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MONITORING OF COAGUALATION PROCESS OF SOYMILK BY AN INTEGRATED ELECTRICAL SENSING AND CONTROL SYSTEM

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ABSTRACT Coagulation of soymilk is the most important process in tofu processing. The aim of this study is to develop a monitoring method which can detect the coagulation of soymilk by electrical impedance spectroscopy (EIS) to control the temperature of soymilk in a vessel accurately and uniformly by Ohmic heating. Monitoring and heating were completed with the same electrodes in at different time intervals. Soymilk coagulation process was investigated by electrical impedance spectroscopy and rheological analysis of tofu break stress, break strain and Young modulus. The impedance amplitude of soymilk decreased at frequencies of 42 to 200 Hz and increased over 200 Hz with the progress of the coagulation of the soy milk, splitting method indicated that the electrode capacitance might increase and that electrical conductivity of medium (soymilk) might decrease during coagulation. Volume resistivity of soymilk at 10 kHz showed a positive linear correlation with the rheological properties. Therefore, EIS can provide a simple, safe and rapid approach for predicting both tofu physical quality and the coagulation degree of soymilk. The developed method is capable of controlling the coagulation degree during a soymilk coagulation process and estimating the tofu textural properties.

Keywords: Electrical impedance spectroscopy (EIS), Ohmic heating, volume resistivity, splitting method, break stress

INTRODUCTION A basic procedure of tofu-making involves two heating processes: thermal denaturation of soy protein in raw soymilk as a pre-requisite for tofu-gel formation and tofu-gel formation of soymilk-coagulant mixture. In the first heating process, the major soy proteins composed of Glycinin and β-conglycinin will be completely denatured and unfolded in heating up to 95º (Tang et al., 2007). As a result, the hydrophobic regions of the protein molecules, which are located inside the protein in the native state, are exposed into soymilk solution in the denatured state. The denatured soy protein is negatively charged because the –SH, S-S, and hydrophobic amino acid side chains are exposed (Liu et al., 2004, Kohyama et al., 1995). When a coagulant is added to the denaturated soymilk, the negative charges on the protein molecules neutralized. Consequently, protein molecules come close owing to the decrease of electrostatic repulsion and the –SH/S-S interchange reactions and hydrophobic interactions becomes more predominant, which facilitate protein aggregations leading to the formation of tofu gel with a three-dimensional network structure (Liu et al., 2004). Therefore, the denaturation temperature and heating rate should be carefully controlled to induce the soy proteins in raw soymilk into a certain intermediate state between the native and denatured one, which consequently it is possible to adjust and control tofu gel's textural properties by promoting and hindering S-S interchange reactions. Liu et al. (2004) noted that ohmic heating in two steps, (e.g. combination of 75 for 5min and 95° for 5min) increased soymilk viscosity by 150% and tofu's apparent Young's modulus by approximately 20% comparing single-step heating (95° for 5min). In addition, it was emphasized that the viscosity of soymilk and the gel hardness of tofu were more highly related with the heating rate than the mode of heating (Tang, 2006). Generated heat by Ohmic heating of soymilk depends on the electrical conductivity of soymilk and the monitoring of the electrical conductivity during Ohmic heating enable precise control of temperature and a heating rate of soymilk (Wang, 2006). In the second heating process, tofu gel-formation is accompanied with the SH/SS interchange reactions between soybean protein molecules in soymilk solution which cause the changes in particle size distribution of oil body and the changes of tofu gel network structure (Guo et al., 2005; Ono, 2008; Ohara et al., 1993), which affects electrical properties of soymilk. Hence, electrical impedance spectroscopy (EIS) is expected to be used to monitor the progress of tofu coagulation during the coagulation process indirectly.

The aim of this study was to characterize the heating process of soymilk protein denaturation and the tofu coagulation process by electrical impedance spectroscopy and to develop a monitoring method to detect the progresses of soymilk heating and tofu coagulation.

