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Dielectric behavior of adulterated milk with urea and water☆

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ABSTRACT

Milk adulteration, especially by adding urea, is one of the major health concerns. In this work, we report a dielectric methodology to detect the existence of polar adulteration like urea and water in milk. The microwave dielectric spectra of whole milk, skim milk and their mixture with varying contents of water or urea was measured. An obvious dielectric relaxation was observed for these systems. The dielectric relaxations were well represented by the superposition of two relaxation processes which are attributable to free water and water adsorbed on protein, urea-water co-clusters and urea-water co-clusters adsorbed on protein for both milk-water systems, urea aqueous solution and both urea-milk systems respectively. The relaxation of lower frequency was identified as the dipole orientation polarization of water or urea-water co-clusters adsorbed on protein in milk mixtures. Significant differences in dielectric parameters, relaxation time and relaxation strength, between these systems were analyzed by comparing between skim and whole milk with different water contents and among water, skim and whole milk with different urea contents. The results show that microwave dielectric spectroscopy may provide a way or a choice for monitoring of water and urea in adulterated milk.

1. Introduction

Milk is an essential component of a healthy diet for all age groups because it contains fat, protein, and mineral, which are the major elements required for growth [1]. Moreover, dairy products as a major food on dining tables also make from milk. Therefore, the assessment of milk quality is of critical importance. Protein and fat contents in milk are important quality parameters that characterize its nutritional value. To some extent, their content determines the value of the milk for payment. Accordingly, in order to obtain more profit, dishonest seller or/and some farmers would adulterate milk with urea or water to increase its protein content or its volume [2–5]. However, milk adulteration not only reduces its nutritive value but also harms consumers, what is more, it may lead to devastating diseases [6].

Several conventional chemical methods have been developed for detecting the contents of fat, protein and urea in milk [7–10]. In recent years, some physical means are also utilized to determine the components of milk, especially fat content, such as, visible light scatter, short-wave near-infrared spectra, digital imaging technology [11–14]. The contents of fat and protein in milk can also be determined by simple and rapid methods, such as laser light scattering technology and quartz crystal microbalance [15,16]. About the adulteration of milk, various detection methods have been reported for a long time [17–20]. The methods mentioned above actually just gave chemical information on milk or adulterated milk, such as, the contents of fat, protein and/or other substances. However, the information on the complex interactions between water and protein, water and impurities has not been provided, and insight into special microstructure of water, like water clusters and special colloidal structures of whole milk cannot be given by chemical methods. However, the answers to such questions is crucial to understanding the metabolic processes, such as lipolysis and absorption of milk fat and postprandial lipemia [21].

Dielectric spectroscopy (DS) is believed to be one of the highly desirable methods to explore the special structures of water cluster and milk colloidal structure, because it is very sensitive to molecular polarization or collective dynamics. In recent years, DS has been widely applied in the field of food research [22–26]. It is well known that milk can be considered as a colloidal dispersion that fat or lipid globule as oil phase dispersed in the water phase. However, a matter worthy of reflection, for such a heterogeneous system with huge phase interface, there is no distinct dielectric relaxation caused by interface polarization can be observed in the radio frequency range. For this reason, the dielectric researches of milk in the radio frequency range were rather limited, and most of dielectric measurements of milk were focused on microwave range [27]. In recent years, Guo and Zhu reported the researches on the dielectric properties of raw cow's and goat's milk and their temperature, protein or water contents dependence [28–30]. They pointed out that the dielectric parameters can help to evaluate milk's quality during the storage of milk and dairy processing.

☆ Practical Application: This work effort describes the influence of water and urea on dielectric property of milk by dielectric relaxation spectroscopy. It may be helpful to the application of dielectric spectroscopy method to detect milk adulteration.

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Milk is colloidal solutions in which fat dispersed in the watery solution of mineral salts and proteins as previously described. Urea [CO $(NH_2)_2$], as an end product of nitrogen metabolism in mammals, is also included in natural milk. This is because it is a small molecule and water soluble and can readily diffuse across mammary tissue into milk [31,32]. Both urea and water are very similar in structure. Understandably, a large number of water molecules in natural milk maybe interacts with very little urea molecules, forming special water-urea cluster structure as shown in Fig. 5b. Such clusters may be cause dielectric responses in the microwave range. The dynamic features of urea–water clusters and bulk-water cluster can be differentiated from the dielectric relaxation time. While the relaxation strength can measure water content in milk [22]. Some relevant studies have been reported [33–36], such as, Hayashi Y. et al. measured aqueous urea solutions of varying concentration at a frequency range from 200 MHz to 40 GHz and attributed the observed two relaxation processes to bulk-water clusters and urea-water co-clusters respectively [33]. They also studied the dielectric properties of the solvent protein/glycine betaine dissolved in aqueous urea solutions and discussed the interactions between urea and protein/glycine betaine [34,35]. There are other many researches that reported the effect of urea on the structural dynamics of water [36,37]. Besides urea, the dielectric properties of low-molecular-weight nonelectrolytes, such as formamide and alcohol, have also been studied [38–40].

From above studies, we get inspiration: whether the urea in milk where water occupies a considerable proportion also affects water-cluster structure and its biochemical functions in protein metabolism. Firstly, what is the differences in dielectric relaxation behavior between urea-water solution and urea-milk solution. In addition, how will add water and urea into natural milk or pure milk from the market changes their dielectric properties? In this paper, the microwave dielectric spectra of whole milk, skim milk and their mixture with varying contents of water or urea has been measured.

