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Soft gels from bovine colostrum

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ABSTRACT

Colostrum puddings are widely popular in India as well as in some European countries. To prepare these desserts fresh colostrum from the first or second day of lactation is heated and flavoured by adding spices and sugar. Depending on the day of lactation the colostrum is mixed with normal milk in order to achieve a thinner consistency. However, no thickening agents like starch or different polysaccharides need to be added since, in contrast to drinking milk, the early milk of cows and other mammals, called colostrum, forms gels without adding gelling agents when heated.

The aim of this study is to understand the mechanisms involved in colostrum gel formation. In order to learn about the fat distribution in colostrum and the melting of the fat, initially, differential scanning calorimetry (DSC) and optical microscopy were performed. Furthermore, the colostrum gel formation was investigated by carrying out rheological measurements. Temperature and time sweep were used to characterize the gel formation of colostrum under heating and an amplitude sweep was performed to understand the forces and molecular interactions involved in the formation of the gel network. Taking into account the different composition of milk and colostrum and comparing their distinct behavior under heating, conclusions could be drawn about which mechanisms cause the formation of a colostrum gel.

The gel formation of colostrum is caused by its significantly higher concentration of different proteins, especially β-lactoglobulin. During the heat treatment these proteins denature and subsequently rearrange themselves. Consequently, soft elastic gels are formed by β-lactoglobulin in combination with other proteins, such as IGF-1 (insulin-like growth factor 1), IGF-2 (insulin-like growth factor 2), N-acetylgalactosaminyltransferase 1 (GALNT1) and lactoferrin.

Introduction

Junnu, *kharvas*, *posu* or *ginna*, all describing the same sweet in different regional languages, are well-known colostrum puddings served in India on different occasions as special treats. The pudding is cut into small pieces and typically served with *idli*, *dosa*, or *roti* (Sarkar et al., 2015). In Northern India these sweet desserts made of colostrum are called *khees* (Poonia and Dabur, 2012). Also European countries know puddings made of colostrum. In Norway it is called *råmelkspudding*, whereas the people in Iceland call their dessert *abrystir*. In England a pudding made from colostrum called *beestings* is served whereas Swedish people call their jiggling pudding *kalvdans* ("calf-dance") (Lopez, 2018). Colostrum puddings are in some cultures traditional foods. However, because of limited availability and legislative restrictions in some countries a large scale production is rather unlikely. Nevertheless, colostrum provides useful insight on the physical aspects of the collective behavior of whey proteins.

Colostrum is the first milk produced by a mammal after giving birth. The mother provides it shortly before and throughout the first 4–5 days after birth (Ternes et al., 2005; Eisenbrand and Schreier, 2006). The color of colostrum appears yellow-brownlike and its consistency is visibly more viscous compared to milk produced later on (Eisenbrand and Schreier, 2006). Besides being the only source of nutrition for the newborn, it is also crucial for the immunological protection of the neonate, especially for ruminants (Stelwagen et al., 2009).

Normal (mature) milk contains about 3.3 % of protein (Töpel, 2016). The proteins present in milk are categorized into two fractions, casein (2.7 %) and whey proteins (0.6 %) (Belitz et al., 2009). The different types of caseins, α_{S1}-, α_{S2}-, β- and κ-casein, form micelles (de Kruif et al., 2012). These proteins are very heat-stable (Fox, 2003) and only start to denature at about 110 °*C* (Sauer and Moraru, 2012). Whey proteins in bovine milk are mainly α-lactalbumin (1.2–1.3 g in 1 kg milk) and β-lactoglobulin (3.1–3.5 g in 1 kg milk). However, also serum albumin (0.4 g in 1 kg milk) and immunoglobulin (0.6–0.8 g in 1 kg milk) are

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present in milk, as well as low concentrations of lactoferrin (0.1 g in 1 kg milk) (Haug et al., 2007; Töpel, 2016). Native whey proteins occur in folded, globular shape (Töpel, 2016). α-lactalbumin contains 142 amino acids including 8 times the amino acid cystein (Bateman, 2019; UniProt, 2020a). Chaplin et al. reported that α-lactalbumin denatures reversibly between 53 °*C*and 70 °*C*. Irreversible heat-denaturation has been reported for higher temperatures (Chaplin and Lyster, 1986). β-lactoglobulin is made of 178 amino acids and contains 7 cystein molecules (Bateman, 2019; UniProt, 2754). β-lactoglobulin denatures between 65 °*C* and 71 °*C* (de Wit and Swinkels, 1980).

The total protein content of colostrum is about 4–6 times higher compared to milk. The whey protein fraction is about 20 times higher (Jensen, 1995; Töpel, 2016) and also the content of immunoglobulins is increased strongly. In bovine milk the dominant immunoglobulin is IgG_1 with a concentration of 0.59 mg/mL. Nevertheless, others like IgG_2, IgA and IgM appear too, although in much smaller concentrations (IgG_2 0.02 mg/mL, IgA 0.14 mg/mL and IgM 0.05 g/mL). The concentration of all of these immunoglobulins is much higher in colostrum compared to normal (mature) bovine milk. In colostrum IgG_1 is present with a concentration of 47.60 mg/mL, IgG_2 with 2.90 mg/mL, IgA with 3.90 mg/mL and IgM with 4.20 mg/mL (Stelwagen et al., 2009). From this follows that in particular, the concentration of IgG_1 is about 80 times higher in colostrum than in milk. The content of lactoferrin is also highly increased in colostrum with 2.0 g in 1 kg colostrum instead of 0.1 g in 1 kg milk for milk (Töpel, 2016). Lactoferrin consists of 708 amino acids, including 35 cystein molecules (Bateman, 2019; UniProt, 2020b). It has been reported by Sánchez et al. that the lactoferrin structure has basically remained unchanged under heat pasteurization (72–74 °*C* for 15 s). However, longer heat treatment of iron-saturated lactoferrin at 65 °*C* resulted in very slow denaturation (Sánchez et al., 1992). Furthermore, the concentration of vitamins and mineral nutrients is increased remarkably in colostrum as compared to milk (Eisenbrand and Schreier, 2006; Samuel et al., 2017; Urukpa et al., 2002). Especially, the concentration of calcium ions is highly increased in colostrum (Tsioulpas et al., 2007).

