Contribution to the Process Development for Lactulose Production through Complete Valorization of Whey Permeate by Using Electro-Activation Technology Versus a Chemical Isomerization Process

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ABSTRACT: Whey permeate (WP) is a co-product of a cheese or casein production process that is regarded as an environmental pollutant because of its high organic load and is creating a major disposal problem for the dairy industry. However, it can be used as a suitable substrate to meet the increasing demand of producing a prebiotic lactulose through the isomerization of lactose present in the WP under adequate alkaline conditions. The goal of this study was to produce lactulose in situ of WP using electro-activation (EA) technology and compare the productivity of EA with conventional chemical isomerization at potassium hydroxide (KOH)-equivalent solution alkalinity in the feed medium. Electro-isomerization was conducted under different current intensities of 300, 600, and 900 mA for 60 min of EA with a 5 min sampling interval using 6, 12, and 18% (w/v) WP solutions. Chemical isomerization was carried out at the KOH-equivalent solution alkalinity to that measured in the EA solution at each 5 min interval using KOH powder as a catalyst. The outcomes of this study revealed that the production of lactulose using the EA approach was current intensity-, WP concentration-, and reaction time-dependent and produced the highest lactulose yield of 36.98% at 50 min of EA-time under 900 mA current intensity using 6% WP as a feed solution, whereas a maximum lactulose yield of 25.47% was achieved by the chemical isomerization at the solution alkalinity corresponding to that of the EA under 900 mA current intensity at 50 min in the 6% WP solution. Furthermore, a greater yield of lactulose was obtained using the EA technique for all reaction conditions compared to the chemical process at the equivalent solution alkalinity. Therefore, the results of this work suggest that the EA can be an emergent sustainable technology for achieving dual objectives of prebiotic lactulose production and concurrent valorization of WP using it as a feed medium.

1. INTRODUCTION

Whey permeate (WP) is the secondary co-product of the cheese and casein making industry. It is the residual material after proteins are extracted from whey by membrane filtration of precipitation.1,2 Casein, which is the main protein of milk, can be precipitated by either pH lowering of heat denaturation. At pH close to 4.6, which corresponds to the isoelectric point of casein, this molecule (micelles) is least soluble and can easily precipitate as agglomerates. This precipitation by pH lowering can be achieved by adding an acid (e.g., HCl or lactic acid). Casein can also be precipitated following heat treatment combined with some minerals such as calcium. The high temperature can induce casein denaturation leading to significant loss of its stability. From the point of view of its proximate composition, WP is a poor raw material and has low commercial and nutritional value. In its liquid form, it is primarily comprised water (~93%), lactose (~5%), minerals (~0.53%), and traces of nitrogen-containing molecules such as free amino acids and peptides.3,4 From environmental considerations, WP constitutes a serious concern because of its high biological (BOD ≈30,000−50,000 mg/L O₂) and chemical (COD ≈60,000−80,000 mg/L O₂) oxygen demand. This particularity can easily cause eutrophication of different ecosystems because of its impact on oxygen depletion of water.2,5 Thus, WP is still a huge challenge for researchers and industries to find rational, efficient, and economical ways of valorization.

WP is mainly dried and sold as a powder for some applications as a feed additive or used as a raw material for lactose production by cold crystallization of saturated solutions after being purified from minerals and N-containing molecules. The processes of drying, demineralization, and purification are costly, which make the end-product of low commercial value. Lactose is then used in its turn as a raw material to produce high costly derivatives such as sorbitol, mannitol, ethanol,
lactobionic acid, and other derivatives. Thus, it can be seen that the industry is multiplying different commercial processes to achieve a product with relatively good commercial value but which is highly expensive if one considers the overall product life cycle.6

Analysis of the current situation of WP pointed out a necessity to investigate other nontraditional ways of valorization. In this context, a sustainable approach was highlighted in the present study with a possibility of valorizing WP as a whole component by adding a net positive added value to this material from both the nutritional and the environmental and economic points of view.6 Thus, by following this innovative way, it would be possible to produce a highly valuable food grade ingredient by the whole valorization of WP by targeting specific modifications without any need of fractionation of the initial material. The target is the effective conversion of lactose (present in the WP) into lactulose, a recognized prebiotic with many applications in the pharmaceutical and food industries.1,7 Thus, it will be possible to convert WP into value-added ingredient rich of lactulose and possibly other valuable molecules and minerals.

