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Whey permeate integral valorisation via in situ conversion of lactose into lactulose in an electro-activation reactor modulated by anion and cation exchange membranes

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ABSTRACT

Whey permeate (WP) was valorised through in situ isomerisation of lactose into lactulose by electro-activation. The effect of electric current intensity and salt ($CaCl_2$, KCl and $MgCl_2$) on the amount of lactose conversion into lactulose using WP (6%, w/w) and pure lactose (5%, w/w) solutions was studied. Lactose was converted into lactulose at a level of 35.1% when KCl at current intensity of 330 mA for 21 min of electro-activation was applied to WP; and 38.66% when KCl at current intensity of 330 mA for 14 min was applied to lactose solution. The use of WP in both the central and cathodic compartments without addition of salt yielded 39.78% lactulose after 35 min of electro-activation at 330 mA. Application of electro-activation to WP and lactose solutions enhanced product antioxidant capacity. Scanning electron microscopy and calculation of the global electrical resistance of the reactor did not reveal any membrane fouling.

1. Introduction

Whey is a co-product of cheese and casein manufacturing, and it represents about 85% of the total milk volume used in these processes (Panesar & Kennedy, 2012; Parashar, Jin, Mason, Chae, & Bressler, 2016; Smithers, 2008). On a dry basis, whey is composed of 75% lactose and 20% proteins. The high lactose content of whey and whey permeate is primarily responsible for the high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of these secondary dairy residues with values of 30,000–50,000 and 60,000–80,000 mg L^{-1} O_2, respectively (Siso, 1996).

Whey proteins are separated from whey by ultrafiltration and the secondary co-product of this technology is the whey permeate (WP), which is mainly composed of lactose (4–5%) and minerals (0.6–0.8%) (Coté, Brown, Cameron, & Van Walsum, 2004). WP has very limited applications, and a major part produced worldwide is discarded as a dairy effluent with serious environmental impacts (Parashar et al., 2016). WP produced by ultrafiltration has a biological oxygen demand of 30,000–45,000 mg L^{-1} O_2 and cannot, therefore, be directly discharged into sewage as wastewater (Puhan & Gallmann, 1981; Qureshi & Manderson, 1995). Thus, a safe way to valorise WP into value-added ingredients is of great interest; indeed, its high lactose content can be used as a cheap source for synthesis of non-absorbable lactose-derivatives such as lactulose, lactitol and lactobionic acid (Paseephol, Small, & Sherkat, 2008).

Lactulose (4-O-β-D-galactopyranosyl-D-fructose) is a disaccharide with proven prebiotic properties, and is composed of fructose and galactose moieties linked by a β(1 → 4) glycosidic linkage. It is a white powder, odourless with a sweet taste of 0.6–0.8 compared with that of sucrose (Aït-Aissa & Aïder, 2014a). Lactulose has a wide range of applications with a great economic importance. It has been used in medicine for the treatment of hepatic encephalopathy since 1966 (Morgan & Hawley, 1987) and for constipation, and it figures on the World Health Organisation Model List of Essential Medicines (WHO, 2017), which is a list of the most important drugs needed in a basic health system (Nagasawa, Sato, & Kasumi, 2017). In the food industry, it can be used as a bifidus-factor, as sweetener for diabetics, and an ingredient in dairy products, cookies and chocolate (Schumann, 2002).

At an industrial scale, lactulose is produced by chemical isomerisation of lactose in alkaline media according to the Lobry de Bruyn-Alberda van Ekenstein (LBAE) transformation (Aider & de

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Halleux, 2007). The process is relatively expensive because of the low reaction yield (20–30%) and a high cost of the purification steps involved; galactose, iso-saccharinic acid and coloured products resulting from the degradation of lactose and lactulose are present in the reaction products (Villamiel, Corzo, Foda, Montes, & Olano, 2002).

The enzymatic synthesis of lactulose is feasible by trans-galactosylation of glucose to fructose using specific enzymes, such as β-galactosidase from *Sulfolobus solfataricus* (Kim, Park, & Oh, 2006) or hyperthermostable β-glycosidase from *Pyrococcus furiosus* (Mayer, Kranz, & Fischer, 2010). The combination of enzymatic and chemical catalysis was applied using cellobiose 2-epimerase from *Caldicellulosiruptor saccharolyticus* in the presence of borate and a yield of 88% of lactulose was achieved (Kim, Kim, & Oh, 2013). However, the high cost of the enzymes used and the difficulty of enzyme recovery render this approach costly at a large industrial scale. Furthermore, the enzyme activity decreases after some time of application (Aït-Aissa & Aïder, 2014a). In addition, lactulose can also act as substrate for α-galactosidase and the lactulose hydrolysis can prevail over the lactulose synthesis, which negatively influence the reaction yield (Silvério, Macedo, Teixeira, & Rodrigues, 2016).

Recently, Aider and Gimenez-Vidal (2012) successfully isomerised lactose into lactulose without added reagents using electro-activation (EA) technology. This technology is based on the use of an electrochemical reactor composed of three compartments separated by cation and anion exchange membranes to maintain high alkaline pH in the cathodic compartment. This is possible using a cationic exchange membrane (CEM) as a separator between the cathodic and central compartments, and an anion exchange membrane (AEM) to separate the central compartment from the anodic one. In addition, Kareb, Champagne, and Aïder (2016) used a similar electro-activation reactor configuration and was able to isomerise lactose in situ in whey into lactulose with a 35% conversion yield; under the conditions applied, it was possible to obtain and maintain pH 11–12 in the cathodic compartment of the reactor. In this context, we demonstrated using sweet whey that electro-activation yielded electro-activated whey that has high antioxidant capacity. This was correlated with Maillard reaction products resulted from the interaction between the reducing sugars and amino groups in the electro-activated sweet whey (Kareb, Champagne, Jean, Gomaa, & Aïder, 2018; Kareb, Gomaa, Champagne, Jean, & Aïder, 2017; Kareb et al., 2016).

The objective of the present work was to achieve the electro-isomerisation of lactose into lactulose directly in situ of whey permeate (WP) using electro-activation (EA) technology with a view to obtain a new high value-added prebiotic with antioxidant properties. Therefore, the effect of the electro-activation reactor configuration, electric current intensity, type of added electrolyte and running time was studied in regard of the lactose conversion yield to lactulose and the amount of the side reaction products formed. In addition, the antioxidant activity of the electro-activated WP was evaluated using the ABTS• and DPPH• radical tests.

2. Materials and methods

2.1. Chemicals and materials

All chemicals (purity ≥ 95%) were of analytical or high performance liquid chromatography (HPLC) grade. Lactose, lactulose, glucose, galactose, and fructose (HPLC grade) were purchased from Sigma–Aldrich (Ottawa, Ontario, Canada); Na_2SO_4 was purchased from Anachemia (Montreal, Quebec, Canada); $CaCl_2$, KCl and $MgCl_2$ were from VWR International (Radnor, PA, USA). The whey permeate (WP) powder used in this study was purchased from Agropur Cooperative (Longueil, Quebec, Canada). Its main components (%) were: 85 ± 0.12 total sugars, 1.93 ± 0.24 total proteins, 6.5 ± 0.11 ash, 6.4 ± 0.13 residual humidity, other components 0.07 ± 0.01. All the solutions were prepared in deionised water. The CMI-7000S cation exchange membrane (CEM) and the AMI-7001S anion exchange membrane (AEM) were purchased from Membranes International Inc. (Ringwood, NJ, USA).

2.2. Electro-activation reactor

The EA reactor used in this study is shown in Fig. 1. It is composed of three compartments made with Plexiglas disposed in the following configuration: anodic compartment, central compartment and cathodic compartment. The anodic compartment is connected to the positive side of the electric current generator (model CSI 12001X, Circuit Specialists; AR, USA) by means of a ruthenium–iridium coated titanium (RuO_2–IrO_2–TiO_2) electrode, whereas the cathodic one was connected to the negative side using a food grade stainless steel electrode with an active area of 50 cm^2. These two compartments are separated from each other by the central one that communicates with the anodic and cathodic compartments via a cation (CEM) and anion (AEM) exchange membrane, respectively, as shown in Fig. 1 (Aider & Gimenez-Vidal, 2012; Aït-Aissa & Aïder, 2014b; Kareb et al., 2016). This configuration of the EA reactor allows high alkaline conditions to be obtained at the cathodic side; a necessary condition for lactose isomerisation into lactulose (Aider & Gimenez-Vidal, 2012).

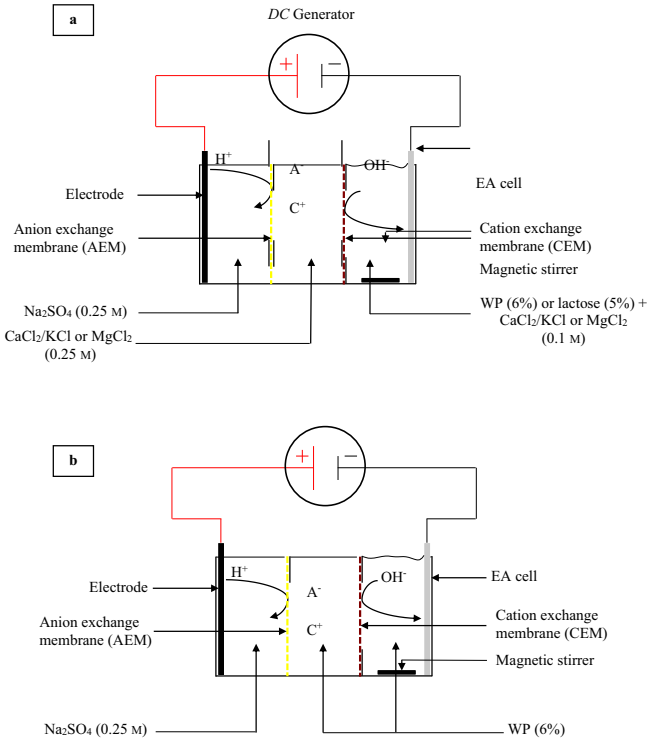

Fig. 1. Schematic representation of the electro-activation (EA) reactor used to isomerize lactose into lactulose: (a) with whey permeate (WP) (6%, w/w) in configuration 1 and lactose (5%, w/w) in configuration 2; (b) with whey permeate (WP) (6%, w/w) in configuration 3.