Moreover, colostrum contains growth factors to promote the development of the newborn. The most abundant are insulin-like growth factor 1 (IGF1) and insulin-like growth factor 2 (IGF2) (Pakkanen and Aalto, 1997). IGF1 consists of 154 amino acids including 10 cystein molecules and IGF2 179 amino acids with 8 cysteins (Bateman, 2019; UniProt, 2020c, 2020d). Also, the enzyme polypeptide N-acetylgalactosaminyltransferase 1 (GALNT1) is found in bovine colostrum. This protein molecule contains 559 amino acids including 16 cysteins (Bateman, 2019; UniProt, 2020e; Elhammer and Kornfeld, 1986).

Whey protein isolate (WPI) can produce similar gels under heat treatment when their concentration is sufficiently high (Aguilera, 1995; Lavoisier et al., 2019). Alternatively, WPI produces cold-set gels when bivalent calcium ions are added after a mild heat-treatment to ensure denaturation and dissolution of the proteins (Kharlamova et al., 2018; Marangoni et al., 2000; Lavoisier et al., 2019; Bryant and McClements, 2000).

The aim of this work is to understand which processes and proteins induce a gel formation in colostrum under increasing temperature whereas milk does not show a similar behavior. Taking into account the different composition of milk and colostrum while comparing their different behavior under heating fosters the understanding of the mechanisms that cause gel formation and the different rheological behavior of colostrum and corresponding raw milk. These mechanisms leading to the gel formation of colostrum under heating make it possible to create dishes like *kharvas* or *kalvdans*.

Materials and methods

Colostrum

The raw milk and colostrum from Jersey cows used in this study was provided by the local farm of Hans-Christoph Gill in Bodenheim close to Mainz, Germany. A phase separation (creaming) naturally occurred in the colostrum; the upper phase appeared yellow in color, the lower phase was significantly whiter. In order to characterize the phase separated colostrum, three different types of sample were investigated: one from the upper phase, one from the lower phase as well as one after stirring and thus mixing upper and lower phase. The last one will be referred to as mixed phase.

Differential scanning calorimetry

The different phases have been investigated by differential scanning calorimetry (DSC). A Mettler Toledo DSC3+/700/453 (Mettler-Toledo, LLC, 1900 Polaris Parkway, Columbus, OH 43240, USA) was used to get information about the thermal properties of each colostrum sample. As a reference milliQ water was used. Both, the sample and the reference, were measured in a 100 μl aluminum pan. During the measurement the temperature was first raised from 20 °*C* to 70 °*C* with a heating rate of 1 K/min and afterwards kept at 70 °*C* for 30 min. For the heating process a liquid nitrogen flow with a flow rate of 30 ml/min was used. DSC measurements were performed once due to the restriction of identical material. The measured data was displayed using OriginPro 2017 software (OriginLab Corporation, One Roundhouse Plaza, Suite 303, Northampton, MA 01060, USA). Integration over the peak area gives the enthalpy change ΔH. The enthalpy change ΔH was calculated for the upper and the mixed phase by fitting the main peak of the measured curves with a Gaussian. For integration and fitting the curves OriginPro 2017 software was used as well.

Light microscopy

To gain more insights and observe the fat droplet behavior during heating, light microscopy was performed using a Carl Zeiss Axio Scope. A1 (Carl Zeiss AG, Carl-Zeiss-Straße 22, 73447 Oberkochen, Germany) microscope. All shown images were taken using polarized light. The used objective lens magnifies 20 x and thus the total magnification was 200 x. The analysis shows that droplets can be distinguished clearly. To observe the behavior of the fat droplets under heating the temperature of the samples was varied from 20 °*C* to 70 °*C* using a heating rate of 1 K/min. Afterwards, the temperature was kept at 70 °*C* for 30 min. Images of the samples were taken at 20 °*C* as well as at 70 °*C* (after waiting for 30 min).

Rheology

Rheology measurements were performed on a DHR3 rheometer from TA Instruments (TA Instruments, 159 Lukens Drive, New Castle, DE 19720, USA), to investigate the gel formation of colostrum. A plate-plate geometry with a diameter of 40 mm and a gap of 500 μm was used for all measurements. First, a temperature sweep in combination with a time sweep was carried out. In this measurement the temperature was increased from 20 °*C* to 70 °*C* with a heating rate of 1 K/min and afterwards the temperature was kept constant at 70 °*C* for 30 min. The oscillation strain for both, the temperature sweep and the time sweep, was 0.01 %. Secondly, an amplitude sweep was done at 70 °*C* and a frequency of 1 Hz. The oscillation strain was varied from 0.001 % to 1000 %. All rheology measurements were performed in duplicate. The measured data was displayed using OriginPro 2017 software (OriginLab Corporation, One Roundhouse Plaza, Suite 303, Northampton, MA 01060, USA).