MATERIALS AND METHODS

Materials Soybeans purchased at a local market, Turunoko variety was used. 100g soybean was soaked in distilled water at the weight ratio of soybean-water of 1:6 for 12h at 25°. The swollen soybeans were ground and the obtained slurry was filtered through a double-layered cheesecloth to yield raw soymilk. The raw soymilk was heated to 95° for 5 min by applying AC 10V/cm at 1000 Hz with the heating system. After the heating treatment, the soymilk was cooled in an ice bath to 4° for the subsequent process. In each experiment, the soymilk was freshly prepared daily.

Experiment setup element An Ohmic heating and electrical impedance measurement system was set up as shown in Fig. 1. The system was composed of an ohmic heating system and an EIS measurement system. The ohmic heating system was composed of a signal generator (WF1943, NF Co., Japan), a bipolar amplifier (HSA4012, NF Co., Japan), a switch unit (Agilent 34970A, Agilent Co LTD.) and a heating vessel with two flat titanium electrodes. The heating vessel was made of a transparent Plexiglas block with 5mm thickness and had the inner dimensions of 5cm length, 5cm width and 5cm depth. In order to minimize thermal dissipation and evaporation from the vessel, the vessel was covered with polystyrene foam in 3 cm thick, and the lid was made of in 3-cm-thick polypropylene block, a signal generator can produce various electric waveforms (sinusoidal, triangular, square and ramp) at frequencies between 50 Hz -10 kHz and the a generated signal was amplified with an amplifier to 30 -100 V and then applied to a coagulation vessel. The impedance measurement system was composed of a LCR impedance analyzer (3532 LCR Hit ester, HIOKI, Japan) and the switch unit connected to the coagulation vessel. The switch unit could switch the electrical line connected to the electrodes from the heating system to the measurement system and vice versa, which can accomplish ohmic heating and impedance measurement in time-sharing mode. A data-logger (HP34870A, HP Inc.) continuously recorded the temperature at the center of the coagulation vessel with a Teflon-coated thermocouple to monitor soymilk temperature. Heating and measurement systems were controlled by a personal computer using developed matlab program.

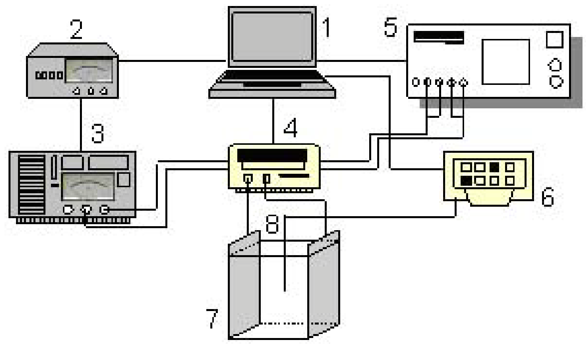


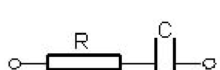
Figure 1 Schematic diagram of Ohmic heating and electrical impedance measurement system: 1) PC, 2) signal generator, 3) bipolar amplifier, 4) switch unit, 5)impedance analyzer, 6) data logger, 7) coagulation vessel, 8) thermocouple

Experiment method 25 ml cooked soymilk was injected into coagulation vessel, then 0.1 ml magnesium chloride solution (coagulant) was added and mixed immediately. The final concentration of magnesium chloride in the soymilk mixture was adjusted to 0.25% (w/w). Soymilk was heated to 80° by Ohmic heating under predetermined heating conditions. During a heating process, the electrical impedance and phase angle of soymilk was measured with impedance analyzer (3532 LCR Hi TESTER) at the predetermined frequency every 5°. Temperature at the center of the vessel was recorded automatically every second. Electrical conductivity of soymilk was determined in the following equation.

$$\sigma = \frac{1}{R}\frac{L}{A} = \frac{1}{\rho} \tag{1}$$

where, σ is conductivity (S/m), R is measured resistance was calculated using the equivalent circuit model, A is cross-sectional area of the vessel, L is electrode distance, ρ is volume resistivity (Ω–m) and L/A is cell constant. .In a coagulation process, temperature of soymilk was controlled at 75, 80, 85 C respectively and electrical impedance was measured about 1 hour at intervals of 10 minutes after coagulant addition.