2.3. Protocol of electro-isomerisation

Three electro-activation reactors configurations were used to achieve the electro-isomerisation of lactose into lactulose. In configuration 1 the anode (+) compartment was filled with 0.25 M Na_2SO_4, the central compartment with 0.1 M $CaCl_2$, KCl or $MgCl_2$ and the cathode (−) compartment with 6% WP + 0.1 M $CaCl_2$, KCl or $MgCl_2$. In configuration 2 the anode (+) compartment was filled with 0.25 M Na_2SO_4, the central compartment with 0.1 M $CaCl_2$, KCl or $MgCl_2$ and the cathode (−) compartment with 5% lactose + 0.1 M $CaCl_2$, KCl or $MgCl_2$. In configuration 3 the anode (+) compartment was filled with 0.25 M Na_2SO_4, and the central and the cathode (−) compartments with 6% WP.

A freshly prepared 6% WP solution or 5% lactose solution was added with salts (0.1 M $CaCl_2$, KCl or $MgCl_2$) and introduced in the cathodic compartment while the central compartment was filled with the corresponding salt solution at 0.1 M (Configurations 1 and 2). In addition, the test consisting of using 6% WP in both the central and cathodic compartments without addition of salt (Configuration 3) was investigated. In all experiments and reactor configurations, the anodic compartment was filled with 0.25 M Na_2SO_4 solution. A current intensity of 110, 220 and 330 mA, equivalent to 2.2, 4.4 and 6.6 mA cm^{-2} of field density, respectively, was applied for 63 min. Sampling from the cathodic compartment of the EA reactor was carried out every 7 min and the pH was immediately measured using a pH meter (Model OAKTON pH 700; EUTECH Instruments, Vernon Hills, IL, USA). The corresponding voltage (V) was also read directly from the electric current generator. All the experiments were carried out in triplicate. The samples were kept at 4–6 °C until analysis.

2.4. Selectivity of the electro-activation process

The selectivity of the electro-isomerisation of lactose into lactulose was determined as type and rate of the by-products formed in the reaction medium. For this, freshly prepared sugar solution (2.5%) (glucose, galactose, fructose, lactulose and lactose) with added salts (0.1 M KCl) was introduced in the cathodic compartment of the EA reactor, while the same salt solution (0.1 M) filled the central compartment. Na_2SO_4 (0.25 M) was maintained in the anodic compartment in all cases. The configuration for this purpose was: anode (+) compartment filled with 0.25 M Na_2SO_4, central compartment filled with 0.1 M KCl and cathode (−) compartment filled with the sugar studied + 0.1 M KCl.

The current intensity was maintained at 330 mA and was applied for 63 min. Samples from the cathodic compartment of the EA reactor were collected every 7 min. The solution pH and sugar content were determined (the latter using HPLC) in each sample. All the experiments were made in triplicate and the samples were kept at 4–6 °C until analysis.

2.5. Global system resistance of the reactor

The global system resistance, R (Ω), of the EA reactor was calculated from the values of the applied current intensity I (A) and the corresponding voltage U (V) using the Ohm's law as follows (Eq. (1)):

$$R = \frac{U}{I} \tag{1}$$

2.6. Energy consumption of the reactor

The energy consumption was calculated according to Eq. (2) (Cifuentes-Araya, Pourcelly, & Bazinet, 2011):

$$EC = \int I(t)U(t)dt \tag{2}$$

where U(t) and I(t) are the voltage (V) and the intensity (A) as a function of time while *dt* is the time variation. EC was expressed in Watts per hour (W h^{-1}).

2.7. Scanning electron microscopy/energy dispersive X-ray spectroscopy

The membrane integrity and eventual fouling were evaluated by the method used by Aït-Aissa and Aïder (2014b). Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) analyses were used. The SEM images of the membranes were taken with an electron microscope (JSM-840A; Joel, Portland, OR, USA) equipped with an EDS spectrometer, PGT Instrument, model Avalon (Princeton, NJ, USA). With a view to make the surface of membranes samples conductive and to allow the free flow of the excess electrons, the membrane samples were primary metallised by coating with a thin gold/palladium layer. The EDS condition was set at 15 kV and the magnifications of 100 and 500 μm were used.

2.8. Determination of sugars composition of the electro-activated WP

Samples (electro-activated WP from configurations 1 and 3, electro-activated lactose from configuration 2) at 7 min intervals were quantified using a Waters HPLC system (Millipore Corp., Milford, MA, USA) equipped with a refractive index detector (Hitachi model L-7490). The column (Waters Sugar Pak-I, 6.5 × 300 mm, Waters) was maintained at 90 °C. The isocratic mobile phase consisting of a solution of 50 mg L^{-1} ethylenediamine tetra-acetic acid was used at a flow rate of 0.5 mL min^{-1}. The injection volume was 50 μL and the running time was set at 30 min per sample. The identification and quantification of mono- and disaccharides was done by comparison of their retention times against standard solutions of lactose, lactulose, glucose, galactose and fructose using a standard curve of each sugar made with solutions concentrations of 1, 0.75, 0.50, 0.250 and 0.125% (w/v). The R^2 of each curve is greater or equal to 0.9999.

2.9. Antioxidant activity

The antioxidant activity (AA) was investigated on the samples with the best lactose conversion yield into lactulose. The AA of the electro-activated whey permeate (EAWP) under configuration 1 and 3 and electro-activated lactose (EALac) under configuration 2 were determined using two methods: $ABTS^{\bullet}$ and $DPPH^{\bullet}$ radical scavenging capacity.

2.9.1. ABTS radical scavenging activity

Evaluation of 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\bullet+}$) radical scavenging activity was based on the ability of antioxidants to inhibit long-life ABTS radical cation, a blue/green chromophore with characteristic absorption at 734 nm, in comparison with that of Trolox. The scavenging of $ABTS^{\bullet+}$ is assumed an electron transfer process (Li, Wang, Chen, & Chen, 2011) (Eq. (3)):

$$ABTS^{\bullet+} + e^{-} \rightarrow ABTS \tag{3}$$

The procedure used in the present work was that described by Re et al. (1999).

2.9.2. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity of the EAWP and EALac was evaluated by the method adapted from the study of Kareb et al. (2018), as follows: An aliquot of each sample (250 μL) was added to 1 mL 0.1 mM DPPH solution freshly prepared in methanol. The reaction mixture was then vortexed and kept in the dark at a temperature of 22 ± 2 °C for 30 min. The decrease of the absorption was measured at 517 nm using a spectrophotometer. The control consisted of a mixture of methanol and DPPH solution (Kareb et al., 2017). The percentage of DPPH radical scavenging activity (%) was calculated by the following equation (Eq. (4)):

$$RSA(\%) = \frac{A_c - A_s}{A_c} \cdot 100 \quad (4)$$

where *RSA* is the radical scavenging activity (%), $A_{Control}$ is the absorbance of the control (containing all reagents except the tested sample at $t = 0$ min), and A_{Sample} is the absorbance of the tested EAWP and EALac solution ($t = 30$ min).

2.9.3. Evaluation of Maillard reaction products and browning index

The quantity of the intermediate and final Maillard reaction products generated during the EA of WP in configurations 1 and 2 was estimated by measuring the absorbance at 294 and 420 nm, respectively (Kareb et al., 2017), while the browning index of the EALac-configuration 2 was measured at 420 nm.

2.10. Statistical analysis

Statistical analysis was performed using a full factorial experimental design with repeated measurements (time). The independent variables were the following: applied current intensity at 3 levels (110, 220 and 330 mA), type of salt used at 3 levels ($CaCl_2$, KCl and $MgCl_2$) and reactor configuration set at 3 levels (1, 2 and 3). The dependent variables studied were the pH of medium, lactulose yield synthesised, as well as yield of the side reaction (galactose) that occurred in the cathodic compartment of the EA compartment. Each treatment was carried out in triplicate, and mean values ± standard deviation were used. Differences at $p < 0.05$ were considered to be significant. Analysis of variance (ANOVA) of the data was performed using SAS software (V9.3; SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. pH evolution during electro-activation process

The evolution of the pH versus time in the solutions of WP, lactose and WP without added salt (configuration 3) during electro-activation at different current intensities (110, 220 and 330 mA) in the cathodic compartment is shown in Fig. 2. It appears clearly that the current intensity and the type of salt added were the key factors that influenced the alkalinity of the medium in the cathodic compartment. From the curves in Fig. 2a–c, it can be seen that by increasing of the current intensity from 110 to 330 mA, a significant increase ($p < 0.001$) of the pH of the catholyte solution was observed whatever the salt added to the electro-activated solution. Furthermore, it is known that by increasing the electric current intensity, the flow of electrons migrating through the electrochemical reactor is higher and, consequently, induces a higher dissociation amount of water molecules at the electrode/solution interface (Fiegenbaum, Martini, de Souza, Becker, & de Souza, 2013). Consequently, an increase of the concentration of OH^- ions is obtained that leads to the elevation of the solution alkalinity, according to the following reaction (Eq. (5)):

$$\text{Cathode (reduction reaction): } 2H_2O + 2\,e^- \rightarrow H_2\,(g) + 2OH^-\,(aq) \quad (5)$$

The hydroxyl ions act as proton acceptors (Bologa, Sprinchan, & Bologa, 2008) when the isomerisation of lactose into lactulose proceeds by the LBAE transformation which takes place via an enolisation pathway (Speck, 1958). On the other hand, in the anodic side of the reactor an oxidation reaction of water takes place allowing the increase of the H^+ ions inducing lowering of the pH medium according to following reaction (Eq. (6)):

$$\text{Anode (oxidation) } 2H_2O \rightarrow O_2\,(g) + 4H^+ + 4\,e^- \quad (6)$$

However, the use of an anion exchange membrane between the anodic and central compartments avoids the inference of this acidity with the alkalinity generated in the cathodic compartment that is separated from the central compartment by a negatively charged cation exchange membrane. The highest pH values for lactose isomerisation into lactulose under the EA conditions in this study were 11.52, 11.59 and 11.51 for WP-KCl (configuration 1), Lac-KCl (configuration 2) and WP without added salt (configuration 3), respectively, at 330 mA current intensity. The results obtained are in good agreement with those reported by Kareb et al. (2016), who used EA technology with sweet whey as substrate of the electro-activation reaction to give an electro-isomerisation yield of ≅ 35% (Kareb et al., 2016). The results we obtained in the present study were also in good agreement with those reported for chemical isomerisation (≅ 30%) using boric acid with trimethylamine as catalyst (Hicks & Parrish, 1980). However, our results are slightly lower than those reported by Wang, Gasmalla, Tessema, Hua, and Yang (2017) who used sodium aluminate as chemical alkali, obtained pH 12 and an isomerisation yield varying between 73.87 ± 0.50 and 80.44 ± 1.05%.

