Contribution to the production of lactulose-rich whey by in situ electro-isomerization of lactose and effect on whey proteins after electro-activation as confirmed by matrix-assisted laser desorption/ionization time-of-flight-mass spectrometry and sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Ourdia Kareb,*† Claude P. Champagne,†‡ and Mohammed Aïder†§¹

*Department of Food Science, Université Laval, Quebec, Qc, G1V 0A6, Canada

†Institute of Nutrition and Functional Foods (INAF), Université Laval, Quebec, Qc, G1V 0A6, Canada

‡Food Research and Development Centre, Agriculture and Agri-Food Canada, 3600 Casavant, St. Hyacinthe, Qc, J2S 8E3, Canada

§Department of Soil Sciences and Agri-Food Engineering, Université Laval, Quebec, Qc, G1V 0A6, Canada

ABSTRACT

Cheese-whey, a major co-product of the dairy industry, has recently been the subject of many technological applications. We studied the bioconversion of whey into valuable bio-products such as a potential lactulose prebiotic and compounds with antioxidant properties. This paper examines efficiency, safety, and economics of electro-activation as an eco-friendly technology for a maximum valorization of whey. Thus, a bottom-up approach was therefore addressed. The effect of 4 experimental parameters—low working temperatures (0, 10, and 25°C), current intensities (400, 600, and 800 mA), volume conditions (100, 200, and 300 mL), and feed concentrations [7, 14, and 28% (wt/vol)]—on lactose-whey isomerization to lactulose under electro-activation process were studied. Structural characteristics of whey proteins and antioxidant functionality were also investigated. The results showed a compromise to be reached between both parameters. Therefore, the maximum yield of 35% of lactulose was achieved after 40 min of reaction at the working temperature of 10°C under 400 mA electric current field and 100-mL volume conditions with using feed solution at 7% (wt/vol). The isomerization of lactose to lactulose is accomplished by subsequent degradation to galactose, but only at a very small amount. Additionally, whey electro-activation showed significantly elevated antioxidant capacity compared with the untreated samples. The enhancement of antioxidant functionality of whey electro-activation resulted from the synergistic effect of its partial hydrolysis and the formation of antioxidant components that were able to scavenge free radicals. In conclusion, the

findings of this study reveal that the whey treated by the safety electro-activation technology has both lactulose-prebiotic and antioxidant properties and could have a substantial application in the manufacture of pharmaceutical and functional foods.

Key words: prebiotic whey, lactulose, electro-activation, isomerization, antioxidant capacity

INTRODUCTION

Whey is the major co-product of the dairy industry that is removed after the coagulation of casein during cheese manufacturing (Zadow, 1994; Siso, 1996). The global world production of whey was estimated to be 160 million tons per year, showing a constant annual growth of about 2%, whereas in North America, 3.6 million tons of whey is obtained every year during by the cheese manufacturing industry (Guimarães et al., 2010b). Due to its high volume production and pollutant load effects, cheese whey represents a significant problem for the dairy industry (Janczukowicz et al., 2008). Whey exhibits a biochemical oxygen demand of 0.6 to 60 g/L and a chemical oxygen demand of 0.8 to 100 g/L. Lactose, the major component of whey, contributes highly to its organic load matter effect (Kosikowski, 1979; Carvalho et al., 2013). On the other hand, currently whey is not considered only as a waste but as a source of added-value components (Koutinas et al., 2009; de Souza et al., 2010; Guimarães et al., 2010b; Madureira et al., 2010; Tavares et al., 2011; Corzo-Martínez et al., 2013). However, only half of the global whey processing is now transformed into marketable products (Siso, 1996; Panesar et al., 2007; Spălățelu, 2012) and many of them are linked to the protein fraction (Karam et al., 2013). In this respect, other innovative and complete approaches are needed for the valorization of whey based on adding value

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¹Corresponding author: mohammed.aider@fsaa.ulaval.ca

to the lactose together with the protein components (Smithers, 2008; Banaszewska et al., 2014). Whey typically retains 55% of milk nutrients, including lactose (4.5–5% wt/vol), soluble proteins (0.6–0.8% wt/vol), lipids (0.4–0.5% wt/vol), and other trace elements such as B-group vitamins and mineral salts (Kosikowski and Wzorek, 1977; Marwaha and Kennedy, 1988; Prazeres et al., 2012). The qualitative and quantitative contents of whey make it a good raw material for the production of versatile valuable products (Mollea et al., 2013) such as bioactive peptides with substantial functionalities for both human health and food technology applications (Madureira et al., 2007; Smithers, 2008; Pihlanto and Korhonen, 2015). Lactose is presently the most underutilized whey component, mainly because of the huge quantities generated by the cheese/casein manufacturing industry and its limitations in regard to functional foods applications (Paterson and Kellam, 2009; Guimarães et al., 2010a). However, it provides an attractive opportunity to produce different chemicals, pharmaceutical, and functional ingredients for food applications (Gänzle et al., 2008). Growing attention has recently been paid to some prebiotics derived from lactose, such as lactulose, lactilol, lactobionic acid, and galacto-oligosaccharides. They are considered as substrates with a high promoting effect on the growth of beneficial bacteria (probiotics) and improve overall gut health (Saarela et al., 2003; Schaafsma, 2008; Cardelle-Cobas et al., 2011; Gutiérrez et al., 2012; Seki and Saito, 2012). Among these sugars derived from lactose, lactulose is considered as the most promising one in regard to the prebiotic effect.

Lactulose (4-O- β -D-galactopyranosyl-D-fructose) is a disaccharide composed of a moiety of galactose linked to a moiety of fructose by a β (1–4) glycosidic linkage (Panesar and Kumari, 2011). It is recognized as a potential prebiotic because it beneficially affects the growth of health-promoting bacteria in the gastrointestinal tract (Roberfroid, 2007). In the pharmaceutical field, lactulose is mainly used as a gentle laxative (Schuster-Wolff-Bühning et al., 2010). For food considerations, it is of both technological and nutritional interest because it has higher sweetening power than lactose and is more soluble (Huebner et al., 2007; Olano and Corzo, 2009; Aït-Aissa and Aïder, 2014a). The sweetness power of lactulose is 48 to 62% of that of sucrose, which is referred as the standard in measuring sweetness. The sweetness of lactose versus sucrose is only about 17 to 20%. Lactulose has been introduced in various dairy and confectionery products and sweetener for diabetics (Aider and Halleux, 2007; Schuster-Wolff-Bühning et al., 2010; Panesar and Kumari, 2011; Seki and Saito, 2012).