Results

Differential scanning calorimetry

In Fig. 1 the DSC results of upper phase (red curve), mixed phase (purple curve) and lower phase (blue curve) of the colostrum are shown. The grey line which shows a linear slope first and is constant afterwards indicates the temperature ramp. It illustrates how the temperature was raised first from 20 °*C* to 70 °*C* and then kept at 70 °*C*.

For the upper phase a pronounced endothermic peak occurs at about 1100 s. From comparison with the temperature ramp it can be assigned to about 38 °*C* (minimum of the peak, the peak starts already at about 33 °*C*). For the mixed phase a smaller endothermic peak occurs at the same temperature (minimum of the peak at about 38 °*C*, start of the peak at about 31 °*C*). However, no peak can be seen for the lower phase at this temperature. Milk fat is a mixture of different lipids with different melting temperatures. The mixture of lipids in milk fat melts at temperatures just below 37 °*C* (Töpel, 2016; Williams et al., 1997). If a similar lipid composition is assumed to be present in colostrum, the endothermic peaks in the upper phase and mixed phase can be assigned to the melting of the fat present in colostrum. Integration of the area under the fit resulted in an enthalpy change of $\Delta H \approx 8.8$ J/g for the upper phase and $\Delta H \approx 2.2$ J/g for the mixed phase. For the upper phase the value of the enthalpy change is higher than for the mixed phase. Thus, a higher enthalpy is needed in the upper phase for melting the solid parts of the fat than in the mixed phase. This implies that the upper phase contains a higher concentration of fat. Moreover, this behavior can be explained by the fact that the lipids in milk have a lower density than water, which causes the creaming of milk. Despite the high protein contents and higher viscosity, colostrum also shows strong creaming behavior which is known from native milk as well.

Light microscopy

Fig. 2 shows pictures taken with a light microscope using polarized light. The images on the left were taken at 20 °*C* (before heating the samples) and the ones on the right at 70 °*C* (after heating and waiting for 30 min).

Fat crystals and droplets can clearly be distinguished from the whey (grey background). On the left side a lot of white spots can be seen clearly in all three phases. Fat crystals appear as white spots under polarized light since the crystals show typical polarization patterns. So as expected from the DSC measurement the fat is solid at 20 °*C*. On the right side in contrast, no white spots are visible, but spherical and ellipsoid droplets. The polarization effects have vanished. As supported by the DSC measurement, it can be concluded here as well that after heating the milk to 70 °*C* the solid parts of the milk fat are melted. Moreover, it is observed that in the upper phase there are significantly more fat crystals (at 20 °*C*) respectively fat droplets (at 70 °*C*) than in the mixed phase or lower phase. The fat crystals in the upper phase are densely packed. The lower phase shows the least amount of fat crystals respectively droplets. These microscopic observations underline the findings of the DSC measurement that the fat droplets are concentrated in the upper phase and thus colostrum shows creaming behavior.

Rheology

The results of the temperature and time sweep are shown in Fig. 3. The curves of upper phase, mixed phase as well as lower phase of colostrum are displayed. Additionally, the result of raw milk is shown for comparison. The grey line indicates the temperature ramp of the measurement (temperature vs. time). It shows a linear slope first which represents the temperature sweep followed by a constant line which illustrates the time sweep.

All three colostrum samples show steeply rising moduli first, followed by a plateau. The loss modulus and storage modulus of the upper phase rises much earlier (inflection point at about 26 min) than the moduli of the other two phases. The mixed phase follows at about 40 min and the lower phase at about 53 min. From comparing these times with the grey line of the temperature ramp the temperatures of the sol-