However, as mentioned above, the most common adulterated milks are done by adding water or urea, scientifically this is because both water and urea are polar substance and similar in structure, and they tend to mingle with milk which is made up primarily of water. Therefore, the similarity in dielectric behavior of urea-milk and water-milk may make it difficult to detect and distinguish them quantitatively. The main objectives of this study are to evaluate the effect of urea content on dielectric parameters and thereby detect the adulterating degree and try a new way for monitoring of urea in adulterated milk. We also hope this study can provide an important piece of information on the relaxation dynamics of these food systems which have never been studied by dielectric spectroscopy by investigating the interactions between the water/urea and protein in milk.

2. Materials and methods

2.1. Materials and reagents

Whole milk or defatted milk (skim milk) bought from a local market is the most popular types produced and consumed in China. Their water content is about 88% and 92% respectively estimated by the ingredients on the label of milk. Urea was analytical grade and obtained from Beijing Chemical Co. Ltd., China. The water used in this work is deionized which was produced by RiOs-water system (Millipore Corp., America).

2.2. Preparations of samples

2.2.1. Preparations of milk-water mixtures

A series of the "milk-water mixture solution" with following volume ratio of milk (whole milk and skim milk) to water were prepared: 1:0, 3:1, 2:1, 1:1, 4:5, 2:3, 4:7, 1:2, 1:3, 1:4, 1:5, 1:8, 1:10, 0:1. The samples were placed for about 30 min before dielectric measuring.

2.2.2. Preparations of milk-urea and water-urea mixtures

Milk-urea mixture and water-urea mixture with different urea contents, including 15, 30, 75, 150, 300, 750, 1500 mg/100 mL respectively, were prepared by adding urea into the whole milk, skim milk and water. Before dielectric measurements, the solutions were well shacked and placed for 15 min.

2.3. Dielectric measurements

Dielectric measurements were performed in a frequency range from 100 MHz to 40 GHz by Agilent E8362B PNA series network analyzer (Agilent Technologies, made in America) equipped with an Agilent 85070E open-ended coaxial probe (Agilent Technologies, made in America), All measurements were carried out in the temperature of laboratory about 288 K (±0.2 K) during the experiment. The permittivity ε and total dielectric loss ε'' were automatically calculated as functions of frequency by the built-in software of this measuring system, which was calibrated in accordance with the procedures recommended by the manufacturers.

2.4. Analysis of dielectric data

In an applied electric field of angular frequency ω ($=2\pi f$, f is measuring frequency), the dielectric property of a material, including aqueous solutions can generally be characterized in terms of the complex permittivity by the Cole-Cole equation:

$$\varepsilon^*(\omega) = \varepsilon'(\omega) - j\varepsilon''(\omega) = \varepsilon_h + \sum_g \frac{\Delta\varepsilon_g}{1 + (j\omega\tau_g)^{\beta_g}} \tag{1}$$

where j is the imaginary unit, ε_h is the high-frequency limit of permittivity, $\Delta\varepsilon_g$ and τ_g ($=1/(2\pi f_{0g})$, f_{0g} is the characteristic relaxation frequency) indicate the relaxation strength and relaxation time of the gth relaxation, respectively; $\beta_g (0 < \beta_g \le 1)$ is the Cole–Cole parameter related to the distribution of relaxation time.

The curve-fitting was carried out by the Levenberg-Marquardt method to minimize the sum of the residuals for the real part $\varepsilon'(\omega)$ and the imaginary part $\varepsilon''(\omega)$ of the complex permittivity:

$$\chi = \sum_i [\varepsilon'_e(\omega_i) - \varepsilon'_t(\omega_i)]^2 + \sum_i [\varepsilon''_e(\omega_i) - \varepsilon''_t(\omega_i)]^2 \tag{2}$$

where the subscripts e and t respectively refer to experimental and theoretical values, and ω_i is ith angular frequency.

The $\varepsilon'(\omega)$ and $\varepsilon''(\omega)$ in Eq. (1) are the real and imaginary part of complex permittivity, ε'' also known as dielectric loss, they are described as, respectively:

$$\varepsilon'(\omega) = \varepsilon_h + \frac{(\varepsilon_l - \varepsilon_h)\left[1 + (\omega\tau_0)^{\beta}\cos(\pi\beta/2)\right]}{1 + 2(\omega\tau_0)^{\beta}\cos(\pi\beta/2) + (\omega\tau_0)^{2\beta}} \tag{3}$$

$$\varepsilon''(\omega) = \frac{(\varepsilon_l - \varepsilon_h)(\omega\tau_0)^{\beta}\sin(\pi\beta/2)}{1 + 2(\omega\tau_0)^{\beta}\cos(\pi\beta/2) + (\omega\tau_0)^{2\beta}} \tag{4}$$

3. Results and discussions

3.1. Dielectric spectra of milk-water mixtures

Figs. 1 and 2 show the typical dielectric spectra for whole milk and skim milk of adding different water content. The volume fraction of adding water to the milk-water mixture change from 25% to 91%, 0% refers to pure whole milk or skim milk, 100% represents pure water. From the two results, a single dielectric loss peak for Figs.1(b) and 2(b) and a relaxation platform for Figs. 1(a) and 2(a) around 16 GHz can be easily