Industrial production of lactulose is commonly performed by 3 methods: (1) chemical catalysts in alkaline media via a Lobry de Bruyn-Alberda van Ekenstein rearrangement in which the glucose of lactose isomerizes to fructose (Montgomery and Hudson, 1930; Martinez-Castro et al., 1986), (2) Amadori rearrangement in which the carbonyl group of reducing lactose interacts with free amino groups from proteins or AA (Adachi and Patton, 1961; Andrews, 1986; Bologa et al., 2009), and (3) biocatalysis with β -galactosidase and glycosidase enzymes (Adamczak et al., 2009; Gänzle, 2012). However, the aforementioned processes present some drawbacks, which include the low catalysis efficiency, cost effectiveness, low yield of lactulose, and complexity of purification steps (Aït Aissa and Aïder, 2013b). Currently, lactulose was successfully synthesized by using a safe and eco-efficient process, which is the electro-activation technology (Aider and Gimenez-Vidal, 2012; Aït Aissa and Aïder, 2013b, 2014b). Electro-activation is an emerging technology based on electrolysis of aqueous solutions (Bologa et al., 2009). Electro-activation has been used for enhancing the antioxidant activity of enzymes, maple beverage stabilization and starch hydrolysis (Aider et al., 2012). In regards to whey valorization, one of the most promising way consists of converting part of lactose into lactulose in situ by submitting whey to electro-activation. Consequently, a new product, lactulose enriched whey, can be produced and can be used as a valuable ingredient with proven prebiotic effect. This approach will enhance the use and valorization of whey in a complex form without need for its fractionation. Furthermore, whey proteins could be subjected to the Maillard reaction with reducing sugars and partial hydrolysis under the conditions occurred using the electro-activation and improved the antioxidant capacity of the final product (Oh et al., 2013).

In the present work, the first objective was to study the process of lactose conversion into lactulose in situ of sweet cheese whey by using the electro-activation technology. The effect of 4 processing parameters such as the working temperature (0, 10, and 25°C), current electric field intensity (400, 600, and 800 mA), whey volume (100, 200, and 300 mL), as well as initial whey concentration (7, 14 and 28%, wt/vol) on lactose electro-isomerization yield into lactulose was studied. The second objective of this study was to investigate the structural changes of whey proteins after electro-activation in the cathodic side of the reactor by SDS-PAGE and matrix-assisted laser desorption/ionization (MALDI)-time of flight (TOF) analysis and to evaluate the antioxidant capacity of the product by the oxygen radical absorbance capacity (ORAC)

Figure 1. Schematic representation of the experimental setup for lactose electro-isomerization in the in situ conditions of whey. Color version available online.

assay to target find dual functionality such as potential prebiotic and natural antioxidant effect.

MATERIALS AND METHODS

Chemicals

All chemicals used in this study were of analytical grade. Whey powder was obtained from Agropur (Agropur Cooperative, St-Hubert, Canada). The composition of the whey (on a wet basis) is as follows: water content: 4.28%, lactose: 75.50%, total proteins: 12.15%, ash 7.41%, and residual fat 0.85%. Lactose monohydrate was purchased from Avantor Performance Materials (Center Valley, PA). Standard sugars of lactulose, glucose, galactose, and fructose (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO). Sodium sulfate (Na_2SO_4) was purchased from Anachemia (Montreal, Canada). All solutions were prepared in deionized water.

Electro-Activation Reactor and Configuration

The electro-activation apparatus used in this work was designed as shown in Figure 1. The electro-activation cell was immersed in the refrigerated heat exchanger bath equipped with circulating system (Isotemp 1016S, Fisher Scientific, Ottawa, Canada) to keep the working temperature at the desired values as specified for this parameter (0, 10, and 25°C). The bath was filled with Polycool MIX-25 silicone heat fluid (PolyScience,

Niles, IL) to maintain the required temperature. The electro-activation cell is composed of 3 compartments: the anodic, central, and cathodic. The central compartment was connected to the anodic and cathodic ones by anion and cation exchange membranes, respectively. The used anion exchange membrane was the AMI-7001S and the cation exchange membrane was the CMI-7000S. Both the membranes were purchased from Membranes International Inc. (Ringwood, NJ). This configuration was chosen to maintain acidic conditions in the anodic side while the alkaline conditions were kept in the cathodic compartment in which the lactulose formation was targeted. To ensure the passage of external electric current, 2 food-grade stainless-304 electrodes were introduced in the anodic and cathodic compartments and connected to the positive and negative poles of a DC-electric generator, respectively. The cathodic compartment was initially filled with whey solution, whereas the central and anodic compartments were filled with 0.5 M Na_2SO_4 solution.

Electro-Activation Procedure

The experiments were carried out by modifying the following parameters: operating temperatures (0, 10, 25°C), current electric field intensity (400, 600, 800 mA), whey volume (100, 200, 300 mL), and whey concentrations (7, 14, 28% wt/vol). For each experiment, freshly prepared solutions were placed into the cathodic compartment in which the targeted reaction occurred. The solution was gently stirred by using an agitator RW

20 DS1 (IKA, Wilmington, NC) at 200 rpm. Samples from the cathodic compartment were collected every 10 min until the end of the electro-activation process, which was set for a total of 60 min. For each of these samples, the values of the corresponding voltage and pH were measured by using a digital multimeter (Keithley, Inerga Series, Cleveland, OH) and a pH meter (model Oakton pH 700, Eutech Instruments, Cole-Parmer, Montreal, Canada). All samples were kept under refrigerated conditions until analysis. All experiments were done in duplicate.

HPLC Analysis of the Sugars

Sugar contents were determined in all samples before and after the electro-activation treatment by Agilent HPLC system (Agilent Technology, Millipore Corp., Milford, MA) equipped with a refractive index detector. The column chosen was 300 × 6.5 mm Carbohydrate Analysis (Waters Co., Milford, MA). Before introduction into the HPLC system, the samples were centrifuged at 10,000 × *g* for 5 min at ambient temperature (21 ± 1°C). The supernatant was diluted 10-fold, and an aliquot of 25 µL was injected. The running time was set at 30 min per sample. Calibration curves were prepared using each sugar standard in a range of 5 to 20% (wt/vol) concentration. Once the separation of sugars was achieved, peaks were identified by comparing their retention time with the standard sugars.

The yield of reacted (converted) lactose, which is the fraction of the initial lactose transformed during the reaction, was calculated by the following equation:

$$\text{lactose yield (\%)} = \frac{A_{\text{lactose initial}} - A_{\text{lactose}}}{A_{\text{lactulose initial}}} \times 100, \quad [1]$$

where A_{lactose} is the peak area of the lactose at time τ , corresponding to the time at which the treated solution was sampled, and $A_{\text{lactose initial}}$ is the peak area of the standard of lactose samples.

Lactulose yield, which is the fraction of lactulose formed in the total carbohydrates in cheese whey, was calculated by the following equation:

$$\text{lactulose yield (\%)} = \frac{A_{\text{lactulose}}}{A_{\text{lactulose initial}}} \times 100, \quad [2]$$

where $A_{\text{lactulose}}$ is the peak area of lactulose at time τ , corresponding to the time at which the treated solution was sampled, and $A_{\text{lactulose initial}}$ is the peak area of the standard of lactulose samples given by the HPLC.