During lactose isomerisation into lactulose, some authors reported that this latter (lactulose) undergo degradation into galactose, and acidic compounds such as saccharinic and formic acids (Dendene, Guihard, Nicolas, & Bariou, 1994) causing the lowering of the pH medium (Paseephol et al., 2008). In our work, this phenomenon did not occur and the curves of pH versus time followed a two-stage evolution, as previously reported in the literature (Aït-Aissa & Aïder, 2014c; Kareb et al., 2016). The first stage consists of a drastic increase of the pH (0–21 min) and the second stage was characterised by a slight pH increase until the end of the reaction process (21–63 min). In the first stage, a high level of OH^- was generated following intensive water electrolysis, whereas in the second stage, the solution becomes saturated with OH^- with a lower rate (intensity) of pH increase.

In configuration 1, the pH reached values greater than 10 after 14 min of EA in the case of $CaCl_2$ and KCl as electrolyte at 330 mA current intensity (Fig. 2a–c). The pH increased gradually to reach 11.12, 11.72, 12.02 and 11.55, 12.21, 12.61 in the case of $CaCl_2$ and KCl as electrolyte at 110, 220 and 330 mA, respectively, while in the case of $MgCl_2$ as electrolyte, the pH of the catholyte did not exceed 10 whatever the current intensity applied during all the process of electro-activation.

The comparison between salt effectiveness indicated that KCl was the best salt that contributed to rapid increase of the pH and, thus, created high alkalinity of the medium. At the same time, the data obtained showed that $MgCl_2$ was inefficient in terms of its impact on pH evolution in the cathodic compartment (pH did not exceed 10 regardless the applied current intensity). Indeed, the classification of the pH of the catholyte at any time gave the

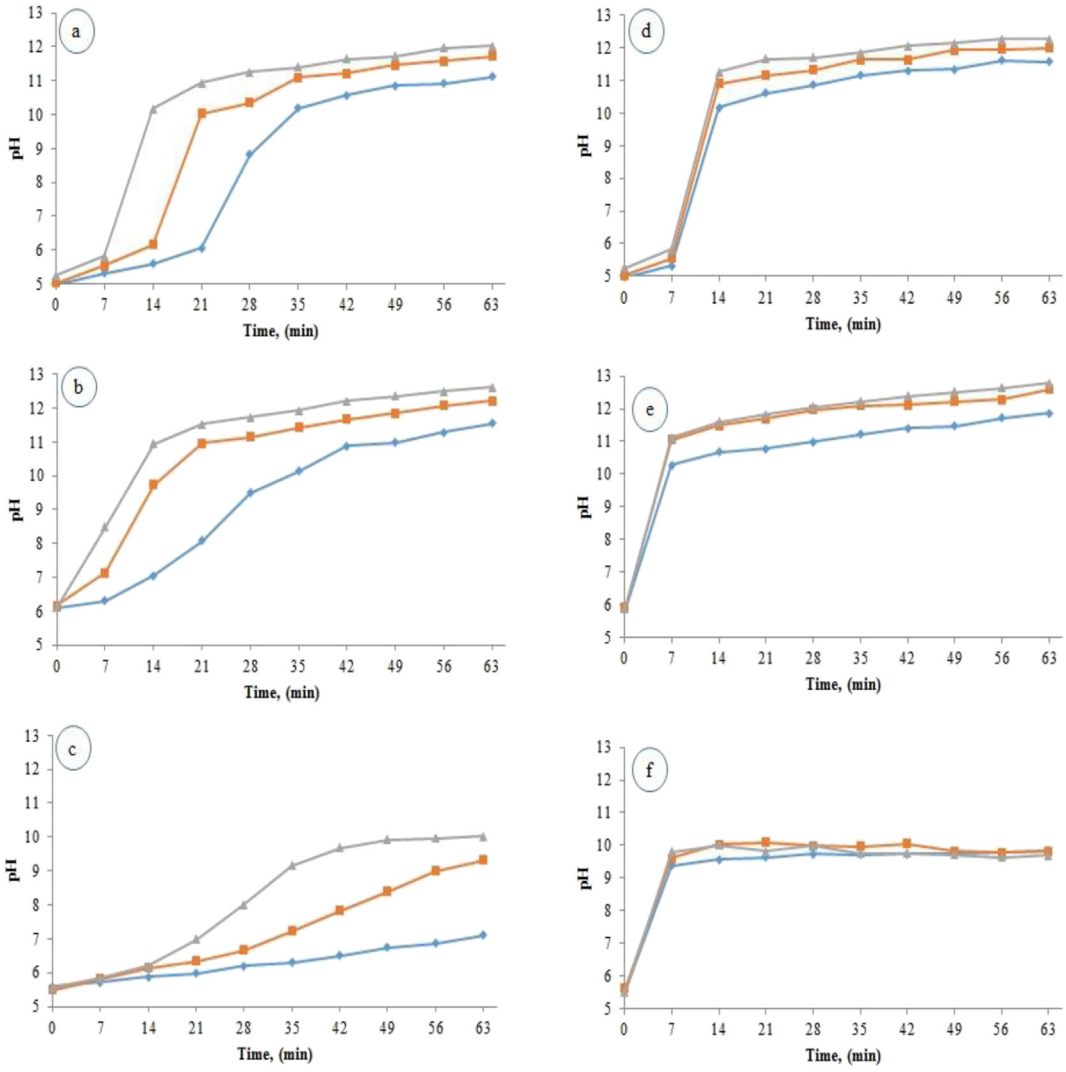

Fig. 2. pH evolution as a function of electro-activation time of WP (6%) (a–c) and lactose solution (5%) (e–f) under different current intensities (◆, 110; ■, 220; ▲, 330 mA) and salt type: (a, d) $CaCl_2$, (b, e) KCl and (c, f) $MgCl_2$.

following order [salt, I (mA)]: KCl, 330 > KCl, 220 > $CaCl_2$, 330 > $CaCl_2$, 220 > KCl 110 > $CaCl_2$, 110 > $MgCl_2$, 330 > $MgCl_2$, 220 > $MgCl_2$, 110. A significant difference ($p < 0.001$) was observed between the pH generated by KCl (330 mA) and all the other salts and current intensities. On the other hand, no significant difference ($p > 0.05$) was observed between the pH values obtained when $CaCl_2$ (330 mA) and KCl (220 mA) were added.

In the case of pure lactose as feed solution in the EA reactor (configuration 2), the pH of the medium reached a value greater than 10 after 7 min regardless of the applied current intensity when KCl was used as electrolyte (Fig. 2d–f). The alkalinisation of the medium in this case was faster than that of WP. This difference can be explained by the buffering effect of the proteins and/or other miscellaneous constituents contained in WP, which slowed down a pH change of the medium, in contrast to pure lactose. This phenomenon was also observed previously for milk concentrate permeate (Paseephol et al., 2008). When $CaCl_2$ was used as electrolyte, the pH of the medium reached a value greater than 10 only when the applied current intensities of 220 and 330 mA were applied. As in the case of WP, $MgCl_2$ was the inefficient electrolyte in terms of generating high alkalinity in the cathodic side of the EA reactor when pure lactose was used as feed solution. Indeed, the pH of the medium did not reach 10 when 110, 220 and 330 mA current intensities were applied. Moreover, a slight decrease of the pH was observed during the process, in particular when 220 and 330 mA current intensity were applied. This decrease of pH may be due to the acidification of the reactional medium caused by the competition of the reaction of water dissociation and $Mg(OH)_2$ formation at the membrane-solution interface (reactions 7, 8).

