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Rapid freshness evaluation of cow milk at different storage temperatures using *in situ* electrical conductivity measurement

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ABSTRACT

The present study investigated the efficacy of *in situ* electrical conductivity measurement was evaluated to estimate the freshness of cow milk. Accordingly, the same for the refrigerated milk (5 °C) gradually increased from 0.505 to 0.610 S/m during 42 days, whereas that for the milk stored at room temperature (20 °C) promptly increased from 0.708 to 1.195 S/m during 30 days. In the empirical model, the electrical conductivity freshness index (EF_i) presented a good correlation between pH and microbial growth with the freshness parameters. In the pH analysis, the EF_i could predict the pH decline in spoiled milk with a non-linear curve. Likewise, the growth of total aerobic bacteria (TAB) at 20 °C exhibited a good correlation with EF_i ($ß_2$ coefficient and R^2 values of 9.330 and of 0.977, respectively). This study thus demonstrated the practical application of *in situ* electrical conductivity measurement for rapid prediction of milk freshness during storage.

Industrial relevance text: In the cold change system of milk, rapid assessment of freshness has its significance for food safety. Conventional evaluation of milk freshness required the analytical equipment, trained technician, labor, and time. Electrical conductivity measurement could represent the freshness of foods associated with pH changes and microbial growth. This study proposed the potential of electrical conductivity measurement for rapid assessment of milk freshness.

1. Introduction

Cow milk is a biological fluid with unique components and complexity containing abundant nutrients with bioactive components, such as proteins, lipids, lactose, minerals, and vitamins, that is popularly consumed by infants and children worldwide (Liang et al., 2021; Yang et al., 2016; Yang et al., 2017). Thus, it is extremely important to estimate and appropriately manage the freshness of milk to prevent its spoilage and subsequent food positioning following the consumption of spoiled milk. Cow milk is highly perishable and frequently prone to the occurrence of spoilage reactions during shelf life such as microbial activity, oxidation of lipids and proteins, and lipolysis (Bylund, 2015; Romero, Sharp, Dawson, Darby, & Cooksey, 2021). Milk is an emulsion or colloid of butterfat globules within a water-based fluid that contains dissolved carbohydrates and protein aggregates along with minerals (Jost, 2007; Loffi et al., 2021). United State Department of Agriculture (USDA) recommends the storage temperature of milk in the range of 0.0–4.4 °C. Consequently, the cow milk is distributed and stored in the cold chain systems to guarantee its safety and freshness (James & James, 2010; Mercier, Villeneuve, Mondor, & Uysal, 2017; Zhang, Wei, Yuan, & Yue, 2019).

However, it is often subjected to frequent temperature misapplication occurring at the distribution stage, retail stores, and home refrigerators. Thus, monitoring the freshness of milk is critical in order to maintain food safety and human health. In addition, there is an urgent need for the development of fast, sensitive, reliable, and cost effective methods and sensor systems for the rapid assessment techniques that enable detecting the growth of bacteria at an early stage (Poghossian, Geissler, & Schöninga, 2019). Typically, the freshness of cow milk is estimated using physicochemical analysis (acidity, specific density, fatty acid) and microbial growth assays (total aerobic bacteria, psychrotrophic bacteria, lactic acid bacteria, *etc.*). However, these conventionally used assessment techniques for milk freshness requires the analytical equipment, trained technician, labor, and time. Especially, the microbial analysis requires a long periods (~ 48 h) for incubation and subsequent enumeration.

As a result, there is a pressing need for the development of rapid and real-time freshness evaluation methods of milk freshness that can be

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conveniently employed during its distribution and storage. Electrical conductivity measurement is one of the potentially suitable candidates that can be conveniently used for the rapid freshness evaluation of milk. Electrical conductivity is the ability of a material that allows the passage of electric current through it (Park, Balasubramaniam, & Sastry, 2013). Once the food is spoiled, its physical status as well as chemical composition is altered. Thus, it is rational to hypothesize that food spoilage might influence the electrical conductivity of food materials. In previous studies, electrical conductivity measurements have been demonstrated to provide information about the freshness of vegetable products during storage and processing (Kuang & Nelson, 1998; Palta, Levitt, & Stadelmann, 1977a, 1977b; Park et al., 2013). Moreover, the electrical conductivity has been reported to enable the estimation of cellular damage in fruits and vegetables during Ohmic heating (Sarang, Sastry, & Knipe, 2008).

Thus, this study aimed to evaluate the potential of electrical conductivity measurements for the freshness assessment of cow milk. Here, the *in situ* electrical conductivity of milk was numerically expressed as the *in situ* electrical conductivity freshness index, which facilitated the comparison between the increasing electrical conductivity of spoiled and fresh milk during storage at different temperatures. Finally, the interrelation between electrical conductivity and instrumental analysis of freshness were mathematically modelled with microbial growth. Successful development of *in situ* electrical conductivity based freshness assessment method will enable the real-time management the food freshness at on-site of production, distribution, re-tail store and home refrigerant storage.