Lactulose is classically produced following the chemical isomerization of lactose under strong alkaline conditions requiring heat and catalysts.5,9 This process is generally costly because of the low process efficiency caused by the necessity of different purifying and concentrating steps.10,11 To overcome the inconveniences of the chemical isomerization process of lactose into lactulose, recently electro-activation (EA) technology has been shown to be highly effective to convert lactose into lactulose by exploiting water electrolysis at the cathode–solution interface in an adequately designed EA reactor. The reaction of water electrolysis combined with adequate ion exchange membrane disposition permits to create strong alkaline conditions without using chemical alkalinizing agents and catalysts.3,12 Right now, the reported studies showed a conversion of lactose into lactulose to be in the range of 35–45% which is considered to be very good and highly promising for large scaling of the EA process.3,12,13 However, because of the fundamental and process design differences between the chemical and EA methods of lactose isomerization into lactulose, it is difficult to state which of these processes is the most efficient. Thus, a structured and objective comparison of the isomerization of lactose into lactulose using a conventional chemical process versus the method based on the EA technology (electro-isomerization) for WP integral valorization is necessary, which is the key objective of the present study.

Specifically, this study was intended to compare the efficiency of lactose isomerization into lactulose between the EA process and the chemical method based on the equivalent solution alkalinity using potassium hydroxide (KOH) as a catalyst and WP as a substrate of the isomerization process.

2. MATERIALS AND METHODS

2.1. Reagents and Chemicals. Highly pure (purity ≥95%) reagents and chemicals were purchased from various suppliers. Lactulose, lactose, galactose, glucose, and fructose of high-performance liquid chromatography (HPLC)-grade were acquired from Sigma-Aldrich (Ottawa, Ontario, Canada). The analytical grade hydrochloric acid (HCl), potassium sulfate (K2SO4), phenolphthalain (C20H14O4), and potassium hydroxide (KOH) were purchased from Fisher Chemical (Geel, Belgium), Sigma-Aldrich Co. (St. Louis, MO, USA), Fisher Chemical (Fair Lawn, NJ, USA), MAT Laboratory Inc. (Quebec, Canada), respectively. The WP powder was procured from Agropur Co-operative (Longueil, Quebec, Canada). The major components (%) of WP are as follows: total sugars: 85 ± 0.12; total proteins: 1.93 ± 0.24; ash: 6.5 ± 0.11; residual humidity: 6.4 ± 0.13; other components: 0.07 ± 0.01. The AMI-7001S anion-exchange membrane (AEM) and the CMI-7000S cation-exchange membrane (CEM) were obtained from Membrane International Inc. (Ringwood, NJ, USA).

2.2. Protocol for EA. The EA reactor used in this work consists of three chambers, namely, anodic (5.5 × 2.2 × 10 cm), central (5.5 × 2.2 × 10 cm), and cathodic (6.5 × 5.5 × 10 cm) compartments (Figure S1). The cathodic compartment was connected to the negative side of a direct current (DC) CSI12001X power generator (Circuit Specialists, Tempe, AZ, USA) through a stainless steel (food grade) electrode (12 × 5 cm), while the anodic compartment was tied to the positive side using a ruthenium-iridium-coated titanium electrode (12 × 4 cm). The central compartment divided the cathodic and anodic compartments, and it was communicating with them through the CEM and AEM, respectively. The 350 mL of WP solutions (6, 12, and 18% w/v) was introduced in a cathodic chamber, while the central and anodic chambers were loaded with the solution of 0.1 M K2SO4 (Figure 1). The solution of K2SO4 was used as an anolyte in a EA reactor to avoid chlorine formation at the anode surface following the oxidation reaction. The EA experiments were performed under different current intensities (i.e., 300, 600, and 900 mA) for a reaction time of 60 min at ambient temperature (22 ± 2 °C). Aliquot samples were collected from the cathodic and central chambers in a regular interval (i.e., 5 min) and preserved at 4 °C for further analysis.

2.3. Assessment of pH, Alkalinity, Potassium Ion, Temperature, and Oxidation–Reduction Potential. The WP solution pH, total alkalinity, oxidation–reduction potential (ORP), and temperature, prior to and during the EA process, were evaluated at 5 min intervals during the 60 min of EA. An Oakton pH 700 pH meter equipped with a standard pH probe (Oakton, Vernon Hills, IL, USA) was used to determine the medium pH while an Ultrapen ORP meter (Myron L Company, Carlsbad, CA, USA) was used to measure ORP and temperature. The concentration of the potassium ion (K+) was measured in a central chamber in 5 min intervals using an AAAnalyst 200 atomic absorption spectrometer (PerkinElmer Instrument, Boston, MA, USA). The total alkalinity of the electro-activated WP solutions (i.e., catholytes) was evaluated using a standard titration method with phenolphthalain as an indicator according to Karim and Aider.12 The total alkalinity was
of the solutions was determined following eq 1 and was sated in mmol/L.