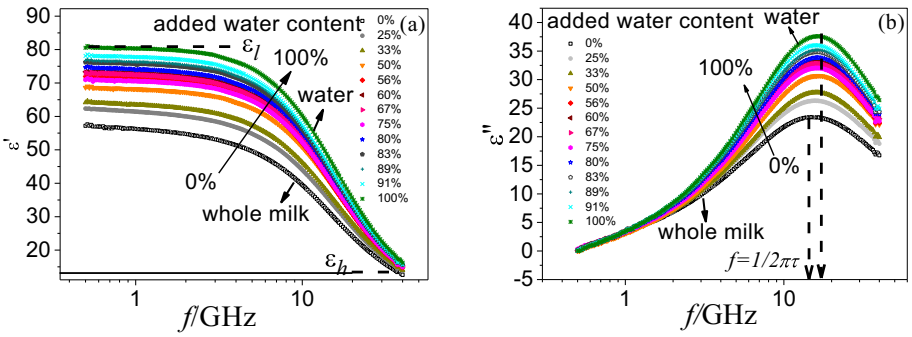

Fig. 1. Dielectric spectra of whole milk-water mixture with varying added water contents at 288 K. (a) and (b) are the real and imaginary parts of the complex permittivity respectively.

seen at first sight for all water contents. The locations of the loss peaks were slightly shifted toward higher frequencies with added water content increasing, and the static (low-frequency) permittivity significantly (whole milk) or slightly (skim milk) increased with adding water. That is, their relaxation time shortened and relaxation strength increased with adding water. The characteristic relaxation frequency f_0 of both systems are basically the same, indicating the dielectric relaxation for the two milk-water mixture systems occurred in the same mechanism, or due to the same relaxation dynamics. The value of f_0 is about 16 GHz for both milk systems (slightly lower than 20 GHz of pure water at 20 °C), corresponding to a relaxation time of 10 ps which is slightly longer than 9.67 ps of pure water at 288 K [41]. It was reported the relaxation time of free water increases if solute molecules reduce the free volume of free water [42]. Therefore, this may be the result of the contribution of water adsorbed on the other constituents of milk i.e., proteins, lactose etc., to this relaxation [43]. This will be discussed later.

The dielectric spectra for pure whole and skim milk are close to that of pure water with dilution of pure whole and skim milk respectively, this is reasonable enough and in good agreement with that reported by A.C. Nunes [27]. What is interesting is the difference between whole milk and skim milk. For whole milk (Fig. 1), the strength and relaxation time of both changed clearly with the dilution, while the change was not obvious for skim milk (Fig. 2). Fig. 3 shows the difference of the relaxation strength $\Delta\varepsilon$ between whole and skim milk, which is obtain by fitting the data in Figs. 1 and 2. It was found that $\Delta\varepsilon_{skim}$ (67) of skim milk is much larger than $\Delta\varepsilon_{whole}$ (48) of whole milk. It may be because the whole milk contains fat globular which is mainly composed of weak-polar molecules. The permittivity of fat globular is much smaller than that of water. The presence of fat globular in whole milk dilutes the water's permittivity [27]. And it was reported that such effects are nonlinear to the volume fraction of the fat globular [44–50]. There is another reason which may be due to the water content of whole milk smaller than that of skim milk. The permittivity values of whole milk obtained here were a little lower than those reported by A.C. Nunes et al. [27], Xinhua Zhu et al. [51]. The difference might be caused by other different compositions. It was also observed $\Delta\varepsilon_{whole}$ increased more rapidly than $\Delta\varepsilon_{skim}$ as added water content increasing, and when water content reached to 100%, both $\Delta\varepsilon_{skim}$ and $\Delta\varepsilon_{whole}$ are equal, about 75. It indicated the difference of water and fat content in both systems are reduced with adding water content.

The relaxation strength for both whole and skim milk increased as adding water. This may be because when the water's molar fraction of the system is reached 83%, the structure of water cluster will be changed from chainlike cluster to cyclic cluster and the relaxation intensity will significantly increases with the increase of water content [52,53]. And the water's molar fraction of both milk systems is >83%,hence the main structure of water cluster in milk may be cyclic cluster as shown in Fig. 5a. Furthermore, according to Cavell equation [54] which describes the relation between dielectric strength and concentration of the dipoles in the system, the relaxation strength closely relates to the number of dipole moments, i.e. the water molecule in unit volume.

$$\frac{2\varepsilon_l + 1}{\varepsilon_l}\Delta\varepsilon_i = \frac{N_A c_i}{k_B T \varepsilon_0}\mu_i^2 \tag{5}$$

where ε_l is the permittivity at low-frequency limit; ε_0 is the permittivity of vacuum; N_A, k_B, and T are the Avogadro constant, Boltzmann constant, and absolute temperature, respectively; c_i and μ_i is the molar concentration and dipole moment of the species i in mixtures, respectively. Therefore, the relaxation strength $\Delta\varepsilon_f$ caused by free water is proportional to the amount of free water c_f in milk:

$$\frac{c_f}{c_w} = \frac{\Delta\varepsilon_f}{\Delta\varepsilon_w} \tag{6}$$

where $\Delta\varepsilon_w$ and c_w (g/cm3) are the relaxation strength and density of pure water respectively. Base on Eq. (6), we can deduce the concentration of water molecules contributing to the bulk water relaxation [55,56]. The number of free water per unit volume in milk increased