Galactose yield, which is the amount of galactose formed in the total carbohydrates, was calculated by the following equation:

$$\text{galactose yield (\%)} = \frac{A_{\text{galactose}}}{A_{\text{galactose initial}}} \times 100, \quad [3]$$

where $A_{\text{lactulose}}$ is the peak area of galactose at time τ , corresponding to the time at which the treated solution was sampled, and $A_{\text{lactulose initial}}$ is the peak area (given by HPLC) of the standard of galactose samples.

Total Protein Content

The total nitrogen of whey samples was measured by using a Leco analyzer (Truspec, Leco, St. Joseph, MI) according to Dumas nitrogen method (Bremner and Mulvaney, 1982). The corresponding total protein was calculated by multiplying the nitrogen content by a factor 6.38 (Finete et al., 2013).

Gel Electrophoresis (SDS-PAGE)

Sodium dodecyl sulfate-PAGE with and without β -mercaptoethanol of whey proteins submitted to electro-activation was performed using mini-protein electrophoresis system (Bio-Rad Laboratories, Hercules, Canada) according to the method of Laemmli (1970). Briefly, the 1% (wt/vol) protein solutions were diluted 1:1 ratio with a SDS-PAGE sample buffer and then heated at 95°C for 5 min. A 10-µL aliquot of each sample was loaded into the gels. Electrophoresis running was conducted at constant electric current of 30 mA. After running, the proteins bands were stained with Coomassie Brilliant Blue R-250 (0.2%) in 40% methanol and 10% acetic acid for 2 h and destained overnight in 10% methanol and 10% acetic acid.

MALDI-TOF-MS Assessment

To achieve a better resolution and to get a more detailed insight of electro-activated whey proteins, the protein profiles were analyzed by MALDI-TOF-MS. Prior to analysis, a 10-µL aliquot of each protein sample was diluted 50-fold in 0.1% trifluoroacetic acid. The matrix mixture was prepared by combining α -cyano-4-hydroxycinnamic acid and 2,5-dihydroxybenzoic acid solutions in 1:1 volume ratio as described previously by Fenaille et al. (2006). A 1-µL aliquot of a protein sample was mixed with the same volume of the matrix mixture, and 1 µL of the resulting solution was then deposited on a MALDI target and analyzed using a MALDI-TOF mass spectrometer instrument.

(4800 MALDI-TOF/TOF, AbSciex, Concord, Canada) equipped with the nitrogen laser 335 nm. The laser-desorbed positive ions were analyzed after acceleration by 20 kV under linear mass range of 4,000 to 30,000 Thomson (Th) for the proteins and under the reflector mode in the mass range of 650 to 4,000 Thomson (Th) for the peptides. Mass calibration in linear mode was achieved using the proteins mixture composed of insulin, thioredoxin, and apomyoglobin. The external calibration in reflector mode was performed using the peptide mixture composed of the adrenocorticotrophic hormone fragments (1–17, 18–39, and 7–38) and angiotensin I, provided by AbSciex (Concord, Canada). A total of 2,000 individual shots of each spot samples were added to produce a MALDI mass spectrum.

Determination of Free Amino Acids by UPLC-UV Fluorescence Detection

The free AA composition of whey after electro-activation was determined after derivatization with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate using the Waters Acquity UPLC system (Milford, MA) equipped with UPLC AccQ·Tag Ultra Column (2.1 mm × 100 mm) and with an Acquity tunable UV and fluorescence detectors as described in an AccQ·Tag Ultra kit (Waters, 2012). Briefly, whey samples were centrifuged for 5 min at $4,500 \times g$ at 4°C. The standards and samples derivatization of AA were prepared by mixing 60 µL of AccQ·Tag borate buffer, 10 µL of samples, 10 µL of internal standard (Norvaline at 100 µM), and 20 µL of AccQ·Tag. Ultra reagents were added into a recovery vial subsequently. The mixture was vortexed immediately for 5 s and allowed to rest for 1 min before heating at 55°C for 10 min. Five microliters of the solution obtained was injected into the Waters HPLC system and monitored using both UV and fluorescence set at 260 and 473 nm, respectively.

Free Radical Scavenging Activity (ORAC) of Electro-Activated Whey

The automated ORAC assay with fluorescein was carried out with the BMG Fluostar Galaxy micro-plate reader (Durham, NC) equipped with fluorescence filters for an excitation wavelength of 485 nm and an emission wavelength of 520 nm. The experiment was performed at 37°C and pH 7.0 using Trolox as the standard (a water-soluble derivative of vitamin E). A mix of 200 µL of fluorescein solution (0.036 mg/L), 75 µL of a 2,2'-azobis-2-aminopropane dihydrochloride solution (8.6 mg/L), and 20 µL of the diluted samples were added to a 96-well plate in triplicate. The analyzer recorded the fluorescence 35 times for 120 s. The ORAC

values of electro-activated whey were expressed as micromoles of Trolox equivalent per liter of whey.

Statistical Analysis

Statistical analysis was performed using a complete randomized factorial design with repeated measurements. The factors considered were the electric current, temperature, volume, concentration, and reaction time. The dependent variables studied were the pH, lactulose yield obtained, as well as yield of by-products (glucose, galactose, and fructose) that occurred in the cathodic compartment of the electro-activation cell. Each treatment was carried out in triplicate, and mean values ± standard deviation were used. The antioxidant capacity was performed using the regression model. Statistical analysis (ANOVA) of the data was performed using SAS software (V9.3, SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Process of Whey Solution Electro-Activation: Evolution of Whey Solution Alkalinity

The evolution of the whey solution pH in the cathodic compartment in which the electro-isomerization of lactose into lactulose is anticipated under varying experimental conditions is shown in Figure 2. The effect of 3 current intensities (400, 600, and 800 mA) on the pH evolution of whey at 10°C and 100 mL conditions are summarized in the Figure 2a. The results showed that electro-activation is effective in terms of enhancing the alkalinity of whey without adding alkalizing reagents such as NaOH. To achieve this pH increase up to 10 and more, the results showed that the current intensity is an important parameter because it is necessary to achieve the electrochemical metastable state of aqueous solutions and to make it able for electrocatalysis (Bologa et al., 2008; Sprinchan et al., 2011). Indeed, the whey solution possesses the necessary properties for the rapid and efficient accumulation of actively charged particles (Bologa et al., 2008). Unlike the lactose solution, which suffers from lack of conductivity, whey contains sufficient amount of mineral salts that ensure the passage of electric current (Aït Aïssa and Aïder, 2013a). Thus, under the influence of the applied external electric field, electrolytic dissociations of aqueous solutions giving charged electrolytes in the anodic and cathodic compartments occur. Under electro-activation, a water oxidation takes place in the anodic compartment, leading to the formation of H⁺ cations according to the reaction (equation 4):

