Study of the Electro-Activation Process of Calcium Lactate, Calcium Ascorbate Solutions, and Their Equimolar Mixture: Assessment of Their Physicochemical Properties

Pierre Emerson Cayemitte, Natela Gerliani, Philippe Raymond, and Mohammed Aïder*

ABSTRACT: The aim of this study was to prepare electro-activated solutions (EAS) from calcium lactate, calcium ascorbate, and an equimolar mixture of these two salts to obtain their corresponding acids and to study their physicochemical characteristics, in particular, pH, titratable acidity, pKₐ, and antioxidant activity. Indeed, the solutions were electro-activated in a reactor comprising three compartments (anodic, central, and cathodic) separated by anionic and cationic exchange membranes, respectively. The electric current intensities used were set at 250, 500, and 750 mA for a maximum period of 30 min. In general, the EAS obtained at 750 mA for 30 min showed the lowest pH (2.16, 2.08, 1.94) and pKₐ (3.13, 3.07, 2.90) values and the highest titratable acidity (0.107, 0.102, 0.109 mol/L) for calcium lactate, the mixture, and calcium ascorbate, respectively. In addition, the obtained results have demonstrated that the pH, titratable acidity, and pKₐ of the EAS varied proportionally and significantly (p < 0.001) with the duration of the experiment and the intensity of the electric current applied. To evaluate the migration of calcium (Ca²⁺) between the central and the cathodic compartments of the reactor, the concentration of Ca²⁺ was determined especially in the cathodic section by inductively coupled plasma optical emission spectroscopy (ICP-OES). The results showed that the migration of Ca²⁺ varied proportionally with the electric current intensity. In this context, analysis by Fourier transform infrared (FTIR) spectroscopy, high-performance liquid chromatography (HPLC), and differential scanning calorimetry (DSC) have confirmed the production of lactic acid and ascorbic acid compared to standards. In addition, analysis by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging technique confirmed high antioxidant activities of >90 and >83% for calcium ascorbate and the mixture, respectively, in comparison to the standard ascorbic acid (85%). Overall, this research has clearly demonstrated the eventual potential of electro-activation to produce highly reactive organic acids from their conjugated salts. These EAS can become excellent antimicrobial and sporicidal agents in the food processing industry.

1. INTRODUCTION

Electro-activation in solution is a branch of applied electrochemistry that studies the reactivity of aqueous solutions following their excitation by an electric field to modify the activation energy required for the chemical reactions occurring there.¹ Electro-activation (EA) of aqueous solutions is a technology that is based on water electrolysis and has already been experimented for over 100 years on the basis of Faraday’s works on electrochemistry. Since then, further studies on this technology have been conducted in the past decades, particularly in the former Soviet Union (USSR), to better understand the nonconventional chemical changes of aqueous solutions (including water) and their possible utilization in...
different fields. In this context, Bakhir, a Russian engineer, found in 1972 that slightly salted aqueous solutions could have specific physicochemical properties, resulting in acidic or alkaline media when submitted to electric current. Meanwhile, the EA technology was also recognized to be well experimented in Japan before it becomes popular in many other developed countries, such as Canada, China, or the United States. Among the processes (biological, chemical, and physical) capable of activating aqueous solutions and inducing their nonequilibrium thermodynamic state, EA is considered as one of the most effective. In fact, the electro-activation technique is based on several electrochemical reactions, particularly the electrolysis phenomenon, which is linked to Faraday’s laws. Basically, during the electrolysis process, the electric field induces a migration of anions and cations to the positive (anode) and negative (cathode) electrodes of an electrochemical cell, respectively. Furthermore, the physicochemical reactions that occurred close to the solution–electrode interfaces resulted in two different phenomena called reduction and oxidation, occurring at the cathodic and anodic sections. They are responsible for the acidification and alkalinization of the anolyte and catholyte, respectively (eqs 1 and 2). During the reduction reaction, electrons (e⁻) are transferred from the cathode to cations such as hydrogen (H⁺), to produce gaseous hydrogen (H₂) illustrated as follows.

**reduction reaction at the cathode:** \[ 2\text{H}_2\text{O}_{(\text{liquid})} + 2\text{e}^- \rightarrow \text{H}_2(\text{gas}) + 2\text{OH}^-_{(aq)} \] (1)

**reduction reaction at the cathode:** \[ \text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2(\text{gas}) \] (2)

Simultaneously with the reduction phenomenon, the oxidation reaction occurred with the transfer of free electrons (e⁻) from anions, such as oxygen (O²⁻) or hydroxide (OH⁻) to the anode, producing oxygen gas (O₂) that can be illustrated as follows:

**oxidation reaction at the anode:** \[ 2\text{H}_2\text{O}_{(\text{liquid})} \rightarrow \text{O}_2(\text{gas}) + 4\text{H}^+ + 4\text{e}^- \] (3)

**oxidation reaction at the anode:** \[ 4\text{OH}^-_{(aq)} \rightarrow \text{O}_2(\text{gas}) + 2\text{H}_2\text{O}_{(\text{liquid})} + 4\text{e}^- \] (4)

Indeed, during the electro-activation process, the aqueous solutions were electrically excited, and spontaneous chemical reactions occurred in the reactor, resulting in mass transfer of anions and cations throughout semipermeable ion-exchange membranes. According to Rahman et al. (2016), when a saline solution (e.g., NaCl) is submitted to an electric current field, the molecules are separated into ions (Na⁺, Cl⁻, H⁺, and OH⁻). Consequently, different compounds are formed, e.g., O₂, HCl, HClO, ClO⁻, or NaOH, producing simultaneously some acidic (anolytes) and alkaline (catholytes) solutions in the anodic and cathodic compartments, respectively. In terms of characteristics, it has been reported that anolytes may have acidic pH of about 2–6.5 and positive oxido-reduction potential (ORP) ranging from +200 to +1200 mV under specific conditions. On the other hand, the electro-activated catholytes are rather characterized by alkaline pH, ranging from 7 to 13 and negative ORP between −80 and −900 mV according to the experimental conditions. Moreover, several studies have reported that the significant changes induced by electro-activation in pH and ORP of aqueous solutions may be due to the metastable state of such solutions, which increased their reactivity and rendered them potentially usable as an antimicrobial disinfectant for many fields, namely, food processing and safety, medicine, and biotechnology. In this context, the factors responsible for the antimicrobial effects of electro-activated solutions (EAS) have been the subject of many investigations in the scientific community. Thus, some scientists think that low pH and high ORP are responsible for such biocidal activities, whereas other researchers believe that chlorinated residues or a mixture of all of these factors are the root cause of the antimicrobial effects. Aider et al. (2012), who reviewed the topic, have mentioned that when aqueous solutions are electro-activated, they became saturated with oxidizing components, namely, ozone (O₃), hydrogen peroxide (H₂O₂), and active oxygen, including chlorine components that may be generated from available chloride ions of chlorinated salts such as NaCl, KCl, or CaCl₂. Then, these EAS could acquire significant antimicrobial properties. It was reported that the biocidal ability of EAS was related to chlorine compounds that are in metastable state, while Len et al. rather believed that chlorine gas from EAS was the cause of the antimicrobial activity, which contributed to food preservation.