2. Materials and methods

2.1. Sample preparation

Low temperature long time (LTLT) pasteurized cow milk samples were purchased at a local market (Emart, Seoul, Republic of Korea). The freshness of the milk samples were assured as they were purchased within two days of production and distributed through cold chain system.

2.2. Electrical conductivity measurement system

A custom-developed electrical conductivity measurement system was used in this study (Fig. 1). The *in situ* electrical conductivity measurement system was equipped with an arbitrary wave form generator (33500B; Keysight Technologies, CA, USA), rectangular type electrical conductivity cell (78 mm × 55 mm × 35 mm), square type titanium electrodes (55 mm × 30 mm), current sensing resistor (50 Ω; JEIL ELECTRONICS CO., LTD., Gwangju, Republic of Korea) and a data acquisition (DAQ) system (34970A, Keysight Technologies, CA, USA). Titanium electrodes were installed at both ends of acrylic conductivity cell maintaining a distance of 53 mm. The conductive cell and current sensing resistor (50 Ω) was serially connected to measure the current flow using Ohm's law with provided electric field strength.

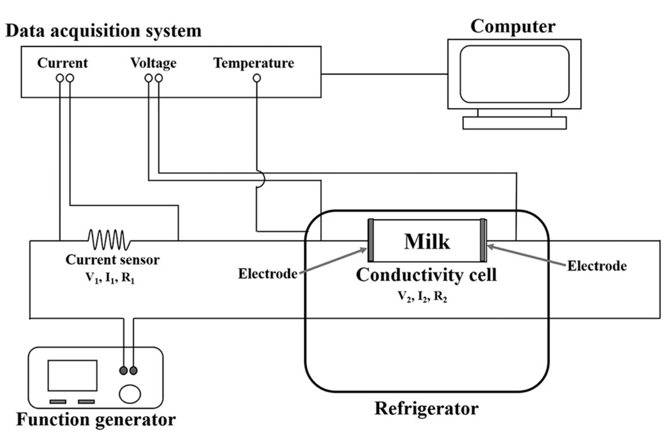

Fig. 1. Schematic diagram of electrical conductivity system of cow milk[1].
[1]Drawn by Jeong Hyeon Hwang and Ah Hyun Jung for master thesis in 2022 at Seoul National University of Science and Technology, copyright is shared for Hwang and Jung.

2.3. Sample loading into conductivity cell and in situ electrical conductivity measurement

In this study, the sample loading of cow milk into the conductivity cell was performed in the biosafety cabinet (SCB-I15; SAMIN SCIENCE CO. LTD., Seoul, Republic of Korea) to assure the aseptic conditions. The conductivity cell and electrodes were sanitized with 70% ethanol solution before conducting the experiment. A 50 ml sample of cow milk was poured into the *in situ* electrical conductivity cell (78 mm × 55 mm × 35 mm) and then it was sealed with parafilm (PM-996; Bemis Company, Inc., WI, USA) to prevent contamination and evaporation of sample during storage. An electric field of 0.70 V/cm at 100 kHz was applied to the milk sample using the arbitrary waveform generator (33500B; Keysight Technologies). The assay electric field strength and frequency allowing stable current flow in the milk sample were determined in the preliminary experiments. The current sensing resistor was connected to the conductivity cell to measure the magnitude of current through milk using Ohm's law. The electrical conductivity was calculated using measured voltage, current, and cell constant of the conductivity cell as shown in Eq. (1) (Park et al., 2013; Rieger, 1994).

$$\sigma = \frac{L \cdot I}{A \cdot V} = k \cdot \frac{I}{V} \tag{1}$$

where L is the distance (m) between electrodes, A is the area (m^2) of electrode, I is current flow (A) across the sample, V is the measured electric potential (V) across the sample, and k is the cell constant (m^{-1}) of the conductivity cell.

In this study, the k was estimated as 57 m^{-1}. The changes in the electrical conductivity of milk were measured every 5 min at two different storage temperature of 5 °C and 20 °C until 6 weeks.

2.4. Determination of in situ electrical conductivity freshness index (EF_i)

The electrical conductivity data were converted into *in situ* electrical conductivity freshness index (EF_i). The EF_i was calculated from the changes in the electrical conductivity of spoiled milk samples during the indicated storage time as compared to that of fresh sample as described in the Eq. (2). This equation was previously utilized as the tissue disintegration index (Z) of vegetables to estimate the tissue damage in vegetables after heat treatment (Bazhal, Lebovka, & Vorobiev, 2003; De Vito, Ferrar, Lebvoka, Shynkaryk, & Vorobiev, 2008; Lebovka, Bazhal, & Vorobiev, 2002; Park et al., 2013).

$$EF_i = \frac{\sigma_{is} - \sigma_{fs}}{\sigma_{sp} - \sigma_{fs}} \tag{2}$$

where σ_{is} is the *in situ* electrical conductivity, σ_{fs} is the electrical conductivity of fresh sample, and σ_{sp} is the electrical conductivity of totally spoiled sample during storage at the storage temperature. The fresh milk shows the EF_i value of 0 that increases up to 1 when milk is totally spoiled.

