Effect of Sodium Caseinates Addition on the Rheological Properties of Kefir during Gel Formation

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Abstract

The effect of Sodium Caseinates (SCN) addition on the rheological behavior of kefir during gel formation was monitored by means of a dynamic rheometer of novel design (U-tube Rheometer). Kefir samples were prepared from homogenized and pasteurized full fat (3.5% w/w) bovine milk with or without the addition of SCN at varying concentrations (1%, 2% or 3% w/w). A fermented milk inoculum derived from kefir grains was inoculated into the heat-treated milk and incubated at 25°C until the pH dropped to 4.6. According to the results, the fermentation time required for the onset of gelation, as well as for the pH value to reach 4.6 increased with increasing SCN concentration. The beginning of cross-linking of proteins towards aggregates that leads to the formation of a three-dimensional protein matrix, took place at higher pH values with increasing SCN concentration. The values of elastic modulus and loss tangent that correspond to this point decreased with increasing SCN content. The increase in SCN concentration caused the values of elastic modulus during gel formation to increase and those of loss tangent to decrease. The addition of caseins into the milk increased the number and the strength of the protein-protein interactions causing the elastic behavior of the samples to increase. The presence of SCN into the system of kefir greatly affected the formation of the kefir gel.

Keywords: kefir, sodium caseinates, gel formation, U-tube rheometer, viscoelastic properties

1. Introduction

Kefir is a fermented dairy product produced by the fermentative activity of kefir grains consisting mainly of lactococci, lactobacilli and yeasts in a protein-polysaccharide matrix (Koroleva, 1988; Farnworth, 2005). As kefir is reported to be beneficial to health and due to its nutritional and favorable organoleptic characteristics (Otles & Cagindi, 2003), the study of the factors affecting the rheological properties of the gel during formation is of great importance since they affect the quality of the final product and thus its acceptance by the consumers.

Among the many factors studied, chemical composition (mainly concentration of proteins) of the milk used affects the formation of the protein matrix and thus the rheological behavior of the final product. The addition of skim milk powder, sodium caseinates (SCN) or whey proteins concentrates is usually aims in increasing the dry matter content of the milk and of course in improving its consistency and texture of fermented milk products (Lucey, 2004a; Tamime & Robinson, 2007). According to Dimitreli and Antoniou (2011), SCN addition caused the apparent viscosity of kefir samples to increase and their flow behavior index values to decrease. Caseins possess an important role in the fermentation process of kefir, since they form the continuous matrix that holds together all the constituents of milk.

The study of the gelation process of yogurt (Haque, Richardson, & Morris, 2001; Lee & Lucey, 2006; Ozcan-Yilsay, Lee, Horne, & Lucey, 2007; Lazaridou, Serafeimidou, Biliaderis, Moschakis, & Tzanetakis, 2014; Ozcan, Horne, & Lucey, 2015), other fermented dairy products (Lucey & Singh, 1998; Peng, Horne, & Lucey, 2010; Kristo, Miao, & Corredig, 2011) or glucono-δ-lactone induced acid milk gels (Lakemonda & van Vliet, 2008) was made so far, by using the concentric cylinder geometry of an oscillating rheometer for the *in situ* preparation of the samples and the measurement of their viscoelastic properties. Nevertheless the gel of fermented milk products is extremely fragile and especially during fermentation structure formation could be

easily disrupted if the gel matrix suffered a certain mechanical disturbance. Additionally, the rather small quantity of sample contained in the measuring unit of an oscillating rheometer combined with the possible slip effects, due to whey separation, may lead minute phenomena, taking place especially at the early stages of the gelation process, to go unnoticed.

Bearing in mind the above, a novel custom made pneumatic dynamic U-tube rheometer (Xu & Raphaelides, 2005) was constructed to monitor the structure formation of the kefir gel. The rheometer, based on the U-tube technique of Saunders and Ward (1953), consists of two identical limbs where the sample is loaded, an air chamber whose pressure is measured by two transducers and a reciprocating piston creating a driving pressure resulting in to sample deformations and consequently to pressure increments in the air chamber on the other side of the U-tube. From these pressure measurements the rheological characteristics of the sample can be determined. An important asset of the design is the adaptive nature of the acting pressure arising from a back pressure, which allows measurements to be made on liquids as thin as water yet keeping the maximum strain within the limit of linear viscoelasticity, by adapting its value according to the rigidity of the material exhibited at the moment of measurement. Finally, the interior of the sample holder has been profiled to a screw thread shape for preventing sample slippage. This instrument has already been used in order to study the structure development of set-type yogurt (Xu, Emmanouelidou, Raphaelides, & Antoniou, 2008).