Even if several chemical products to control pathogenic microorganisms are available on the market, foodborne illness is still a concern for the food industry worldwide. For this purpose, scientists are still developing methods that are not only more efficient for food processing but also less harmful for the human health and the environment. In this context, electro-activation, as an innovative and nonthermal technology requiring mainly electric current, food-grade salts, and water as raw materials, is becoming an undeniable asset to further enhance the control of pathogenic and spoilage microorganisms to reduce foodborne diseases and reinforce food preservation. Meanwhile, many studies to better comprehend the effects of EAS on various types of microorganisms, such as bacteria, fungi, viruses, algae, protozoans, and nematodes, have also been reported. However, almost all of these studies were carried out with sodium chloride (NaCl) solutions that could generate chlorinated gas or other chemical compounds (e.g., HClO, ClO⁻) that might be harmful to industrial workers, consumers, as well as the environment. In addition to the aforementioned drawbacks, chlorinated residues could produce unwanted defects during food processing such as vegetables bleaching. Moreover, they could also affect some sensitive nutrients such as antioxidants (phenolic compounds) and precursor of vitamins (e.g., β-carotene), which results in a reduction of food quality. Owing to these substantial disadvantages, it is of utmost importance, by the present study, to substitute chlorinated compounds by organic salts such as calcium lactate (Ca₇H₁₄CaO₆), calcium ascorbate (C₆H₁₄CaO₁₂), and a mixture of them to produce EAS that would be more convenient for human health and the environment. Recently, different studies carried out at the Laval University by Dr. Aider’s team have demonstrated the potential of the electro-activation technology to produce EAS from salts of organic acids to control pathogens and assure food preservation, as well as to improve food processing efficiency.
Thus, the aim of this research was to study the electroactivation process of solutions prepared with calcium lactate, calcium ascorbate, and their equimolar mixture, as well as to evaluate the physicochemical properties of the conjugated acid forms generated, in particular lactic acid (C₃H₆O₃), ascorbic acid (C₆H₇O₆), and their mixture. In fact, we also expected that the generated acidic components will be highly reactive and useful as antimicrobial and sporicidal agents.

2. MATERIALS AND METHODS

2.1. Chemicals and Materials. Among the chemical products used in this study, standard (conventional) lactic acid and ascorbic acid as well as calcium L-lactate hydrate and calcium L-ascorbate dihydrate were purchased from Sigma-Aldrich (St. Louis, MA). Sodium chloride (NaCl) was bought from VWR International Co. (Mississauga, Canada). The sodium hydroxide (1 N NaOH) solution was ordered from Fisher Chemical (Fair Lawn, NJ), and the phenolphthalein indicator was obtained from Laboratoire MAT INC (Montreal, Canada). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was procured from Sigma-Aldrich (St. Louis, MA). Indeed, the salts were dissolved in distilled water to finally obtain different solutions with concentrations of 10 ppm calcium lactate and ascorbate, 0.25 M calcium lactate and ascorbate, and 0.1 M sodium chloride solution, to be used in the anodic, central, and cathodic compartments of the electro-activation reactor, respectively. The anticorrosion electrodes (anode: ruthenium−iridium-coated titanium; cathode: stainless steel food grade) used in the reactor were ordered from Qixin Titanium Co. (Baoji, China). Two types of ion-exchange membranes (anionic [AMI-7001] and cationic [CMI-7000]) were purchased from Membranes International, Inc. (Ringwood, NJ). All chemicals and equipment used in the experiments complied with the standards of the Laval University laboratories.

2.2. Configuration of the Electro-Activation Reactor and EAS Production. The electro-activation of the salted solutions was carried out in a reactor designed with tree Plexiglas compartments (Figure 1). The dimensions of each compartment were: 5 cm length × 2 cm width × 11 cm depth, for a volume capacity of 120 mL. Additionally, the dimensions of the anode were 12 cm length × 4 cm width, while the cathode measured 12 cm length × 5 cm width, and they were placed at a distance of 8.5 cm from each other in the reactor. The anodic compartment was isolated from the central compartment by an anionic exchange membrane (AEM) (positively charged), whereas a cationic exchange membrane (negatively charged) separated the central to the cathodic compartment. The membranes used, through which ions could selectively flow, had a standard thickness of about 0.45 ± 0.025 mm for an exposed transfer surface of 3 cm × 7 cm. Thus, the anionic exchange membrane (AEM) installed close to the anode prevented the exit of cations (e.g., H⁺, Ca²⁺) and facilitated anions transfer (e.g., lactate ion (C₃H₅O₃⁻), ascorbate ion (C₆H₇O₆⁻)) between the anodic and central sections. The cationic exchange membrane (CEM) avoids the transfer of undesired anions from the cathodic to the other compartments, and consequently, anolytes with distinctive properties (pH, pKₐ, ORP, etc.) could be produced.⁴ Then, as shown in Table 1, the anodic compartment of the reactor was filled with 120 mL of calcium lactate solution or calcium ascorbate, or their equimolar mixture, with a concentration of 10 ppm at time zero of the electro-activation process. The concentration of 10 ppm at time zero in the anodic compartment was selected at the lowest concentration that allowed the use of the selected highest electric current intensity of 1000 mA. Likewise, the central compartment was also filled

| Table 1. Aqueous Solutions Comprising the Electro-Activation Reactor Compartments |
|---------------------------------|---------------------------------|---------------------------------|
| anodic compartment | central compartment | cathodic compartment |
| (1) calcium lactate (C₆H₁₀CaO₆): 10 ppm | calcium lactate (C₆H₁₀CaO₆): 0.25 M | sodium chloride NaCl: 0.1 M |
| (2) calcium ascorbate (C₁₂H₁₄CaO₁₂): 10 ppm | calcium ascorbate (C₁₂H₁₄CaO₁₂): 0.25 M | sodium chloride NaCl: 0.1 M |
| (3) mixture: (C₆H₁₀CaO₆ + C₁₂H₁₄CaO₁₂): 10 ppm | mixture (C₆H₁₀CaO₆ + C₁₂H₁₄CaO₁₂): 0.25 M | sodium chloride NaCl: 0.1 M |

with 120 mL of the aforementioned solutions with a concentration of 0.25 M. Finally, the cathodic compartment was filled with 120 mL of a NaCl solution at 0.1 M concentration.

Once filled with the specific solutions, the electro-activation reactor (Figure 1) was connected to a DC electric generator (Circuit Specialists CSI 12001X, Tempe) monitored consequently to provide the reactor with current intensities of 250, 500, and 750 mA. Under each intensity, the electro-activation process was performed for a limited time of 30 min and the targeted acidic solutions (anolytes) were generated in the anodic compartment. Meanwhile, samples were collected every 5 min for a maximum of seven samples (0, 5, 10, 15, 20, 25, and 30 min). For each EAS sample, measurement of pH, titratable acidity, and pK_a was carried out. In addition, the composition of EAS in calcium ion (Ca^{2+}) in the cathodic compartment was investigated by inductively coupled plasma optical emission spectroscopy (ICP-OES) to evaluate the migration of this ion after the electro-activation process. Furthermore, Fourier transform infrared (FTIR), high-performance liquid chromatography (HPLC), and differential scanning calorimetry (DSC) analyses were also performed to validate the chemical structure of lactic acid and ascorbic acid produced in comparison to standards under the same conditions. Moreover, the antioxidative activity of ascorbic acid and the mixture has also been validated by the use of DPPH free-radical scavenging assay.

2.3. Physicochemical Analyses of Electro-Activated Solutions. 2.3.1. pH, Titratable Acidity, and pK_a.

2.3.1.1. Monitoring of pH. The pH of electro-activated solutions (EAS) was measured every 5 min with a pH meter Model SR 601 C SympHony (VWR Scientific, Montreal, Canada), calibrated with pH buffers before the experiments. The measures of EAS pH were carried out in triplicate.