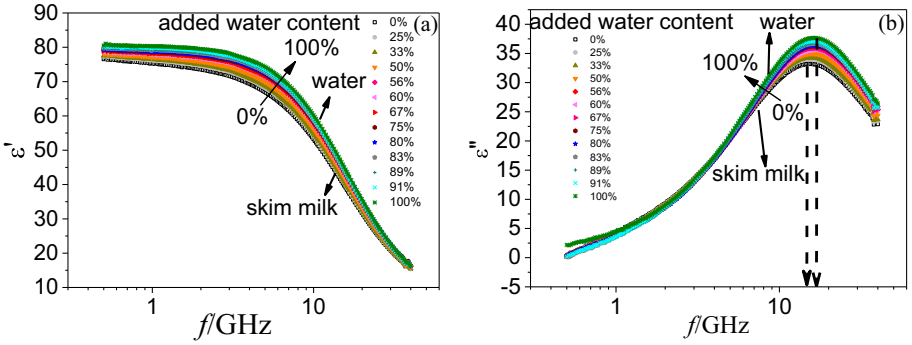

Fig. 2. Dielectric spectra of skim milk-water mixture with varying added water contents at 288 K. (a) and (b) are the real and imaginary parts of the complex permittivity respectively.

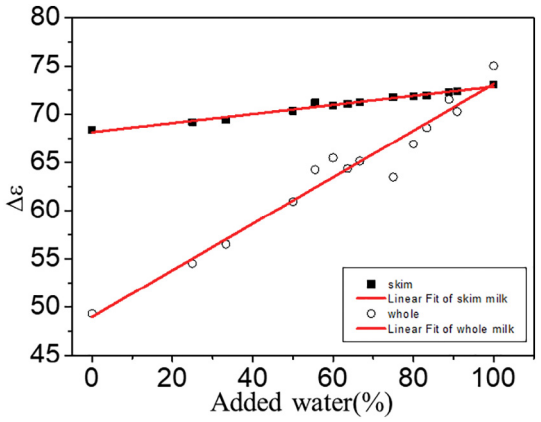

Fig. 3. Added water-content dependence of the relaxation strengths for whole and skim milk.

with the addition of water, leading to the increase of c_f, thereby $\Delta\varepsilon_f$ also increased and eventually approached to that of pure water.

3.2. Interaction between water and protein

The dielectric spectra of Figs.1 and 2 showed an imperfect Debye relaxation: the permittivity-frequency curves demonstrated a small distribution and the dielectric loss peaks are somewhat asymmetrical. To make clear the reasons and clearly demonstrate the difference between whole and skim milk-water mixture systems, the curve fitting for both systems with the assumption of two Debye-type relaxation processes given in Eq. (1) was carried out. Fig. 4 shows the typical results of curve fitting using Eq. (1) for whole and skim milk with different water-content.

As seen in Fig. 4, the dielectric loss spectra of both milk mixture systems contain lower-frequency relaxation process located between about 2 and 8 GHz and its relaxation strength much less than that of main relaxation at 17 GHz. Both relaxation process may be ascribed to the orientation polarization of the water adsorbed on protein and free water, respectively [43,57,58]. It is obvious that the relaxation frequency (or relaxation time) of the main relaxation for both milk systems is the same irrespective of water-content, but the relaxation strength of whole milk is lower than that of skim milk for the same added water-content. This may be because the water content of pure whole milk is lower than pure skim milk. The number of free water in whole milk therefore was less than that of skim milk. Some studies also report the relaxation strength of free water is usually increase with the water content. [56,59] Besides, fat having much a smaller dipole moment than water, the presence of fat in whole milk may be dilutes the permittivity of free water. [27] And because of that, the difference was reduced with the dilution of milk and tended to the same. The water molecules adsorbed on proteins responded to the ac electric field is more slowly and the sub-relaxations from the orientation polarization appear in lower frequency range as seen in Fig. 4 (a detailed comparison is in Supporting Information (SI) A).

The proteins have a wider variety of chemical groups, a larger molecular weight and various polar and apolar groups in close proximity [60]. And it has been speculated that this will simultaneously and effectively restrain water motions [61]. There are many studies reported that the relaxation process ascribed to coupled protein–hydration water motions has characteristic frequency from ~10 MHz to ~10GHz at room temperature [55,62–65]. Therefore, we believe the sub-relaxation may be mainly cause by the water molecules adsorbed on the protein of milk [43,57,58]. It can be observed that the strength and time of this sub-relaxation change with the dilution of the milk. Peculiarly, the relaxation time was shortened (relaxation frequencies shifted to the higher frequency as indicated by arrows) with adding water to the