The aim of the present work was to study the effect of SCN addition on the rheological behavior of kefir during gel formation. The structure formation of the kefir gel was monitored, by the use of the dynamic U-tube rheometer of novel design. The elastic modulus and loss tangent were recorded as a function of time and pH. The development of the elastic modulus will contribute to the study of the effect of SCN addition on the structure formation of kefir as well as on the pattern of its gel formation. Loss tangent will give an indication of whether a phase transition is occurring to the system (from liquid to gel) during fermentation.

2. Materials and Methods

2.1 Materials

The kefir samples were prepared with full-fat (3.5% w/w) pasteurized and homogenized bovine milk purchased from the local dairy, kefir grains obtained from a household and SCN (MIPRODAN 30; Arla Food Ingredients, Viby J., Denmark). The chemical composition of SCN (% w/w) was: moisture ≤ 6.0; proteins 88.0-93.5; fat 1.5; ash 4.0; lactose 0.3.

2.2 Preparation of Kefir Samples

The SCN were added into the milk before its heat-treatment at varying concentrations (1%, 2% or 3% w/w), and dissolved in a Grant GLS400 shaking water-bath (Grant Instruments Ltd, Cambridge, G.B.) under continuous stirring for 20 min at 35°C. The milk with or without the addition of SCN was heated at 85°C for 15 min under continuous stirring (Grant GLS400) and then cooled down at room temperature (25°C). Following heat-treatment, the milk was inoculated with a fermented milk inoculum, at 3% (w/w) ratio, derived from the household kefir grain culture (the kefir grains were inoculated into the milk at the same ratio and fermented until a pH of 4.6 was achieved). A quantity of approximately 100 mL of the inoculated milk was placed inside the measuring unit of the U-tube rheometer, while a smaller one was poured into a beaker (for pH measurements), which was placed into a very small (25 × 30 × 25 cm) custom made incubation chamber and kept at the same temperature as the rheometer's measuring unit. The samples were incubated at 25°C until the pH dropped to 4.6. All samples were replicated two times.

2.3 Rheological Measurements

The free surface of the sample placed inside the measuring unit of the U-tube rheometer was covered with a thin layer of liquid paraffin oil to avoid possible evaporation. The sample was incubated by setting the rheometer's heating unit at 25°C. The frequency of oscillation was set at 0.1 Hz and every 10 min, measurements of elastic modulus (G'), loss tangent (tanδ), incubation temperature and pH were taken and automatically recorded via the rheometer's microprocessor control unit. The data were shown and stored in a PC which was connected to the rheometer. From the data recorded, the fermentation time required for the onset of gelation (G' started to increase) (t_g), the pH value at the onset of gelation (pH_g), the maximum value of tanδ ($\tan\delta_{max}$), the fermentation time at $\tan\delta_{max}$ ($t_{\tan\delta max}$), the pH at $\tan\delta_{max}$ ($pH_{\tan\delta max}$), the G' at $\tan\delta_{max}$ ($G'_{\tan\delta max}$), the fermentation time for the pH to reach the 4.6 value (t_{total}) and the maximum value of G' (G'_{max}) were evaluated.

2.4 Determination of the pH

The pH measurements were carried out by inserting a combined pH electrode into the sample contained in the beaker which was placed inside the incubation chamber that was kept at the same temperature as the rheometer's

measuring unit. Both the incubation temperature and the pH measuring unit were controlled by the rheometer's microprocessor.

2.5 Statistical Analysis

The experimental data were subjected to one-way ANOVA, while the Tukey multiple comparison test determined whether statistically significant differences occurred among means. The statistical analysis of the experimental data was performed using Minitab 16.0 statistical software.