2.5. pH analysis

The pH changes were measured using a glass electrode pH meter (ORION STAR A211, ThermoFisher Scientific Inc., MA, USA) every 3 days. Briefly, a 30 ml of milk sample was poured into a 50 ml conical tube. The pH was measured at a constant temperature of 5 °C. The pH meter was calibrated using standard buffer solutions at pH 4.0, 7.0, and 10.0 before each analysis.

2.6. Microbial growth analysis

In this study, three representative microbial communities was assayed which included total aerobic bacterial (TAB), lactic acid bacterial (LAB), and psychrotrophic bacterial (PCB) counts. The microbial growth in milk was counted every three days during the storage period at 5 °C and 20 °C. In the TAB and LAB count, 1 ml of the milk sample was sequentially diluted 10-fold with sterilized 0.85% saline solution. 100 μL diluted milk solution was aseptically spread on used milk count agar (MB cell, Seoul, Republic of Korea) and de Man, rogosa and sharpe (MRS) agar (MB cell, Seoul, Republic of Korea) for TAB and LAB, respectively. The LAB were incubated on milk count agar and incubated for 7 days at 7 °C as per the previously described protocols (Hantsis-Zacharov & Halpern, 2007; Zhang, Palmer, Teh, & Flint, 2020).

2.7. Empirical model fitting

The empirical model was developed to estimate the interrelation between EF_i and milk freshness, which included the pH as well as the TAB, LAB, and PCB counts. The first and second polynomial regression functions were fitted using SAS, 9.1.3, software (SAS Inst. Inc., Cary, NC, USA) as shown in Eqs. (3), (4), (5), and (6).

$$pH = \beta_0 + \beta_1 \cdot EF_i + \beta_2 \cdot EF_i^2 \pm \varepsilon \quad (3)$$

$$TAB = \beta_0 + \beta_1 \cdot EF_i + \beta_2 \cdot EF_i^2 \pm \varepsilon \quad (4)$$

$$LAB = \beta_0 + \beta_1 \cdot EF_i + \beta_2 \cdot EF_i^2 \pm \varepsilon \quad (5)$$

$$PCB = \beta_0 + \beta_1 \cdot EF_i + \beta_2 \cdot EF_i^2 \pm \varepsilon \quad (6)$$

where EF_i is the *in situ* electrical conductivity freshness index, β_0 is the intercept coefficient of the polynomial regression, β_1 is the slope coefficient of first order term, β_2 is the slope coefficient of second order term, ε is the standard error in Y. The goodness of model fit was determined with coefficient of determination (R^2) and probabilities of each coefficient (*Pr* value). Thus, the empirical model fitting enabled the prediction of pH changes and microbial growth from the assessment of *in situ* electrical conductivity changes in milk. The goodness of model fit was determined using coefficient of determination (COD, R^2) and confidence interval ($Pr > |t|$). In the empirical model fitting, confidence interval was determined as 90% ($P < 0.10$) since two different qualitative parameters were compared.