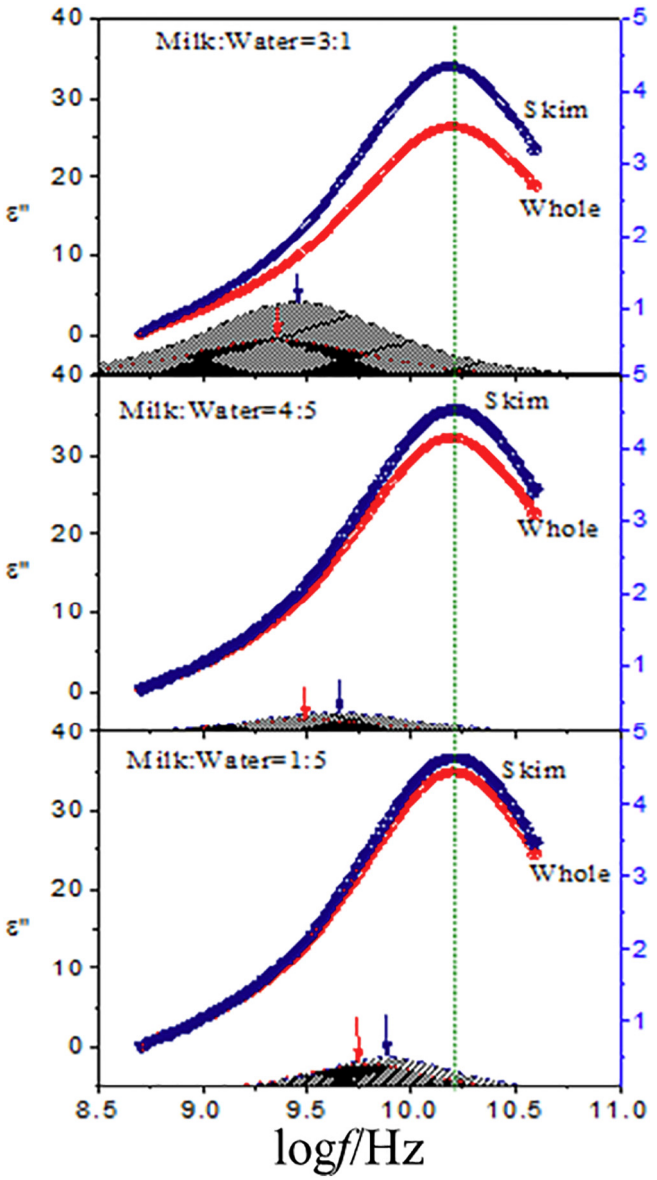

Fig. 4. Curve fittings results using Eq. (1) for whole and skim milk with different added water-content. The red and blue plots show the experimental data of whole milk and skim milk respectively; the red and blue dotted lines and arrows show the relaxation processes for corresponding bound water respectively. The dielectric loss of bound water is presented by the ordinate in right side.

milk, showing that the orientation motion of the bound water became freer. Other research also supports the relaxation time of bound water is reducing along with the increasing of water content [60,66–68]. This indicated that the rotation of the bound water became easy and time for dipole orientation was reduced because of the viscosity of both systems lowered by adding water. In addition, the strength of the sub-relaxation for skim milk is slightly larger than that of whole milk for the same water content, and so does the relaxation frequency. Since the sub-relaxation is mainly caused by water linked with the non-condensed hydrophilic residues of protein through hydrogen bonds [43] as shown in Fig. 5d.The milk used in this study is homogenized pasteurized milk. In whole milk, some casein and whey proteins may get embedded in fat globular surface after homogenization as shown in Fig. 5c and d [69]. And the total surface area of protein contact with water molecules is reduced compared to skim milk. This also shows that the dipole orientation polarization of water adsorbed on protein occurs more easily in skim milk than in whole milk at a given temperature. Because the hydrophilic of protein in skim milk is stronger