\[
\text{Total alkalinity}_{eq} = \frac{C_{\text{titrant}} \times V_{\text{titrant}}}{V_{\text{sample}}} \times 1000
\]

where, \(C_{\text{titrant}}\) is the concentration (in mol/L) of the titrant (i.e., HCl); \(V_{\text{titrant}}\) is the total volume (in mL) of the titrant, that is, 0.1 M HCl required for titration; \(V_{\text{sample}}\) is the total volume (in mL) of the sample; and the total alkalinity_{eq} is the equivalent concentration of KOH/NaOH in mmol/L.

2.4. Chemical Isomerization by KOH. The conventional chemical isomerization process was conducted using similar WP solutions (6, 12, and 18%; w/v) as a feed medium. The isomerization reactions were carried out by creating an equivalent solution alkalinity corresponding to that obtained in the EA process by adding KOH as a catalyst to the WP solution. Briefly, the equivalent amount of KOH (mg/L) corresponding to the total solution alkalinity using the EA process was determined each 5 min interval. Thereafter, the KOH powder was added in 5 min intervals to achieve an equivalent solution alkalinity as the one formed during the EA process. The feed medium was constantly mixed at an ambient temperature and the medium pH and ORP were measured during the isomerization reaction. The samples were collected at regular 5 min intervals and maintained at 4 °C for further analysis.

2.5. Analysis of Carbohydrate Composition. The analysis of the carbohydrate composition of electro-activated WP was performed by using a Hitachi L-7000 HPLC system series (Hitachi High-Tech, Japan). A Waters refractive index detector and a Sugar Pak-I 300 × 6.5 mm carbohydrate analysis column (Waters Corp., Milford, MA, USA) were used in the HPLC system. A solution of 50 mg/L Ca-ethylenediamine tetra-acetic acid (50 mg/L) was used as a mobile phase at a flow rate of 0.5 mL/min. The column temperature was maintained at 80 °C. The running time and injection volume were set to 30 min and 50 μL per sample, respectively. The composition of the targeted carbohydrates (lactose, lactulose, glucose, galactose, and fructose) were identified and quantified comparing their retention times with the corresponding standard solutions.

2.6. Statistical Analysis. Statistical analysis was carried out following a complete randomized factorial design with repeated measurements. The studied independent variables were: WP concentration (6, 12, and 18%), electric current intensity (300, 600, and 900 mA), and reaction time (5−60 min at 5 min interval). The dependent variables were the electro-activated WP solution pH, total alkalinity, ORP, temperature, and migration of K+ ions from the central chamber to the catholyte, the yield of lactulose, and other carbohydrates (residual lactose, glucose, galactose, and fructose). The experiments were repeated three times and the mean values ± standard deviations were considered for the analyses. The differences at \(p < 0.05\) were termed as significant. Analysis of variance of the obtained data was conducted by using SAS software V9.3 (SAS Institute Inc., Cary, NC, USA).

3. RESULTS AND DISCUSSION

3.1. Evaluation of Medium pH and Alkalinity. The evolution of medium pH in the cathodic compartment was assessed during the 60 min EA process under several current intensities (i.e., 300, 600, and 900 mA) using WP solutions (i.e., 6, 12, and 18%) and presented in Figure 2. It appeared that the current intensity, solution concentration, and EA time significantly affected (\(p < 0.001\)) the pH evolution during electro-isomerization of WP. The higher pH was achieved for 6% WP solution than for the 12 and 18% WP solutions and reached the maximum values of 10.86 ± 0.04 and 11.36 ± 0.02 at 60 min under 300 and 600 mA current intensity, respectively. However, it reached a plateau (11.37 ± 0.06) at
45 min, and then it decreased to 11.22 ± 0.06 at 60 min when 900 mA current intensity was used. Likewise, the maximum pH of 9.92 ± 0.04 and 10.90 ± 0.03 was obtained under 300 and 600 mA for 12% WP solution, respectively; while it reached plateau (10.90 ± 0.02) at 50 min before slightly deceased to 10.86 ± 0.02 at the end (i.e., 60 min) of the EA process under 900 mA current intensity. However, this phenomenon was not observed for 18% WP solution rather a pH reached maxima of 8.33 ± 0.05, 10.47 ± 0.08, and 10.70 ± 0.02 at the end of the reaction under 300, 600, and 900 mA current intensity, respectively.