Electrical equivalent circuit model Measured impedance of soymilk in a coagulation vessel includes the effects of electrode polarization at low frequency and bulk resistance and it was necessary to split those effects. Thus, an electrical equivalent circuit model was adopted and applied to the measured data of electrical impedance as follows: the



circuit was composed of a capacitor and a resistor which were connected in series with a capacitor. Resistance of a resistor was defined as $Z_M = R$ and reactance of a capacitor as $Z_E = \frac{1}{\pi fc}$ and the amplitude of measured impedances as $|Z| = \sqrt{Z_M{}^2 + Z_E{}^2}$

, f is frequency (Hz).

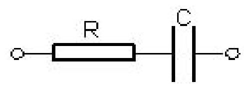


Figure 2 Electrical Equivalent circuit model

Rheological measurement In a coagulation process, soymilk was held during the coagulation period of 2, 5, 10, 20, 30, 40, 50, 60 min at 75, 80, 85 C and tofu gel was formed. Then, tofu gel was kept at room temperature (25 ^{O}) 60 min for aging and tested for textural measurement. Rheological properties of tofu gels were measured using a rheometer (CR-2000, SUN Scientific co., LTD, Japan). A cylindrical shaped specimen of tofu gel 30 mm in diameter and 20 mm in height was tested for an uniaxis compression test with an 8 mm cylinder plunger at a 60 mm/min compression rate. Apparent breaking stress σ_F of tofu-gel specimen, apparent breaking strain ε_F and Young's modulus E_F were determined as textual indices of tofu gel (Mohsenin 1986; Prabhakaran and Perera 2006). The experiment procedure is shown in Fig. 3.

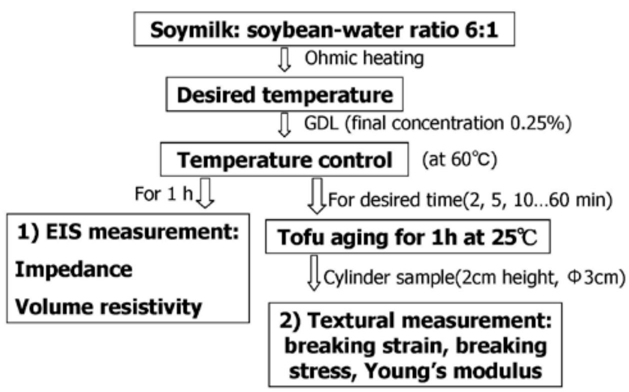


Figure 3 experiment procedure of coagulation

RESULTS AND DISCUSSION

Effect of temperature on electrical conductivity of soymilk

Electrical conductivity of soymilk at 10 kHz presented a linear correlation with temperatures between 40 and 90 C (Fig. 4.b). The conductivity at lower frequency, e.g. 60 Hz, showed a large disturbance in Fig. 4.a, however, the linear correlation at 10 kHz was little affected by electrical filed strength and wave form. Sarang et al. (2008) reported that the electrical conductivity of various fruits and meats increased linearly with temperature during ohmic heating at constant electrical field strength, which meant that the electrical conductivity was a linear function of temperature. Therefore, electrical conductivity at higher frequency can be used to estimate the temperature of heated materials in Ohmic heating.