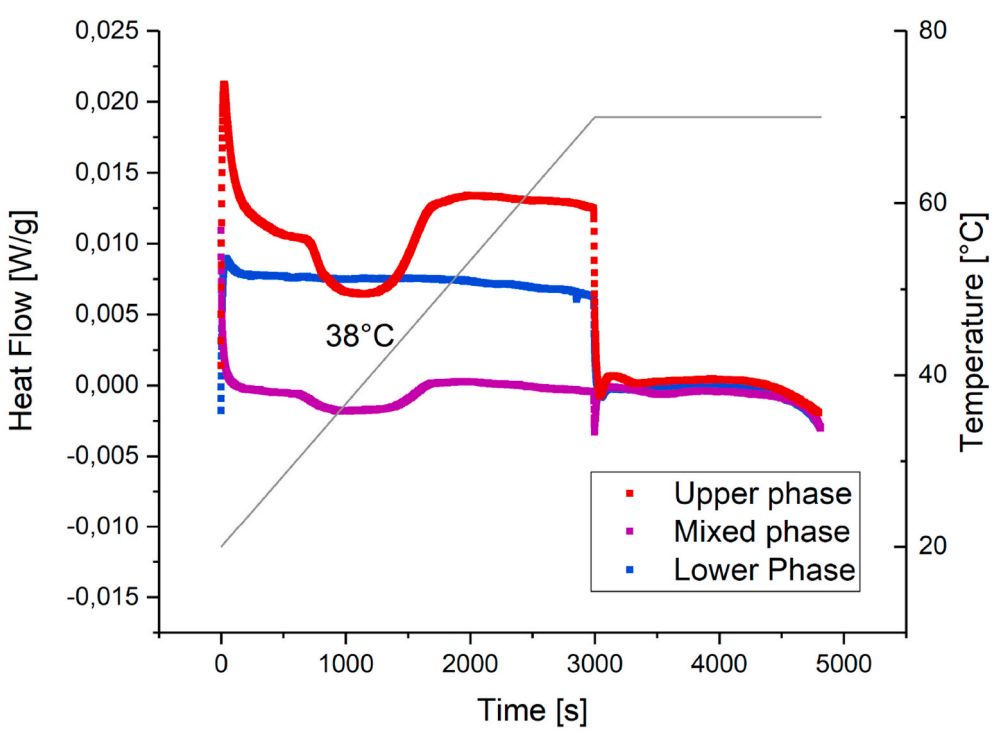
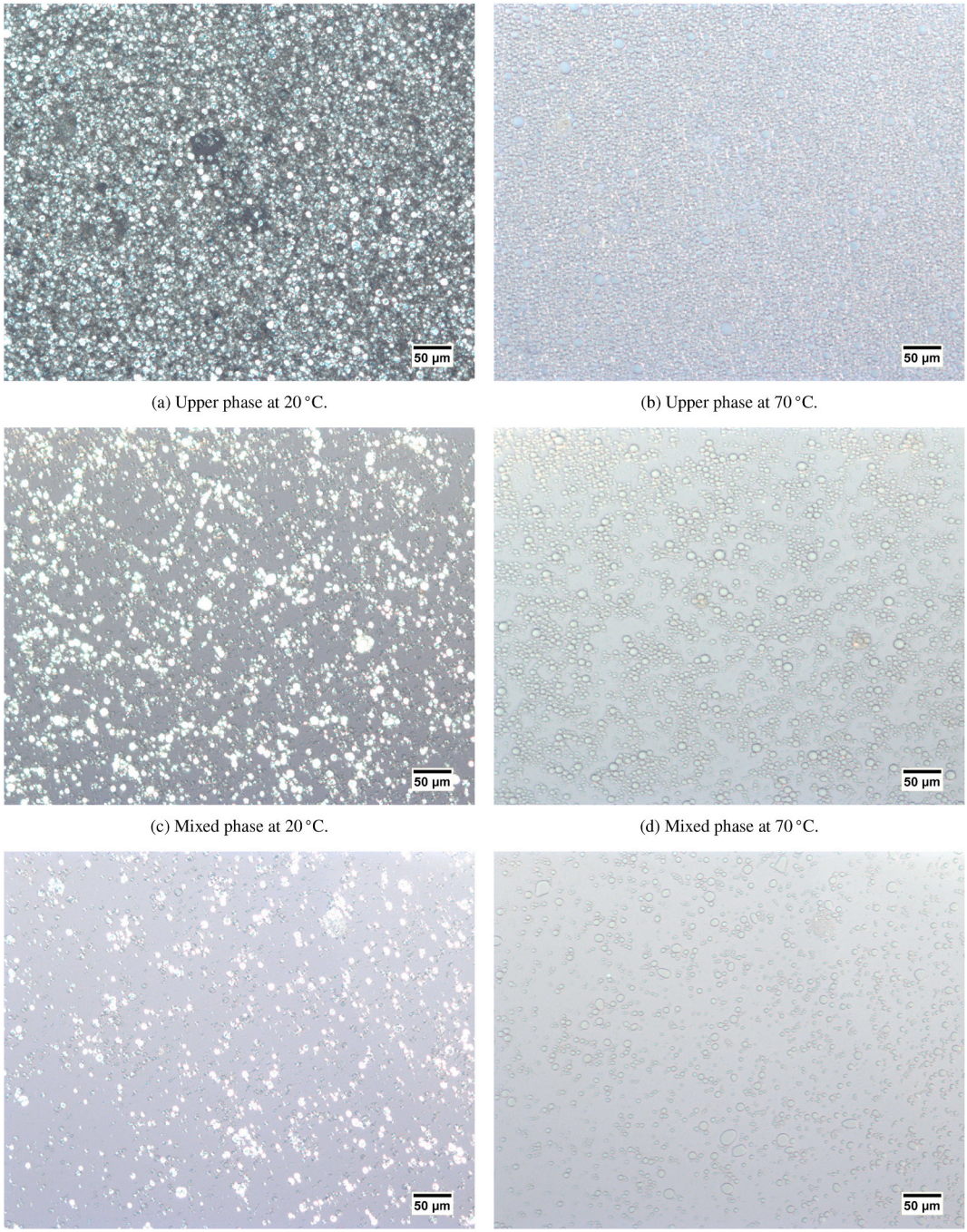


Fig. 1. Differential scanning calorimetry (DSC) of upper phase (red), mixed phase (purple) and lower phase (blue) of colostrum. The temperature ramp is indicated as grey line. Upper and mixed phase show a peak at about 1100 s. From comparison with the temperature ramp this peak can be assigned to 38 °*C*. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



(a) Upper phase at 20 °C.

(b) Upper phase at 70 °C.

(c) Mixed phase at 20 °C.

(d) Mixed phase at 70 °C.

(e) Lower phase at 20 °C.

(f) Lower phase at 70 °C.