The evolution of solution alkalinity expressed as KOH-equivalent was also measured during the EA process under 300, 600, and 900 mA current intensities using various WP solutions (6, 12, and 18%), as shown in Figure 3. Like solution pH, the development of solution alkalinity was significantly (p < 0.001) affected by the solution concentration, current intensity, and EA time and showed a strong correlation with pH evolution. Alkalinity was observed to increase with rising current intensity and reaction time, while it was seen to decrease for the higher solution concentrations. The maximum alkalinity was achieved for 6% WP solution, and it reached 20.67 ± 1.15 and 38.00 ± 2.00 mmol/L at a reaction time of 60 min under current intensities of 300 and 600 mA, respectively. Nevertheless, the solution alkalinity achieved a plateau of 40.00 ± 1.46 mmol/L at 45 min under 900 mA, and then it decreased to 36.67 ± 1.15 mmol/L at 60 min for 12% WP solution, the utmost alkalinity of 7.33 ± 1.15 and 30.00 ± 0.00 mmol/L were obtained at the end, while it reached a plateau (32.67 ± 1.15 mmol/L) at 50 min and remained unchanged until the end. A maximum of 2.67 ± 1.15, 21.33 ± 1.15, and 32.00 ± 2.00 mmol/L alkalinity were observed under 300, 600, and 900 mA at the end of EA for 18% WP solution, respectively.

The higher pH and alkalinity were achieved for greater current intensity whatever the solution concentration was used. The concentration of KOH increased as the EA process progressed because of the continuous formation of OH\textsuperscript{−} ions in the cathodic compartment and the electro-migration of K\textsuperscript{+} ions from the central compartment to the cathodic compartment of the EA reactor. Thus, the simultaneous increase of OH\textsuperscript{−} and K\textsuperscript{+} ions yielded an increase of the KOH concentration in the cathodic side as the EA process progressed. Moreover, this phenomenon was dependent on the applied electric current intensity. Also, this is because the movement of electrons (e\textsuperscript{−}) through the electro-chemical system was enhanced because of the application of higher current intensity. As a result, a greater splitting of water molecules was instigated at the solution–electrode interface which resulted in the generation of more hydroxyl ions (OH\textsuperscript{−}) in the cathodic chamber. Indeed, a reduction reaction transpired in the cathodic chamber allowing the generation of hydrogen gas (H\textsubscript{2}) and OH\textsuperscript{−} ions through intensive water dissociation \[2\text{H}_2\text{O} (l) + 2\text{e}^- \rightarrow \text{H}_2 (g) + 2\text{OH}^{-} (\text{aq.})\]. The rate of water electrolysis is directly proportional to the applied electric field. Subsequently, the OH\textsuperscript{−} ions were increased in the medium that led to the increase of the solution pH and alkalinity. At the very beginning of the EA, the rate of pH increment was sluggish, and it was more apparent for higher WP concentrations. This is probably due to the initial resistance of the feed solutions and the resistance of the system for its intrinsic resistance of the ion-exchange membranes used. Thereafter, pH and alkalinity increased exponentially because of the continuous production of the high amounts of OH\textsuperscript{−} ions following rigorous water splitting for permitting the current transmission in the solution–cathode interface. In the third stage, the rate of pH increase showed an incurring behavior with a lower rate of pH increase, indicating that it reached a plateau because the solution become saturated with enough OH\textsuperscript{−} ions.

The lower rise of solution pH and alkalinity for higher solution concentrations (6 > 12 > 18% WP solution) ascribed
to the higher buffering capacity of the more concentrated WP solutions that was retarding the pH evolution in the medium by absorbing/desorbing the OH\(^-\) ions.\(^{16}\) Furthermore, the low rate of water electrolysis for higher WP solutions might be owing to the greater concentration (i.e., greater solid/water ratio) of WP, thus a lower quantity of OH\(^-\) ions were created at the solution–cathode interface. In some cases, particularly, for 6% WP solution under 900 mA, pH and alkalinity were found to decrease after a duration of 45 min of EA attributed to the migration of some H\(^+\) ions to the cathodic chamber from the central chamber, and perhaps inducing acidification of the feed medium. Indeed, more H\(^+\) and OH\(^-\) ions would have been produced through water dissociation at the solution–CEM interfaces, especially, at the interface that is facing the central chamber when the reaction reached a limiting current density, to compensate ion depletion in the central chamber.\(^{12}\)
3.2. Evaluation of Potassium Ion Migration. During the EA, the concentration of K⁺ ion in the central chamber was studied under several current intensities (i.e., 300, 600, and 900 mA) using 6, 12, and 18% WP solutions and is demonstrated in Figure 4. The concentration of the K⁺ ion was declining over running time no matter what the current intensities and solution concentrations used. The decreasing rate was comparatively higher for greater current intensities and slightly lower for more concentrated WP solutions.