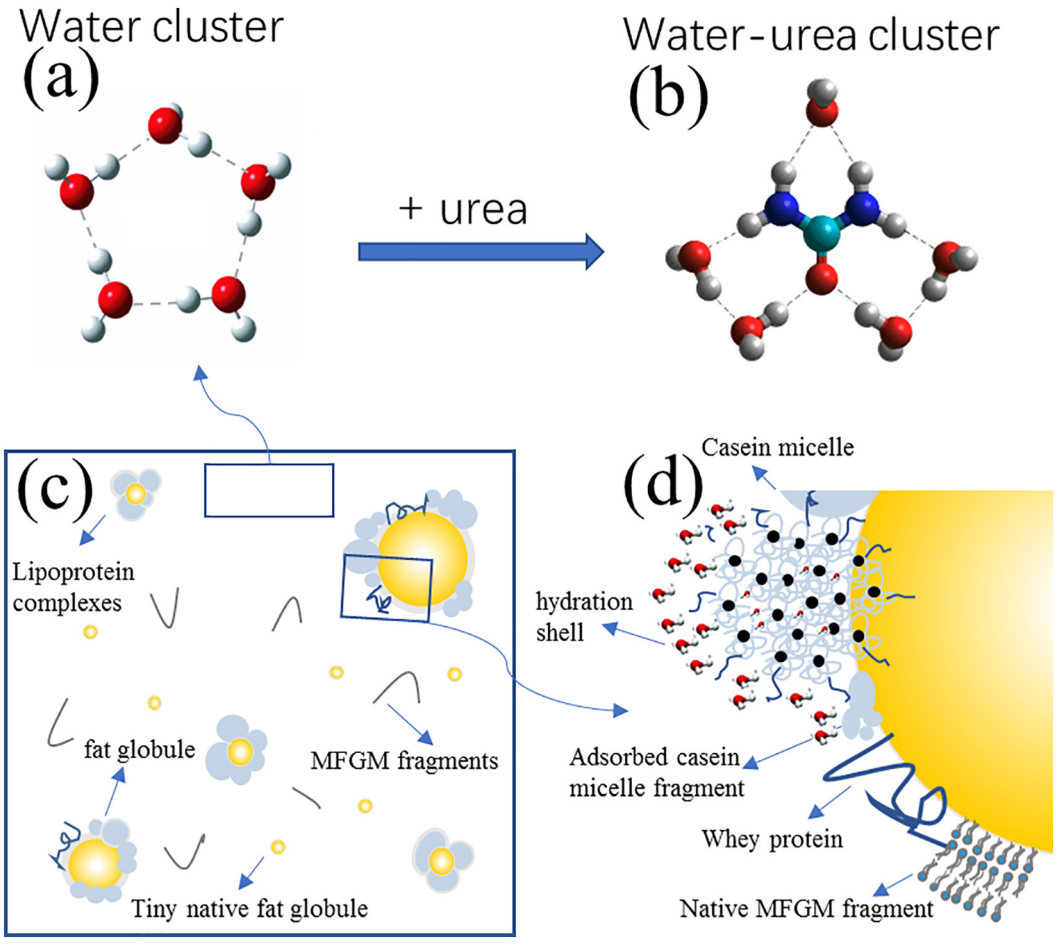

Fig. 5. (a) the structure of water clusters in milk, (b) the structure of water-urea clusters, (c) organization of homogenized whole milk, (d) interfacial organization of fat globular in homogenized whole milk.

than that of embedded in the fat globular surface in whole milk, it makes the water clusters smaller and the water molecules rotate more easily in skim milk because of the increase in hydrogen-bond defect. [70]

3.3. Dielectric spectra of milk-urea mixtures

Figs. 6, 7 and 8 are the dielectric spectra for the whole milk-urea, skim milk-urea and urea aqueous solution, respectively. Similarly, with the milk-water mixture, the three systems also show an unsymmetrical relaxation at around 16GHz. It is obvious that the relaxation frequency of the three systems basically remains unchanged when the compositions of the mixed systems were changed, the dielectric relaxation strength of whole milk-urea system is proportional to the urea concentration (Fig. 6) and the other two systems are independent of the urea concentration (Figs. 7 and 8). This difference maybe comes from the protein embedded in fat globular surface in whole milk.

3.4. Interaction between protein and urea-water co-clusters

To figuring out the causes of the dielectric relaxation behavior shown in Figs.6–8, the dielectric loss data of the three systems with different urea contents were fitted by using Eq. (1). Fig. 9 shows the comparison between the three systems with the same urea content, which may look very similar in both of its peak value and position to that of milk-water mixture. The relaxation positions, i.e. relaxation time, of

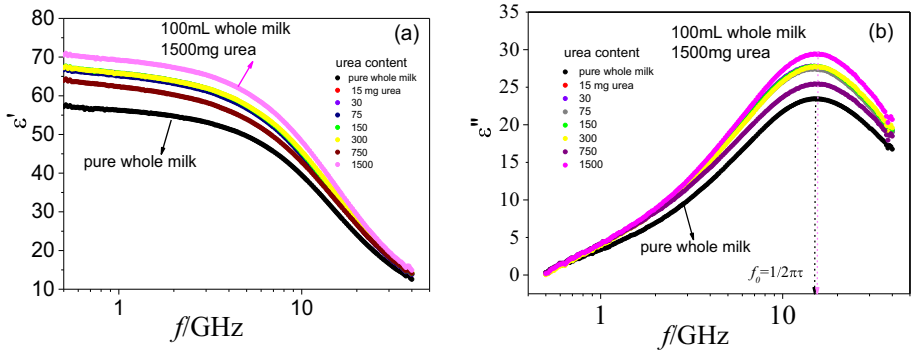

Fig. 6. Dielectric spectra of whole milk-urea mixture with different urea contents in wt%. (a) and (b) are the real and imaginary parts of the complex permittivity respectively.

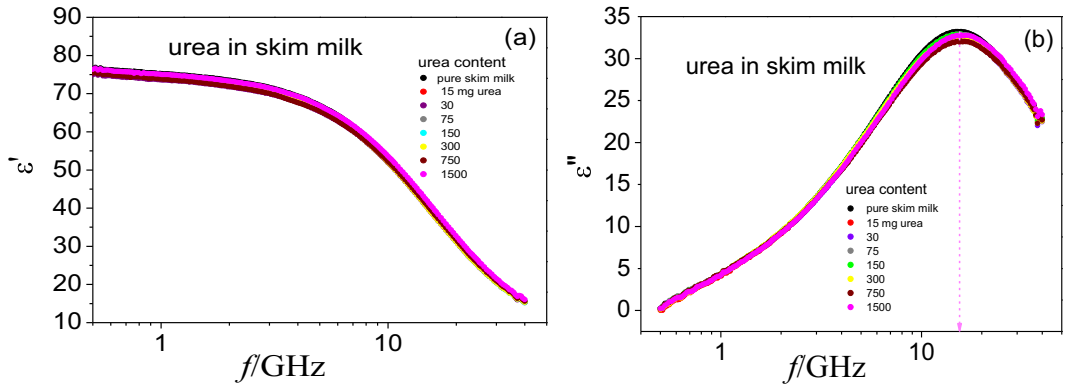

Fig. 7. Dielectric spectra of skim milk-urea mixture with different urea contents in wt%. (a) and (b) are the real and imaginary parts of the complex permittivity respectively.