2.3.1.2. Titratable Acidity. The conventional acid–base titration technique was used to measure the percentage of acid (titratable acidity) in EAS samples collected every 5 min in the anodic compartment. The titratable acidity of EAS samples collected every 5 min in the anodic compartment was measured by the conventional acid–base titration technique using phenolphthalein and 0.1 M NaOH. The equivalent concentration of acid in EAS (mol/L(eq)) was determined as described by the following equation

\[
\text{acid(t)} = \frac{\text{concentration of NaOH} \times \text{volume of NaOH used}}{\text{volume of EAS used}} \tag{5}
\]

2.3.1.3. Monitoring of pK_a. As an acid dissociation constant, pK_a is a good parameter that helps us to better comprehend the behavior of some chemical products.\(^8\) In this sense, the pK_a values of EAS as well as standard acids were determined to make a comparison. Since the dissociation of weak acids is not complete, the dissociated and nondissociated forms coexisted in solution (eq 6) and the constant K_a reduces the concentration ratio of the respective components (eq 7). The pK_a value of the aforementioned chemical products (eq 8) was calculated using the Henderson–Hasselbalch equation (eq 9)

\[
\text{pK}_a = -\log_{10} K_a \tag{8}
\]

\[
\text{pH} = \text{pK}_a + \log_{10} ([A^-]/[HA]) \tag{9}
\]

2.3.2. Calcium Concentration Analysis by ICP-OES. To evaluate the effects of electric current intensity and time on the migration of calcium ions (Ca^{2+}) between the central and cathodic compartments, the concentration of calcium in the cathodic compartment was determined by inductively coupled plasma optical emission spectroscopy (Agilent 5100 SVV ICP-OES, Agilent Technologies, Victoria, Australia). The analyses were performed according to a protocol previously used in ref 24, and measurements were made at 422.673 nm wavelength.

2.3.3. Comparison of EAS and Standard Acids by FTIR, HPLC, and DSC Analyses. 2.3.3.1. FTIR Spectroscopy. The molecular composition of the obtained EAS was investigated using Fourier transform infrared (FTIR) spectroscopy. For this purpose, samples of electro-activated calcium ascorbate were prepared as follows: 10 mL of samples was put in a 15 mL tube to be dried in a SpeedVac Concentrator (Savant SPD131DDA, Thermal Scientific, IN). The SpeedVac was set at 45 °C, and the samples stayed in it for 2 days until they dried. Thereafter, 2 mg of the dried sample was combined with 300 mg of potassium bromide (KBr) to have a fine powder. Then, the fine powder was compressed with a Hydraulic Press (Fred S. Carver, Inc., Wabash, IN) that exerted 9000 pounds of force on samples to form thin pellets that were placed into the IR beam of the FTIR spectrometer (Nicolet 6700 FT-IR, Thermo Scientific, IN) for examination by transmission in the range of 4000–400 cm\(^{-1}\). Then, a mean of 32 scans were taken for every spectrum at a 4 cm\(^{-1}\) resolution using a deuterated triglycine sulfate (DTGS) detector. An attenuated total reflectance (ATR) accessory was used to implement the analysis of electro-activated calcium lactate and standard lactic acid due to the fact that they started melting at around 17 ± 1 °C while we were working at room temperature of 22 ± 1 °C. To do so, a few microliters of samples were placed on a diamond crystal to perform FTIR analysis. The spectrometer was also equipped with an interferometer to capture all of the wavelength at the same time by the detector when samples were placed under IR beam. Afterward, FTIR used interferometry to obtain information regarding samples that was transformed in spectra. Subsequently, the spectra generated from FTIR were compared with reference to identify specific functional groups presented in the EAS and standard acids.

2.3.3.2. HPLC. To compare electro-activated calcium lactate and calcium ascorbate solutions to standard lactic acid and ascorbic acid, a high-performance liquid chromatograph (HPLC) (Phenomenex, Inc., Torrance) assembled with a column, a pumping device, a UV–vis detector, and chromatographic software, was used to perform the analyses. The chromatographic elution (separation) was done through a Kinetex column (Phenomenex, Inc., Torrance), measuring 150 × 4.6 mm². Hence, the isocratic mobile phase was composed of 20 mM (v/v) potassium phosphate monobasic (KH₂PO₄) with a pH of 1.59, flowing through the column at a rate of 1.25 mL/min. Then, the analysis was performed by injecting into the column the samples (EAS and standard acids), and the absorbance was measured using a UV–vis detector previously adjusted at 210 nm. All of the analyses were performed at
room temperature (22 ± 1 °C), and the retention time and curves of the samples were recorded for interpretation.

2.3.3.3. DSC. The differential scanning calorimetry (DSC) analytical technique was used to identify, particularly the melting and crystallization temperatures of electro-activated calcium lactate and calcium ascorbate, to make a comparison with standard acids under similar conditions. Thus, the analyses were performed using a DSC Q1000 (TA Instruments, Tokyo, Japan) that was set up in the temperature range of −80 to 250 °C. Thereafter, 8–10 mg of dried matter (EAS or standards lactic acid/ascorbic acid) was weighted in pans before they were hermetically sealed and introduced in the DSC analyzer for around 1–2 h. Then, the changes provoked in the samples by the heat flow were interpreted based on typical DSC transition curves. Indeed, the operating principle of DSC is based on the difference in energy provided to a sample and a reference according to the temperature. Given that the variation of the sample energy depends on temperature, specific physical and/or chemical phase transitions may appear as curves to be analyzed.25

2.3.4. Antioxidant Activity by DPPH Assay. The antioxidant activity of the electro-activated calcium ascorbate, the standard ascorbic acid, and their mixture has been evaluated by DPPH free-radical scavenging method, based on a protocol previously described with some specific modifications. To perform the test, 100 mL of methanol was combined with 3.9 mg of DPPH® free radicals to make a solution. Then, 250 μL of samples previously diluted with methanol was poured into aliquots in which 750 μL of DPPH solution was added and mixed by a vortex. Meanwhile, a sample without EAS (blank) was also made with 250 μL of methanol to make a comparison. Following the aforementioned manipulations, the aliquots were incubated at room temperature (22 ± 1 °C) for a maximum of 1 h in the dark. Thereafter, a UV−vis spectrophotometer (BIO-RAD, xMark Microplate Spectrophotometer, Mississauga, Canada) was used to measure the absorbance at 517 nm wavelength. When DPPH® free radicals reacted with antioxidants (hydrogen donors) from the samples, they were neutralized (reduced), and a decolorization of such analytes was noted that changed from purple to slightly yellow.26 Then, the percentage of DPPH inhibition was calculated according to the following equation.

antioxidant activity (%) = \frac{\text{absorbance of control} − \text{absorbance of EAS}}{\text{absorbance of control}} × 100 \tag{10}

2.4. Statistical Analysis. The data collected from the experiments were analyzed with the software SPSS (IBM SPSS Statistics 25). Given that the experiments were performed in three replicates, the results were presented as mean ± standard deviation. To compare the mean values of samples, an analysis of variance (ANOVA) with post hoc Tukey test calculator was used and differences were considered significant when p-values were below the 5% threshold level of significance (p < 0.05).