At the same time, in the cathodic compartment, a water reduction into hydrogen with release of HO^- ions takes place according to the following reaction (equation 5):

The formed hydroxyl ions OH^- increase the alkalinity of the medium, which is a key parameter in the lactose electro-isomerization into lactulose. Indeed, the hydroxyl ions formed as a result of water dissociation at the solution/cathode interface play the role of proton acceptors when the isomerization of lactose into lactulose occurs according to the Lobry de Bruyn-Alberda van Ekenstein rearrangement. To maintain this condition at the cathodic side, a negatively charged cation exchange membrane was used to repulse the hydroxyl ions that can be transported by the current flow. In our study, the results showed a similar tendency of the evolution of pH as a function of time under the different currents applied (Figure 2a). The curves obtained can be divided into 2 stages. During the first stage, typically in the first 20 min of electro-activation, a rapid increase of pH occurred in the solution due to the high dissociation of water with the subsequent generation of high amount of hydroxyl ions OH^- . In the second stage, the pH solution continued to increase gradually but at a lower rate than in the first stage, which means that the solution was highly charged by hydroxyl ions OH^- . It can be also seen that the average pH of solution increases sharply with increasing the current intensity applied to the electro-activation reactor. This behavior can be explained by the proportional increase of water electrolysis due to the increase of the current intensity. The optimum pH of lactose isomerization to lactulose under electro-activation for this study was 11 ± 0.3 . The results published by previous authors using chemical catalysis such as boric acid and sodium hydroxide are in good agreement with those here described (Zokaei et al., 2002; Hashemi and Ashtiani, 2010). Some works reported the decline of pH solution as the reaction of isomerization of lactose to lactulose proceeds at the end (Angel de la Fuente et al., 1999; Montilla et al., 2005). According to the studies of Berg and Van Boekel (1994) and Olano and Martinez-Castro (1981) in heated milk, the decrease of pH resulted from the hydrolysis of lactulose into galactose and acid compounds such as isosaccharinic and formic acids. Contrary to these works, the pH profile of the whey submitted to electro-activation during 60 min did not show any decreasing phase in all experimental conditions. Thus, it can be assumed that under the effect of electro-activation, high alkaline conditions were maintained until the end of the reaction process. Thus, subsequent degradation

of lactulose into organic acids did not occur in these conditions. This result is very important in terms of the differentiation between the conventional chemical lactose isomerization into lactulose and the process based on the reagentless electro-activation technology. Similar results were obtained when lactose aqueous solution was used to synthesize lactulose by using an electro-activation reactor (Aider and Gimenez-Vidal, 2012; Aït Aissa and Aïder, 2013a). The alkalinity of whey solution under different temperatures was also studied and the results are shown in Figure 2b. The variation of temperatures did not affect the evolution of the whey pH submitted to electro-activation at the cathodic compartment (Figure 2b). On the other hand, when the volume conditions at the cathodic compartment were varied, one can observe a different behavior of pH evolution. As a function of running time, the ANOVA analyses showed a high significance level ($P < 0.0001$) of the volume variation on the evolution of pH solution at the cathodic compartment. As shown in Figure 2c, the maximum alkaline conditions were obtained when a whey volume of 100 mL was used. Further increase of the whey volume resulted in a slower increase of the solution pH. For example, after 20 min of electro-activation, the pH of solution reached a value of 10.65 ± 0.4 , 9.10 ± 0.90 , and 7.70 ± 0.57 when the whey volumes of 100, 200, and 300 mL were used, respectively. The difference of the alkalinity of whey solution is related to the concentration hydroxyl ions formed as well as to the solution electric conductivity and buffering capacity (Hill et al., 1985; Al-Dabbas et al., 2011).

Assessment of Lactulose Formation During Whey Electro-Activation

Figure 3 showed the results obtained when whey and lactose, at similar lactose content, were electro-activated under 400 mA of current electric field at 10°C in 100-mL volume conditions. The obtained results showed that the conversion of lactose to lactulose was faster by using lactose aqueous solution compared with whey solution. The lower level of lactulose formed in whey in the early stage of reaction until 20 min is due to the retarding of pH increase caused by the buffering action of whey proteins and other compounds such as citrate and phosphate (Paseephol et al., 2008). Furthermore, lactulose was not detected in whey at earlier reaction when pH was in the range of that of milk and it was formed only when alkaline conditions were reached in the cathodic compartment. Thus, one can conclude that lactulose did not remove as a result of lactulosyl-lysine hydrolysis. These results agreed with previous

Figure 2. Profile of pH as a function of running time in whey ($C_{\text{whey}} = 7\%$ wt/vol), at different experimental conditions (a) current intensities (400, 600, and 800 mA) at 10°C and 100 mL, (b) working temperatures (0, 10, and 25°C) at 400 mA and 100 mL, and (c) volume conditions (100, 200, and 300 mL) at 10°C and 400 mA. Error bars represent standard deviations. Color version available online.

Figure 3. Profile of lactulose yield as a function of reaction time using different feed solutions. The experimental conditions were as follows: whey ($C_{\text{whey}} = 7\%$ wt/vol), lactose ($C_{\text{lactose}} = 5\%$ wt/vol) at 10°C, 400 mA, and 100 mL conditions. Data represent the mean $\pm$ standard deviation of 3 replicates.

reports which showed that protein did not interact to increase the lactulose concentration in heated milk (Martinez-Castro et al., 1986; Andrews and Prasad, 1987). Thus, it can be concluded that lactulose was formed exclusively via the (LA) rearrangement using whey as lactose source under electro-activation conditions.

It was reported that in milk or whey, lactulose is subsequently formed by different reaction mechanisms in 2 main forms as free lactulose and covalently bound to amino groups of proteins as lactulosyl-lysine (Adachi and Patton, 1961; Andrews, 1986). Under alkaline conditions, the free lactulose was mostly formed via the Lobry-de-Bruyn-van-Ekenstein transformation (LA), in which the glucose moiety of lactose is isomerized to fructose via an intermediate enolization reaction (Martinez-Castro et al., 1986; Andrews and Prasad, 1987). The rearrangement of lactose to lactulose requires proton acceptors, which are ensured by achieving high alkaline medium in the cathodic compartment of electro-activation cell (Aider and Gimenez-Vidal, 2012). Regarding the lactulosyl-lysine, it is formed by the Amadori rearrangement in which the carbonyl group of lactose condenses with the free amino groups of the protein (Adachi and Patton, 1961). Some workers suggested that in the second mechanism, free lactulose can be also removed as a result of the hydrolysis of bound lactulosyl-lysine. The conversion of lactose to lactulose in milk during heating at 110 to 150°C during 20 min was studied by Berg and Van Boekel (1994) and

the authors concluded that the major lactulose formed followed the LA rearrangement and only 20% was by the Amadori pathway.