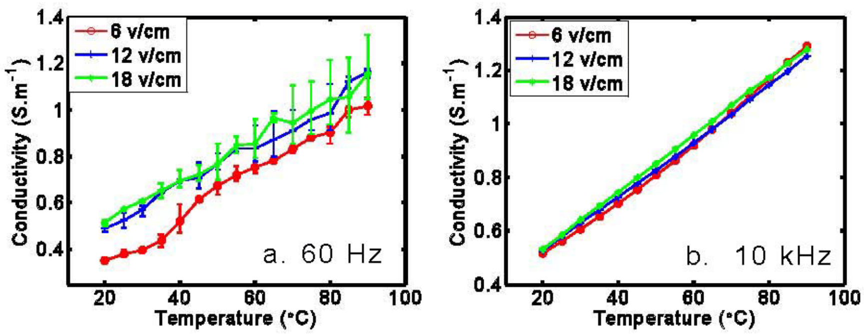


Figure. 4 Electrical conductivity of soymilk-coagulant mixture at three electric field strength 6, 12, 18 V/cm at frequency a. 60 Hz and b. 10 kHz.

Electrical impedance characteristics of soymilk in a coagulation process Typical impedance characteristics of the equivalent model depicted in Fig. 2 shows relaxation phenomena at low frequency and stable values at higher frequency in Bode diagram. The measure impedance of the soymilk-coagulant mixture decreased sharply with an increase in frequency at low regime between 42 Hz and 400 Hz and it approached to a constant value with the frequencies beyond 200 Hz in Fig. 5.a. Therefore, the measured impedance of soymilk-coagulant mixture showed a similar characteristic to that of the model. A relaxation phenomena at lower frequency was caused by electrode polarization and the stable values of the impedance at higher frequency would be attributed to the characteristics of electrical conductivity (or volume resistivity) of soymilk-coagulant mixture. Therefore, the impedance measured at high frequency can be used to analyse a coagulation process. With the progress of coagulation, a curve of the impedance amplitude shifted in Fig. 5.a.: at a low frequency range between 42 Hz and 200 Hz, the impedance decreased with the progress of coagulation time and the impedance shows a rise at a higher frequency range than 200 Hz.

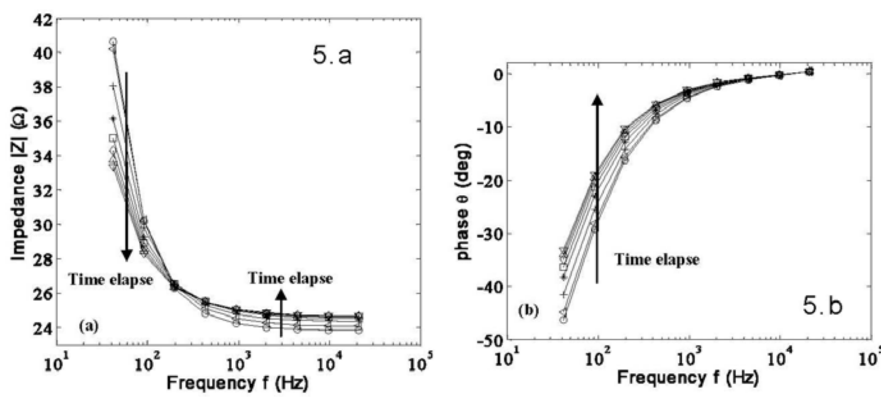


Figure 5 Bode diagram of variation of impedance (5.a) and phase (5.b) with the progress of coagulation at 80 C.: coagulant time 0 min (O), 3 min (◁), 10 min (+), 20 min (*), 30 min (□), 40 min (◇), 50 min (△), 60 min (▽).

Cole-Cole plot (complex-plane plot) is available to investigate the resistance and the capacitance of the equivalent model separately because x- and y-coordinates correspond to the real and imaginary parts of the complex impedance of the equivalent model respectively.

The Cole-Cole plot of the same impedance as in Fig. 5 was shown in Fig. 6. The upper right side symbols indicates the low frequency impedance and a remarkable time variance can be observed in both electrode reactance (y-coordinate) and medium resistance (x-coordinate). High frequency impedance at the lower left side in the figure presents an increase in resistance and constant values in reactance. Consequently, the high frequency impedance in Cole-Cole plot presented the resistance of the mixture. Thus, resistance Z_M at 10 kHz was considered to be useful to analyze a coagulation process.