3. RESULTS AND DISCUSSION

3.1. Characteristics of Electro-Activated Solutions: pH, Acidity, and pHK a. The results obtained from the electro-activation processes demonstrated that the electric current intensity and time had significant effects on the pH, acidity (titratable), and pHK a of the treated solutions (one-way ANOVA: p < 0.001), as presented in Figures 2–10. Regarding the electro-activated calcium lactate, the posteriori test of Tukey showed a significant difference, essentially between time zero and the other treatment times for pH and pHK a (p < 0.001), whereas no significant pH difference (p > 0.05) was observed between 5, 10, 15, 20, 25, and 30 min. The difference in titratable acidity was significant, except for the times 0 & 5, 5 & 10, 10 & 15, 15 & 20, 20 & 25, and 25 & 30 min, respectively. Statistically significant differences were observed in pHK a except for 10 & 15 and 20 & 25 min (p > 0.05). According to the electro-activated calcium ascorbate, the differences between 10, 15, 20, and 25 min were not statistically significant for pH and pHK a (p > 0.05). Moreover, the titratable acidity was not statistically different for treatments under times 0, 5, and 10 min (p > 0.05). Concerning the mixture, the difference in pH was not statistically significant for 10, 15, 20, 25, and 30 min (p > 0.05). No significant difference was observed in the titratable acidity between times 0, 5, and 10 min (p > 0.05). Furthermore, the difference in pHK a was not statistically significant between 10 and 15 min. Nevertheless, the interactions pH*time, acidity*time, and pHK a*time were statistically significant (p < 0.001) for calcium lactate, calcium ascorbate, and their mixture. However, the interactions between pH*intensity and pHK a*intensity were not significant for the three EAS (p > 0.05). Furthermore, the interaction between acidity*intensity was not statistically significant for the calcium lactate, whereas it was significant for the calcium ascorbate and the mixture (p < 0.001).

Generally, the lowest pH and highest titratable acidity of the electro-activated solutions were generated with the current intensity of 750 mA for 30 min. At this intensity, the pH values decreased from 6.08 ± 0.01 (time zero) to 1.94 ± 0.15 for the calcium ascorbate (Figure 2), from 6.20 ± 0.04 to 2.08 ± 0.05 for the mixture (Figure 3), and from 6.18 ± 0.06 to 2.16 ± 0.01 for the calcium lactate (Figure 4).

Meanwhile, for the same intensity and time (750 mA and 30 min, respectively), the obtained titratable acidity increased as follows: from 0.002 (time zero) to 0.109 ± 0.001 mol/L for the calcium ascorbate (Figure 5), from 0.002 to 0.107 ± 0.007 mol/L for the calcium lactate (Figure 6), and 0.002 to 0.102 ± 0.001 mol/L for the mixture (Figure 7).

However, EAS treated at 250 mA and 30 min mainly showed the least acidic property in terms of decreasing pH, which ranged from 6.10 ± 0.09 (time zero) to 2.66 ± 0.10 for calcium lactate (Figure 4), from 6.07 ± 0.04 to 2.39 ± 0.03 for the calcium ascorbate (Figure 2), except for the mixture where
the pH value decreased from 6.17 ± 0.05 to 2.08 ± 0.05, surprisingly similar to the pH also recorded at 750 mA and 30 min (Figure 3). In fact, the results demonstrated that when calcium lactate and calcium ascorbate solutions were combined, the decrease in pH at 250 and 750 mA intensities was equivalent during 30 min. Basically, the electrolysis of water provoked essentially an accumulation of hydrogen ions (H+) close to the anode interface that contributed to the acidification of EAS, as well as the appearance of a few reactive species, e.g., hydrogen radical (H•), hydroperoxyl radical (HO2•), hydrogen peroxide (H2O2), ozone (O3), superoxide radical (O2−•), etc., which presumably increased the energy of EAS and contributed to their oxidative property.7 In addition, it has been reported that electrolysis of water could also generate hydroxyl radicals (•OH), which are considered as the most reactive oxygen radicals known to date27 and the most toxic for some specific biological macromolecules.28 Indeed, these radicals could combine with other metastable compounds, resulting in the formation of other organic components (e.g., CH3OO•) that may possibly contribute to the metastable state of such EAS.7 In a recent work, Liato has reported that the presence of free gaseous oxygen (O2) generated during the electro-activation process obviously contributed to the decrease of EAS pH while the acidification increased in the medium.4 Meanwhile, the lowest acidity (titratable) values of EAS were observed at 250 mA and 30 min, increasing from 0.002 (time 0) to 0.065 ± 0.002 mol/L, from 0.002 to 0.025 ± 0.001 mol/L, and from 0.000 to 0.025 ± 0.001 mol/L for calcium lactate (Figure 6), calcium ascorbate (Figure 5), and mixture (Figure 7), respectively. Interestingly, the results also demonstrated that only 5 min was enough to provoke a significant decrease in pH values by more than a half regardless of the intensity used for all of the EAS. For instance, the pH of the calcium lactate decreased from 6.10 ± 0.09 (time 0) to 2.92 ± 0.21, from 6.03 ± 0.05 to 2.71 ± 0.26, and from 6.18 ± 0.06 to 2.56 ± 0.21 under current intensities of 250, 500, and 750 mA at 5 min, respectively. Nevertheless, for solutions electrically excited under 500 mA, pH showed intermediate values, between those at 250 and 750 mA. As expected, the elevations in the acidity recorded after 30 min were essentially between those at 250 and 750 mA.
Likewise, all of the $pK_a$ values obtained were proportionally affected by time and current intensity, with lower decreasing for treatments carried out under 250 mA compared to those performed at 500 and 750 mA. For instance, the decrease of $pK_a$ for calcium lactate varied as follows: from 8.88 (time zero) to 3.85, from 8.81 to 3.52, and from 8.96 to 3.13 for 250, 500, and 750 mA at 30 min, respectively (Figure 8). According to ascorbic acid, treatments under 250 mA and 15 min, 500 mA and 10 min, or 750 mA and 5 min were enough to obtain EAS with $pK_a$ very close to the standard value: 4.21, 4.11, and 4.28, respectively. Overall, the $pK_a$ value obtained in this study was in good agreement with $pK_a$ values (3–5) previously reported by Ho (2017) for the majority of organic acids. However, it is well known that weak acids such as lactic acid and ascorbic acid have dissociated and nondissociated forms that coexist in solution. In this regard, it was noted that the pH values obtained were all lower than the $pK_a$ for the EAS after electro-activation under 250, 500, and 750 mA during 30 min. These results suggested that the acidic state of such EAS was predominant after the electro-activation process.

In general, the results gathered from this study were in accordance with the first law of Michael Faraday published in 1834 on electrolysis, which is stated as follows: “The mass of substance liberated on the electrode during the electrolysis process is directly proportional to the quantity of electricity passed through the electrolyte including time”, as reported in ref 31. In addition, the results also showed that the type of solution used could differently behave to current intensity and time, which could have significant effects on the properties (e.g., pH, $pK_a$) of EAS, as previously reported. Indeed, our results also complied with those obtained by Liato et al., who reported a pH decrease from 7.07 ± 0.08 (time zero) to 2.82 ± 0.10 for EAS–potassium acetate, from 7.53 ± 0.12 to 2.13 ± 0.09 for EAS–potassium citrate, and from 6.18 ± 0.10 to 2.26 ± 0.15 for EAS–calcium lactate under variable current intensities during a maximum time of 180 min.7 Latterly, El Jaam et al. (2017) studied the electro-activation of potassium acetate and potassium citrate solutions, who have demonstrated that the decrease of pH was time- and current-intensity-dependent. For instance, they found an initial pH of 8.49 for potassium acetate solution (time zero) that gradually decreased to 3.77, 3.60, and 3.18 when treated under 200, 300, and 400 mA during 60 min, respectively. In a more recent study using electro-activation to extract proteins and carbohydrates from soybean meal, Gerliani et al. (2020) have found a direct correlation in the evolution of EAS parameters (e.g., pH, acidity) and the increase in the current intensity and time during the electro-activation process, which is totally in agreement with our results.

In fact, the obtained results clearly demonstrated that by controlling the electro-activation parameters, particularly time and current intensity, we could produce EAS with specific characteristics in terms of pH, titratable acidity, or $pK_a$, which are expected to be powerful enough and useful as antimicrobial agents in food processing and preservation.