Analysis of the HPLC Chromatograms of Lactulose and the Other By-Products

The conversion of lactose into lactulose was usually followed by its degradation into various by-products as was reported by Berg and Van Boekel (1994) for milk and Montilla et al. (2005) for whey. The isomerization of lactose to lactulose and formation of other by-products such as glucose, galactose, and fructose were investigated by HPLC chromatograms as shown in Figure 4. In the present study, experiments carried out with a 7% (wt/vol) whey solution treated by electro-activation at 10°C in a 100-mL volume under a 400 mA electric current field showed that as the electro-activation processed, the formation of lactulose (lactose conversion yield) increased gradually with a slight decrease at the end of the treatment. A maximum conversion of lactose into lactulose of $36 \pm 0.2\%$ was achieved after 50 min of electro-activation. However, this high conversion was followed by an increase of the galactose formation, which was observed more at the early stage of the electro-activation treatment. At the same time, analysis of the HPLC chromatograms did not show any formation of glucose or fructose (a few traces in some cases). Thus, these results clearly indicate that galactose is the only by-product formed after electro-activation of lactose. This means that lactulose formation by electro-activation is quite different from the other chemical ways used for this purpose. Our results are in accordance with those previously reported by Ait-Aissa and Aïder (2013a) who used electro-activation, but do not agree with the findings of Olano and Martinez-Castro (1981) who used the chemical conversion method and showed that lactulose was more easily degraded than lactose in alkaline solution. Moreover, only minimal traces in the round of $0.17 \pm 0.02\%$ of glucose and fructose were detected at the advanced stages of the electro-activation reaction in strong alkaline medium. Similar results were reported by Aider and Gimenez-Vidal (2012) under other experimental conditions. In agreement with our results, no glucose or fructose has been previously detected in heated milk or whey (Olano et al., 1989; Montilla et al., 2005). Mendez and Olano (1979) reported that because no fructose and glucose were detected, it can be assumed that the disaccharides lactose and lactulose were degraded into galactose and acid compounds. However, our results did not show any pH decrease in all cases such as shown in Figure 2. Thus, it could be suggested that under conditions that occurred in electro-activation, glucose

and fructose could be isomerized to galactose or take part in the formation of lactulose after the ring opening of the lactose molecule (Figure 5). Furthermore, a brown color was not observed, which suggests that the advanced Maillard reactions were not formed at the low temperatures used in this study. Thus, the main route of lactose conversion under electro-activation was its isomerization into lactulose with subsequent formation of some galactose as the sole by-product of the reaction.

Effect of Temperature

In contrast with the previous studies, the isomerization of lactose into lactulose under refrigerated conditions until 0°C has been addressed in the study of (Aït Aissa and Aïder, 2013a) for the first time. By using lactose aqueous solution, the authors investigated the effect of 6 temperatures (0, 5, 10, 15, 20, and 30°C) as a function of time on lactulose synthesis under electro-activation in the cathode section of the reactor. Then, it is worthwhile noting that they obtained approximately 25% lactulose yield with a purity of 95% at low temperatures (0–10°C) during 2 min of reaction time. Based on the above results, the low temperatures are great suited for lactulose isomerization under electro-activation reactor because it limited the formation of by-product to only galactose in a small amount, a fact which constitutes a real advancement in the knowledge and technology of lactulose synthesis. Therefore, 3 temperatures (25, 10, and 0°C) were selected for the present study. Figure 6 shows the effect of the different temperatures in a volume of 100 mL of whey under a 400 mA electric field on lactose conversion into lactulose and galactose formation as a by-product. The profiles of lactulose yield obtained were similar at all the temperatures. A maximum yield of $34.57 \pm 1.37\%$ with low galactose formation was obtained at the working temperature of 10°C after 40 min of electro-activation. The formation of galactose was slightly lower in the range at 0 and 10°C than at 25°C. On the other hand, further decrease of temperature down to 0°C resulted in moderate decline of lactulose formed ($32.18 \pm 0.61\%$). Therefore, the temperature of 10°C can be considered as a key parameter for the optimal synthesis of lactulose by using the electro-activation process. These results were in good agreement with those reported by Ait-Aissa and Aïder (2013b) using lactose aqueous solution. Because the synthesis reaction of lactulose is kinetically controlled by temperature, these results suggest that using electro-activation; the activation energy required for lactulose isomerization is markedly lower than for the chemical and enzymatic methods. Besides, values of lactulose yield obtained in these experimental conditions are slightly higher within the ranges usually

reported in the literature. Thus, the present process of lactulose synthesis is significantly more sensitive, rapid, and simple than those routinely used.

Based on the findings of several researchers, the alkaline isomerization of lactose to lactulose via LA rearrangement was usually carried out at high temperatures in the range of 50 to 130°C combined with different reaction times (Martinez-Castro and Olano, 1980; Geier and Klostermeyer, 1983; Andrews, 1986; Andrews and Prasad, 1987; Olano et al., 1992; Zokaee et al., 2002; Montilla et al., 2005; Paseephol et al., 2008; Song et al., 2013; Sakkas et al., 2014). Using egg shell, a food grade catalyst and cheese whey permeate, Corzo-Martínez et al. (2013) obtained a yield of 16.1% of lactose conversion into lactulose by heating at 98°C during 150 min of reaction time. A similar lactulose

yield of 18% was reached by using milk ultrafiltrate within 60 min (Montilla et al., 2005). The synthesis of lactulose from milk permeate using calcium carbonate-based catalysts was also achieved. The lactulose yield reached did not exceed 21% by a heat treatment at 96°C during 120 min of running time (Paseephol et al., 2008). Recently, Seo et al. (2015) combined cheese whey as lactose source and sodium carbonate (Na_2CO_3) as catalyst for lactulose synthesis. The authors obtained only 3 to 4% of lactose to lactulose conversion yield at 60°C. When the temperature was increased up to 90°C, the lactulose yield increased up to 29.6% after 20 min of reaction time. In fact, Andrews and Prasad (1987) showed that the increasing of temperature from 90 to 130°C results in shortness reaction time from 120 to 4 min with positive effect on lactulose yield conversion.

Figure 4. Chromatogram profiles of whey sugars after electro-activation at 7% (wt/vol) concentration, temperature, $T = 10^\circ\text{C}$, current intensity, $I = 400\text{ mA}$, and volume, $V = 100\text{ mL}$: (a) $t = 0\text{ min}$, (b) $t = 10\text{ min}$, (c) $t = 20\text{ min}$, (d) $t = 30\text{ min}$, (e) $t = 40\text{ min}$, (f) $t = 50\text{ min}$, (g) $t = 60\text{ min}$. The x-axis shows HPLC running time; the y-axis shows signal intensity.

Figure 5. Mechanism of the electro-isomerization of lactose to lactulose in whey and the possible galactose formation pathways under low temperature using the electro-activation process.

