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Impact of the Anodic and Cathodic Electro-Activation Treatment on the Physico-Chemical and Antioxidant Capacity of Red Beetroot Juice

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ABSTRACT: The aim of the present work was to study the feasibility of using electro-activation as a nonthermal treatment to produce stable beetroot juice. Specifically, red beetroot juice was electro-activated under two different reactor configurations by using three electric current intensities (100, 200, and 300 mA) during 120 min. Different parameters of the juice were measured such as the pH, redox potential, juice titratable acidity, Brix degree and total dry matter, color, betalain and polyphenolic contents, and antioxidant capacity of the electro-activated juice. By using the reactor Configuration A in which the targeted juice was electro-activated in the anodic compartment of the used reactor, acidic juice with pH 4 and 5 as well as a redox potential close to +300 mV was obtained. The Brix degree, color, dry matter, and phenolic content were not significantly influenced by this electro-activation. However, the treatment permitted increasing the antioxidant capacity of the juice as measured by the DPPH and ABTS assays. By using the reactor Configuration B in which the targeted beet juice was electro-activated in the cathodic compartment of the used compartment, a juice with an alkaline pH of approximately pH 9 and a reducing redox potential of −697 mV was obtained. With this reactor configuration, the Brix degree and total dry matter were not affected, but the color and total polyphenolic content changed. The betalains and polyphenolic compounds were degraded under the alkaline conditions of this electro-activation treatment, which had a negative consequence on the juice quality by decreasing its antioxidant capacity. In conclusion, this study demonstrated that anodic electro-activation of a beet juice can be technologically feasible since this treatment permitted producing stable juice as well as maintaining the main physico-chemical properties of the juice, enhancing its antioxidant capacity, and keeping the juice color at high level.
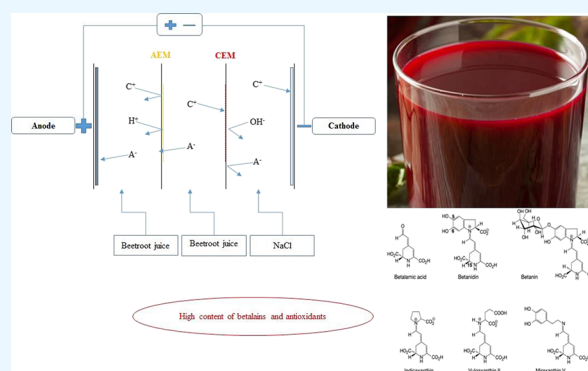
1. INTRODUCTION

Red beetroot, *Beta vulgaris*, is a vegetable widely produced in the world. The most productive region was Europe with a production of approximately 160 million tons of beets in 2009. North America produced 2,743,690 tons and Canada produced 657,700 tons in 2009.[1] In Canada, the beet is produced in different regions, but most of its production is located at the Bas-Saint-Laurent region. The red beet is a vegetable rich in vitamins, minerals, and fibers and contains high amounts of different bioactive compounds such as phenolics, nitrate, and betalains. The phenolic compounds and betalains, the pigments responsible for the color of the beets, contribute to the nutritional value and antioxidant activity of this vegetable.[2] The high content of nitrates in the beet is also an intrinsic added value since consumption of naturally occurring nitrite in beet is considered as a contributing factor to reduce the risk of cardiovascular disease.[3] The beets are vegetables with antioxidant, anti-inflammatory, and vascular effects. Their consumption is highly beneficial to human health.[2,4] Moreover, it has been also reported that beetroot juice has been shown to reduce blood pressure at rest and improve several performance parameters during exercise.[5]