Fig. 2. Optical microscopy under polarized light conditions with a magnification of 200 x.

gel transition can be determined. The point where the loss modulus and the storage modulus of one sample cross is called gelation point. At this point the material undergoes a sol-gel transition and starts to gel. The upper phase which starts to gel first has a sol-gel transition temperature of about 46 °*C*. For the mixed phase a temperature of about 60 °*C* could be determined for the gelation point. The lower phase only started to gel after reaching 70 °*C*. Raw milk showed a completely different behavior. First, the moduli and viscosity are much smaller. Second, native milk does not form large scale gels during heating, and no rise of the moduli values can be observed. The reason for the different behavior lies in the different composition of normal milk as compared to colostrum. As mentioned earlier, colostrum is much richer in protein, especially immunoglobulins and whey proteins which denature under heating at about 70 °*C*. In addition, a higher calcium content in colostrum than in normal milk has been reported by McGrath et al. in (McGrath et al., 2016). The content of free calcium ions also has a significant impact on the gel formation since they are bivalent positively charged ions and thus bind to the negatively charged lateral groups of amino acid residues of whey proteins. The ionic links formed lead to aggregation. As a consequence the viscosity increases. Furthermore, the casein fraction in colostrum is enhanced as compared to normal milk and larger casein micelles are formed (McGrath et al., 2016). These reasons explain why colostrum undergoes gel formation under heating whereas normal milk does not.

Fig. 4 summarizes the results of the amplitude sweep that was performed to understand the gel network properties.

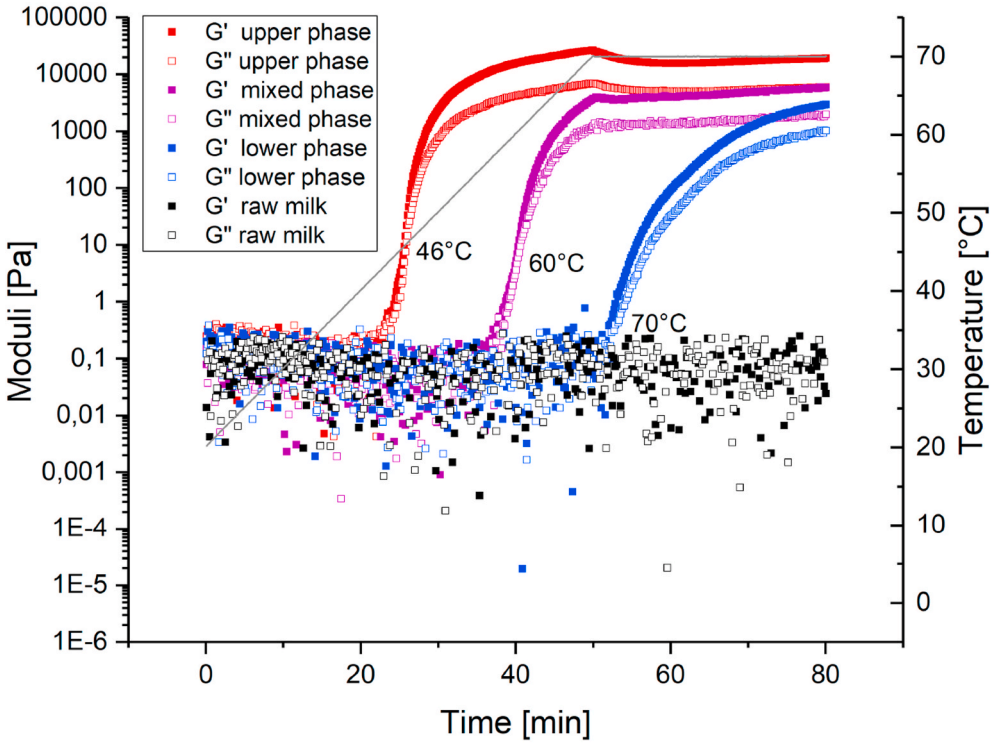


Fig. 3. Temperature sweep and time sweep of the upper phase (red), mixed phase (purple) and lower phase (blue) of colostrum. The temperature ramp is indicated as a grey line. At the cross-over point of storage modulus and loss modulus the sample started to gel. The corresponding gelation temperature was determined by superimposing the cross-over point of the moduli with the temperature ramp. For comparison, raw milk (black) is shown, which does not gel under heat treatment. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