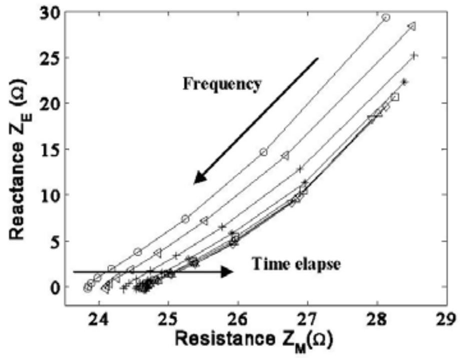


Figure 6 Cole-Cole plot at various coagulation time. Coagulation time 0 min (○), 3 min (◁), 10 min (+), 20 min (*), 30 min (□), 40 min (◇), 50 min (△), 60 min (▽).

Characterization of tofu coagulation process by EIS Time variation of volume resistivity derived from resistance, Z_M in Fig. 7 was shown at three coagulation temperature experiments. Volume resistivity increased rapidly at initial several minutes and subsequently increased gradually.

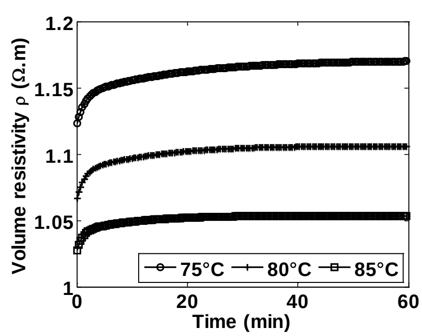


Figure 7 Time variation of volume resistivity (at 10 kHz) at 75, 80, 85 C during coagulation.

Normalized parameter ϕ of volume resistivity was defined as $\phi = \frac{\rho_i - \rho_0}{\rho_\infty - \rho_0}$ and applied to the measured data. 0, ∞ indicates initial and finial values.

Furthermore, the time variation of the normalized parameter could be approximated with a successive reaction equation as below through the analysis about the relation between $d\phi/dt$ vs. ϕ as shown in Fig. 8.

$$\phi = 1 - a_1 \exp(-k_1 t) + a_2 \exp(-k_2 t) \quad (2)$$

Where, a_1, a_2 are constants, t is time (min^{-1}), ϕ is normalized parameter, k_1 and k_2 are the rate constant of the first and second reaction stage.

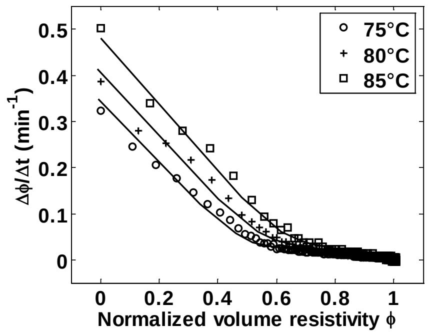


Figure 8 Relation between ϕ *and* $\Delta\phi/\Delta t$

Equation (2) agreed well with the time variation of ϕ at 70, 75, 80 C as shown in Fig. 8. Rate constants of first and second stages k_1 and k_2 were formulated by Arrhenius equation in Fig. 9.