In spite of that, the rapid degradation of lactulose in favor of galactose by-product constituted a limit of the process. It was concluded that the amount of galactose

increases with elevated temperature due to the greater activation energy formed and which pushed the reaction on the other side pathways (Calvo and Olano, 1989;

Figure 6. Profiles of lactulose and galactose produced under electro-activation as a function of running time at different processing temperatures (0, 10, and 25°C), whey concentration 7% (wt/vol), temperature $t = 10^\circ\text{C}$, current intensity $I = 400\text{ mA}$, and volume $V = 100\text{ mL}$. Error bars represent standard deviations. Color version available online.

Olano and Calvo, 1989; Troyano et al., 1992; Hashemi and Ashtiani, 2010).

Effect of the Electric Current Intensity

The effect of different current intensities on lactulose synthesis was also studied as shown in Figure 7. The statistical analysis of obtained data showed a high significance level ($P < 0.0001$) of the electric field intensity on lactulose yield. As it can be seen in Figure 7a, when the electro-activation reaction was carried out at 25°C , the isomerization of lactose (in the whey) into lactulose was accelerated at 600 and 800 mA compared with 400 mA as a function of running time. After 10 min of elec-

tro-activation, the lactulose yield achieved at 400 mA was only $4.48 \pm 0.6\%$. At the same time, mean values of 8.23 ± 0.76 and $16.35 \pm 1.3\%$ were recorded at 600 and 800 mA, respectively. It can be noticed that lactulose yield increased in a linear fashion when the current intensity was increased from 400 to 800 mA. As the reaction time was increased and after 30 min of electro-activation, lactulose yield in the cathodic compartment still increased and reached the maximum value of $35.78 \pm 0.8\%$ at the current intensity of 800 mA. In similar conditions and by using a low current intensity of 200 mA, Aider and Gimenez-Vidal (2012) reported only a value of $5.71 \pm 0.31\%$ lactulose produced from whey permeate as feed solution. Thus, these results are in agreement with previously reported data on the synthesis of lactulose using electro-activation process, where the increasing lactulose yield was clearly related to the increase of the current intensity (Sprinchan et al., 2011; Aider and Gimenez-Vidal, 2012; Aït Aïssa and Aider, 2014a,b). Nevertheless, a gradual decrease of lactulose yield, which could be attributed to its degradation into galactose after 30 min of electro-activation, was observed at elevated current intensities compared with 400 mA. Regardless of pH solution, these results are in good agreement with the fact that the increase of electric field intensity implied the Joule heating effect. During the passage of electric current through whey solution, some resistance occurred and the temperature increased in the range of 3 to 6°C in the reactor. The heated energy dissipated is proportional with electric current applied and described as the Joule effect (Aït Aïssa and Aider, 2014b).

In the same conditions with the working temperature of 10°C as seen in Figure 7b, high lactulose yield values were also achieved. As a function of the reaction time, lactose conversion into lactulose still increased until 40 min of electro-activation in all experimental conditions. The maximum conversion yield of $33.65 \pm 0.2\%$ of lactose into lactulose was obtained after 30 min under 800 mA current electric field. After 40 min of electro-activation, one can distinguish 2 distinct behaviors on lactulose evolution: at 400 mA, lactulose synthesis was still predominant and reached the value of $34.57 \pm 0.79\%$ with significant high purity, whereas at higher current intensities higher by-products, mainly galactose, decreased the synthesis. However, it can be noted that the rate of lactulose yield decrease at 10°C occurred more slowly and was less important than at 25°C , due the fact that the Joule effect was quite negligible. Moreover, depending on the pH value of the reaction medium and after 40 min of electro-activation, the pH reached the values of 11.15 ± 0.2 , 11.85 ± 0.1 , and 11.95 ± 0.05 under the effect of 400, 600, and 800 mA, respectively. In fact, the rate of water electrolysis

Figure 7. Profiles of lactulose yield as a function of reaction time at different current intensities (400, 600, and 800 mA). The concentration of feed whey (C_{whey}) and volume (V) were fixed at $C_{\text{whey}} = 7\%$ (wt/vol) and $V = 100$ mL, respectively. The working temperatures were varied at (a) 25°C, (b) 10°C, and (c) 0°C. Error bars represent standard deviations. Color version available online.

is directly proportional to the electric current applied. On the other hand, preliminary results of pH effect on lactulose synthesis indicate that the pH 11 ± 0.3 was more suitable for lactulose synthesis, which is in accordance with the results obtained at 400 mA after 40 min of electro-activation. The same effect was also reported by other authors (Zokaee et al., 2002; Hashemi and Ashtiani, 2010). Figure 7c showed the profiles of lactulose yield obtained under different electric current applied at 0°C. The reaction rate of isomerization of lactose into lactulose was slightly lower. However, as expected, the drop of lactulose yield was slowest and less significant under these conditions. When the desired temperature range is determined, the corresponding parts of the current intensity-time profile can be used to extract the best current intensity field for isomerization of lactose into lactulose by electro-activation. In these assays (experiments), because the compromise between the highest lactulose yield and the lowest by-product was carried out with the lowest electric field applied of 400 mA, this parameter was chosen to ensure the optimal lactulose yield and to reduce the energy expenditure of the process.

Effect of the Volume Reaction

The curves described in Figure 8 show the change of the lactulose yield values as a function of the processing time with varying the volume of the reacted whey (100, 200, and 300 mL) in the cathodic compartment of the electro-activation reactor. All of the experiments were carried out at 10°C. Analysis of the obtained data showed a general trend toward an increased lactulose yield with decrease of the volume of the electro-activated whey in all cases. Moreover, when the current electric field was increased, an important increase in lactulose yield was observed at 100 mL compared with the result obtained when the higher whey volumes were used (200 and 300 mL). The ANOVA analysis showed that the effect of the volume on lactulose yield as a function of the running time was highly significant ($P < 0.0001$). As it can be seen in Figure 8a, when the cathodic compartment was filled with 100 mL of whey solution and submitted to 400 mA, the evolution of lactulose yield showed 2 distinguished zones: in the first one, lactulose yield still increased until 40 min, and in the second part, it was constant until the end of electro-activation reaction. By using the volumes of 200 and 300 mL, the rate of the electro-isomerization reaction was slower and the lactulose yield obtained was low compared with 100 mL. After 40 min of electro-activation, the lactulose yield reached the values of 34.57 ± 0.79 , 20.43 ± 0.81 , and $7.08 \pm 1.07\%$ by using 100, 200, and 300 mL of whey, respectively. The difference observed can be explained

by the fact that at any given constant electric current, an increase of the treated volume results in lower pH increase due to the similar amount of OH^- ions generated by electrolysis of water at the cathode/solution interface. Thus, the lactulose yield is directly related to the amount of hydroxyl ions formed, which play the role of proton acceptors and ensure the optimum pH for lactose isomerization into lactulose. On the other hand, according to the Nernst law, an increase in current intensity is required for decreasing the minimum voltage of water decomposition.