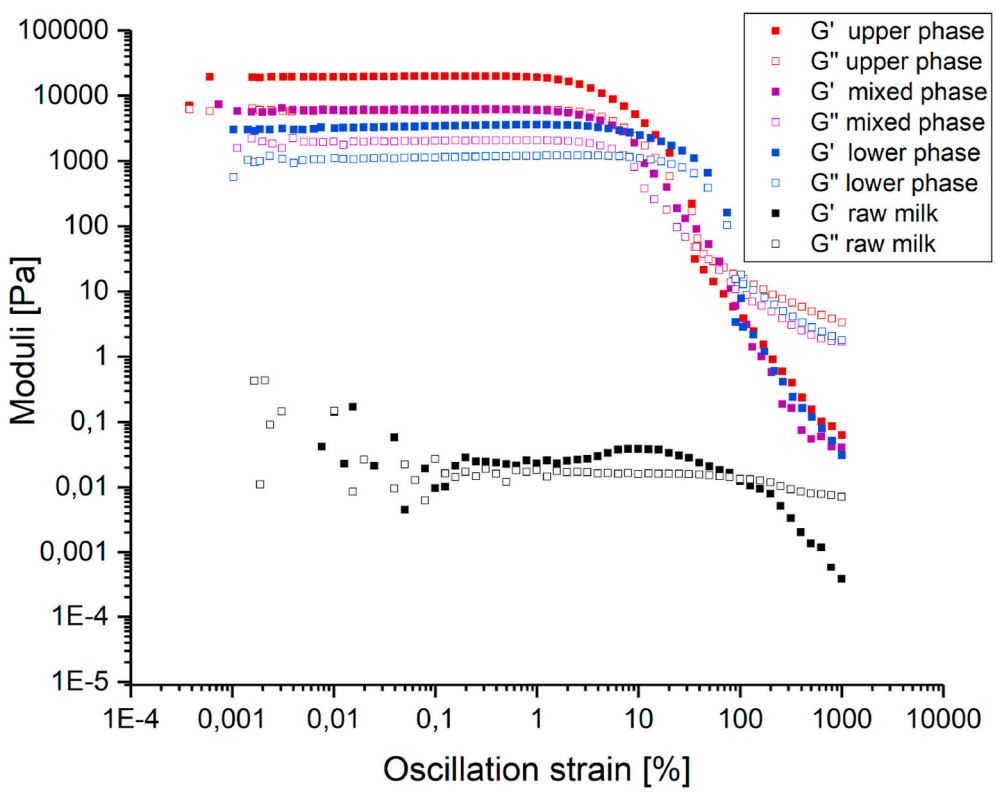


Fig. 4. Amplitude sweep of upper phase (red), mixed phase (purple) and lower phase (blue) of colostrum. The temperature was kept constant at 70 °*C*. For comparison the behavior of raw milk (black) is shown as well. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 4 shows the typical breakdown of the gel. During the linear viscoelastic region the storage and loss moduli remain constant up to approximately 10 % deformation. At larger strains the gel structure breaks as shown by the decreasing storage modulus at corresponding amplitudes. The large scale gel structure is broken at amplitudes, when the loss modulus becomes larger than the storage modulus. The breaking

of the gel structure occurs first for the upper phase, then for the mixed phase and finally for the lower phase. Thus the breakdowns show the same trend as the moduli of the respective phases.

Comparing the values of the moduli of the different colostrum phases, it is noticed that the moduli of the upper phase show higher values than the ones of the other phases. Hence, it has the highest gel strength and viscosity. This behavior can be explained by its higher fat content. During gelation the fat droplets become trapped in the network and thus enhance the viscosity of the gel. For the mixed phase the moduli show smaller values than for the upper phase. The lower phase shows even lower values for the moduli. Consequently, with decreasing fat content the viscosity of the gel decreases. The amplitude sweep for raw milk is shown also for a direct comparison. The storage and loss moduli measured in this case are significantly smaller (about 5 orders of magnitude). When heating normal milk no bulk gel formation is observed. It stays liquid under heating.

Discussion

As a first result it should be noted that a pronounced long time creaming is observed and confirmed by DSC and polarized light microscopy as indicated by Figs. 1 and 2. However, the creaming velocity for colostrum is reduced by the pronounced higher viscosity compared to native milk (see Fig. 4).

Comparison of microscopy images at 20 °*C* with those at 70 °*C* showed that the milk fat was solid at 20 °*C* whereas the lipids melted after heating to 70 °*C*. The observed polarizibility at 20 °*C* vanished at 70 °*C*. This was the case for all three phases of the colostrum. Comparing the upper, mixed and lower phase with each other, it stands out that there are much more fat droplets present in the upper phase. The lower phase shows the least amount of fat droplets. The different concentrations of fat droplets in the upper, mixed respectively lower phase suggest a creaming behavior of colostrum.

Moreover, it was observed that colostrum undergoes a gel formation under heat treatment whereas milk does not. To characterize the gel properties of the colostrum gel rheological measurements were performed. The upper phase of colostrum starts to gel earlier and shows a stronger gel than the mixed or lower phase, which can be explained by the effect that a higher fat content enhances the gel formation. For a higher fat content the fat droplets are packed more closely. After starting to gel the fat droplets are trapped inside the gel network. Comparing the different rheological behavior of milk and colostrum under heating and regarding their different composition leads to understanding the mechanisms causing the gel formation in colostrum. The reasons for the formation of strong gels are manifold.

The first reason for the formation of a colostrum gel with relatively high moduli has mainly its reasons in the relative high concentration of the ensemble of proteins compared to native milk. When colostrum is heated, most of the proteins denature and form partly unstructured chain molecules which can easily interact with each other, since the total protein concentration is larger than their overlap concentration (de Gennes, 1979). As in normal milk, also the proteins in colostrum can be divided into two fractions: casein and whey proteins. From casein and casein micelles it is well-known that the denaturation temperature is higher than the temperature range in the present experiment (Töpel, 2016). Caseins do thus not contribute to the gel formation, in contrast to the whey proteins, α-lactalbumin, which consists of 142 amino acids and β-lactoglobulin, containing 178 amino acids. Especially β-lactoglobulin denatures at temperatures between 65 °*C* and 71 °*C* (de Wit and Swinkels, 1980). It is also reported, that the denaturation temperature of β-lactoglobulin depends on the presence of the concentration of α-lactalbumin (Boye and Alli, 2000), which needs to be taken into account for comparisons between colostrum and native raw milk. Other important proteins in colostrum involve soluble polypeptide N-acetylgalactosaminyltransferase 1 (GALNT1) containing 559 amino acids (Elhammer and Kornfeld, 1986) and insulin-like growth factors 1 and 2 (IGF1, IGF2) with 154 and 179 amino acids respectively (Francis et al., 1988).