the main relaxations for the three systems are identical at about 17 GHz, which is independent of both type of the mixture and urea content as seen in Fig. 9. This main relaxation obviously comes from the contribution of free water although it is slightly less than that of pure water. Kaatze,U etc. also reported that dielectric relaxation on 1 and 2 M solutions of urea has indicated a slight slowing down of the water dynamics [71]. While, the lower frequency relaxation time significantly depended on the kind of mixtures and somewhat on the urea content, and their relaxation strengths are much smaller than that of free water. It is easily perceived that this sub-relaxation can be attributed to the contribution of the water molecules interacting with urea [72] or some substances in milk [43]. The relaxation times of the three mixed systems (τ_{whole} for whole milk-urea, τ_{skim} for skim milk-urea and τ_{water} for water-urea) were observed in the following order, under the same urea concentration, $\tau_{whole} > \tau_{skim} > \tau_{water}$ as shown in Fig. 9 (note that the peak position indicated by the arrows is relaxation frequency that is inversely proportional to the relaxation time). This may be interpreted as the number of hydrogen bonding defects shows a smaller value in the whole milk, because the hydrophilic of proteins embedded on the surface of fat is reduced, and the water clusters larger and the water molecules rotate more difficult [70] compare to skim milk.

What is interesting is that the low-frequency relaxation of urea aqueous solution showed a very large relaxation strength when urea content in water was 1500 mg/100 mL in Fig. 9. In addition, the relaxation time of urea solution depended strongly on the urea content, increasing with the concentration of urea and gradual away from that of free water as indicated by the orange arrows in Fig. 9. Such behavior is similar to that reported for urea aqueous solutions [38]. This maybe because the water neighbors of urea-water cluster as shown in Fig. 5b are replaced by one or more sites of the urea molecules [73]. It indicated a larger urea-water co-clusters formed and its orientation polarization becomes slow. This speculation is in line with that reported by Y. Hayashi [33]. (a detailed description is provided in the Supporting Information (SI) B).

3.5. The difference in interaction between urea in skim milk and whole milk

In Fig. 10, a double logarithmic plot of the low-frequency relaxation time τ as a function of urea concentration is presented for two urea-milk mixtures and one urea aqueous solution. It is well known that although numerous studies were performed to discuss whether urea is a structure breaker or not [33], there is no evidence of a breakup of the pure water cluster by adding urea [36,37,73]. However, it was reported that a small fraction of the water molecules turns out to be strongly immobilized by urea, these water molecules may be engaged in specific urea–water complexes, and its orientational dynamics is slower than that of bulk water [36]. We consider that the low frequency dielectric relaxations for urea-water and both urea milk systems may be separately caused by the dipole orientation polarization of urea-water cluster [74] and urea-water clusters interacting with protein surface [34]. According to the following Stokes-Einstein-Debye equation [72,75,76] which describes the relationship between the macroscopic properties like viscosity η and temperature T, and microstructure like effective volume of polarization unit V and molecular dipole orientation which is expressed by relaxation time τ of the system

$$\tau = \frac{3\pi\eta R^3}{k_B T} = \frac{3\eta V}{k_B T} \tag{7}$$

where k_B is the Boltzmann constant. We assume the effective volume of urea-water cluster in the three systems are similar and equal approximately to 11.7 ± 0.2 Å^3 [72]. The viscosity η of urea-water, urea-skim milk and urea-whole milk systems are separately about 1.1373 mP, 1.790 mP [77] and 2.1275 mP [78]. The relaxation time τ of urea-

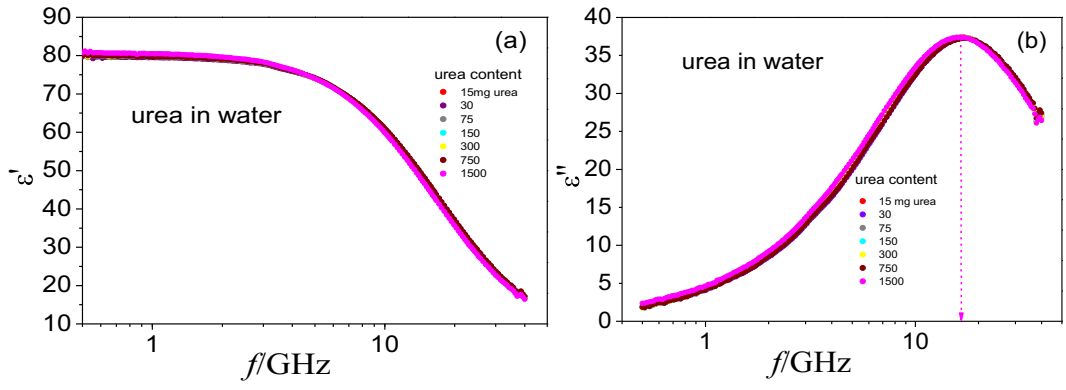

Fig. 8. Dielectric spectra of urea aqueous solution of different urea contents. (a) and (b) are the real and imaginary parts of the complex permittivity respectively.