Figures 8b and 8c present the comparison of the values of lactulose yield with varying the volume of the electro-activated whey at 600 and 800 mA, respectively. The results showed a strong dependence on the applied current electric when the reacted volume was increased. The increasing of current electric applied resulted in a faster decomposition of water, which ensures an increasing of required alkalinity for lactulose synthesis. Highest lactulose yield was obtained when the target compartment was filled with 200 mL, reaching values of 34.85 ± 1.09 and $35.95 \pm 0.7\%$ at the end of reaction process under 600 and 800 mA, respectively. However, lactulose yield and purity obtained with increasing the whey volume and applied electric current did not differ noticeably from the corresponding values of lactulose yield obtained in 100 mL ($34.57 \pm 0.79\%$) under 400 mA electric current applied. Thus, the optimum volume in which high lactulose yield was recovered was 100 mL.

Effect of the Feed Whey Concentration

To assess the potential of the electro-activation process for eventual large scale production level, synthesis of lactulose was studied as a function of the feed solution concentrations. Having selected previously the optimum conditions for lactulose production, the effect of 3 initial concentrations of the whey [7, 14, and 28% (wt/vol)] was studied. The results showed that the conversion of lactose to lactulose is feasible using different feed concentrations under the electro-activation process. However, the lactulose yield did not increase with increasing the substrate concentration in all the cases. As shown in Figure 9a, when 400 mA of electric field was applied, the lactulose production in a 7% (wt/vol) whey solution reached the maximum of $34.57 \pm 0.79\%$ after shorter time compared with the case when higher concentration of whey was used. Under these conditions, it was necessary to increase the reaction time to reach the maximum lactulose concentration due to an incomplete reaction. Figures 9b and 9c showed that with increasing the current electric intensity, the both concentrations of 7 and 14% were suitable for lactulose production. Thus, it was possible to maintain a high

Figure 8. Profile of lactulose yield produced as a function of electro-activation time at different volume conditions (100, 200, and 300 mL). The concentration of feed whey and temperature were fixed at $C_{\text{whey}} = 7\%$ (wt/vol) and temperature = 10°C , respectively. The current intensities were varied at (a) 400 mA, (b) 600 mA, and (c) 800 mA. Error bars represent standard deviations. Color version available online.

yield of lactulose production in an average value of $36 \pm 0.54\%$. These results agree with those reported by Montilla et al. (2005) who obtained a similar percentages of conversion by using whey ultrafiltrate concentrated up to 5.2- and 7.6-fold. Further increase of the feed solution up to 28% (wt/vol) was not suitable for lactulose synthesis by using the electro-activation process. At this concentration, the rate of the reaction was the slowest despite increasing the applied current intensity, and the lactulose yield reached was lowest compared with the other concentrations. These results could be explained by the high buffering capacity of the whey solution at this concentration. This was corroborated by the lowest pH increase in the cathodic compartment when 28% whey solution was used compared with the other concentrations. Thus, it can be concluded that the suitable whey concentrations for lactulose production that can be used are 7 and 14% (wt/vol).

Proteomic Analysis of the Electro-Activated Whey

SDS-PAGE Profiles of Whey Proteins After Electro-Activation. The profile of whey proteins after electro-activation could be changed as a result of the variation of pH and the compositions of aqueous medium in the cathodic compartment of the reactor. Therefore, the changes in whey proteins during simultaneous isomerization of lactose into lactulose treated at 10°C and 400 mA were investigated by SDS-PAGE analysis under the both reducing and nonreducing conditions (Figure 10). The SDS-PAGE patterns of whey proteins after electro-activation revealed a decline tendency of the level and intensity of protein bands as the reaction time progressed. The 3 prominent native whey proteins including α -LA (14.4 kDa), β -LG (18.3 kDa), BSA (67 kDa), and a faint band of Ig fraction in the upper part of the separating gel clearly appeared in the untreated samples. After 20 min of electro-activation, the band intensity of the β -LG fraction was significantly diminished, whereas a new band with molecular weight of approximately 34 kDa appeared. The appearance of high molecular weight fractions in the protein patterns could have resulted from protein aggregates formed through disulfide linkages (Monahan et al., 1995; Lillard et al., 2009) or a conjugated protein-carbohydrate achieved by Maillard reaction products (Chevalier et al., 2001; Diftis and Kiosseoglou, 2006). To confirm whether whey proteins conjugated with the sugars present in the cathodic compartment, the SDS-PAGE pattern was investigated under reducing conditions (Figure 10b). A larger broader spectral smearing zone was still detected under the reducing conditions, but of minor intensity, which indicates that the both covalently disulfide bonds and protein cross-links with sugars occurred.

Figure 9. Profiles of lactulose yield produced as a function of electro-activation time at different whey concentrations [7, 14, and 28% (wt/vol)]. The temperature (t) and volume (V) conditions were fixed at $t = 10^\circ\text{C}$ and $V = 100\text{ mL}$, respectively. The current intensities were varied at (a) 400 mA, (b) 600 mA, and (c) 800 mA. Error bars represent standard deviations. Color version available online.

The conjugation demonstrated by SDS-PAGE was also supported by MALDI-TOF-MS analysis as discussed below. After 40 min of electro-activation, the characteristic bands gradually disappeared but some traces were still observed. Thus, the less perceptibility of the bands is interpreted by the partial hydrolysis of whey proteins to smaller products than the limit detection of the gel electrophoresis. The combined Maillard reaction products with whey proteins and hydrolysates has been previously reported (Oh et al., 2013), and the authors suggested that these products can contribute to enhance the antioxidant capacity of the product. Therefore, the changes in whey proteins after electro-activation could result in improved antioxidant functionality.

MALDI-TOF-MS Analysis of Whey Proteins After Electro-Activation

The MALDI-TOF-MS analysis is widely used to monitor the main modifications induced in whey proteins by thermal processing, including their glycation pattern in the presence of lactose and sugars (Meltretter et al., 2009; Carulli et al., 2011). Furthermore, no studies have been reported so far on applying MALDI-TOF-MS to investigate the modifications in whey proteins submitted to the electro-activation process. In this study, MALDI-TOF-MS analysis was performed for screening the structural modifications of whey proteins submitted to electro-activation in the presence of a mixture of different sugars such as lactose, lactulose, and galactose. It is well known that the interaction of reducing sugars with proteins might lead to the formation of protein bound carbonyls as early Maillard reaction products. Covalent bounds of lactose and galactose mix with proteins whey resulted in 324 and 162 Da increments (Carulli et al., 2011). The results of MALDI spectrum of the native whey proteins showed the main signals at m/z 14,189 and 18,362 Da, which corresponded to the expected mass of α -LA and β -LG, respectively. Another peak signal exhibiting mass values of 6784 Da, which could be attributed to proteose-peptone, was also detected. The profile of whey proteins electro-activated during 40 min at temperature of 10°C and current electric field of 400 mA is shown in Figure 11b. The MALDI mass spectra were characterized by a Gaussian peak shape including new peaks due the great heterogeneity of the conjugated-proteins with sugars formed as described in other work (Corzo-Martínez et al., 2008). A decrease of the signal intensity was also observed due the aggregation and partial hydrolysis of the native proteins under the alkaline conditions occurred in the cathodic compartment of the electro-activation reactor. Furthermore, the native and electro-activated whey proteins