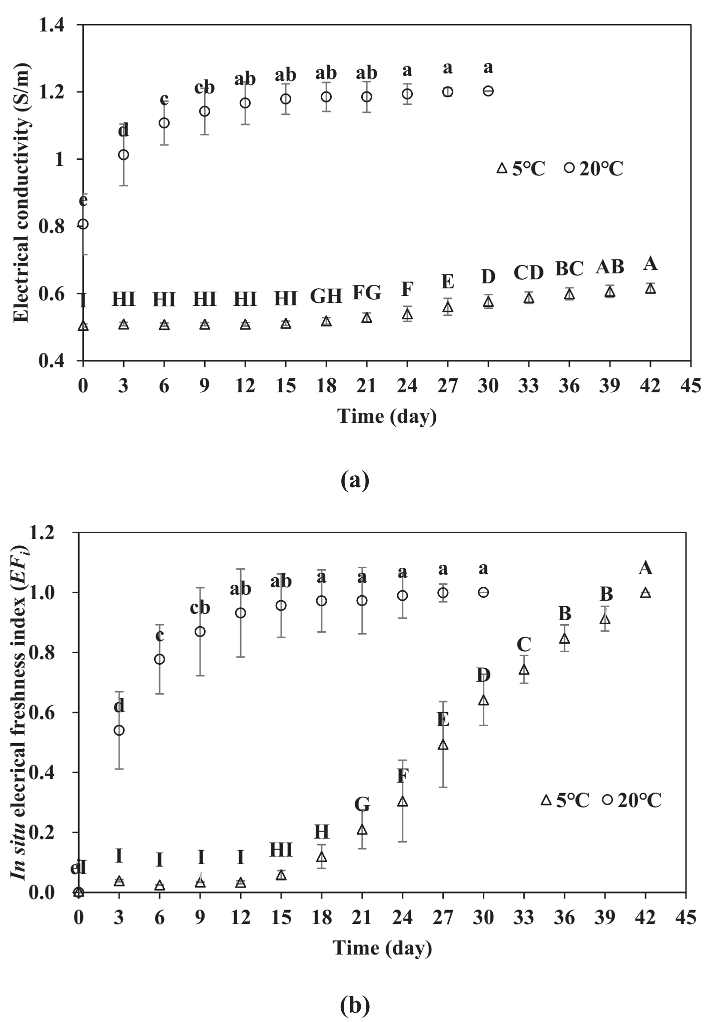

Fig. 2. Evolution of (a) electrical conductivity and (b) *in situ* electrical freshness index (EF_i) of milk stored during refrigerated (5 °C) and room temperature (20 °C) storage. [A-I]Means (±Standard deviation) with a different letter are significantly different at 5 °C ($P < 0.05$). [a-e]Means (±Standard deviation) with a different letter are significantly different at 20 °C ($P < 0.05$).

2.8. Statistical analysis

Measurement and analysis of electrical conductivity, pH, and microbial growth were performed in triplicate. The statistical significance among the different treatment groups was determined through analysis of variance (ANOVA). The Fisher's least significant difference (LSD) was estimated at a 95% confidence interval using the Statistical Analysis System (SAS) software (version 9.1.3, SAS Inst. Inc., NC, USA).

3. Results and discussion

3.1. The in situ electrical conductivity (σ_{is}) and in situ electrical freshness index (EF_i) of cow milk

The evolution of *in situ* electrical conductivity and electrical freshness index of milk at refrigerated (5 °C) and room temperature (20 °C) according to the storage time are presented in Fig. 2. The initial *in situ* electrical conductivity of the milk samples was estimated to be 0.505 S/m and 0.806 S/m at 5 °C and 20 °C, respectively. Furthermore, a higher initial electrical conductivity of fresh milk samples was detectable at 20 °C as compared to that of 5 °C. This finding might be attributable to the increased movements of ions in the liquid food and subsequent amplification of electrical conductivity with increasing temperature (Jittanit et al., 2017; Jo & Park, 2019; Shirsat, Lyng, Brunton, & McKenna, 2004). In the milk samples stored refrigerated at 5 °C, the *in situ* electrical conductivity gradually increased from 0.505 to 0.616 S/m during the 42 days of storage. However, the room temperature storage (20 °C) of milk samples resulted in a more rapid and higher increment of milk electrical conductivity as compared to that of the refrigerated milk samples. Particularly, the *in situ* electrical conductivity of the milk samples increased from 0.806 to 1.202 S/m during the 30 days storage at room temperature. Moreover, the rapid increase of the *in situ* electrical conductivity of the milk samples up to 1.142 S/m was detected within 10 days of storage at room temperature followed by the slight increment to the final level of 1.202 S/m.

The estimated *in situ* electrical freshness index (EF_i) of milk is presented in Fig. 2(b). The application of EF_i enabled a relatively fair comparison of the electrical conductivity changes, since it was calculated based on the initially detected different electrical conductivity values of fresh milk samples at 5 °C and 20 °C. The fresh milk showed the EF_i value of 0 at both refrigerated (5 °C) and room temperature storage (20 °C). Moreover, the EF_i value of the refrigerated milk samples at 5 °C did not alter from 0 until 16 days of storage, which indicated that the milk samples were not spoiled until then. Subsequently, the EF_i value gradually increased from 0.120 at 18th day to 1.000 at 42nd day. In the scope of EF_i, the freshness of milk was assured until 18 days of storage at refrigerated condition of 5 °C. In general, the shelf life of refrigerated milk has been reported to be 10 to 15 days (Al-Hilphy, Abdulstar, & Gavahian, 2021; Juff & Deeth, 2007). The EF_i thus, facilitated the evaluation of freshness in refrigerated milk since its value increased after 15th day of storage, which is quite close to the conventionally recommended shelf life of refrigerated milk.

In contrast, the EF_i of the milk samples increased to 0.030 at 3rd day of storage at room temperature (20 °C) followed by a stark rise to 0.931 at the 12nd day of storage. In the refrigerated storage at 5 °C, the EF_i of 0.059 was considered as the initiation point of milk spoilage at the 15th day of storage. However, the room temperature (20 °C) stored milk samples showed the initiation point of spoilage at 3rd day of storage, which was much earlier than that detected for the refrigerated milk samples. These findings are further deliberated upon in the context of instrumental freshness analysis and microbial count studies in the subsequent sections.