It is well known that WP is rich in minerals (salts) ($\approx$0.6%). This characteristic can be used as an advantage in the proposed approach of the present work by discarding the use of salts as electrolyte in the two compartments (central and cathodic) of the EA reactor. Configuration 3 was proposed and investigated to use WP as both a substrate for electro-activation and an electrolyte in the central compartment without addition of any salt. Fig. 3 shows the evolution of pH versus time of the WP without any salt added in the cathodic compartment; when current intensities of 220 and 330 mA were applied, the pH of the reactional medium exceeded 10 at after 14 and 21 min of electro-activation, respectively. The final pH at 63 min reached values of 11.74 and 11.62 when 220 and 330 mA current intensities were applied, respectively, without any

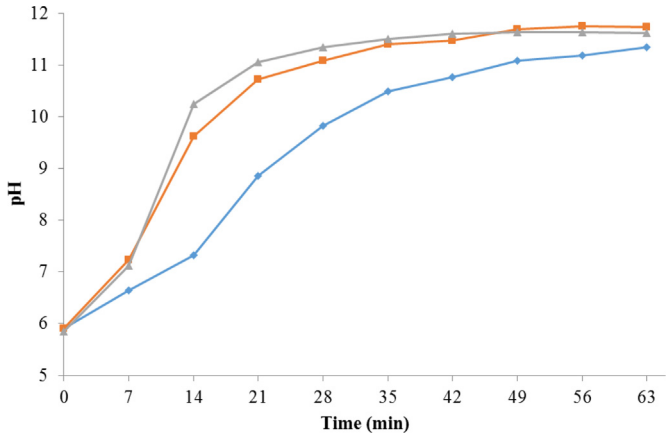

Fig. 3. pH evolution as a function of electro-activation time of WP (6%) without added salts (configuration 3) under different current intensities (◆, 110; ■, 220; ▲, 330 mA).

significant difference ($p > 0.05$) between the effect of these two currents intensities. Once current intensity of 110 mA was applied, the catholyte reached a pH greater than 10 at 35 min of electro-activation and the final pH obtained (at 63 min) was 11.36. The results obtained with configuration 1 showed a significant difference ($p < 0.001$) of pH when KCl and I = 330 mA were applied in comparison with the three current intensities applied when configuration 3 was used, but no significant difference ($p > 0.05$) was observed between WP without added salts (I = 330 mA) and $CaCl_2$ (I = 330 and 220 mA) in the case of configuration 1. Therefore, using WP without added salt as electrolyte was equivalent to the effect obtained with $CaCl_2$ and better than that obtained with $MgCl_2$ in terms of pH of the catholyte.

3.2. Global electrical resistance and energy consumption

The electrical resistance results in dissipation of energy in the form of heat. This property is called the Joule effect. In the present study, it is considered as undesirable since the reactor solutions must be conductive of the current to allow a greater water dissociation and create high alkalinity in the cathodic side of the EA reactor (Eq. (5)). In membrane process, the global electrical resistance is due to the intrinsic resistance of the membranes, the resistance of the treated solutions and the presence of fouling (Cifuentes-Araya et al., 2011; Lee, Moon, & Tsai, 2002). The global electrical resistance (R) of the reactor as a function of time in configurations 1 and 2 was constant except when $CaCl_2$ at 110 mA current intensity was used in configuration 1. In addition, it decreased with the increase of the current intensity and varied as a function of the salt used as electrolyte. The classification of the global electric resistance (R) when WP was used as substrate (configuration 1) is as follows [salt, I (mA)]: KCl, 330 < $CaCl_2$, 330 < $MgCl_2$, 330 < KCl, 220 < $CaCl_2$, 220 ≈ $MgCl_2$, 220 < KCl, 110 < $MgCl_2$, 110 < $CaCl_2$, 110. Thus, it can be concluded that KCl at 330 mA applied electric current provided the lowest global resistance while $CaCl_2$ at I = 110 mA provided the highest.

When lactose was the feed solution in the cathodic compartment (configuration 2), the classification of the global electric resistance of the reactor (R) is as follows [salt, I (mA)]: KCl, 330 < $MgCl_2$, 330 < $CaCl_2$, 330 < KCl, 220 < $MgCl_2$, 220 < $CaCl_2$, 220 < $MgCl_2$, 110 < KCl, 110 < $CaCl_2$, 110. As in configuration 1, KCl (I = 330 mA) provided the lowest global resistance and $CaCl_2$ (I = 110 mA) the highest. In contrast to configurations 1 and 2, the global resistance of the reactor in configuration 3 was high from the beginning of the process and increased as a function of running time. From 0 to 35 min, the classification of the global resistance is (I, mA): 110 > 220 > 330 and from 35 to 63 min the classification is 220 > 110 > 330. As in configurations 1 and 2, the highest applied current intensity of 330 mA gave the lowest global resistance of the reactor.

According to these results, we can conclude that the three configurations investigated are conductors of electricity at high current intensity. This characteristic allowed generation of a higher pH level in the cathodic side that was needed to isomerise lactose into lactulose with good energy efficiency. Comparing the global electrical resistance of the three configurations, there is a significant difference ($p < 0.001$) between configurations 1 and 2 compared with configuration 3. Indeed, configuration 3 has a mean global resistance 4.5-fold higher than those of configurations 1 and 2. This difference can be explained in part by the resistance of WP solution present in the central and cathodic compartment in contrast to configurations 1 and 2 because of the higher content of electrolyte than configuration 3. Furthermore, the cations of WP in the central compartment migrated through the CEM and the anions migrated toward the anode cross the AEM. This migration phenomenon demineralised the central compartment and decreased its conductivity. Consequently, an increase of the resistance of the system was observed.

Energy consumption (EC) of the proposed configuration was estimated and the obtained results showed that the EC of configuration 3 was greater than that of configurations 1 and 2. This observation confirms the positive effect of addition of electrolyte in the central and cathodic compartments in terms of electric current conductivity. Moreover, the influence of salts was highlighted and a significant difference ($p < 0.001$) between the three studied salts was observed. Indeed, KCl provided the lowest EC than $CaCl_2$ and $MgCl_2$ whatever the applied current intensity in configurations 1 and 2. Furthermore, no significant difference ($p > 0.05$) was observed between configurations 1 and 2 in terms of EC when KCl was used as electrolyte at the three applied current intensities.

3.3. HPLC sugar analysis

With a view to optimise the isomerisation process of lactose into lactulose, three salts ($CaCl_2$, KCl and $MgCl_2$), three intensities (110, 220 and 330 mA) and three configurations were investigated. The results of the isomerisation yield of lactose into lactulose versus running time in the cathodic compartment of the EA reactor (configuration 1 and 2) are summarised in the Fig. 4a while those of configuration 3 in Fig. 4b. As it can be observed, the reactor configuration, current intensity, type of salt added and running time had a significant effect ($p < 0.001$) on the rate (yield) of lactose conversion into lactulose. Moreover, the results obtained showed that only galactose was generated as a side reaction product; no fructose, glucose, tagatose and epilactose as impurities were found in the reaction medium.

In the chemical based process, the conversion of lactose into lactulose in an alkaline medium is generally followed by a rapid degradation of lactulose into galactose, tagatose, epilactose and formic acid (Dendene et al., 1994; Montilla, Del Castillo, Sanz, & Olano, 2005; Olano & Martinez-Castro, 1981; Schuster-Wolff-Bühring, Fischer, & Hinrichs, 2010). The results we obtained in the present study were in good agreement with those previously reported (Aït-Aissa & Aïder, 2014b,c; Kareb et al., 2016). Furthermore, the generation of galactose increased linearly with the amount of lactulose until the end of the process of electro-activation.

In commercial lactulose (as syrup), the maximum levels of other sugars tolerated by the US pharmacopeia are 12% lactose, 16%

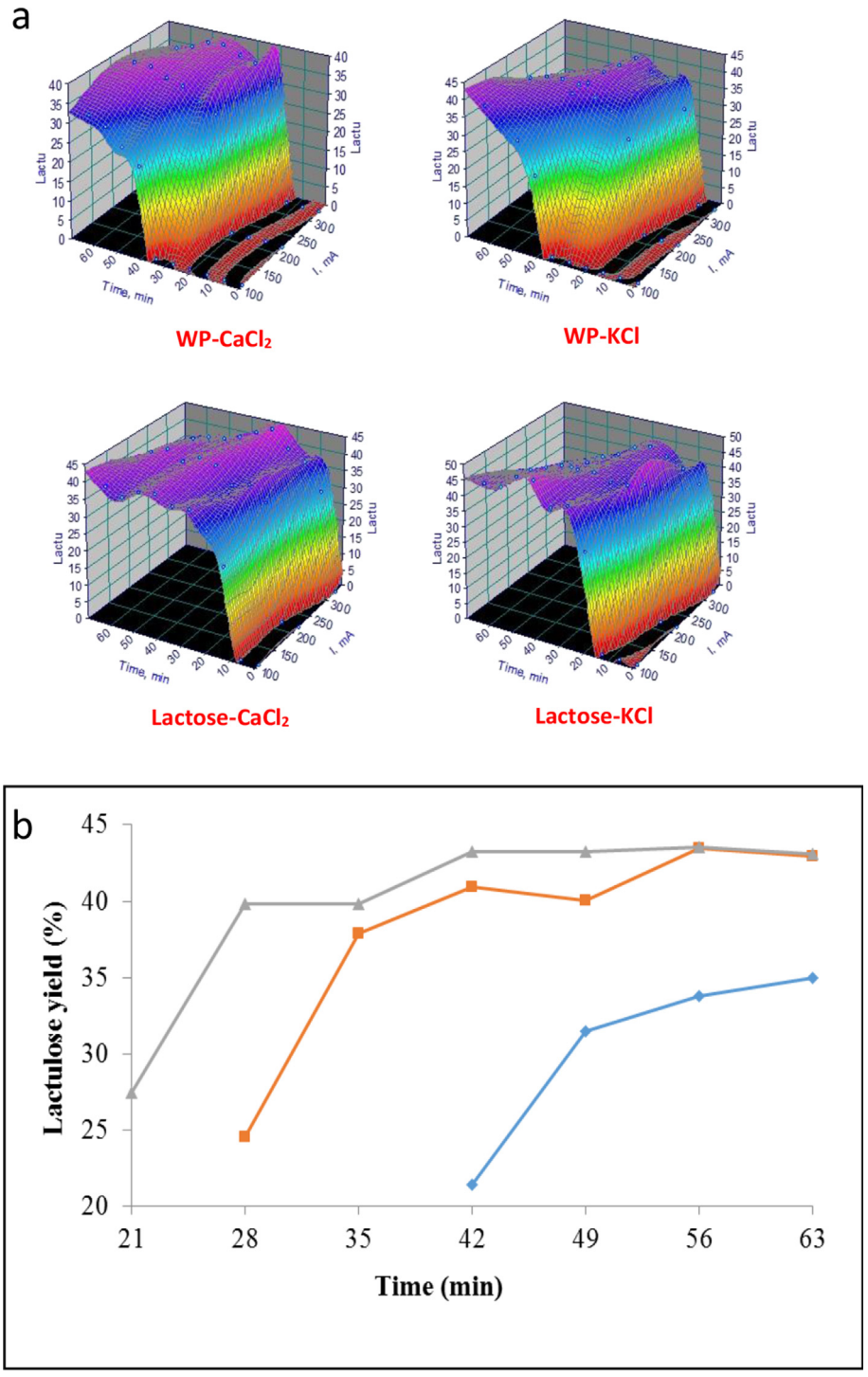

Fig. 4. Evolution of the lactulose yield as a function of the electro-activation time time and current intensity: (a) WP (6%) –configuration 1 with lactose (5%) –configuration 2 and (b) WP (6%) –configuration 3 (◆, 110; ■, 220; ▲, 330 mA).