The concentration of K⁺ ions decreased in the central chamber over running time because of the migration of positively charged K⁺ (cations) toward the negatively charged cathode through the CEM. The migration rate was more rigorous at the beginning of the EA and slowed down at the end, which could be ascribed to the concentration polarization phenomena. Indeed, the concentration polarization phenomena was generated because of a variation between ion transfer numbers in the membrane and solution, leading to a change in the electrolyte concentration near the membrane surface and a significant potential drop in the polarized region (i.e., Nernst layer), consequently, diminishing migration of ions. At this stage, the global system resistance of the reactor would have increased (Figure S2), and water splitting might be ensued at the CEM–solution interfaces to recompense the deficiency of current exporters toward the cathode. As a result, more OH⁻ and H⁺ ions would have been generated, and H⁺ ions competed with the K⁺ ions for electro-migration toward the cathodic side because of the higher electrophoretic mobility of H⁺ ions in solution. Thus, the migration of K⁺ ions was decelerated at the end point of the EA technique, particularly, for the 900 mA current intensity.

3.3. Temperature Evolution in the EA Reactor. The evolution of temperature in the cathodic chamber was monitored throughout the EA process using several WP solutions (6, 12, and 18%), as shown in Figure 5. The increase in temperature was mainly reliant on the current intensity and EA-time throughout the 60 min of EA, and it steadily rose till the end for all current intensities and WP concentrations used.

A relatively higher temperature was observed for 6% WP solution whatever the current intensities used. The maximum values of temperature were 27.00 ± 0.10, 35.00 ± 0.44, and 42.63 ± 0.15 °C for 6% WP at the reaction time of 60 min for 300, 600, and 900 mA current intensities, respectively. The highest temperature was achieved under a current intensity of 900 mA no matter what solution concentrations were used and reached the maximum values of 42.63 ± 0.15, 42.87 ± 0.60, and 41.63 ± 0.67 °C at 60 min for 6, 12, and 18% WP solutions, respectively. The increase in temperature was considerably sharp for higher current intensities for the reason that the greater intensities produced increased heat in the medium and the heated energy dissipated in the solution. In fact, the rise in the temperature during the EA process is mostly due to the Joule effect in the electrodes and based on the Joule’s law: "the rate of increment is directly proportional to the electric current and electric tension". Indeed, the Joule heating at the electrodes occurs in any electrochemical device. Indeed, applying electric current to an electrode (anode and cathode) dissipates heat to the surrounding solution according to Joule’s law, leading to an interfacial temperature that is much higher than that of the bulk solution. This phenomenon is known as the interfacial Joule heating effect. Furthermore, the demineralization phenomena as a result of ion migration resulted in the intensified resistance of the system that could be another possible reason for the temperature rise in the EA. Additionally, the initial resistance of the feed solutions and ion-exchange membranes may have, to some extent, contributed to the temperature increase.

3.4. Evolution of ORP. The change of ORP in the cathodic chamber was evaluated throughout the EA process using 6, 12, and 18% WP solutions and is presented in Figure 6. The ORP values were decreased drastically at the beginning...
of EA and reached −450 to −650 mV in the first 5 min of the EA process no matter what the current intensities and WP concentration used. Later, they reduced in a different way depending on the current intensities and WP concentrations, and then achieved a quasi-steady state.

The ORP drastically reduced within the first 5 min ascribed to the excessive e\(^{-}\) generation and other active reducer formation resulted from rigorous electrolysis of the solution.\(^{12}\) Indeed, a negative ORP in the cathodic electro-activated solutions perhaps correlated to the training effect of excess e\(^{-}\) that produced after the electro-chemical activation of the WP solutions.\(^{19}\) Besides, being a dynamic electrochemical process, EA caused the production of many radicals and ionic species, such as the generation of extremely active reducers including OH\(^{-}\), H\(^{+}\), ·OH, ·H, ·O\(^{-}\), ·O\(_2\)\(^{-}\), H\(_2\)O\(_2\)\(^{-}\), · and HO\(_2\)\(^{-}\), leading to the high reduction potentials.\(^{10}\) The ORP values were greater (more reduced) for higher current intensities as the amount of water electrolysis was more vigorous for greater current intensities, and consequently, the number of negative charge concentration was increased through the accumulation of OH\(^{-}\).