3.2. pH analysis

The changes in the pH and *in situ* electrical freshness index (EF_i) of the refrigerated (5 °C) and room temperature (20 °C) stored milk samples were simultaneously plotted with electrical conductivity freshness index (EF_i) (Fig. 3). pH of milk showed the inverse interrelation to EF_i. pH is the one of the representative indices for milk freshness (Romero et al., 2021) and electrical conductivity of milk enable to predict the pH changes of milk. The results show that in both the storage temperatures, the pH of milk decreased with prolonged storage time (Table 1). Specifically, the initial pH value of milk at 5 °C was determined to be 6.98 ± 0.01. Moreover, there was no significant change in the pH of milk until the 18th day of storage ($P < 0.05$) and then it dropped to 6.81 ± 0.02 by 0.14 units at 21st day of storage. Remarkably, the pH drop at the 18th day of storage was concomitant with increment in the electrical conductivity of the milk samples on the 18th day. Contrastingly, the room temperature stored milk samples showed the drop in pH quite earlier around the 3rd day of storage. This was practically in accordance with the changes in electrical conductivity detected at 2nd day storage at 20 °C. Thus, the storage temperature of 20 °C results in a more rapid reduction of pH as compared with that in case of storage at 5 °C. Moreover, it has been reported that the rate and extent of pH decrease was higher at elevated storage temperature when the milk was stored at 5, 20, 37, and 45 °C (Al-Saadi & Deeth, 2008). Studies have shown that the polymerization-induced coagulation of milk casein micelles is favored at higher storage temperature, which reduces the pH of milk below 6.7 (Deeth & Lewis, 2016; Karlsson et al., 2019). Furthermore, the pH reduction of milk is attributable to the Maillard reactions and subsequent acid formation, dephosphorylation of phosphorylated caseins, and proton release concomitant with prolonged storage (Al-Saadi & Deeth, 2008; Venkatachalam, McMahon, & Savello, 1993). Additionally, the reduction of pH from the industrial standard of 6.5 to 4.5 has been documented to cause the settling of casein micelles and subsequent curding (Lu & Wang, 2017; O'Connor, 1995). Inverse interrelation between pH and EF_i demonstrated the potential of electrical conductivity measurement as real-time freshness management of milk during storage.

3.3. Microbial growth analysis

In the present study, the microbial growth was also analyzed in the cow milk samples stored at refrigerated (5 °C) and room temperature (20 °C) *via* assessment of the total aerobic bacterial (TAB), psychrotrophic bacterial (PCB), and lactic acid bacterial (LAB) counts (Table 2). The results demonstrated that the TAB count in the milk samples reached the limit of detection (LOD) by the 3rd day of storage at both the assayed temperatures. In the refrigerated milk samples stored at 5 °C, the TAB count was detected to be 2.56 ± 0.06 log CFU/mL at the 6th day, which gradually increased to 7.44 ± 0.03 log CFU/mL during the 36th day of storage period. Furthermore, the TAB showed no further growth after the 36th day with the same reduced to below 7 log CFU/mL at the 39th and 42nd day of storage. This stark reduction in the TAB count might be attributed to the curdling of milk, which can result in experimental error during serial dilution and subsequent plating. The Pasteurized Milk Ordinance (PMO) recommends that the total aerobic bacteria count in pasteurized milk should be below 20,000 CFU/mL, which is accountable to 4.3 log CFU/mL (Martin, Ranieri, Wiedmann, & Boor, 2011). In this study, the refrigerated milk samples showed the TAB count below 4.3 log CFU/mL until the 12th day of storage at 5 °C. In contrast, the TAB count in the milk samples stored at the room temperature of 20 °C reached the LOD by the 3rd day of storage. Moreover, it sharply increased up to 5.79 ± 0.06 log CFU/mL at the 6th day of storage, which renders it unfit for consumption. Likewise, the TAB count continuously increased to 7.55 ± 0.12 log CFU/mL at the 30th day of storage at room temperature. Overall, our results suggest that the TAB count exhibits a positive association with the increase in electrical conductivity, which is further elaborated the empirical model section.