Besides, even when electro-activation solutions from the central compartment were not principally targeted in this study, we have analyzed the pH and acidity of samples collected from this section to make comparisons. For 30 min treatment under 250 and 500 mA, pH showed different behavior from these recorded at 750 mA. For instance, the pH of the electro-activated calcium lactate increased unexpectedly from 6.65 ± 0.02 (time zero) to 10.50 ± 0.12 and from 6.56 ± 0.13 to 10.57 ± 0.23 after 30 min for 250 and 500 mA,

Therefore, the $pK_a$ values measured for standard lactic acid ($C_3H_6O_3$) and ascorbic acid ($C_6H_8O_6$) were 3.08 and 4.2, respectively. Thus, the obtained results demonstrated that 750 mA and 30 min corresponded to an ideal treatment to produce an electro-activated calcium lactate solution with a $pK_a$ (3.13) that was almost similar to the one of the standard lactic acid. According to ascorbic acid, treatments under 250 mA and 15 min, 500 mA and 10 min, or 750 mA and 5 min were enough to obtain EAS with $pK_a$ very close to the standard value: 4.21, 4.11, and 4.28, respectively. Overall, the $pK_a$ value obtained in this study was in good agreement with $pK_a$ values (3–5) previously reported by Ho (2017) for the majority of organic acids. However, it is well known that weak acids such as lactic acid and ascorbic acid have dissociated and nondissociated forms that coexist in solution. In this regard, it was noted that the pH values obtained were all lower than the $pK_a$ for the EAS after electro-activation under 250, 500, and 750 mA during 30 min. These results suggested that the acidic state of such EAS was predominant after the electro-activation process.
respectively, whereas the pH gradually decreased from 6.71 ± 0.09 to 5.36 ± 0.08 under 750 mA and 30 min. The increase of pH at 250 and 500 mA could possibly be explained by an accumulation of cations in the central compartment due to the selective transfer through the membranes. For instance, given that lactate ions were moving to the anodic section after the dissociation of calcium lactate, some Ca2+ ions, which remained in the central section, could possibly generate some alkaline species that were the root cause of pH increase. On the other hand, when the current intensity was as high as 750 mA, the transfer of cations from the central to the cathodic section could be facilitated and resulted in a slightly acidic medium after 30 min treatment. Surprisingly, the electro-activated calcium ascorbate and the mixture showed a different behavior with their pH that essentially varied from slightly acidic to neutral regardless of the intensity used (data not shown). Furthermore, the titratable acidity of the calcium lactate decreased from 0.002 mol/L (time zero) to 0 at 250 mA and 30 min, increased from 0.002 to 0.096 mol/L at 500 mA and 30 min, and remained constant around 0.002 mol/L under 750 mA and 30 min. Nevertheless, the behavior of the lactate lactate under 500 mA seemed not to be very common, not only the pH increased but also the acidity showed the same trend. It seemed that at 500 mA, formation of alkaline components and accumulation of acidic species in the central compartment occurred simultaneously. This aspect needs to be further investigated for a better understanding of the phenomenon. However, the titratable acidity of the calcium ascorbate and the mixture was stable (around 0.002 mol/L) under any treatment (data not shown).

3.2. Calcium Concentration Analysis by ICP-OES. The calcium (Ca2+) concentration of EAS from the cathodic compartment of the electro-activation reactor was investigated by the inductively coupled plasma optical emission spectroscopy (ICP-OES) technique. The obtained results demonstrated that the migration of calcium from the central to the cathodic section was time- and current intensity-dependent. As expected, the 750 mA current intensity showed a higher level of calcium transfer in all of the EAS compared to 500 and 250 mA intensities, respectively (Figures 11−13). These results are in agreement with Faraday’s first law that linked the electrolysis phenomenon. In addition, the transfer of calcium at 750 mA was higher when calcium lactate was used in comparison to the respective calcium ascorbate and the mixture. As such, these results suggested that not only the dissociation of calcium lactate could have been done easier at 750 mA compared to the other solutions but also the calcium migration was facilitated in the lactate medium.

Calcium is driven to the cathodic side by electromigration. Indeed, in the solution of the central compartment, calcium lactate or calcium ascorbate is dissociated into Ca2+ cations and lactate or ascorbate anions. The Ca2+ cations migrated through the cation exchange membrane to the cathodic side because of their positive electric charge and the cation selectivity property of this membrane, which is negatively charged. Regarding the precipitation on the cathode, it did not occur because the Ca12+ cations formed CaOH2 by the combination of calcium cations with the formed hydroxyl anions following water electrolysis at the cathode−solution interface.

3.3. Comparison of EAS and Standard Acids by FTIR, HPLC, and DSC Analyses. 3.3.1. FTIR Analysis. Fourier transform infrared (FTIR) spectroscopy was performed to compare EAS with standard ascorbic and lactic acids. Therefore, distinctive functional groups from such samples were identified using the peaks exhibited on the spectra (Table 2). According to the electro-activated calcium ascorbate, the outcomes of FTIR spectra showed the best match with standard ascorbic acid at 92.93, 91.78, and 90.64% for treatments under 500 mA and 30 min, 250 mA and 30 min, and 750 mA and 20 min, respectively. In addition, other very good matches between such EAS and standard ascorbic acid were also recorded, e.g., 90.03 and 86.82% for treatments under 500 mA and 20 min, and 750 mA and 30 min, respectively. To make assignments of functional groups, several
Table 2. FTIR Spectral Results (cm\(^{-1}\)) and Assignment Bands of the Standard Ascorbic Acid, the Standard Lactic Acid, and the Electro-Activated Solutions

| assignments                      | SAA  | AA\(_{250mA-30min}\) | AA\(_{500mA-30min}\) | AA\(_{750mA-20min}\) | SLA  | LA\(_{50mA-30min}\) | LA\(_{50mA-30min}\) | LA\(_{50mA-30min}\) |
|---------------------------------|------|------------------------|------------------------|------------------------|------|----------------------|----------------------|----------------------|
| OH stretching                   | 3525s, 3409s, 3315s, 3216s, 3028s-br | 3527s, 3411s, 3316s, 3218s, 3037s-br | 3526s, 3411s, 3316s, 3218s, 3037s-br | 3334s | 2917m | 2991m, 2942m | 2987m | 2987m |
| CH stretching                   | 2916m, 2745m | 2916m | 2916m | 2917m | 2991m, 2942m | 2987m | 2987m |
| C=O stretching                  | 1754s, 1673vs | 1756s, 1673vs | 1756s, 1673vs | 1754s, 1672vs | 1715vs | 1717vs | 1715vs |
| CH bending                      | 1497m, 1457m | 1497m | 1497m | 1497m | 1463m | 1454m | 1454m |
| COO\(^{-}\) sym. stretch        | 1334s  | 1334s  | 1334s  | 1334s  | 1375m | 1299m |
| CH\(_{3}\) deformation & C–O–H bending | 1388m  | 1388m  | 1388m  | 1385m  | 1375m | 1299m |
| CH bending                      | 1321s  | 1321s  | 1321s  | 1321s  | 1321s |
| C–O–C stretching                | 1276s, 1248m, 1222s, 1199s, 1140vos, 1121vos, 1113vos | 1276s, 1247m, 1222s, 1199s, 1140vos, 1121vos, 1112vos, 1113vos | 1276s, 1247m, 1222s, 1199s, 1140vos, 1121vos, 1112vos, 1113vos | 1276s, 1222s, 1199s | 1202s, 1118vs, 1203s, 1117vs, 1207s, 1116vs, 1209s, 1116vs |
| C–O–C & CH stretching           | 1076m, 1067m, 1044m | 1077m, 1067m, 1044m | 1077m, 1067m, 1044m | 1077m, 1067m, 1044m | 1046s | 1040s | 1040s |
| C–O–H bending                   | 1026vs | 1026vs | 1026vs | 1025vs | 1025vs |
| CH bending                      | 989s  | 989s  | 989s  | 989s  |
| C–C stretching & C–H bending    | 870m, 821m | 870m, 821m | 870m, 821m | 870m, 821m | 919m |
| OH & CH out-of-plane deformation | 757s  | 756s  | 756s  | 756s  | 815m | 818m | 817m |
| CH\(_{2}\) rocking              | 721w  | 721w  | 721w  | 721w  | 740m |
| C–C bending & OH out-of-plane deformation | 685w  | 685w  | 685w  | 685w  |
| C–O–H bending                   | 629w  | 629w  | 629w  | 629w  | 603m-br |
| C–O–H out-of-plane deformation  | 567w, 495w | 566w, 472w | 566w, 472w | 566w, 472w |