galactose, 8% epilactose and 1% fructose (Nelofar, Laghari, & Yasmin, 2010). Regarding these specifications, the results obtained in the present study are very promising, since only galactose was detected by HPLC as by-product at low concentration. Furthermore, the remaining lactose can be easily eliminated from the reactional mixture by crystallisation. Moreover, to avoid any possible negative effect of the alkaline pH during the crystallisation process of lactose, the pH of the electro-activated solution can be easily lowered down to pH 7 without any impact of the qualitative and quantitative composition of the electro-activated whey permeate solution (Panesar & Kumari, 2011). Although the effect of temperature has not been the subject of this study, it seems to be very important to point out that the synthesis of lactulose without heat is possibly contrary to what has been reported in the literature where temperatures ranging from 50 to 130 °C were used in the process involving the use of chemical alkalinisation for lactose conversion into lactulose (Hashemi & Ashtiani, 2010; Zokaee, Kaghazchi, Zare, & Soleimani, 2002). Finally, it can be concluded

Table 1
Galactose formation (%) and galactose/lactulose ratio in the EAWP according to reactor configuration 1.[a]

Type of salt added	Time (min)	Galactose (%)			Galactose/lactulose ratio		
		110 mA	220 mA	330 mA	110 mA	220 mA	330 mA
$CaCl_2$	7	0	0	0	/	/	/
	14	0	0	0	/	/	/
	21	0	0	5.36	/	/	0.17
	28	0	2.41	8.15	/	0.08	0.23
	35	0	6.82	12.47	/	0.21	0.34
	42	1.96	7.22	18.68	0.09	0.21	0.48
	49	2.65	11.49	23.09	0.10	0.32	0.60
	56	2.63	15.4	28.38	0.09	0.40	0.78
	63	5.88	20.51	30.56	0.18	0.53	0.87
KCl	7	0	0	0	/	/	/
	14	0	2.06	0	/	0	0
	21	0	3.33	7.88	/	0.12	0.22
	28	0	4.82	11.51	/	0.14	0.31
	35	0	6.91	16.29	/	0.19	0.41
	42	2.68	9.58	22.28	0.12	0.24	0.61
	49	4.06	13.25	26.72	0.13	0.33	0.79
	56	4.79	17.27	31.4	0.14	0.44	1.07
	63	6.14	20.43	37.06	0.16	0.53	1.49

[a] With $MgCl_2$, there was no lactulose and galactose formation.

Table 2
Galactose formation (%) and galactose/lactulose ratio in the EAWP according to reactor configuration 2.[a]

Type of salt added	Time (min)	Galactose (%)			Galactose/lactulose ratio		
		110 mA	220 mA	330 mA	110 mA	220 mA	330 mA
$CaCl_2$	7	0	0	0	/	/	0
	14	0	0	7.44	0	0	0.20
	21	0	6.98	13.09	0	0.18	0.33
	28	3.81	9.74	15.51	0.10	0.27	0.35
	35	0	14.34	21.24	0.00	0.35	0.50
	42	7.32	15.28	25.62	0.18	0.36	0.64
	49	9.44	18.57	28.22	0.24	0.46	0.73
	56	11.95	22.06	30.91	0.32	0.52	0.81
	63	13.88	24.15	33.63	0.35	0.59	0.92
KCl	7	0	0	0	/	/	0
	14	0	0	7.23	/	0	0.19
	21	0	7.62	12.08	0	0.18	0.29
	28	0	9.75	18.67	0	0.24	0.43
	35	0	13.09	22.66	0	0.30	0.54
	42	6.27	17.38	26.44	0.13	0.41	0.68
	49	8.12	20.27	30.39	0.17	0.47	0.83
	56	10.04	23.38	34.3	0.23	0.57	1.03
	63	12.73	26.78	36.36	0.29	0.68	1.19

[a] With $MgCl_2$, there was no lactulose and galactose formation.

Table 3
Galactose formation (%) and galactose/lactulose ratio in the EAWP according to reactor configuration 3.

Time (min)	Galactose (%)			Galactose/lactulose ratio		
	110 mA	220 mA	330 mA	110 mA	220 mA	330 mA
7	0	0	0	/	/	/
14	0	0	0	/	/	/
21	0	0	0	/	/	0.00
28	0	0	7.1	/	0.00	0.18
35	0	5.8	9.27	/	0.15	0.23
42	0	7.59	10.99	0.00	0.19	0.25
49	3.99	9.99	12.82	0.13	0.25	0.30
56	6.23	13.34	12.95	0.18	0.31	0.30
63	7.46	13.01	14.7	0.21	0.30	0.34

that the process developed for lactose electro-isomerisation into lactulose in situ of whey permeate can be considered to be highly effective since only a low level of galactose was formed as a reaction by-product (Tables 1–3).

3.3.1. *Effect of cell configuration on lactulose yield*

In this work, we attempted to convert lactose into lactulose in situ in whey permeate (WP) using the electro-activation (EA) technology. Moreover, electro-isomerisation of lactose into lactulose was compared between WP and pure lactose solution. The optimum yield obtained (%) with a high purity (galactose < 10%) and in a short time are: 35.1, 38.66 and 39.78% for configurations 1, 2 and 3, respectively, with a significant difference ($p < 0.001$) between the three configurations of the EA reactor (Fig. 4a,b). Moreover, considering that the use of KCl at 330 mA current intensity yielded the best effect, the results obtained with the three configurations will be discussed only for these operating conditions.

According to configuration 1, the conversion of lactose into lactulose started from 14 min (28.1%) to reach a maximum of 40.1% at 35 min. After that, a decrease of lactulose content was observed (24.87%) at 63 min of EA. The formation of galactose started to be observable at 21 min (7.88%) and evolved linearly to reach a maximum value of 37.06% at 63 min of EA. In configuration 2, the lactulose formation was observed at 7 min with a yield of 34.69%

and increased to a maximum of 43.66%. Subsequently, it decreased to 30.55% at the end of the EA process. In this configuration, the formation of galactose, as a reaction side product, started after 14 min (7.23%) of EA and increased linearly to achieve a value of 36.36% at the end of the EA process. In contrast to configurations 1 and 2, lactulose formation in configuration 3 (Fig. 2d–f) started at 21 min (27.44%) and increased to a value of 43.54% at 56 min of EA, and then decreased slightly down to 43.12% at the end of the EA process. The formation of galactose during this experience in this configuration was less than that of configurations 1 and 2. Indeed, a maximum of galactose formed (14.7%) was reached at the end of the process (63 min) while its detection in the reaction medium started at 28 min (7.1%) of EA process.

In recent years, researchers have tried to substitute the chemical conversion of lactose into lactulose using green and reagent-less methods. Using eggshell powder as catalyst, Montilla et al. (2005) obtained a conversion yield of 18% of lactose into lactulose (1.18 g 100 mL^{-1}), whereas Corzo-Martínez, Copoví, Olano, Moreno, and Montilla (2013) and Montilla et al. (2005) obtained a conversion yield of 16% This method had an advantage of the easiness for catalyst elimination by centrifugation, but the reaction yield was low. In the same way, Paseephol et al. (2008) obtained a conversion yield of 21% of lactose into lactulose using calcium carbonate (from shells at 96 °C over 120 min) in milk permeate as raw material. Sepiolite with alkaline ions is also another proposed substitute to the chemical alkaline synthesis of lactulose; a yield of 20% was achieved after 150 min reaction time at 90 °C using milk permeate as source of lactose (Villamiel et al., 2002). Furthermore, the search for new efficient chemical catalysts is still a topic. In fact, Seo, Park, and Han (2015) have isomerised lactose contained in the cheese whey into lactulose and they obtained a conversion yield of 29.6% using sodium carbonate as catalyst over 20 min at 90 °C. According to this overview, although these methods have some advantages, the reaction yield remains low and different reaction by-products are generated together with the targeted molecule (lactulose). This fact requires eventual purification steps. Moreover, these methods still need catalysts and heat, which leads to high production costs. In contrast, EA technology seems to be more effective since it can be considered as reagent-less, having low energy consuming and with high reaction yield and specificity.

3.3.2. Effect of salt type added on lactulose yield

The type of the added salt influenced significantly ($p < 0.05$) the isomerisation rate of lactose into lactulose in the three investigated configurations. Indeed, when $MgCl_2$ is used as electrolyte in configurations 1 and 2, no lactulose was found in the reactional medium at any time and whatever the current intensity applied. These results demonstrate that the choice of the added salt is very important. Indeed, isomerisation of lactose into lactulose in the tricompartmental reactor requires a pH of the reactional medium close or greater than 11 such as in chemical-based isomerisation reaction. Also, between $CaCl_2$ and KCl, a significant difference was observed ($p < 0.05$). KCl was more effective than $CaCl_2$ in terms of lactose conversion. For example, lactulose was detected at 14 min in the case of WP-KCl-I = 330 mA, while in the case of using $CaCl_2$, lactulose was detected only at 21 min. In addition, when lactose was used as feed solution in the cathodic compartment of the EA reactor, the yield obtained at 7 min was 29.39% and 34.69% for $CaCl_2$ and KCl, respectively, with an applied current intensity of 330 mA. This difference can be explained by the fact that KCl is a strong electrolyte than $CaCl_2$. Indeed, salts with monovalent cations dissociate more effectively and faster than those with divalent cations.