Figure 7. Formation of lactulose in the EA process during 60 min of EA time for (a) 6, (b) 12, and (c) 18% WP as feed solutions.

Figure 8. HPLC chromatograms of lactose electro-isomerization into lactulose in situ of the 6% WP solution during the EA: (a) initial feed solution and (b) feed solution under 900 mA current intensity.
groups in the electro-activated feed solution. After a certain period of EA, the ORP achieved a quasi-steady stage as the reaction medium turned out to be saturated with the OH ions and other charged species. At the end of the EA, a minor upsurge in the ORP values means decrease in reactivity, attributed to the electro-migration of H+, H3O+, H3SO4−, and so forth, toward the cathodic side from the central chamber.

3.3. The temperature rise in the EA reactor may resulted from global system resistance, as discussed in the Sections 3.1 and 3.2.

Likewise, a maximum of 28.85 ± 0.95% lactulose was produced for 12% WP solution at 50 min under 900 mA current intensity (Figure 7b) and then somewhat reduced at the end of EA (27.57 ± 0.84%). While, a maximum lactulose of only 24.98 ± 0.95% was produced at the end of the EA (i.e., at 60 min) under 600 mA current intensity. It can be observed that the lactulose yield of the higher WP solution (i.e., 12% WP) was less than that obtained for 6% WP solution under a similar current intensity and reaction time. This might be due to the retarding pH evolution and low alkalinity in a feed solution with higher solid concentration because of a higher resistance and buffering capacity of the solution. Indeed, the solution alkalinity and pH significantly affected (p < 0.001) the formation of lactulose in the EA. For 18% WP solution, the lactulose formation was started at 50 (13.72 ± 0.40%) and 35 min (16.50 ± 0.51%), afterward gradually increased to 18.37 ± 0.76 and 23.42 ± 0.84% at the end for 300 and 600 mA, respectively (Figure 7c). However, no lactulose was produced for 300 mA in the 12 and 18% WP solutions. This could be attributed to the lack of sufficient solution alkalinity. Adequate alkalinity is usually essential for lactulose formation because the rearrangement of the molecules for lactose isomerization into lactulose demands proton acceptors, that is, OH−, that can be obtained by reaching a higher alkaline condition in the feed solution.

The results achieved in the present study were corroborated with those obtained from a study by Kareb, et al., where they produced a maximum lactulose yield of 35% at 40 min of EA under a current intensity of 400 mA using 100 mL of 7% (w/v) whey solution. In another recent study, Djouab and Aider achieved a lactulose yield of 39.78% after 35 min of EA under 330 mA using a solution of 6% (w/v) WP in both cathodic and central compartments. However, the configuration and geometrical parameters of the reactors and the reaction conditions were quite different than those used in the present study. Beside the lactulose production, the production of galactose was linearly augmented over running time in the electro-isomerization process because the lactulose that was produced was hydrolyzed into glactose. It can be noted from Figures 8 and 9 that the maximum galactose of 1.73 ± 0.12, 7.04 ± 0.37, and 13.38 ± 0.41% was produced in the 6% WP solution for 300, 600, and 900 mA current intensities, respectively. Higher amount of galactose was formed for a greater current intensity, which might be due to the higher temperature increase in the reactor resulted from global system resistance, as discussed in the Section 3.3. The temperature rise in the EA reactor may
probably be forcing the reaction to the other side pathways because of the formation of greater activation energy.\textsuperscript{16,22} However, the maximum galactose of 10.41 ± 0.37% was produced at 50 min under a current intensity of 900 mA in the 6\% WP solution, while the highest lactulose of \textasciitilde 37\% was obtained. Whereas, the commercial lactulose syrup is accepting galactose of up to 16\% as per United States Pharmacopeia.\textsuperscript{23} Nevertheless, only galactose was produced as a byproduct in the EA reactor, and no other impurities such as tagatose, glucose, epilactose, and so forth, were observed. This finding was comparable to those obtained by Djouab and Aider\textsuperscript{1} and Karim and Aider.\textsuperscript{12} Djouab and Aider\textsuperscript{24} and Aissa and Aider\textsuperscript{17} argued that the only byproduct during the EA of whey/lactose was definitely galactose unlike various acidic compounds and impurities in chemical isomerization. Because fructose and glucose were not observed in the medium (only trace amounts fructose in some cases), they might be isomerized into galactose as a final product as explained in a recent study by Djouab and Aider.\textsuperscript{24} After all, being a more stable carbohydrate, galactose remains intact in the EA reactor (Figure 10). Therefore, the purification step in the electro-isomerization process could be simplified, which is an economical and environmental benefit for industrial application. Indeed, this is one of the most important advantages of the EA, that it is more selective compared to the conventional chemical isomerization process.