*SAA: standard ascorbic acid, AA: ascorbic acid, mA: milliampere, min: minute, SLA: standard lactic acid; LA: lactic acid, br: broad, m: medium, s: strong, v: very, w: weak.*
documents and tables of functional groups were consulted on the Sigma-Aldrich website.\textsuperscript{29} If we analyzed in detail the spectra of calcium ascorbate at 750 mA and 20 min (in agreement with the Beer–Lambert law) and the standard ascorbic acid (Figure 14), we observed the following similarities: For instance, the strong absorption peaks observed at 3526, 3411, and 3317 cm$^{-1}$ were associated with OH stretching, which may be explained by moisture being present in the samples.\textsuperscript{32,33} The medium band appeared at 2917 cm$^{-1}$ was related to CH stretching. In addition, the strong band located at 1754 cm$^{-1}$ and the very strong peak at 1672 cm$^{-1}$ were attributed to C=O stretching vibrations that may essentially come from conjugated acid (Sigma-Aldrich, 2019). CH$_3$ symmetric deformation were observed at the medium band 1385 cm$^{-1}$. CH bending was responsible for the strong band appeared at 1321 cm$^{-1}$. Additionally, C−O−C stretching was attributed to the strong peak located at 1276 cm$^{-1}$. Moreover, the strong bands observed at 1222 and 1199 cm$^{-1}$ were due to C−O−C stretching. The very strong bands exhibited at 1139, 1122, and 1112 cm$^{-1}$ came from C−O−C

Figure 14. FTIR spectra of (a) electro-activated calcium ascorbate solution (750 mA and 20 min, red color) and (b) standard ascorbic acid (blue color).

Figure 15. FTIR spectra of (a) standard lactic acid (red color) and (b) electro-activated calcium lactate solution (750 mA and 20 min, blue color).
stretching, while the very strong peak located at 1025 cm\(^{-1}\) was associated with C–O–H stretching. Additionally, the strong band located at 989 cm\(^{-1}\) corresponded to C–H bending, while the strong peak appeared at 756 cm\(^{-1}\) was due to OH and/or CH out-of-plane deformation. Overall, the spectrum generated for the standard ascorbic acid was quasi-similar to those obtained for the EAS, especially when samples of higher concentrations were used. However, when we used a lower concentration of EAS (to respect the Beer–Lambert law: eq 11), some peak values that were visible in the standard ascorbic acid did not appear in the EAS spectra, as presented in greater detail in Figure 14. According to eqs 3–11, we believed that the problem was due to a reduction in the absorbance, which is directly proportional to the concentration of the samples used during analysis.

\[
A = \varepsilon l c
\]

where \(A\) is the absorbance, \(\varepsilon\) is the molar attenuation coefficient (absorptivity), \(l\) is the optical path length, and \(c\) is the concentration of attenuating species.

Based on the high percentage of match found between the standard ascorbic acid and the electro-activated calcium ascorbate, we can confirm that ascorbic acid could be produced when its conjugated salt was submitted to an electro-activation process.

No FTIR spectra library was available for standard lactic acid to compare to the electro-activated calcium lactate. In this case, a “peak-by-peak” comparison has been made. The analysis of the spectra demonstrated significant similarity between the standard lactic acid and the electro-activated calcium lactate considering the functional groups observed, as presented in Figure 15. Particularly, the FTIR intensity at 3334 cm\(^{-1}\) in the standard lactic acid also seen in the EAS sample treated under and 250 mA and 20 min (data not shown) was linked with stretching vibrations of OH, which may be due to the presence of water in the samples used. The peak located at 3228 cm\(^{-1}\) in the spectrum of EAS 750 mA and 20 min was attributed to OH stretching. In addition, CH stretching bands were observed at 2991 and 2986 cm\(^{-1}\) in standard lactic acid and EAS 750 mA and 20 min, respectively. The peak located at 2942 cm\(^{-1}\) in the standard lactic acid (2941 cm\(^{-1}\) in EAS 750 mA and 20 min) was mainly due to CH\(_3\) symmetric and asymmetric stretching. The CH stretching band was observed in EAS 750 mA and 20 min at 2636 cm\(^{-1}\). The very strong band observed at 1715 cm\(^{-1}\) (the most prominent) was associated with C=O stretching. The medium peak located at 1463 cm\(^{-1}\) (1454 cm\(^{-1}\) in the EAS sample) was associated with CH bending, especially CH\(_2\) and CH\(_3\) antisymmetric/symmetric deformation. COO\(^{-}\) symmetric stretching was observed at the weak band of 1418 cm\(^{-1}\) (only seen in the standard lactic acid). The medium bands located at 1375 cm\(^{-1}\) (1373 cm\(^{-1}\) in EAS) and 1299 cm\(^{-1}\) (visible only in the standard) were likely due C=O–H bending and/or CH\(_3\) deformation. In addition, the strong and very strong bands observed in the region of 1202 cm\(^{-1}\) (1207 cm\(^{-1}\) in EAS) to 1118 cm\(^{-1}\) (1116 cm\(^{-1}\) in EAS) could be linked to C–O–C stretching vibrations. The strong peak located at 1046 cm\(^{-1}\) (1040 cm\(^{-1}\) for EAS) was mainly due to C–O–C stretching. Also, the medium band observed at 815 cm\(^{-1}\) (817 cm\(^{-1}\) in EAS) was originated by the CH out-of-plane deformation. However, the medium bands at 919 and 740–603 cm\(^{-1}\) were mainly attributed to C–C stretching, CH\(_2\) rocking, and C–OH out-of-plane deformation, respectively.

In this context, the most prominent peak observed at 1715 cm\(^{-1}\) (in both EAS and standard lactic acid) was considered as characteristic of lactic acid. These results are in agreement with those reported by Huang et al. (2018) concerning the characteristic peaks of lactic acid (1760 and 873 cm\(^{-1}\)). Moreover, Păucean et al. who studied lactic acid in a previous work had also reported this prominent peak located at 1730 cm\(^{-1}\), as well as Vodnar et al. (2010) who reported similar results. Based on the aforementioned information, we can conclude that lactic acid was generated when conjugated salt was submitted to an electro-activation process. Moreover, the FTIR spectrum of the initial calcium lactate used was different from that of the standard lactic acid when compared together (data not shown).

3.3.3.1. Chromatographic Analysis (HPLC). Regarding the standard ascorbic acid, the chromatogram obtained by the HPLC technique showed the characteristic peak of this acid at a retention time of about 1 min 33 s. The results indicated that the characteristic peak of the standard ascorbic acid (Figure 16a) and the retention time were similar to those recorded for the electro-activated calcium ascorbate (Figure 16b). Based on these results, we can conclude that ascorbic acid has been produced by the electro-activation of its conjugated salt.