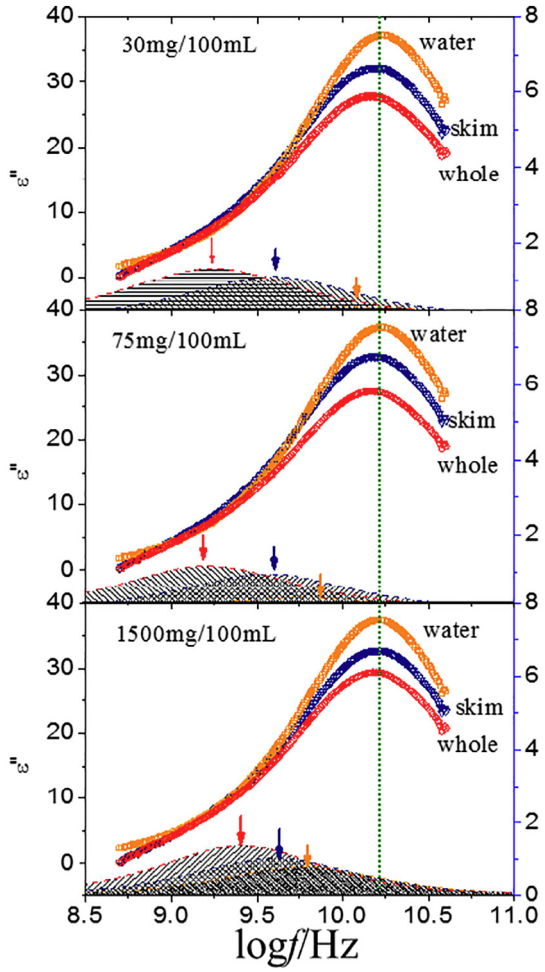

Fig. 9. A comparison of dielectric loss spectra for three urea mixture systems (urea-whole milk (red plots), urea-skim milk (blue plots) and urea aqueous solution (orange plots)) with different urea contents. The red, blue and orange dotted lines and arrows show the relaxation processes for corresponding bound water respectively. The dielectric loss of bound water is presented by the ordinate in right side. (the detailed description, see Supporting Information (SI) B).

water, urea-skim milk and urea-whole milk systems with lowest urea content calculation by Eq. (7) are as follows $\tau'_{water} \approx 12.1$ ps, $\tau'_{skim} \approx 15.8$ ps, $\tau'_{whole} \approx 18.8$ ps, and $\tau'_{whole} > \tau'_{skim} > \tau'_{water}$. These values are reasonable to be compared with the experimental values showed in Fig. 10. Therefore, we consider the difference of low frequency relaxation time of the three systems may be due to the differences in their viscosity.

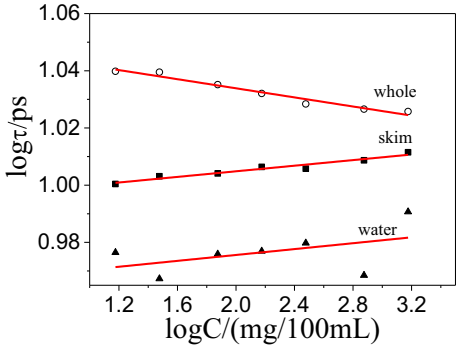

Fig. 10. Urea concentration dependence of relaxation time for urea aqueous solution, urea-skim milk and urea-whole milk systems.

In addition, the increase of relaxation time τ with urea concentration for urea-water and urea-skim milk systems means that the cluster structure of urea-water [73] and urea-water adsorbed on protein increase slowly and their orientation polarization rate slow down. For the urea-whole milk system, the different changing trend indicates the fluctuation of the dynamics of water or water-urea cluster caused by larger interactions with protein molecules in fat globules may be enhanced in whole milk. Because adding urea destroyed the fat globules structure in whole milk [2,3], this makes the hydrophilic of protein embedded in fat globules surface increasing. And increasing hydrogen-bond defect makes the water or water-urea clusters smaller and the water molecules rotate more easily [70]. This led to the result that its relaxation time was shortened with the adding urea to the whole milk. In summary, adulterated milk with urea may be monitored by the dielectric spectra of varying urea contents.

4. Concluding remarks

Adulterated milk systems with water and urea was studied by microwave dielectric spectroscopy. And this is the first time to study the dielectric relaxation behavior of adulterated urea in milk in the microwave frequency range. The dielectric relaxation behavior of pure whole milk and skim milk adulterated without and with urea was investigated by comparing with that of water-milk mixtures and urea aqueous solution respectively. An obvious relaxation was clearly observed for these systems. The relaxations were well represented by the superposition of two relaxation processes which are attributable to free water or bulk water, and the water bounded to protein, the urea-water cluster and urea-water cluster interacted with protein for both milk-water systems, urea aqueous solution and both urea-milk systems respectively. The relaxation strength of the latter is much less than that of free water and its relaxation time varies with the addition of water or urea, while relaxation time of free water basically remain the same.

Total relaxation strength and sub-relaxation time in water-milk mixture are sensitive to the water content in the milk. And the characteristic frequency of urea-water cluster interacted with protein is also sensitive to urea content in the milk. In addition, both of urea-skim milk and urea-whole milk systems were distinguishable through their dielectric parameters. From these results, therefore, the dielectric analysis method presented in this work can be expected as a monitoring means of detecting milk adulteration with water or urea. However, it must be noted that all components of milk are slightly different from cow's own factors, such as dairy breed, lactation period, age parity, etc., and extrinsic factors, such as milking interval, milking process, feeding level, season etc. Therefore, the results given in this study are just as a recommended method for dielectric measurement research.

Author contributions

Yuan Liu and Qi Zhang contributed equally.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molliq.2018.09.133.

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