3. Results and Discussion

3.1 Structure Development

Figure 1 shows the development of G' of kefir samples, prepared with or without the addition of SCN, until the pH dropped to 4.6. As it can be seen the fermentation time required for the onset of gelation as well as for the pH value to reach 4.6 increased with increasing SCN concentration. The increase in fermentation time with increasing SCN concentration is due to the increased buffering capacity of the system because of the presence of caseins. Caseins possess high buffering capacity (Salaün, Mietton, & Gaucheron, 2005) that results in more lactic acid production in order to reduce the pH of the samples, resulting in increased fermentation times. Furthermore, the increasing SCN concentration resulted in increased values of the G' (Figure 1). The addition of caseins into the milk system increases the possibility of either bigger or more casein clusters being formed during fermentation resulting in increased elasticity of the protein matrix. According to Walstra, Wouters and Geurts (2006), there is a power-law relation between the casein concentration and the consistency of the casein-containing systems.
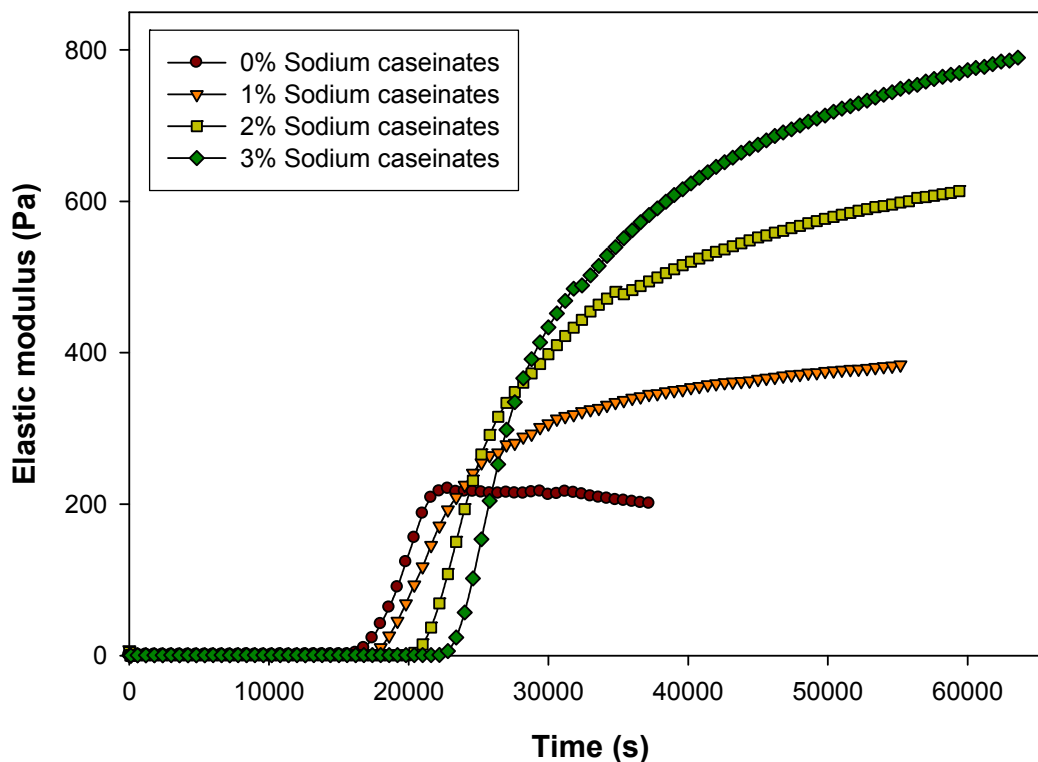

Figure 1. Development of the elastic modulus at 0.1 Hz, with time (to final pH value 4.6), for kefir samples prepared with or without the addition of sodium caseinates

As it can be seen in Figure 2, at the initial stages of gel formation tanδ increases to a maximum value, which indicates the dissociation of colloidal calcium phosphate from the casein micelles. The subsequent reduction in tanδ reflects the cross-linking of proteins and the formation of aggregates that increase in number and size leading to the formation of a three-dimensional protein matrix (the values of tanδ are almost being stabilized). The stabilization of tanδ values indicates that the system being formed is rather stable and no structure rearrangement is occurring. According to Mellema, Walstra, van Opheusden and van Vliet (2002), higher values