According to the HPLC chromatogram of standard lactic acid (Figure 17a), its retention time (around 1 min 30 s) and its characteristic peaks were also similar to those of the electro-activated calcium lactate (Figure 17b). In this regard, it can be considered that lactic acid was generated by the electro-activation of the calcium lactate. However, it is important to underline here the presence of some smaller peaks, essentially exhibited latterly on the electro-activated calcium lactate chromatogram (b). In a previous work related to lactic acid, Xavier has reported the production of lactide and poly-lactic acid (by polycondensation) from lactic acid. In this regard, because lactide is a cyclic dimer derived from the lactic acid, it is possible that some lactide forms (d-lactide and/
or \( L\)-lactic acid and/or meso-lactide) have been generated after the production of lactic acid by the electro-activation process. Moreover, other species like poly-lactic acid (due to polymerization of lactic acid) could have also been generated under the electro-activation conditions. In the future, it would be of interest to identify these components and to investigate their contribution to the antimicrobial effectiveness of the EAS.

3.3.3.2. Thermal Analysis by DSC. In this study, it was essential to comprehend the thermal behavior of electro-activated solutions (EAS) in comparison to standard ascorbic and lactic acids. In this sense, differential scanning calorimetry (DSC) was performed to identify, especially the crystallization and melting temperature of the aforementioned products. For instance, the DSC curve of standard ascorbic acid revealed a glassy state followed by an endothermic peak (melting) at 195.5 °C with an enthalpy of 1150 J/g, as shown in Figure 18a. Moreover, a very small endothermic peak was noted at 216.4 °C followed by an exothermic reaction at 229.6 °C which can be considered as thermal decompositions. On the other hand, the initial calcium ascorbate solution (Figure 18b) showed a glass-transition state followed by an endothermic reaction at 162.8 °C, which could be associated with melting. Moreover, an exothermic reaction was observed at 186.6 °C followed by a strong peak at 212.3 °C, which may likely be related to crystallization, oxidation, or decomposition reaction. The electro-activated calcium ascorbate treated at 750 mA and 20 min (Figure 18c) demonstrated a melting point that was lower than the standard ascorbic acid, which may be explained by the metastable state of such EAS. Its DSC curve exhibited a glass-transition state followed by two exothermic reactions at 100.9 and 121.4 °C likely corresponding to crystallization. Another endothermic point was observed at 127.2 °C, which could be associated with melting.

In the DSC curves of standard lactic acid (Figure 19a), glass-transition state that alternated with three different endothermic peaks, \( \approx 52, 173, \) and 207 °C, could be observed. These points corresponded likely to the melting process of lactic acid that possibly contained different components (e.g., \( L\)-lactic acid, \( D\)-lactic acid). Aissa (2015) has already reported a melting point for lactic acid at around 53 °C, which is close to our findings \( (\approx 52 \, \text{°C}) \). Particularly, when \( D\- \) & \( L\)-lactic acid formed a stereocomplex, its melting point could approximately reach 220 °C. Nevertheless, it should be underlined here that no typical crystallization peak was observed for the standard lactic acid. Concerning the electro-activated calcium lactate treated at 750 mA and 30 min (Figure 19c), a glassy state followed by an exothermic reaction at 140.9 °C was observed. Then, its DSC curve showed an endothermic reaction until it exhibited a clear melting point at 170.7 °C, a thermal behavior that was close to the second melting point recorded for the standard lactic acid. That result was in good agreement with previous work in which Komesu et al. (2017) reported an endothermic point at \( \approx 174 \, \text{°C} \) for lactic acid. In comparison, the initial calcium lactate (Figure 19b) showed four endothermic peaks at 91.5 and 114.5 °C likely linked to a melting process, which suggested somehow that the calcium lactate used was not pure. Likewise, exothermic reactions were also observed at 132.5 and 149.4 °C. In theory, elements of endothermic heat flow could include evaporation, decomposition reactions, and melting or reduction reactions, while cross-linking, crystallization, oxidation, or decomposition reactions are more related to exothermic effects.

3.3.4. Antioxidant Activity by DPPH Assay. The antioxidant activity of the standard ascorbic acid, the electro-activated calcium ascorbate, and the mixture was determined by DPPH free-radical scavenging assay, which is based on the reduction of DPPH\(^{+}\) radicals. The obtained results confirmed a high antioxidant activity for the electro-activated calcium ascorbate (Figure 20a–c) as well as for the mixture (Figure 21a–c) in comparison to the standard ascorbic acid (Figure 22). In particular, the electro-activated calcium ascorbate exhibited an antioxidant activity ranging from 90.33 ± 0.36 to 81.60 ± 1.38 and 80.81 ± 2.18% for treatments under 750 mA and 30 min, 250 mA and 30 min, and 500 mA and 30 min, respectively.

Regarding the mixture, the antioxidant activities recorded were 83.39 ± 0.49, 82.34 ± 0.51, and 81.11 ± 1.27% for treatments carried out at 750 mA and 30 min, 500 mA and 25 min, and 250 mA and 25 min, respectively. Surprisingly, the antioxidant activity obtained for the electro-activated calcium ascorbate (750 mA and 30 min) was higher than that recorded for the standard ascorbic acid (85.33 ± 2.12%). In addition, the antioxidant activity of both electro-activated calcium ascorbate and the mixture was higher with the treatment at 750 mA and 30 min, indicating that the inhibition of DPPH\(^{+}\) radicals in EAS seemed to be time- and current intensity-dependent. Particularly for the mixture, the increase of antioxidant activity (DPPH inhibition) seemed to be affected during the first 10 min, suggesting that the stability of DPPH could have been impacted by some reactive radicals (e.g., \( O^{• -} \), \( O_{2}^{• -} \)) present in the EAS.

4. CONCLUSIONS

This work demonstrated the potential of the electro-activation to be used as a nonthermal technology in the production of two organic acids, ascorbic acid and lactic acid, from their conjugated calcium salts, as well as a mixture of them. The main properties of the produced electro-activated solutions were time- and electric current intensity-dependent. The electro-activation process was carried out for 30 min at 250, 500, and 750 mA nominal electric currents. The
electro-activated aqueous solutions were characterized by low pH values of 2.16, 2.08, 1.94 for the electro-activated calcium lactate, mixture of calcium ascorbate and calcium lactate, and calcium ascorbate, respectively. The solution’s titratable acidity also increased as the electro-activation process progressed to reach the final highest values of 0.107, 0.102, and 0.109 mol/L.

**Figure 18.** DSC curves: (a) standard ascorbic acid, (b) initial calcium ascorbate solution, and (c) electro-activated calcium ascorbate treated at 750 mA and 20 min.
respectively. Regarding the electro-activated solution’s $pK_a$ values, they were characteristic of lactic acid and ascorbic acid with final values at 30 min of electro-activation of 3.13, 3.07, and 2.90, for electro-activated solutions of calcium lactate, the mixture, and calcium ascorbate, respectively. All of these results supported the research hypothesis that electro-
activation is effective to produce lactic and ascorbic acids from their conjugated calcium salts. Regarding the antioxidant activity, the obtained results demonstrated that after anodic electro-activation, the resulted electro-activated solutions from calcium ascorbate and the mixture of calcium lactate and calcium ascorbate have high antioxidant capacity, as measured by the DPPH assay. The electro-activated calcium ascorbate exhibited an antioxidant activity of 90.33 ± 0.36%, when treated under 750 mA for 30 min, while the mixture was characterized by an antioxidant activity of 83.39 ± 0.49% for the same EA treatment.