These long and partially thermally denaturing proteins contain all a significant number of the amino acid cystein, which can form and reform disulphide bonds and connect different proteins by permanent cross-links. During denaturation, the arrangement of the protein structure is changed. The initial links between the amino acids of the whey protein are broken and new ones can be formed (McSwiney et al., 1994; Visschers and de Jongh, 2005; Lavoisier et al., 2019). Therefore, it is very likely, that the formation of disulphide bridges between different proteins contribute to the strong increase of the modulus. Indeed, interactions between different proteins in colostrum can be addressed to the thermal properties of insulin-like growth factors and whey proteins (Ollikainen and Riihimäki, 2012). Obviously, the permanent cross-linking can only take place at sufficiently high temperatures, above 65 °*C*, which corresponds to the final stages of the moduli shown in Fig. 3. However, the gelation of the lower, mixed and upper phase appear at different time scales. For example the onset of the gelation for the fat rich upper phase begins after about 26 min at about 46 °*C*, whereas the lower phase starts to gel at about 53 min at 70 °*C* only. Additional mechanisms for gel formation must therefore be present.

It is well-known from the ion induced cold gelation of β-lactoglobulin, that bivalent calcium ions are able to form ionic cross-links between negatively charged amino acids, glutamic acid and aspartic acid (Veerman et al., 2003; Lavoisier et al., 2019). Colostrum contains a higher concentration of calcium ions. Especially in the fat rich upper phase, where the concentration of proteins and bivalent calcium ions in the continuous water phase is larger, ionic cross-links appear with higher probability. The growth of such clusters leads to a gel formation and thus an increased viscosity. The continuous phase may cross-link partially already at a lower temperature, which explains the early rising of the modulus. In addition, stronger interactions between different charges on the surface of native proteins cause contributions to an "electrostatic cross-linking". In the mixed phase, both effects seem to contribute to the gelation.

These ideas are supported by the strain (amplitude) sweeps shown in Fig. 4. The gelled upper phase at 70 °*C* shows the lowest linear viscoelastic regime. It ranges up to 2 % deformation, whereas the linear viscoelastic regime for the fat poor lower phase ranges up to 10 %. This observations fit into the developed picture. The shorter linear regime and the breakdown of the structure at lower deformation of the upper phase can be understood with higher local deformation of the continuous phase, whereas in the fat poor phase the denatured and cross-linked "unfilled" network of entropic chains support larger deformations (Vilgis et al., 2009).

To support the mentioned explanation that the thermal denaturation of the whey proteins, especially β-lactoglobulin, leads to the heat-induced gel formation of colostrum, the average distance of β-lactoglobulin molecules and the size of one globular β-lactoglobulin molecule are estimated in the following. To calculate the average distance of β-lactoglobulin molecules the particle number density is used.

$$c = \frac{N}{V} \tag{1}$$

where c is the concentration of β-lactoglobulin, N is the number of β-lactoglobulin molecules and V is the total volume. The concentration of β-lactoglobulin is given as 3.1–3.5 g in 1 kg milk (Töpel, 2016). In this calculation its value is assumed as 3.3 g. The mass of a β-lactoglobulin molecule is given as 19,883 Da (Bateman, 2019). It holds: $19,883\ \mathrm{Da} = 19,883\ \mathrm{u} = 3.3 \cdot 10^{-23}$ kg, where $\mathrm{u} = 1.66 \cdot 10^{-27}$ kg is the atomic mass unit. From these values follows the number of β-lactoglobulin molecules in 1 kg milk: $N_m = \frac{3.3\ \mathrm{g}}{3.3 \cdot 10^{-20}\ \mathrm{g}} = 10^{20}$. If the density of milk is assumed to be 1 g/cm^3 the volume of 1 kg milk is 1000 cm^3. It follows for the concentration

$$c_m = \frac{N_m}{V_m} = 10^{17}\text{cm}^{-3} \tag{2}$$

where c_m is the concentration of β-lactoglobulin molecules in milk, N_m is the number of β-lactoglobulin molecules in 1 kg milk and V_m is the volume of 1 kg milk. For the average distance of β-lactoglobulin molecules in milk follows:

$$d_m \sim c_m^{-1/3} \approx 22 \text{ nm}. \tag{3}$$

If the concentration of β-lactoglobulin molecules in colostrum is assumed 20 times higher than in milk (Töpel, 2016), it follows for the concentration and average distance of β-lactoglobulin molecules in colostrum:

$$c_c = 20 \cdot c_m = 20 \cdot 10^{17}\text{cm}^{-3} \tag{4}$$

$$d_c \sim c_c^{-1/3} \approx 8 \text{ nm}. \tag{5}$$

According to Erickson (2009) all globular proteins have approximately the same density und thus approximately the same partial specific volume $\nu_2 = 0.73\text{cm}^3/\text{g}$. If the shape is assumed to be a sphere the size of a globular protein can be estimated by the scaling relation of collapsed polymers,

$$R_{min} = (3V/4\pi)^{1/3} = 0.066M^{1/3} \tag{6}$$

where R_{min} is the radius of the globular protein in nm, V is its volume and M is the mass of the globular protein in Da (Erickson, 2009). Thus the radius of a globular β-lactoglobulin molecule (undenatured protein) can be estimated as $R_{min} = 1.8$ nm.

When denatured, the protein molecule is elongated. The ranges for scaling the size have been given as $M^{2/5} < R < M^{3/5}$ (Hong and Lei, 2009; Vilgis, 2015), where the lower limit describes partially denatured proteins, the upper limit corresponds to self-avoiding walks, well-known from polymers in good solvent (de Gennes, 1979). Estimates of the radius in the denatured states, show their size grows significantly by a factor between 1.9 and 14. β-lactoglobulin denatures completely, which means that in the high concentrations of colostrum the overlap concentration is easily reached, as depicted in the schematic Fig. 5.