of tanδ result in higher rate of rearrangements. During milk acidification, the reduction in pH results in colloidal calcium phosphate dissociation from the casein micelles into the milk serum (Walstra et al., 2006). This causes the reduction in casein micelles size, which in turn results in more liquid-like behavior of the aggregates formed, indicated by the high values of tanδ, at the initial stages of gel formation. The magnitude of tanδ is a measure of the liquid-like behavior of a casein gel (Mellema et al., 2002). When all the amount of the colloidal calcium phosphate has been dissociated from the micelles around pH 5.0 (Lucey, 2004b), no reduction in casein micelles size is occurring. Simultaneously, the successive decrease in pH, close to the isoelectric point of proteins, increases the number of interactions formed as well as their strength. This results in increasing the size of the aggregates formed and thus the solid-like behavior of the samples (the values of tanδ are being decreased). The stabilization of tanδ values probably takes place when a rather increased number of aggregates have been formed.
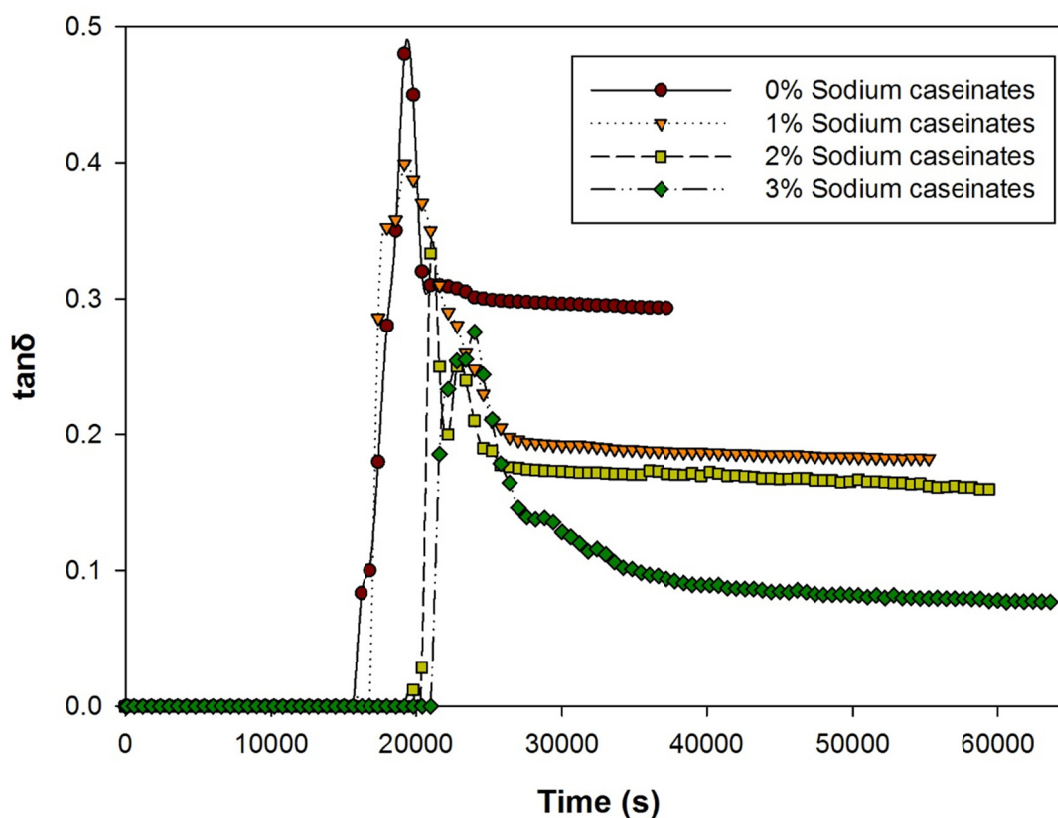

Figure 2. The values of tanδ at 0.1 Hz, with time (to final pH value 4.6), for kefir samples prepared with or without the addition of sodium caseinates

3.2 Rheological Properties and Parameters during Gel Formation

The rheological properties and parameters derived from the dynamic measurements during gel formation (t_g, pH_g, $tan\delta_{max}$, $t_{tan\delta max}$, $pH_{tan\delta max}$, $G'_{tan\delta max}$, t_{total} and G'_{max}) are shown in Table 1. Statistical analysis of the experimental data confirmed the findings discussed in section 3.1. According to ANOVA, all the variables examined were significantly affected by SCN addition ($p<0.001$).

Particularly, increasing SCN concentration resulted in increased t_g and t_{total} values due to the increased buffering capacity of caseins. The Tukey test showed that both t_g and t_{total} increased in the following order: 0% SCN < 1% SCN < 2% SCN < 3% SCN. Similar results, as it concerns the fermentation time required for the pH to reach 4.6, have been reported for stirred yogurt made from buffalo milk and enriched with different concentrations of SCN (Petridis, Dimitreli, Chrysalidou, & Akakiadou, 2013).

The pH_g at the onset of gelation decreased with increasing SCN concentration. According to Tukey test, the pH_g values decreased in the following order: 1% SCN = 0% SCN > 2% SCN = 3% SCN. The onset of gelation is due to the charge reduction of protein molecules resulting in protein-protein interaction, as the molecules come in

contact with each other. In heat-treated milks due to the denatured whey proteins that form complexes with caseins (Lucey, Munro, & Singh, 1998a), the destabilization of proteins takes place at higher pH values, when compared to unheated milk (Lucey, Tamehana, Singh, & Munro, 1998b). This is caused by the higher isoelectric point of the whey proteins (pH 5.2) when compared to caseins (pH 4.6) (Lucey et al., 1998b). As the concentration of SCN is increased, the casein micelles are increased in number resulting in decreased ratio of casein-whey protein complexes to free caseins. This means that the destabilization of proteins will start at lower pH due to the increased casein molecules.