The PCB count in the present study reached the LOD until 6th day when the milk samples were stored at 5 °C, which later continuously increased from 3.41 ± 0.06 log CFU/mL at the 9th day to the maximum

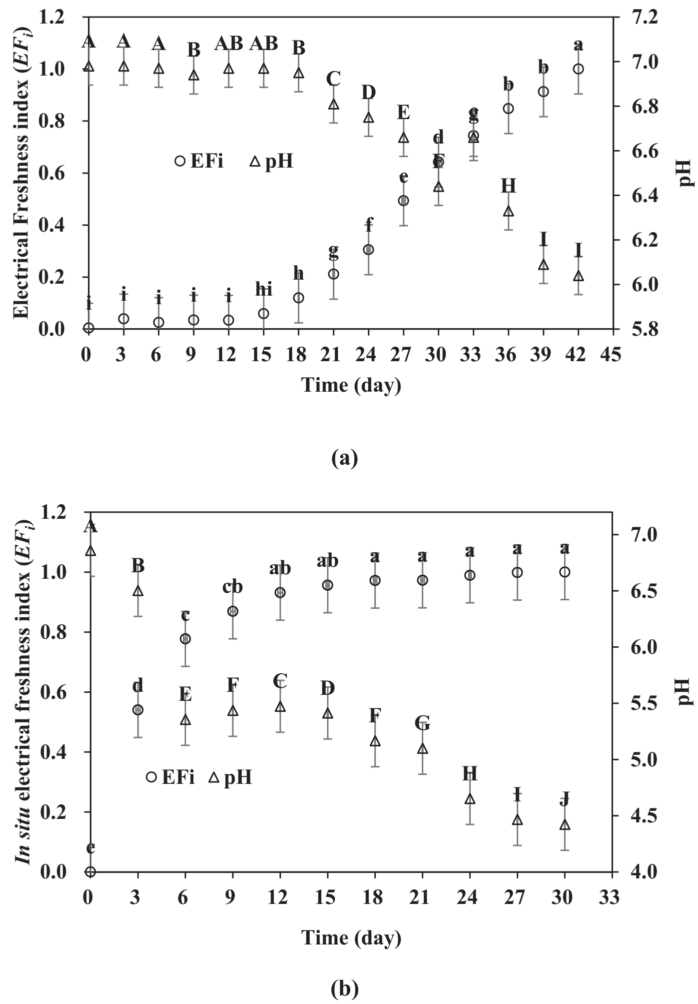

Fig. 3. pH *versus in situ* electrical freshness index (EF_i) of milk during refrigerated storage at 5 °C and room temperature storage at 20 °C. $^{A\text{-}I}$Means (±Standard deviation) with a different letter are significantly different for pH ($P < 0.05$). $^{a\text{-}i}$Means (±Standard deviation) with a different letter are significantly different for EF_i ($P < 0.05$).

Table 1
pH values of milk during refrigerated storage at 5 °C and room temperature storage at 20 °C.

Time (day)	pH	
	5 °C	20 °C
0	6.98 ± 0.01 ^{a}	6.98 ± 0.01 ^{a}
3	6.98 ± 0.01 ^{a}	6.86 ± 0.03 ^{c}
6	6.97 ± 0.01 ^{a}	6.50 ± 0.01 ^{i}
9	6.94 ± 0.03 ^{b}	5.36 ± 0.02 ^{o}
12	6.97 ± 0.01 ^{ab}	5.44 ± 0.01 ^{p}
15	6.97 ± 0.01 ^{ab}	5.47 ± 0.01 ^{m}
18	6.95 ± 0.01 ^{b}	5.41 ± 0.03 ^{n}
21	6.81 ± 0.02 ^{d}	5.17 ± 0.01 ^{p}
24	6.75 ± 0.02 ^{e}	5.10 ± 0.02 ^{q}
27	6.66 ± 0.02 ^{f}	4.65 ± 0.01 ^{r}
30	6.44 ± 0.01 ^{g}	4.47 ± 0.02 ^{s}
33	6.66 ± 0.02 ^{h}	
36	6.33 ± 0.02 ^{j}	
39	6.09 ± 0.02 ^{k}	
42	6.04 ± 0.03 ^{l}	

count of 7.41 ± 0.08 log CFU/mL at the 33rd day of storage at 5 °C. The cow milk reportedly contains psychrotrophic microorganisms that produce thermoresistant exoproteases and lipases, which may compromise the quality of the processed fluid milk and dairy products during storage (Ribeiro Júnior, de Oliveira, Tamanini, de Oliveira, & Beloti, 2018). As per the EU criteria, the recommended PCB count in milk should be <4.22 log CFU/mL (Cempírková, 2002; Zhang et al., 2020). During the storage of milk at room temperature storage (20 °C), the PCB count showed a slower growth as compared with those noted in the milk samples under refrigerated storage as expected. The important characteristics of the PCB are their abilities to grow at low temperatures (3–7 °C) in addition to their ability to hydrolyze and use large molecules of proteins and lipids for growth (Ledenbach & Marshall, 2010).