Denatured β-lactoglobulin molecules in colostrum overlap, and thus form a gel by forming new disulphide bridges between different molecules. The effect is enhanced by the other protein molecules present.

Normal, mature bovine milk with its natural protein content stays liquid during heating. Nevertheless, local gels form, when whey proteins cross-link by forming permanent disulphide bridges between denatured β-lactoglobulins, well-known from milk skin formation. β-lactoglobulin molecules form dimers in milk, which is for simplicity not drawn in Fig. 5, but this does not change the general conclusion.

Summary

Different countries like India and Scandinavian countries know colostrum puddings that are served as sweet desserts and special treats. The question that was investigated in this study was about why such puddings can be made from colostrum without adding any food thickening agents whereas pudding from milk can only be made by adding starch or polysaccharides that cause the milk to thicken under heat-treatment.

It was shown that colostrum shows creaming behavior. Furthermore, the gel formation can be explained by the high protein content of colostrum and the denaturation of the proteins under heat-treatment. Especially, the whey protein β-lactoglobulin, which denatures between 65 °C and 71 °C plays a key role in forming the gel network as well as the insulin-like growth factors IGF-1 and IGF-2 and the proteins polypeptide N-acetylgalactosaminyltransferase 1 (GALNT1) and lactoferrin all containing a significant number of cystein, which forms disulphide bonds and thus can permanently cross-link different protein chains. These cross-links contribute to the formation of a gel network. The formation of the gel network is enhanced by ions present in colostrum like bivalent calcium ions that are able to form ionic cross-links between negatively charged amino acids, glutamic acid and aspartic acid. Furthermore, the

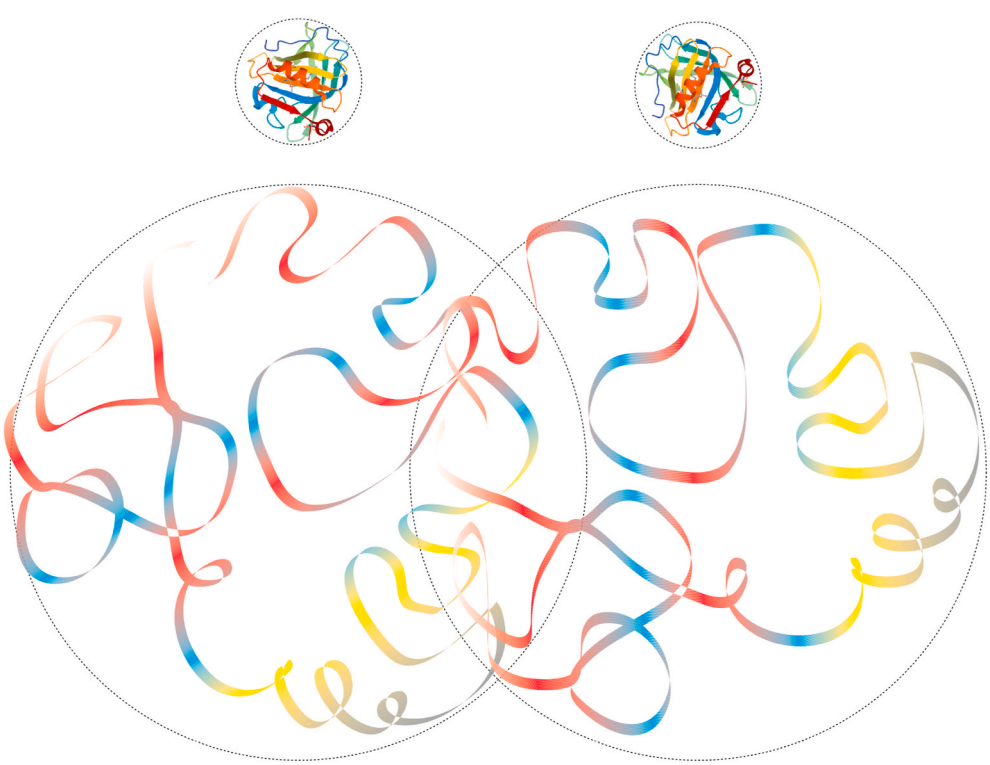


Fig. 5. The radius of β-lactoglobulin increases significantly by denaturation and thus the denatured molecules overlap throughout the entire sample in colostrum. The molecules in the top row show undenatured β-lactoglobulin. The ones below symbolize denatured β-lactoglobulin molecules.

strength of the gel network is enhanced by including fat droplets into the network. Consequently, the unique texture and gel forming properties under heat treatment of colostrum puddings can be explained by the special composition of colostrum.

Colostrum gels are traditionally used for sweet dishes in different cultures, however, the general mechanism of the gel formation allows slightly salty dishes as well. Onion and garlic infused colostrum, spiced with salt and smoked pimenton, combined with olive oil can be a basis for new ideas. Moreover, the results offer possibilities for reconstructions of colostrum milk in gastronomy, when native (untreated) cow milk is enriched with appropriate concentrations of whey proteins which show similar gelling properties with alike mechanical and fracture properties and hence, a similar mouthfeel.

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CRediT authorship contribution statement

Judith Hege: Investigation, Visualization, Writing - original draft, preparation. **Marta Ghebremedhin:** Investigation, Writing - review & editing. **Bhagyashri L. Joshi:** Investigation, Visualization, Writing - review & editing. **Christine Schreiber:** Investigation, Writing - review & editing. **Thomas A. Vilgis:** Supervision, Writing - review & editing.

Declaration of competing interest

All authors have read and agreed to the published version of the manuscript. The authors declare no conflict of interest.

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