Table 1. The mean values of the fermentation time required for the onset of gelation (t_g), the pH at the onset of gelation (pH_g), the maximum tanδ (tanδ_{max}), the time at tanδ_{max} ($t_{tan\delta max}$), the pH at tanδ_{max} ($pH_{tan\delta max}$), the elastic modulus (G') at tanδ_{max} ($G'_{tan\delta max}$), the fermentation time for the pH to reach the 4.6 (t_{total}) and the maximum elastic modulus (G'_{max}). The standard deviations are given in parenthesis

Sodium Caseinates concentration (% w/w)	t_g (s)	pH_g	$tan\delta_{max}$	$t_{tan\delta max}$ (s)	$pH_{tan\delta max}$	$G'_{tan\delta max}$ (Pa)	t_{total} (s)	G'_{max} (Pa)
0	16200 (±283)	5.48 (±0.01)	0.480 (±0.004)	19200 (±293)	5.13 (±0.01)	90.2 (±0.6)	36000 (±324)	220 (±9)
1	17400 (±141)	5.52 (±0.01)	0.399 (±0.008)	19400 (±424)	5.30 (±0.01)	45.6 (±1.0)	55200 (±310)	384 (±4)
2	20400 (±141)	5.38 (±0.01)	0.333 (±0.008)	21000 (±350)	5.33 (±0.01)	14.7 (±0.8)	58200 (±141)	610 (±10)
3	22800 (±145)	5.34 (±0.01)	0.275 (±0.007)	24000 (±275)	5.35 (±0.01)	7.0 (±1.0)	63000 (±473)	786 (±3)

The tanδ_{max}, which indicates the beginning of the conversion of a liquid-like system into a gel, was decreased with increasing SCN concentration. The Tukey test showed that tanδ_{max} decreased in the following order: 0% SCN > 1% SCN > 2% SCN > 3% SCN. The increased in SCN concentration resulted in increased number of casein micelles and thus in increased possibilities of interactions to be formed between destabilized molecules, even at higher pH values. The increased number of caseins required less charge reduction in order the molecules to come closer and thus the reduction in tanδ values started at higher pH values. Indeed according to ANOVA, the $pH_{tan\delta max}$ increased with increasing SCN concentration. The Tukey test showed that $pH_{tan\delta max}$ increased in the following order:

0% SCN < 1% SCN 2% SCN 3% SCN.
_____

The lines indicate the overlapping differences. The decreased values of tanδ_{max} are due to the larger size of casein micelles, because the aggregation of proteins started at higher pH values, where the amount of colloidal calcium phosphate dissociated from the casein micelles into the milk serum was lower.

The increased values of $t_{tan\delta max}$ with increasing SCN concentration are due to the increased buffering capacity of caseins, which results in more lactic acid production for pH reduction. The $t_{tan\delta max}$ values increased in the following order: 0% SCN = 1% SCN < 2% SCN < 3% SCN.

The $G'_{tan\delta max}$ reduced with increasing SCN concentration. According to Tukey test, the $G'_{tan\delta max}$ decreased in the following order: 0% SCN > 1% SCN > 2% SCN > 3% SCN. This can be attributed to the earlier destabilization of proteins (at higher pH values), where the repulsion forces among molecules are still strong and the interactions formed are weaker.

Finally, the G'_{max} increased with increasing SCN concentration. The Tukey test showed that G'_{max} increased in the following order: 0% SCN < 1% SCN < 2% SCN < 3% SCN. Similar results were reported for kefir samples enriched with SCN. According to Dimitreli and Antoniou (2011) the apparent viscosity of kefir samples was increased with increasing SCN concentration. Yovanoudi, Dimitreli, Raphaelides and Antoniou (2013) reported increased values of elongational viscosity and apparent viscosity for kefir-type samples, when the amount of SCN was increased. Caseins, due to their increased size (micelles) and their increased number of active side groups, play the most important role during gel formation. When the number of casein molecules is increased, more interactions can be formed that result in a dense matrix with increased elasticity and thus increased

solid-like behavior.

4. Conclusion

The presence of SCN into the system of kefir greatly affected the formation of the gel and thus the properties of the final product. SCN increased the fermentation time required for the onset of gelation as well as for the pH to reach 4.6. The beginning of cross-linking of proteins towards aggregates that leads to the formation of a three-dimensional protein matrix, took place at higher pH values with increasing SCN concentration. The values of G' and tanδ that correspond to this point decreased with increasing SCN content. SCN addition enhanced the elasticity of the protein matrix and reduced the liquid-like behavior of the final products resulting in increased values of G'_{max} and decreased values of tanδ.

Acknowledgments

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