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## 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

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### 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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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- $\beta$ -D-galactopyranosyl-D-fructose) is a disaccharide composed of a moiety of galactose linked to a moiety of fructose by a  $\beta$  (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; Ait-Aissa and Aider, 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; Bologna et al., 2009), and (3) biocatalysis with  $\beta$ -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 (Ait Aissa and Aider, 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; Ait Aissa and Aider, 2013b, 2014b). Electro-activation is an emerging technology based on electrolysis of aqueous solutions (Bologna 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)

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