Our results are contrary to those of Aït-Aissa and Aïder (2013) who found that $CaCl_2$ salt gave the best lactulose yield than NaCl and KCl. This difference can be explained by the configuration used by the authors. They used Na_2SO_4 in the anodic and central compartment at a concentration of 0.3 M. In addition, in another study, Aït-Aissa and Aïder (2014c) obtained an optimum conversion of lactose into lactulose at 200 mA current intensity at a temperature of 10 °C using $CaCl_2$ as electrolyte in the cathodic compartment. According to these authors, Ca^{2+} ions have a positive catalytic effect during the isomerisation of lactose into lactulose, information that was also reported in the literature (Hashemi & Ashtiani, 2010). With regard to our results, it seems to be that K^+ ions have the higher catalytic effect than Ca^{2+} ions.

In case of using $MgCl_2$ as electrolyte in the central compartment of the EA reactor, the isomerisation of lactose into lactulose did not take place regardless of the current intensity applied. This can be explained by the pH of the system that did not reach 10 at any time (Fig. 2a–f), which was not sufficient to isomerise lactose into lactulose. Indeed, isomerisation of lactose into lactulose requires a high pH (≈11 and more). This limited pH can be explained by the fact that in an alkaline medium, Mg^{2+} ions react with OH^- ions to form $Mg(OH)_2$, which is insoluble, as shown in Eq. (8). This reaction causes a decrease of the concentration of OH^- ions in the cathodic compartment and then slows down the pH rise of the catholyte. Another possible cause is the fouling of the membrane caused by the $Mg(OH)_2$ formed at the cathodic compartment, which is known to be a scaling of CEM in alkaline media. This phenomenon reduces the flow of the electric current and by the way causing the water dissociation, as shown in Eq. (7), at the interface between the CEM and solution in the central compartment. Attracted by the cathode, the formed H^+ ions migrate through the CEM to the cathodic compartment causing acidification of the solution.

$$2H_2O \rightarrow 2H^+ + 2OH^- \quad (7)$$

$$Mg^{2+}{}_{(aq)} + 2OH^-{}_{(aq)} \rightarrow Mg(OH)_{2\,(sol)} \quad (8)$$

3.3.3. Effect of the current intensity on lactulose yield

Electrical current is the precursor of the electrolysis phenomenon of water on which electro-activation (EA) is based. Current intensity influences significantly ($p < 0.001$) the lactulose yield. Indeed, the increase of the intensity from 220 to 330 mA was positively correlated with the lactose conversion into lactulose. As shown in Fig. 4a, in general the current intensity of 330 mA gave the highest yield of lactulose and in less electro-activation times than the other intensities (110 and 220 mA). However, the increase of the current intensity increased the level of the by-product (galactose) formation. For example, the intensity of 330 mA with WP-KCl-configuration 1 gave a yield of 28.1% of lactulose at 14 min, while lactulose was not yet synthesised when the intensities of 110 and 220 mA were applied. The same effects were also obtained with [yield (%), time (min)]: WP-$CaCl_2$ (31.71, 21); lactose-$CaCl_2$ (29.31, 7); lactose-KCl (34.69, 7) and WP without added salt (27.44, 21.0). In addition, as shown in Fig. 2a–c, the increase of the current intensity caused the increase of the pH at any time with any salt allowing the formation of appropriate conditions to isomerise lactose into lactulose. However, the highest current intensity applied for long time can induce lactose and lactulose degradation on other undesirable product. Furthermore, at low current intensity, the degradation of lactulose and formation of galactose is less pronounced than that of the highest intensities.

3.4. Selectivity of the electro-activation process

It is well known that monosaccharides in alkaline media are subjected to both reversible and irreversible reactions (De Bruin, 1986). The reversible reactions include ionisation, mutarotation, enolisation, and isomerisation, resulting in the formation of the enediol anions that are the intermediates in the isomerisation reactions of monosaccharides (Coca, Garcı, González, Peña, & Garcı, 2004). As for the irreversible reaction it consist of the degradation and organic acids are the final products (De Bruin, 1986). Thus, this work was devoted to exploring the effect of EA of aqueous solutions of mono and disaccharides in alkaline media which is generated in the cathodic compartment of the EA reactor.

EA of mono and disaccharides in basic media was studied in this work as a function of the running time under a current intensity of 330 mA using KCl as electrolyte. These conditions allow to obtain a high pH medium with a high level of lactose conversion to lactulose. The results followed Eqs. (9–18):

Lactose → lactose + lactulose + galactose (9)

Lactulose → lactulose + lactose + galactose (10)

Glucose → glucose + fructose + galactose (11)

Fructose → fructose + glucose + galactose (12)

Galactose → galactose (13)

By superimposing the different reactions above the following can be deduced:

Lactose ↔ lactulose (14)

Glucose ↔ fructose (15)

Galactose → galactose (16)

Glucose → galactose (17)

Fructose → galactose (18)

The degradation of D-glucose in alkaline solution was studied and explained using the Lobry de Bruyn-Alberda van Ekenstein (LBAE) rearrangement theory and the results showed the presence of D-mannose and D-fructose in the reaction mixture (Angyal, 2001). However, in previous work on lactose isomerisation into lactulose by electro-activation, as well as in the present study, no D-mannose was detected in the reaction medium by the used HPLC method (Aït-Aissa & Aïder, 2014b, 2013; Kareb et al., 2016). This result is very important because it explains why epilactose (4-*O*-β-D-galactopyranosyl-D-mannose) has never been detected in the reaction medium, in contrast to the chemical and enzymatic synthesis of lactulose. Similarly, galactose ends up in an equilibrium between tagatose and talose when it is subjected to alkali such as KOH, NaOH, NH_4OH and $Mg(OH)_2$ (Wang, 2010).

In the present study, D-galactose seems to be more stable than the other investigated carbohydrates, a fact which explains the absence of tagatose during the electro-isomerisation of lactose. According to the above reactions, the electro-isomerisation of lactose into lactulose seems to proceed following the proposed scheme (Fig. 5), which is different from the chemical isomerisation. Indeed, lactose is isomerised to lactulose via the LBAE

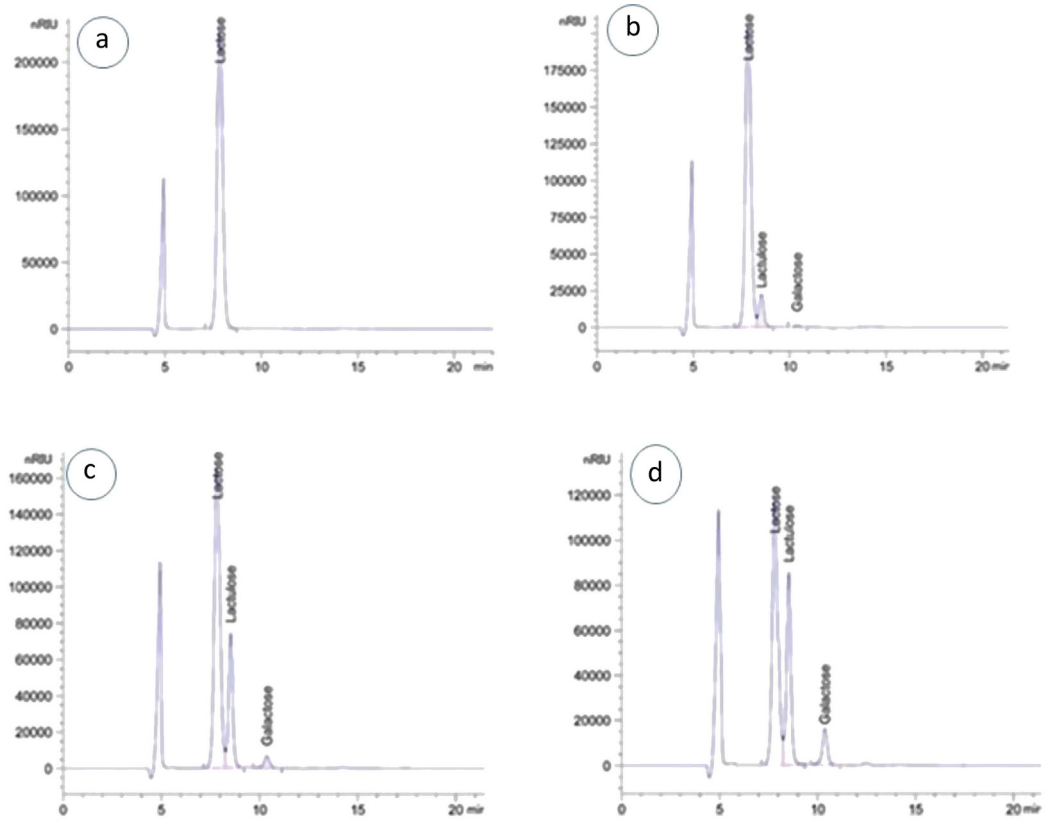

Fig. 5. Selectivity of the used electro-activation technology for lactose electro-isomerisation into lactulose as shown by the HPLC analysis of the formed sugars. (a): initial feed solution, (b): after 5 min EA, (c): after 15 min EA, (d) after 30 min (EA).

rearrangement where the glucose moiety (aldose) is transformed into fructose (ketose) via an 1,2 enediol intermediate (Eq. (9)). The reaction being reversible (Eq. (14)), this may explain, in part, the limited yield of the isomerisation reaction of lactose into lactulose. In the reactional medium, a part of lactose is hydrolysed into glucose and galactose by the rupture of the β(1 → 4) glycosidic bond. Moreover, a part of lactulose gives rise to galactose and fructose in the same way as lactose, while maintaining the presence of lactulose in the reaction medium. The glucose and fructose interconvert according to the Eq. (15). Since glucose and fructose were not present in the medium, they isomerised into galactose as final product according to Eqs. (17) and (18). Finally, galactose being the more stable carbohydrate, it remains intact in the reactional medium (see Fig. 6).