From a global perspective, we expect that their high reactive properties could make them strongly effective as antimicrobial agents, thereby contributing to the reduction of use of several chemicals in food processing and preservation. Furthermore, as pH and acidity have major impact in food preservation, taste, or texture, the electro-activation technology could help industrials to better control these parameters and produce higher-quality foods with longer shelf life. From a chemical perspective, electro-activation could be an efficient asset in the production of highly reactive solutions that can be used, for example, in surface disinfection, as well as in the treatment of drinking water without the aforementioned chlorine inconvenient. In this context, it is of utmost importance to conduct further research to better comprehend the impact of such EAS on pathogenic and spoilage microorganisms in different animal- and plant-derived foods. Therefore, our next specific objective will be to study the antimicrobial and sporicidal effects of these electro-activated solutions in model conditions and in food matrices.

**AUTHOR INFORMATION**

**Corresponding Author**
Mohammed Aider — Institute of Nutrition and Functional Foods (INAF) and Department of Soil Sciences and Agricultural Engineering, Université Laval, Quebec, QC G1V 0A6, Canada; Phone: +1 (418) 656-2131;
AUTHORS

Pierre Emerson Cayemitte — Department of Food Sciences, Université Laval, Quebec, QC G1V 0A6, Canada; Institute of Nutrition and Functional Foods (INAF), Université Laval, Quebec, QC G1V 0A6, Canada

Natale Gerlani — Institute of Nutrition and Functional Foods (INAF) and Department of Soil Sciences and Agri-Food Engineering, Université Laval, Quebec, QC G1V 0A6, Canada

Philippe Raymond — Saint-Hyacinthe Laboratory, Canadian Food Inspection Agency, Saint-Hyacinthe, QC J2S 8E3, Canada

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.1c00345

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank Diane Gagnon and Marie-Michelle Gagnon for the technical assistance in several laboratory activities. The financial support Grant no. CG122306 from The Natural Sciences and Engineering Research Council of Canada (NSERC) is acknowledged. This work was carried out with financial support from the Program Innov’Action agro-alimentaire, a program resulting from the Canada-Quebec Accord for the implementation of the Canadian Partnership for Agriculture and Agri-Food Canada.

REFERENCES

(1) Aider, M.; Gnato, E.; Benali, M.; Plutakhin, G.; Kastychuk, A. Electro-activated aqueous solutions: Theory and application in the food industry and biotechnology. Innovative Food Sci. Emerging Technol. 2012, 15, 38–49.

(2) Al-Haq, M. I.; Sugiyama, J.; Isobe, S. Applications of Electrolyzed Water in Agriculture & Food Industries. Food Sci. Technol. Res. 2005, 11, 135–150.

(3) El Jaam, O.; Fliss, I.; Ben-Ounis, W.; Aïder, M. Acidification of potassium acetate and potassium citrate with/without KCl by electro-activation and impact of the solution on spores of Clostridium sporogenes PA 3679 at ambient temperature. LWT–Food Sci. Technol. 2017, 75, 648–655.

(4) Liato, V.; Labrie, S.; Viel, C.; Benali, M.; Aider, M. Study of the combined effect of electro-activated solutions and heat treatment on the destruction of spores of Clostridium sporogenes and Geobacillus stearothermophilus in model solution and vegetable puree. Anaerobe 2015, 35, 11–21.

(5) Aider, M.; Gimenez-Vidal, M. Lactulose synthesis by electro-isomerization of lactose: Effect of lactose concentration and electric current density. Innovative Food Sci. Emerging Technol. 2012, 16, 163–170.

(6) Liato, V.; Labrie, S.; Aider, M. Study of the antibacterial activity of electro-activated solutions of salts of weak organic acids on Salmonella enterica, Staphylococcus aureus and Listeria monocytogenes. J. Ind. Microbiol. Biotechnol. 2017, 44, 23–33.

(7) Liato, V.; Labrie, S.; Aider, M. Electro-activation of potassium acetate, potassium citrate and calcium lactate: impact on solution acidity, Redox potential, vibrational properties of Raman spectra and antibacterial activity on E. coli O157:H7 at ambient temperature. Springerplus 2016, 5, No. 1760.
Behavior Using Thermoanalytical Techniques. J. Chem. 2017, 2017, 1–7.
(26) Becker, M. M.; Nunes, G. S.; Ribeiro, D. B.; Silva, F. E. P. S.; Catante, G.; Marty, J.-L. Determination of the Antioxidant Capacity of Red Fruits by Miniaturized Spectrophotometry Assays. J. Braz. Chem. Soc. 2019, 30, 1108–1114.
(27) Fernández-Castro, P.; Vallejo, M.; San Román, M. F.; Ortiz, I. Insight on the fundamentals of advanced oxidation processes. Role and review of the determination methods of reactive oxygen species. J. Chem. Technol. Biotechnol. 2015, 90, 796–820.
(28) Kareb, O.; Gomaa, A. I.; Champagne, C. P.; Jean, J.; Aider, M. Impact of electro-activation on antioxidant properties of defatted whey. Int. Dairy J. 2017, 65, 28–37.
(29) Sigma-Aldrich IR Spectrum Table & Chart. https://www.sigmaaldrich.com/technical-documents/articles/biology/ir-spectrum-table.html.
(30) Shimadzu Shimadzu Excellence in Science. pKa and Dissociation Equilibrium. https://www.shimadzu.com/an/hplc/support/lib/lctalk/29/29intro.html.
(31) Gerliani, N.; Hammami, R.; Aider, M. A comparative study of the functional properties and antioxidant activity of soybean meal extracts obtained by conventional extraction and electro-activated solutions. Food Chem. 2020, 307, No. 125547.
(32) Yohannan Panicker, C.; Varghese, H. T.; Philip, D. FT-IR, FT-Raman and SERS spectra of Vitamin C. Spectrochim. Acta, Part A 2006, 65, 802–804.
(33) Yohannan Panicker, C.; Varghese, H. T.; Philip, D.; Nogueira, H. I. S. FT-IR, FT-Raman and SERS spectra of pyridine-3-sulfonic acid. Spectrochim. Acta, Part A 2006, 64, 744–747.
(34) Mayerhöfer, T. G.; Popp, J. Beer’s law derived from electromagnetic theory. Spectrochim. Acta, Part A 2019, 215, 345–347.
(35) Huang, H.; Grün, I.; Ellersieck, M.; Clarke, A. Use of HPLC and FTIR as a tool for analysis of lactic acid in restructured fish products. J. Nutr. Food Res. Technol. 2018, 1, 42–48.
(36) Vodnar, D.; Venus, J.; Schneider, R.; Socaciu, C. Lactic Acid Production by Lactobacillus paracasei 168 in Discontinuous Fermentation Using Lucerne Green Juice as Nutrient Substitute. Chem. Eng. Technol. 2010, 33, 468–474.
(37) Paucan, A.; Vodnar, D. C.; Mureșan, V.; Fetea, F.; Ranga, F.; Man, S. M.; Muste, S.; Socaciu, C. Monitoring lactic acid concentrations by infrared spectroscopy: A new developed method for Lactobacillus fermenting media with potential food applications. Acta Aliment. 2017, 46, 420–427.
(38) Xavier, A. M. M. Study of Lactic Acid Polycondensation and Lactide Production. Master Thesis, 2010.
(39) Di Lorenzo, M. L.; Androsch, R. Influence of α’-/α-crystal polymorphism on properties of poly(l-lactic acid). Polym. Int. 2019, 68, 320–334.