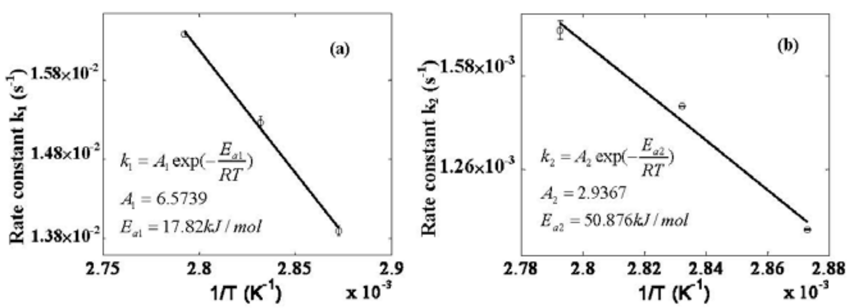
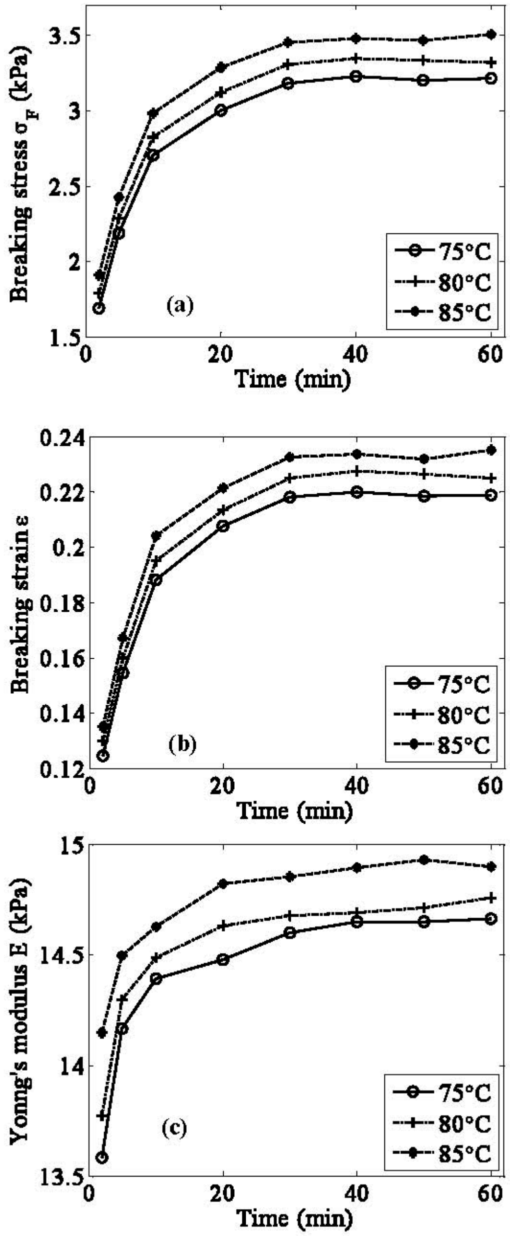


Figure 9 Arrhenius plot of the rate constants for the two stages of coagulation process: (a) rate constant k_1 of the first stage, r=0.998 (b) rate constant k_2 of the second stage, r=0.992

Consequently, impedance variation during a coagulation process could be characterized by Equation (2) which can evaluate the temperature dependence of the soymilk coagulation.

Relation between tofu texture and volume resistivity

Rheological properties such as apparent breaking stress, apparent breaking strain and apparent Young's modulus at various coagulation times were examined and the relation between the properties and coagulation time is shown in Fig.10. Those properties show a similar time variation to each other. They also show larger values at the higher coagulation temperature and the properties at the initial stage are raised more quickly at the higher temperature. Comparing the time variation of the impedance parameter ϕ in Fig. 7, the rheological properties increased more gradually. The linear relationship between the rheological properties and volume resistivity were observed in Fig. 11 and