3.6. Formation of Sugars in the Chemical Isomerization. The conventional chemical isomerization process was conducted using similar feed solutions of 6, 12, and 18\% WP and maintaining equivalent alkalinity in the feed solutions corresponding to those of the EA process, and the lactulose yields are presented in Figures 11 and 12. As it can be seen from Figure 11, the solution alkalinity, WP concentration, and isomerization time significantly affected ($p < 0.001$) the transformation rate of lactose into lactulose. It can be seen from Figure 11a that the maximum lactulose yields of 24.31 ± 0.67 (with 3.99\% galactose) and 25.47 ± 0.71\% (with 5.28\% galactose) were produced at the end of the conventional isomerization for the solution alkalinity of 38.00 ± 2.00 mmol/L and 40.00 ± 3.46 mmol/L using 6\% WP as a feed solution, which are equivalent to that of the EA process under 600 and 900 mA, respectively. Similarly, an optimum yield of 25.40\% lactulose (with 5.58\% galactose as a byproduct) was achieved by Hashemi and Ashtiani\textsuperscript{22} at a pH of 11.00 and 70 °C temperature after 60 min reaction time using 10\% lactose as a feed solution. No lactulose was detected at the solution alkalinity equivalent to that of the EA for 300 mA current intensity (20.67 ± 1.15 mmol/L) in the 6\% WP solution, although pH reached 10.43 ± 0.02 at the end of the reaction (Figure 13). However, it is commonly assumed that a high pH (>10.00) is needed for the isomerization reaction to occur.\textsuperscript{16,22,24} This might be due to inadequate alkalinity in the solution which was essential for an isomerization reaction to occur.

Regarding the pH evolution, a significant variation between electro- and chemical isomerization was noticed. In chemical isomerization, the maximum pH of 10.43 ± 0.02, 11.52 ± 0.02,
and 11.60 ± 0.01 were achieved for 6% WP solution after the completion of reactions for similar solution alkalinity corresponding to the EA process for 300, 600, and 900 mA current intensities, respectively. However, medium pH never reached 10 (pH < 10) for 12 and 18% WP solutions at the equivalent alkalinity corresponding to the EA (Figure 13). Consequently, no lactulose was observed to form for 12 and 18% WP solutions in chemical isomerization (Figure 11b,c). This is obvious that higher solution concentrations were retarding the changes in pH because of the higher buffering capacity of the greater WP concentrations. In fact, greater activation energy was needed for an isomerization reaction to occur in the chemical method. Thus, a higher dosage of catalysts was obliged for producing and adequate degree of alkalinity, while the more concentrated solutions of WP were used as feed solutions.21 Indeed, the catalyst concentration is one of the critical factors to be considered during the conventional lactose isomerization process. In a study by

Figure 12. HPLC chromatograms for the sugar composition during the chemical isomerization using 6% WP solution: (a) initial feed solution and (b) feed solution at equivalent solution alkalinity as in the electro-isomerization under 900 mA current intensity.

Figure 13. pH evolution during the chemical isomerization process at equivalent solution alkalinity to that of the electro-isomerization for (a) 6, (b) 12, and (c) 18% WP solutions.
Seo, et al.,\textsuperscript{25} they achieved a lactulose yield of 29.60% at a solution alkalinity of 79.00 mmol/L by adding 7.6 g/L of \((\text{NH}_4)_2\text{CO}_3\) as the catalyst under 97 °C using sweet whey as the feed solution. In contrast, only 1.83 and 1.80 g/L of KOH were used to achieve the alkalinity of 32.67 ± 1.15 and 32.00 ± 2.00 mmol/L in the 12 and 18% WP solutions as equivalent to the EA under 900 mA current intensity, respectively.

Beside the pH and catalyst concentration, a higher temperature of more than 50 °C (typically, 50 to 130 °C) combined with various reaction times was used for conventional chemical isomerization in most literature studies.\textsuperscript{22,26−28} In the present study, we performed the chemical isomerization without external heating because the EA process was conducted at an ambient temperature. The temperature was increased in the EA process was self-generated in the system because of global electric resistance. Furthermore, the statistical analysis of the acquired data did not indicate a relationship between the temperature increase and lactulose formation. This can be explained by the fact that the temperature raised in the EA (from 22 to 43 °C) was not enough to have any catalyzing effect. It is important to mention here from this result that the lactulose can be generated at an ambient temperature by creating enough solution alkalinity in the feed medium.