This finding is also very important because it explains why acidic compounds were not found in the catholyte during the electro-isomerisation of lactose into lactulose. Corbett and Kenner (1953) reported that the conversion of lactose into lactulose is followed by a fast degradation of the latter into isosaccharinic acids and galactose degraded gradually into acidic products. This part of the present study confirms the hypothesis proposed by Kareb et al. (2016) who stated that glucose and fructose could be isomerised into galactose during the EA process of lactose. From the current study, the only by-product during the EA of lactose, whey, and WP is undeniably galactose. Thus, it can be concluded that EA in solution was more selective than the chemical isomerisation of lactose into lactulose (Fig. 5). Thus, the purification step can be simplified, which is an economical and environmental advantage for an industrial application.

According to these results, the isomerisation of D-glucose into D-fructose (Eq. (11)) using EA opens a new field of research in the production of fructose to synthesis 5-hydroxymethylfurfural (HMF) by dehydration step. Indeed, the dehydration is more effective from D-fructose than from D-glucose which is more available than D-fructose (Despax et al., 2013). The maximum yield obtained by EA in this study was 56.87% of D-fructose after 56 min of electro-activation without any catalyst added.

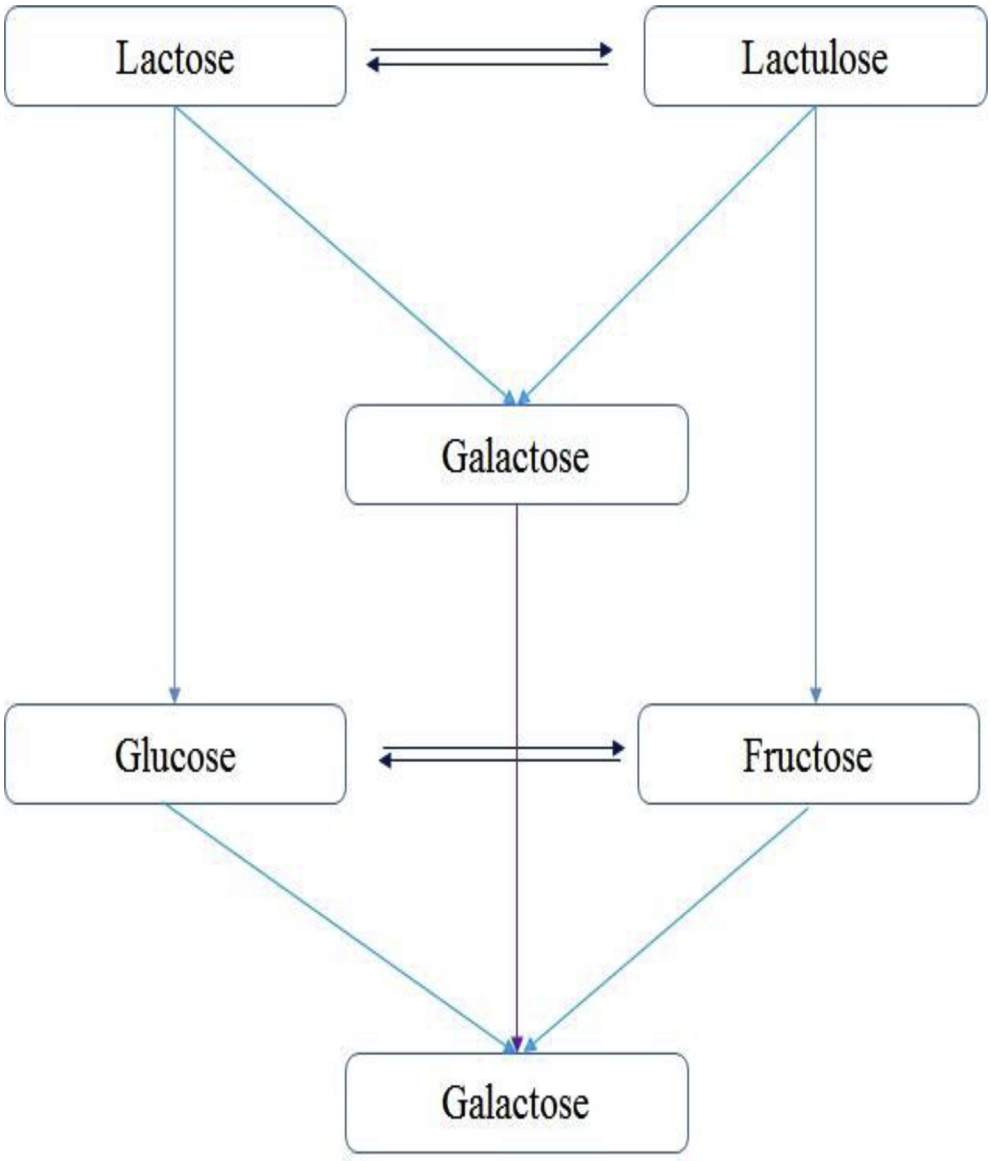

Fig. 6. The possible mechanism pathway of the electro-isomerisation of lactose into lactulose in situ in whey permeate (WP) and the subsequent galactose formation as a reaction by-product by using the electro-activation process.

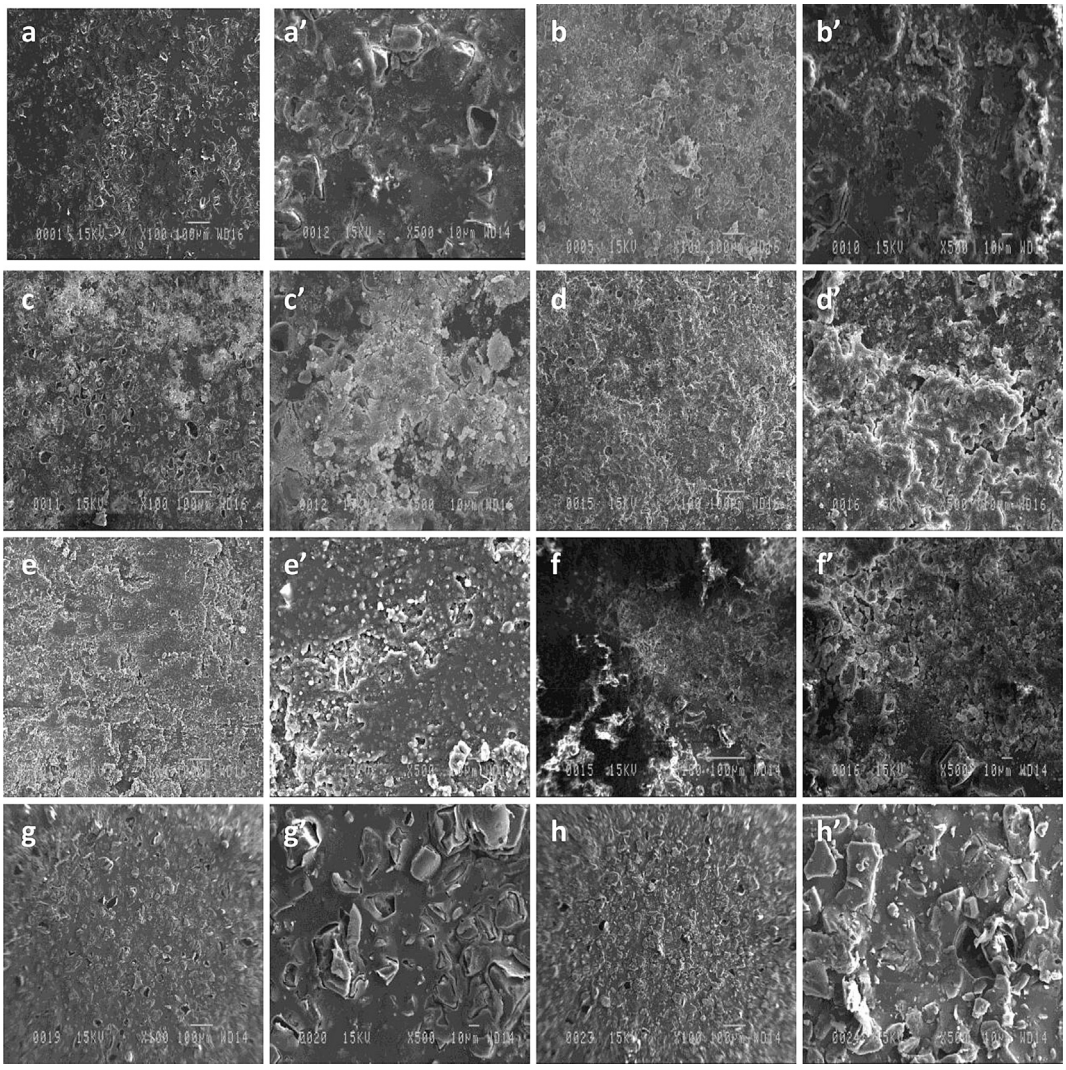

Fig. 7. Scanning electron microscope photographs of the cation exchange membrane (CEM) in the cathodic compartment during electro-activation process given by scanning electron microscopy: reference at (a) 100× and (a') 500× magnification; WP-$CaCl_2$ at (b) 100× and (b') 500× magnification; WP-KCl at (c) 100× and (c') 500× magnification; WP-$MgCl_2$ at (d) 100× and (d') 500× magnification; lactose-$CaCl_2$ at (e) 100× and (e') 500× magnification; lactose-KCl at (f) 100× and (f') 500× magnification; lactose-$MgCl_2$ at (g) 100× and (g') 500× magnification; WP-configuration 3 at (h) 100× and (h') 500× magnification.