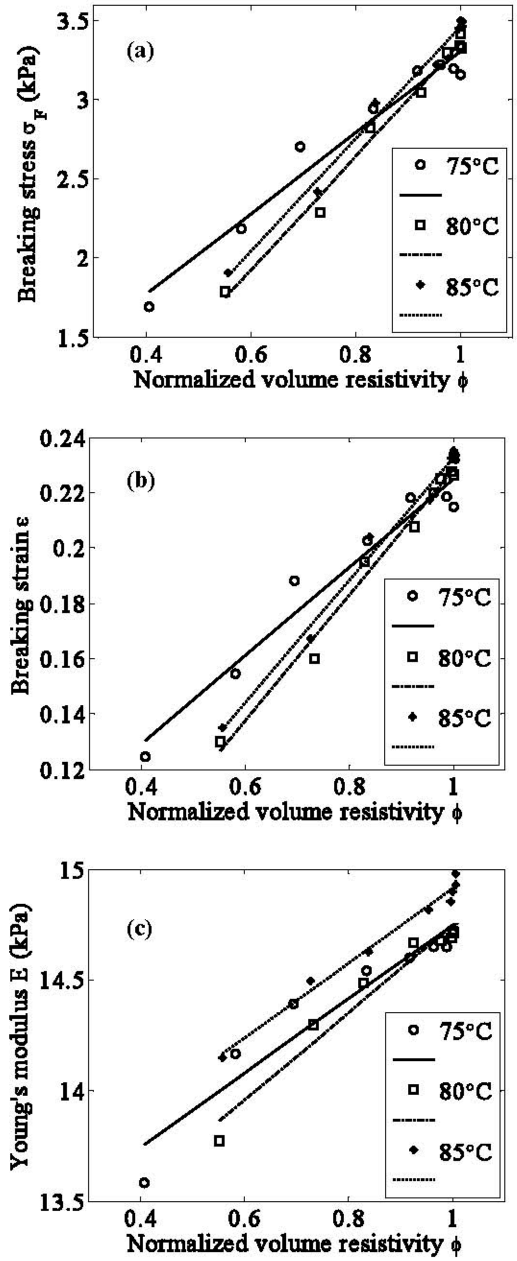


Figure 10 Tofu's rheological properties at various coagulation period and at temperature, (a) apparent breaking stress, (b)apparent breaking strain and (c) apparent Young's modulus

Figure 11 Correlation between volume resistivity and rheological properties at various temperature, (a) apparent breaking stress, (b)apparent breaking strain and (c) apparent Young's modulus

the rheological properties, apparent breaking stress, apparent breaking strain and apparent Young's modulus were expressed as a function of coagulation temperature and volume resistivity by multiple linear regression analysis as follows:

$$\sigma_F = 0.285 + 0.000171T + 3.074\,\phi \quad (R^2 = 0.949) \tag{3}$$

$$\varepsilon_F = 0.0544 + 0.000232T + 0.193\,\phi \quad (R^2 = 0.946) \tag{4}$$

$$E_F = 11.814 + 0.0158T + 1.721\,\phi \qquad (R^2 = 0.921) \tag{5}$$

Their coefficient determinants were higher than 0.92, however, volume resistivity at initial increased more quickly than the rheological properties. Hence, further investigation is needed.

CONCLUSION

In order to develop an on-line and real-time monitoring method to detect the progress of tofu coagulation, characterization of soymilk heating process and tofu coagulation process was conducted by using electrical impedance spectroscopy. Electrical impedance of soymilk-coagulant mixture at lower frequency was insufficient to detect the coagulation because of strong influence by the electrode polarization and the higher frequency impedance at 10 kHz showed a linear correlation with the soymilk temperature. Resistance of the mixture was extracted from the measured complex impedance based on the impedance splitting method in which an electrical equivalent circuit model with a resistor coupled with capacitor in series was assumed. Tofu coagulation process was analyzed by volume resistivity derived from resistance and the experimental equation predicting the resistivity was developed. The rate constants in the equation were determined and the temperature dependence of the rate constants was evaluated by Arrhenius equation. These equations were available to detect the coagulation progress by impedance measurement and to predict the effects of temperature on the coagulation reaction. Besides, relation between rheological properties relating to tofu texture, e.g. apparent breaking stress, apparent breaking strain and apparent Young's modulus and volume resistivity was investigated and the prediction equations of the rheological properties were developed by multiple linear regression analysis. Therefore, EIS can provide a simple, safe and rapid approach for predicting both tofu physical quality and the coagulation degree of soymilk. The developed method is capable of controlling the coagulation degree during a soymilk coagulation process and estimating the tofu textural properties.

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