Furthermore, the LAB count was not detected in the milk samples stored at 5 °C. According to Ledenbach and Marshall (2010), the preferred temperature for the growth of LAB was 8–15 °C. Thus, it was rationalized that LAB failed to grow in the milk samples stored at 5 °C. However, the LAB growth was active in the milk stored at 20 °C with the initial count of 3.25 ± 0.06 log CFU/mL increasing up to 7.48 ± 0.07 log CFU/mL. Additionally, the most abundant LABs detected milk samples have been documented to include *Lactobacillus, Lactococcus, Enterococcus, Streptococcus, Pediococcus, Leuconostoc*, and *Weissella* (Shangpliang, Rai, Keisam, Jeyaram, & Tamang, 2018; Shangpliang & Tamang, 2021; Terzić-Vidojević et al., 2020; Zhong et al., 2016). As a result, the growth of these LABs should be appropriately controlled for safe consumption of milk.

3.4. Empirical model fitting

The Table 3 summarizes the coefficient of empirical model ($\beta_0, \beta_1, \beta_2$)

Table 2
Microbial growth in milk during refrigerated (5 °C) and room temperature (20 °C) storage.

Time (day)	Total aerobic bacteria (TAB) (log cfu/ml)		Psychrotrophic bacteria (PCB) (log cfu/ml)		Lactic acid bacteria (LAB) (log cfu/ml)	
	5 °C	20 °C	5 °C	20 °C	5 °C	20 °C
0	LOD ^{o}	LOD ^{o}	LOD ^{q}	LOD ^{q}	ND ^{h}	ND ^{h}
3	LOD ^{o}	LOD ^{o}	LOD ^{q}	LOD ^{q}	ND ^{h}	3.25 ± 0.06 ^{g}
6	2.56 ± 0.06 ^{n}	5.79 ± 0.06 ^{g}	LOD ^{q}	3.57 ± 0.03 ^{o}	ND ^{h}	6.45 ± 0.01 ^{f}
9	3.54 ± 0.10 ^{m}	7.20 ± 0.03 ^{cd}	3.41 ± 0.06 ^{p}	4.24 ± 0.05 ^{m}	ND ^{h}	6.52 ± 0.08 ^{ef}
12	4.00 ± 0.04 ^{l}	7.20 ± 0.03 ^{cd}	3.66 ± 0.04 ^{n}	5.22 ± 0.07 ^{i}	ND ^{h}	6.58 ± 0.03 ^{de}
15	4.67 ± 0.02 ^{j}	7.19 ± 0.09 ^{cd}	4.43 ± 0.03 ^{l}	5.18 ± 0.06 ^{i}	ND ^{h}	6.61 ± 0.01 ^{de}
18	4.31 ± 0.09 ^{k}	7.21 ± 0.06 ^{c}	5.24 ± 0.03 ^{i}	5.49 ± 0.06 ^{f}	ND ^{h}	6.64 ± 0.02 ^{cd}
21	4.70 ± 0.07 ^{j}	7.32 ± 0.08 ^{ab}	5.03 ± 0.03 ^{j}	5.44 ± 0.02 ^{fg}	ND ^{h}	7.13 ± 0.03 ^{b}
24	4.99 ± 0.02 ^{i}	7.27 ± 0.07 ^{c}	5.41 ± 0.01 ^{gh}	5.36 ± 0.10 ^{h}	ND ^{h}	6.72 ± 0.05 ^{c}
27	5.31 ± 0.06 ^{h}	6.51 ± 0.15 ^{e}	5.80 ± 0.03 ^{e}	5.10 ± 0.04 ^{j}	ND ^{h}	6.54 ± 0.15 ^{ef}
30	7.12 ± 0.03 ^{d}	7.55 ± 0.12 ^{a}	7.11 ± 0.06 ^{b}	4.69 ± 0.05 ^{k}	ND ^{h}	7.48 ± 0.07 ^{a}
33	7.18 ± 0.03 ^{cd}		7.41 ± 0.08 ^{a}		ND ^{h}	
36	7.44 ± 0.03 ^{b}		6.68 ± 0.02 ^{cd}		ND ^{h}	
39	6.32 ± 0.02 ^{f}		6.72 ± 0.03 ^{c}		ND ^{h}	
42	6.57 ± 0.03 ^{e}		6.61 ± 0.05 ^{d}		ND ^{h}	

LOD: Limit of detection.
ND: Not detected.

Table 3
Estimated coefficients and probability test of the fitted second-order polynomial parameters between freshness parameters (pH and microbial counts) and *in situ* electrical freshness index (EF_i) (*$Y = \beta_0 + \beta_1 \cdot EF_i + \beta_2 \cdot EF_i^2 \pm \varepsilon$).