Apart from this, the transformation of lactose into lactulose through the chemical-based processes typically resulted to a quick degradation of lactulose into galactose and tagatose along with some other acidic byproducts including isosaccharinic and formic acids,\textsuperscript{22,24,29−31} resulting in the dropping of pH in a feed medium.\textsuperscript{22,32} In essence, in the conventional isomerization, the use of a high temperature in an alkaline medium triggers isomerization and degradation of lactose and lactulose into epilactose and galactose.\textsuperscript{22,33} However, in this study, this phenomenon was not observed and, subsequently, only galactose was produced as a reaction byproduct.

As can be seen from Figure 14 that the ORP values were decreased to around +200 to −100 mV within the first 5 min in the chemical isomerization. Thereafter, depending on the different reaction conditions, they obtained the highest values of around +200 to −100 mV at the end of the isomerization reaction. It appears that the ORP reached a negative value only for 6% solution, and the highest ORP of −95 ± 4.36 mV was reached at the end of the isomerization reaction at an equivalent solution alkalinity corresponding to 900 mA current intensity in the EA. In contrast, in the EA technique, the ORP values were reduced to around −450 to −650 mV with 5 min of electro-isomerization, no matter what solution concentrations and current intensities used. After that, they reached maximum values of −570 to −870 mV depending on the different reaction conditions (Figure 6). The considerable variation between the ORP values of the electro-isomerization versus the chemical isomerization processes attributed to the fact that: during the EA process, the electrolysis of water produces two key components, that is, \(\text{OH}^-\) ions, which are responsible for the alkalization of the reaction medium and \(\text{H}_2\) gas, which is a powerful reducing agent. As a result, the ORPs were highly reducing in the EA process. However, in the chemical method, the addition of the alkalinizing catalyst, that is, KOH had an effect only in the pH of the feed medium. Consequently, the ORPs in the electro-isomerization process were considerably higher compared to those in the chemical isomerization method, even using the equivalent solution alkalinity.

In the EA system, the highly reduced ORPs made the WP solutions extremely reactive because the applied electric field activates the feed medium to transform into a metastable state. As a result, the reactivity of the medium was substantially enhanced under the EA conditions compared to the normal state;\textsuperscript{12} therefore, the activity of the isomerization reactions might be increased because of the high internal potential energy of the activated solution.\textsuperscript{12} Apart from high ORP, other physico–chemical parameters including high medium pH, critical solution alkalinity, ion migration, and moderate temperature rise in the EA process may probably have intensified the feed medium to reach such conditions, in which the required activation energy for an isomerization

Figure 14. Variation of ORP during the conventional chemical isomerization reactions for (a) 6, (b) 12, and (c) 18% WP solutions.
reaction to occur for transforming lactose into lactulose would have been considerably reduced. Hence, by decreasing the required activation energy, electro-isomerization showed better efficiency for conversion of lactose into lactulose. Subsequently, a higher yield of lactulose was produced within a short reaction time in the electro-isomerization than the conventional isomerization process, although the equivalent alkaline conditions were maintained. To sum up, it can be hypothesized that the main factor, which can explain the difference between the EA process and the chemical one, is that the required activation energy for an isomerization reaction to transform lactose into lactulose in situ of WP solutions to occur is much lower using the EA technique than the chemical method.

**CONCLUSIONS**

The results of the present study suggest that, in contrast to the conventional chemical isomerization process, the EA technology was more efficient in producing a greater amount of lactulose within a short time whatever the reaction conditions. The highest lactulose yield of ~37% was achieved in the EA technique at 50 min under a 900 mA current intensity using a 6% WP solution with a medium pH and alkalinity of 11.34 and 40.00 mmol/L, respectively. Whereas, a maximum of 25% lactulose was obtained in the conventional chemical isomerization for the 6% WP solution as the feed, while the medium pH was 11.60 for an alkalinity of 40.00 mmol/L (equivalent solution alkalinity to 900 mA current intensity as in the EA process). Therefore, the EA technique offers higher potential than the conventional chemical method to produce the prebiotic lactulose through the valorization of the WP, using it as a lactose source. Furthermore, the EA process was carried out under complete autocatalytic conditions, implying that the alkalizing catalysts and external heating are not required in the EA technology. To sum up, the EA could be an attractive, green, and clean technique to achieve the combined target of prebiotic (lactulose) production and sustainable valorization of WP for environmental resilience.

**ASSOCIATED CONTENT**

*Supporting Information*  
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c04178.  
Schematic of the EA reactor with geometrical parameters used for electro-isomerization and evolution of voltage in the EA process using different current intensities and WP solutions (PDF)

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