Figure 10. Electrophoretic pattern of electro-activated whey proteins as a function of running time under nonreducing conditions (a) and reducing conditions (b). The temperature (t) and current intensity (I) were fixed at $t = 10^\circ\text{C}$ and $I = 400$ mA, respectively. Mw = molecular weight of protein standard; IgG = immunoglobulins; β -ME = β -mercaptoethanol.

in the range of 650 to 4,000 Da were also detected and analyzed (Figures 11c and 11d). Compared with the native spectra, new polypeptide peaks were assigned by their mass, which resulted from the partial whey hydrolysis as observed previously by SDS-PAGE analysis.

Total Antioxidant Capacity of Whey Proteins After Electro-Activation

The whey proteins and its hydrolysates have been shown to have antioxidant activities, which can act as free radical scavenging, metal ion chelation, and inhibition of lipid peroxidation (Hernández-Ledesma et al., 2005; Oh et al., 2013; Önay-Uçar et al., 2014). Thus, in addition to the lactose electro-isomerized into lactulose, the total antioxidant capacity (TAC) of electro-activated whey proteins and their capacity to scavenge the peroxyl radicals was measured by the ORAC assays. A significant difference was found in radical-

scavenging activity of whey as the electro-activation time increased ($P < 0.0014$). As shown in Table 1, the TAC of electro-activated whey increased gradually with increase of the reaction time, except at 20 min when a decline was observed. The decrease observed can have resulted from the polymerization of whey proteins as confirmed by the SDS-PAGE pattern (Figure 10). The β -LG in a more folded conformation, which would have prevented the hydrophobic AA residues from the free radicals, hence, reduced the ORAC values. Conway et al. (2013) also found a low capacity of whey proteins concentrate to scavenge the free radicals resulted from thermal denaturation, which induced polymerization. Compared with the control untreated whey (none electro-activated), the whey proteins after electro-activation showed an increase of the TAC up to 324 and 571% after 40 and 60 min of electro-activation, respectively. It is recognized that the antioxidant properties of whey proteins can be enhanced through its hydrolysis to peptides with relatively low molecular mass (<6 kDa) as well as an aromatic and hydrophobic free AA (Pihlanto, 2006). Thus, the potent antioxidant capacity of whey may be partly due to in situ protein hydrolysis

that occurred during electro-activation, which could have produced peptide-derived products with greater exposed ionizable and hydrophobic AA residues leading to act with the free radicals. The present results were in agreement with previous reports where the hydrolysates from the α -LA and β -LG proteins obtained by enzymatic hydrolysis showed strong antioxidant properties (Hernández-Ledesma et al., 2005). Additionally to the presence of protein hydrolysates, other compounds such as reducing sugars and polymer protein-sugars formed under Maillard reaction were also reported to improve the antioxidant action of whey proteins, especially in alkaline pH conditions (Morales and Jiménez-Pérez, 2001; Lertittikul et al., 2007). The hydroxyl groups of reducing sugar and Maillard reaction products were suggested to play an important role through the donation of hydrogen to form stable radical species (Wang et al., 2013). Although Hernández-Ledesma (2005), found that free AA such as tryptophan followed by methionine and cysteine liberating from whey protein hydrolysis have great antioxidant properties because of their capacities to donate hydrogen proton to free radicals. In our present study, the content of free AA

Figure 11. Matrix-assisted laser desorption/ionization (MALDI) mass spectra acquired in the linear mode: (a) untreated whey and (b) after 40 min of the electro-activation. The MALDI mass spectra acquired in the reflectron mode: (c) untreated sample and (d) after 40 min of electro-activation. Color version available online.

Table 1. Antioxidant capacity (in μM Trolox equivalents/L) of the control and electro-activated whey 7% (wt/vol) as a function of running time at 10°C processing temperature and 400 mA current electric field¹

Electro-activated whey (min)	Oxygen radical absorbance capacity (μmol equivalent Trolox/L)
Control	1,383.50 $\pm$ 1.82
10	1,729.90 $\pm$ 3.70
20	1,392.70 $\pm$ 1.88
30	3,055.20 $\pm$ 5.05
40	4,487.60 $\pm$ 4.37
50	4,783.90 $\pm$ 1.67
60	7,160.80 $\pm$ 3.59

¹Data represent the mean $\pm$ standard deviation of 3 replicates. Linear effect from regression model, $R^2 = (0.899)$.

in the cathodic compartment was also investigated. It was found that glutamic acid content was the major AA present followed by proline with fewer amounts of the other AA residues (results not shown). Glutamic acid was reported to act as an antioxidant compound because of its capacity to chelate metal ions (Kim et al., 2007; Salami et al., 2010). Indeed, the reduction of the availability of the other AA residues is due to their reaction with the carbonyl function of reducing sugars present in the reaction medium (Guan et al., 2010). Moreover, it is worthwhile noting that some authors investigated the radical scavenging activity of Maillard reaction products from model and food systems and concluded that the heat treatment resulted in the reduction of the antioxidant properties (Vhangani and Van Wyk, 2013). Therefore, it can be concluded that the enhancement of the antioxidant functionality of whey after electro-activation is the synergistic effect of its partial hydrolysis and generation of mixtures of bioactive compounds with a high ability to scavenge free radicals.

CONCLUSIONS

The main purpose of this work was to evaluate the dual efficiency of electro-activation technology to enhance the lactulose yield and antioxidant properties of whey cheese. The results demonstrated the usefulness of electro-activation for efficient lactose isomerization in situ of whey at low temperatures and short reaction time. In contrast to enzymatic and chemical methods, electro-activation has been proven to be a simple, clean, safe, and green technology that provides a higher lactulose yield with high purity. Whey, a low-value by-product of the cheese industry, was proven to be an excellent raw material for the production of a lactulose-rich product with high antioxidant capacity. The presence of proteins did not show an adverse effect on the isomerization of lactose to lactulose and provide

a great antioxidant capacity. The combination of whey and electro-activation technology reveals a deeper valorization for whey components such as potential lactulose and natural antioxidant. One of the intersecting opportunities was to use whey rich in lactulose in situ with great antioxidant capacity as a functional ingredient to improve the growth and survival of probiotics incorporated in several dairy products. Thus, due to its expected dual prebiotic and antioxidant properties, electro-activated whey could be constituted as a promising functional ingredient in several food formulations. In future study, research on the identification of the antioxidant compounds in whey submitted to electro-activation is needed to elucidate this characteristic.

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