		5 °C		20 °C	
		Coefficients	Pr > |t|	Coefficients	Pr > |t|
pH	β_0	6.970	0.0001	6.855	0.0001
	β_1	−0.314	0.2752	0.791	0.5797
	β_2	−0.584	0.0668	−2.853	0.0540
	R^2 values	0.938		0.860	
	SSEY (ε)	0.089		0.320	
TAB	β_0	2.094	0.0033	−0.278	0.8050
	β_1	12.996	0.0070	−1.236	0.7986
	β_2	−8.607	0.0643	9.330	0.0630
	R^2 values	0.736		0.885	
	SSEY (ε)	1.298		1.095	
PCB	β_0	1.616	0.0237	−0.138	0.8243
	β_1	16.427	0.0027	−2.731	0.3222
	β_2	−11.870	0.0243	8.484	0.0075
	R^2 values	0.748		0.932	
	SSEY (ε)	1.416		0.605	
LAB	β_0	0		−0.147	0.8479
	β_1	0		10.843	0.0063
	β_2	0		−3.840	0.7388
	R^2 values	0		0.959	
	SSEY (ε)	0		0.499	

*Y is either pH, TAB, LAB or YC.

fitting as well as statistical probability of 90% confidence interval and coefficient of determination (COD, R^2) for the electrical conductivity freshness index *(EF_i)* of refrigerated (5 °C) and room temperature (20 °C) stored cow milk. In the model fit of pH, when the milk was stored at the refrigeration temperature of 5 °C, negative correlation was detected between EF_i and pH in which β_1 (first order coefficient) and β_2 (second order coefficient) are −0.314 and − 0.584, respectively. However, no significance of β_1 was determined; whereas, β_2 showed the statistical significance at the 90% confidence level ($P < 0.10$). As a result, the EF_i could predict the reduction of pH in milk with non-linear model according to the prolonged refrigerated storage. In the polynomial model of pH for refrigerated milk, a goodness of model fit was found with high R^2 value of 0.938. The room temperature storage (20 °C) showed the negative coefficient of β_1 as −2.853 with significance ($P < 0.10$). It showed the positive coefficient of β_1 as 0.791; however, no significance was determined for the same ($P > 0.10$). Therefore, the EF_i of room temperature stored milk could predict the linear reduction of pH. In this study, the EF_i thus enabled the prediction of changes in the pH of milk during both refrigerated and room temperature storage.

In terms of the microbial counts, the TAB model showed the positive coefficient of β_1 and negative coefficient of β_2 at both refrigerated (5 °C) and room temperature (20 °C) storage conditions. Furthermore, the magnitude of β_1 was higher than that of β_2, which eventually enabled the EF_i to predict the growth of TAB in milk during both refrigerated and room temperature storage conditions. Moreover, the R^2 value of the empirical model was higher at 20 °C than that at 5 °C, which explains the rapid growth of TAB at 20 °C as compared with that noted at 5 °C. Likewise, the PCB counts showed similar trend to TAB in which β_1 and β_2 showed the positive and negative coefficients, respectively. Similar to the TAB model, the magnitude of β_1 was higher, which thereby enabled the EF_i to predict the increasing count of PCB during storage of milk. Remarkably, a better goodness of model fit was found in case of the room temperature stored milk with higher R^2 value as compared with that of refrigerated milk. This is attributed to rapid growth of microorganism at room temperature.

In the LAB count, no growth was detected at 5 °C storage. As a result the EF_i could not predict the growth of LAB during refrigerated storage. In case of storage at 20 °C, the growth of LAB in the milk samples could be estimated through EF_i with positive β_1 value and a high R^2 value of 0.959. This study, thus demonstrated the potential of electrical conductivity measurement to estimate the growth of microorganism in milk during storage.

4. Conclusions

This study evaluated the potential of electrical conductivity measurement to predict the quality attributes and microbial safety of milk at refrigerated (5 °C) and room temperature (20 °C) storage conditions. The EF_i was calculated based on the comparison between the electrical conductivity changes of milk subjected to a prolonged storage and that of fresh milk. Our experimental findings indicate that the EF_i associated with storage of milk at 20 °C showed stiffer increasing slope *versus* storage time than that observed for storage of milk at 5 °C, suggesting that the electrical conductivity of milk increased with spoilage. The EF_i also correlated with the instrumental freshness of pH and microbial growth parameters (TAB, PCB, LAB) using a second order polynomial model. Overall, our results suggested a positive association between EF_i and pH at both refrigerated (5 °C) and room temperature (20 °C) storage. This finding implies that the reduction of pH in milk could be estimated through the changes in the electrical conductivity of the spoilt milk. In terms of microbial count, the growth of TAB, PCB, and LAB could be predicted through EF_i with good fitness of model except for the growth of LAB at 5 °C. This study thus showed the practical use of *in situ* electrical conductivity measurement for rapid prediction of the quality attributes and microbial safety of milk during storage.

CRediT authorship contribution statement

Jeong Hyeon Hwang: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Ah Hyun**

Jung: Methodology, Writing – review & editing, Visualization. **Seung Su Yu:** Methodology, Writing – review & editing, Visualization. **Sung Hee Park:** Resources, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

Data availability

No data was used for the research described in the article.

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