown in Table 2.

Figure 4 shows the influence of the heat treatment on the creaming of the sterol esters. As we can see in Figure 4, no creaming was present in the samples that had an indirect heat treatment, and a denaturation degree of 79 or 93. Creaming occurred later in the sample that had been heated directly at 150°C compared with 140°C. Thus, gel strength of the carrageenan gel increased in the samples that had been heated more and had a higher protein denaturation degree.

Sedlmeyer and Kulozik (2006) have already shown an effect of processing on carrageenan gel strength. They have measured a larger particle size in carrageenan milk protein gels if the UHT heating temperature was higher. Their results confirm our findings that

Figure 3. The influence of cation concentration on creaming in a milk drink. Creaming is indicated on a scale of 0 to 4, where a value of 0 represents no creaming and a value of 4 represents severe creaming. Scores were given every 4 wk. The milk drinks contain skim milk, 0.5% sterol esters and, respectively, 0.05% hexametaphosphate, 0.07% EDTA, 0.05% CaCl₂, or 0.05% KCl.

the carrageenan gel strength increases if the total heat load is larger (which is the case for indirect heating).

The results of pH change in the milk samples on creaming of sterol esters is shown in Figure 5. As we can see in Figure 5, an increase in pH from 6.2 to 6.5 led to an increase in creaming, indicating that gel strength would decrease at higher pH. A decrease in pH did not affect creaming significantly.

We suggest that the strength of the carrageenan-protein network, and thus the amount of creaming, is related to the coverage of the casein micelles with whey protein. The use of heating methods where the total heat load is larger, such as an indirect UHT system, should result in a greater portion of the whey proteins associating with the micelles than a more sharp heating method such as the direct UHT system (Corredig and Dalgleish, 1996b). Vassbinder et al. (2001) has calculated that at complete denaturation of the whey proteins, 40% of casein micelles are covered by the β -LG. Heating may therefore reduce the possible carra-

Table 2. The denaturation degree (DG) of the whey proteins in the samples with various heat treatments¹

Heating system	Heating temperature (°C)	DG (%)
Direct	140	49
Direct	150	61
Indirect	140	79
Indirect	150	93

¹Composition of the samples was skim milk, 0.5% sterol esters, and 0.015% κ/λ -carrageenan hybrid.

Figure 4. The influence of heating on creaming in a milk drink. Creaming is indicated on a scale of 0 to 4, where a value of 0 represents no creaming and a value of 4 represents severe creaming. Scores were given every 4 wk. The milk drinks contain skim milk, 0.015% κ/ι -carrageenan hybrid, and 0.5% sterol esters. All samples were UHT-treated at 140 or 150°C with the direct or indirect system.

geenan-protein interactions. The carrageenan-carrageenan interactions would hence be increased, leading to an increase in gel strength, which is in accordance with our data.

A similar argument can be given for the effect of pH. Vasbinder and de Kruif (2003) has shown that small increases in pH would lead to an increase in β -LG- β -LG interactions, thus forming more whey protein aggregates and leading to less attachment of whey proteins on the CN surface. Acidification would lead to an increase in κ -CN- β -LG interactions, thus more attach-

Figure 5. The influence of pH on creaming in a milk drink. Creaming is indicated on a scale of 0 to 4, where a value of 0 represents no creaming and a value of 4 represents severe creaming. Scores were given every 4 wk. The milk drinks contain skim milk, 0.015% κ/ι -carrageenan hybrid, and 0.5% sterol esters. The pH of the samples were, respectively, 6.0, 6.2, and 6.5.

ment of whey proteins onto the surface of the casein micelle.

An increase in pH could thus lead to more protein-carrageenan interactions, giving a weaker gel. This is in agreement with our results.

Effect of Storage on Carrageenan Gel Strength

Surprisingly, the gel strength of the carrageenan gel network slowly increased in time. For instance in the sample containing 0.015% κ/ι -hybrid, after 12- to 16-wk storage gel particles were formed. This was also the case in the samples containing 0.03 to 0.05% ι -carrageenan. In the sample containing 0.05% ι -carrageenan, a strong gel was formed after 16 wk of storage. Note that this increase in gel strength cannot be seen in creaming data because of the irreversible nature of the creaming process.

To be able to measure this firming effect, particle size measurements and viscosity measurements were applied to a skim milk sample containing a 0.015% κ/ι -carrageenan hybrid, over a storage period of 26 wk. Results of the particle size measurement are shown in Table 3. A particle size increase may be caused either by the increase in fat droplet size due to creaming or the formation of (protein) aggregates. The table shows particle size ($D_{3,2}$) results before and after an ultrasound treatment. This ultrasound treatment breaks weak bonds in the sample. Droplet size increases in time due to the formation of weak aggregates, such as aggregates of carrageenan with proteins, will thus not be measured after the ultrasound treatment.