3.5. Scanning electron microscopy/energy dispersive X-ray

Since the electro-isomerisation of lactose into lactulose takes place in the cathodic side of the EA reactor, only the surfaces facing the cathode were analysed by SEM and presented in this work (Fig. 7). As it can be observed, there is a difference in the surface between the original and the used CEMs in all cases. Indeed, a deposit was formed on the membranes. X-ray elemental analysis was used to examine the surface mineral profiles of the CEM before and after WP and lactose EA in the three reactor configurations. The spectrum of the CEM before use (reference) showed that it was mainly composed by fluorine, carbon, oxygen, sulphur and a small amount of Na^+. These elements present the elementary composition of the CEM used in this work. Indeed, the CEM used contains a gel of polystyrene cross-linked with divinylbenzene having sulfonic acid as functional group. The presence of gold (Au) and palladium (Pd) were also detected, which are from the coating used to generate a conductive layer on the samples prior to scanning electron microscopy/energy dispersive X-ray (SEM/EDS) analysis.

The main element composing all the CEM layer deposit was silicon (Si) at a high level (silicon was from the used antifoaming agent during the EA process), except when WP-KCl was used (Fig. 7d) for which K^+ ion was detected at low level. It migrated through CEM from the central compartment toward the cathode in the cathodic compartment. According to these results, it can be concluded that EA of pure lactose and WP did not cause membrane fouling. This conclusion was confirmed by the global electrical resistance which did not change during the EA process when configurations 1 and 2 were used. In addition, contrary to the expected results when WP-$MgCl_2$ and Lac-$MgCl_2$ are used in configurations 1 and 2, respectively, Mg^{2+} was not detected on the deposit layer or inside the membranes. It is well known that $MgCl_2$ causes a scaling of CEM by forming a deposit of $Mg(OH)_2$ in basic media. According to our results, it can be assumed that $Mg(OH)_2$ in the cathodic compartment was kept in suspension by mixing the solution in the cathodic side and did not form a deposit on the surface of CEM. Also, the energy dispersive X-ray analyses of a used CEM in configuration 3 showed similar composition. This observation suggests that the fouling phenomenon did not occur in the EA reactor.

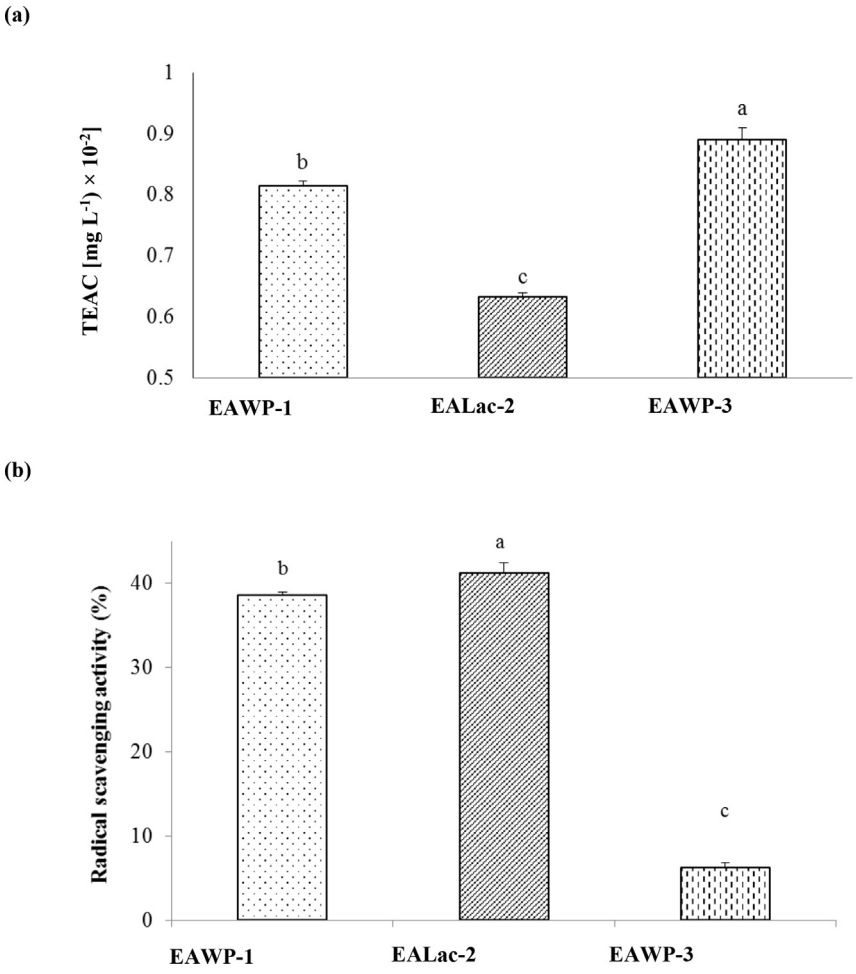

Fig. 8. Antioxidant activities of electro-activated whey permeate (EAWP-1 and EAWP-3: EAWP configurations 1 and 3, respectively) and electro-activated lactose (EALac-2: EALac-configuration 2): (a) against $ABTS^{\bullet+}$ radical, expressed as TEAC values; (b) against $DPPH^{\bullet}$ radical, expressed as radical scavenging activity (in %). Means with different letters are significantly different at $p < 0.05$.

3.6. Antioxidant activity

3.6.1. ABTS radical scavenging activity

Antioxidant activity of the electro-activated whey permeate (EAWP) using reactor configurations 1 and 3, and of the electro-activated lactose (EALac) in the reactor configuration 2 are shown in Fig. 8a. The type of the reactor configuration significantly influences the antioxidant capacity of the electro-activated substrate. EAWP-configuration 3 shows the highest antioxidant activity whereas EALac-configuration 2 shows the lowest; EAWP-configuration 1 was characterised by a medium activity. The observed antioxidant capacity of the electro-activated whey permeate could be a result of different products such as the Maillard reaction products that are formed from the interaction of the amino groups of protein/peptides/amino acids and the reducing sugars present in the medium. Indeed, the production of whey protein concentrate usually uses 10 kDa membranes that allow the oligosaccharides and peptides to cross the membrane to whey permeate (Dallas et al., 2014). Thus, during the EA treatment of WP, the terminal α- or ε-amino groups of lysine residues in peptides or proteins can interact with the carbonyl functions of the reducing sugars to form Maillard reaction products (MRPs), which are known to have a high antioxidant activity (Vhangani & Van Wyk, 2013; Wang, Bao, & Chen, 2013). In case of the electro-activated pure lactose (EALac), the antioxidant activity can be attributed to the coloured products formed through the effect of the Joule heating at the cathode/solution interface in the alkaline medium. Recently, it has been demonstrated that the antioxidant activity of electro-activated whey is due to the MRPs generated by the glycation of peptides with lactose, lactulose and galactose in whey (Kareb et al., 2016).

3.6.2. DPPH scavenging activity

The DPPH radical-scavenging activity indicates the hydrogen-donating abilities of antioxidants (Brand-Williams, Cuvelier, & Berset, 1995; Villano, Fernández-Pachón, Moyá, Troncoso, & García-Parrilla, 2007). The results obtained are shown in Fig. 8b and it can be observed that significant differences between the radical scavenging activities were obtained according to the used configurations of the EA reactor. Indeed, electro-activated lactose solution (EALac) was characterised by the highest scavenging activity, while EAWP-in configuration 3 had the lowest value. EAWP-configuration 1 has an activity close to that of EAWP-configuration 2. There was a significant difference ($p < 0.05$) of the antioxidant activity of EAWP-configuration 1, EALac-configuration 2 and EAWP-configuration 3.

By comparing the two antioxidant activity assays, we can conclude that the three samples act by different mechanisms. Indeed, the mechanism of antioxidant activity of the EAWP obtained with configuration 3 is through an electron donor

mechanism, while the antioxidant mechanism of the EALac-configuration 2 involves hydrogen donor molecules. The antioxidant molecules contained in the EAWP-configuration 1 act simultaneously as electron and hydrogen donors, a phenomenon that generated a powerful antioxidant capacity. Indeed, in the first stage of the Maillard reaction products (MRPs), colourless low molecular weight compounds are formed that did not absorb in the visible spectrum; while the advanced MRPs give rise to the production of high molecular weight compounds, such as melanoidins, with a characteristic maximum absorbance at 420 nm (Delgado-Andrade, Seiquer, Haro, Castellano, & Navarro, 2010).

The spectroscopic analysis of the intermediate and the end MRPs of the EAWP showed that the electro-activation process according the reactor configuration 3 had a great absorbance at 420 nm and low absorbance at 294 nm while in configuration 1 the contrary was observed. This result can be explained by the effect of EA duration which was longer (35 min) in configuration 3 and shorter (only 14 min) in configuration 1. In configuration 2, the EALac gave an absorbance near that of EAWP due to the presence of coloured products. In this case, the molecules responsible for the absorbance were not the MRPs because of the absence of amino acids. In addition, it can be concluded that the antioxidant activity of the samples studied was caused by the intermediate MRPs reaction products that were generated using the EA reactor configuration 3, while in configuration 1 it was mainly due to the final MRPs (Kareb et al., 2017).

4. Conclusion

In the present study, electro-isomerisation of lactose into lactulose was successfully carried out in situ in whey permeate (WP) using electro-activation (EA) technology. Lactose was converted into lactulose at a level of 35.1% when KCl, current intensity of 330 mA for 21 min of electro-activation were applied to WP; and 38.66% when KCl, current intensity of 330 mA during 14 min were applied to lactose solution. The use of WP in both the central and cathodic compartments without addition of salt yielded 39.78% lactulose after 35 min of electro-activation at 330 mA. Thus, WP, which is a low value co-product of the valorisation of cheese whey, was used as lactose source for the production of a new dairy ingredient rich in lactulose that is known to have prebiotic properties. Moreover, the obtained final product was characterised by a high antioxidant activity. This result suggest that it can be used as a functional ingredient in several food formulations to enhance their functionality; especially the prebiotic effect and the antioxidant activity. Moreover, the proposed approach can be considered as effective solution for the dairy industry to achieve the zero waste objectives.

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