Table 3 shows an increase in $D_{3,2}$ over time in all samples. In the samples without carrageenan, no difference is seen in droplet size before and after the ultrasound treatment, so the increase in droplet size is most likely due to droplet coalescence.

In the samples with carrageenan, the particle size increases more than in the samples without carrageenan. The ultrasound treatment reduced the particle size, though the particle size is still larger than at the beginning of the storage. This means that part of the particle size increase is caused by the formation of (weak) aggregates in time.

Figure 6 shows the viscosity of skimmed milk with and without 0.015% κ/ι -carrageenan hybrid. As we can see in this figure, the viscosity of milk with carrageenan increases during the storage time of 26 wk. This indicates that the carrageenan network becomes stronger in time.

We can speculate on the reason why the gel strength of the carrageenan network increases in time in the milk: it may for instance be due to changing calcium equilibria. The milk calcium, which is necessary for

Table 3. Droplet size ($D_{3,2}$) before and after ultrasound treatment of skim milk samples containing 0.5% sterol esters and 0.015% κ/ι -carrageenan hybrid over a storage period of 26 wk

Storage time (wk)	0.015% κ/ι -carrageenan hybrid				No carrageenan
	0	4	10	26	26
$D_{3,2}$ (μm)	1.27	1.36	1.39	1.38	1.33
$D_{3,2}$ after sonication (μm)	1.27	1.34	1.38	1.30	1.33

carrageenan gelation, is mainly present in the casein micelles. These micelles change in time (Walstra, 1999). Calcium may migrate from the micelle into the bulk phase of the milk during this change process, hereby increasing the cation concentration in solution. This will lead to an increase in gel strength.

In 2 samples (containing milk and carrageenan or sterol ester) the calcium concentration in the milk serum (not the calcium in the micelles) was measured in time. The measured calcium included both the calcium ions and the calcium crystals in the bulk phase of the milk. The results are shown in Figure 7.

In this figure we see that the calcium concentration does not change significantly in time. This measurement does not support the above-mentioned hypothesis of the effect of calcium on carrageenan(-protein) gelation.

To estimate the contribution of age gelation to the carrageenan gelation process, proteolytic activity was measured in some gelled milk samples. The samples did not show any proteolytic activity when measured with the Boehringer protease kit. It is thus very unlikely that age gelation, induced by proteolytic activity, has influenced the carrageenan gelation process.

We conclude that the increase in gel strength in the samples in time is most likely related to protein

(-carrageenan) rearrangements, similar to those found in acid milk protein gels (Mellema et al., 2002).

CONCLUSIONS

In this work we studied the influence of ingredient and processing parameters on carrageenan-induced gelation of milk proteins in sterilized milk upon long-term storage. The gelation was more pronounced if κ -carrageenan was used instead of ι -carrageenan because ι -carrageenan forms stronger complexes with (whey) proteins than κ -carrageenan, resulting in more protein-carrageenan interactions, hereby leaving less carrageenan for gelation. The addition of calcium-sequestering agents reduced the gel strength. The use of a more severe heating process, leading to more whey protein denaturation, gave stronger carrageenan gels. It is suggested that the attachment of the whey proteins to the casein micelle surface hinders the attachment of carrageenan to the casein micelle. As a consequence, more carrageenan-carrageenan interactions will be formed. This same effect is seen as a result of pH changes in the milk: acidification leads to an increase in whey protein-casein interactions, finally resulting in a stronger gel.

In time, the viscosity, particle size, and gel strength of the milks containing carrageenan increased. Particle

Figure 6. The viscosity of skim milk with 0.5% sterol esters with or without 0.015% κ/ι -carrageenan hybrid over a storage period of 26 wk: ($\blacktriangle$) $t = 0$ wk carrageenan hybrid; ($\blacklozenge$) $t = 4$ wk carrageenan hybrid; ($\square$) $t = 10$ wk carrageenan hybrid; ($\circ$) $t = 26$ wk carrageenan hybrid (+) $t = 26$ wk no carrageenan. **Figure 7.** Free calcium concentration in time in the milk drinks containing: ($\blacktriangle$) skim milk and 0.5% sterol esters; and ($\square$) skim milk and 0.015% κ/ι -carrageenan hybrid.

size measurements involving ultrasound treatments showed that at least part of the increase in particle size was caused by the formation of weak carrageenan-protein aggregates. The firming of the gels is probably related to slow structural rearrangements of the gel and not to slowly changing calcium equilibria or proteolytic enzymes (age gelation).

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