Currently, the consumption of red beet is limited because of some specific organoleptic properties of the beetroot juice such as a soil-like smell.[6] Beet is mostly consumed in a cooked or canned form, as well as mixed with other vegetables. Some beet-based products have recently emerged such as raw juice, chips, and cereal bar, but they are rare and expensive because of the limited market for such products and the reluctance of the consumers. In the food industry, the red beet is principally
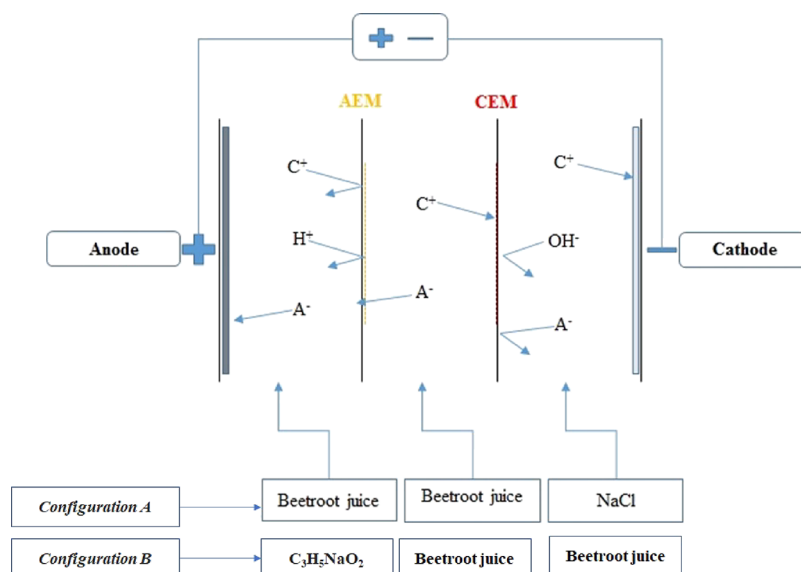
Figure 1. Schematic representation of the used electro-activation reaction (EA-Reactor). AEM: anion exchange membrane; CEM: cation exchange membrane; A^-: anion; C^+: cation; H^+: hydrogen ions; OH^-: hydroxyl ions.

used as a natural colorant.[7] The beet extract is available as powders, which are mostly produced by freeze- or spray-drying, as well as beet juice concentrates.[8] No maximal limitation exists for the use of these colorants, but the Good Manufacturing Practice in the USA and Europe must be respected.[9] The beet colorant is named beet red in Canada and E162 in Europe,[10] and it is used in some desserts, confectioneries, and dairy and meat products.[7,8] Moreover, as a natural colorant, the beet extract can be also used in the pharmaceutical industry and as an alternative to nitrite in meat products like sausage since nitrite is naturally present in this product.[11] Apart from its use as a colorant, beet juice (extract) can contribute to a balanced diet because it contains many bioactive compounds, vitamins, and minerals. Thus, the range of applications of the beet juice can be extended because of its high potential to improve the nutritional properties of foods. However, some improvements of the organoleptic properties of the beet juice (extract) must be realized such as the necessity of eliminating the soil-like smell, which is due to the presence of geosmin in the root. Moreover, the used process must be adequate to limit the product quality degradation. In fact, the mostly used heat treatment to stabilize the beet juice has been reported to decrease the content of some vitamins and phenolic compounds. Thus, it is important to explore alternative and highly efficient technological processes to ensure the stability and quality of the beet juice and its derivatives such as powders and concentrates.[12] In this context, electro-activation technology is highly promising to adequately achieve the aforementioned requirements.

Electro-activation is a nonthermal process based on applied electrochemistry and water electrolysis in which the oxidation–reduction reactions at the electrode–solution interfaces are exploited. The electro-activation treatment is carried out in a reactor in which anion and cation exchange membranes are used to control the physico-chemical and electrochemical properties of different solutions to achieve the targeted objectives following a treatment at a given external electric field intensity. Indeed, when an external electric current is applied to the electrodes (anode and cathode), oxidation reactions occur at the anode–solution interface,

whereas reduction reactions occur at the cathode–solution interface. These reactions generate solutions with acidic-oxidizing properties in the anodic side of the electro-activation reactor and alkaline-reducing properties in the solution of the cathodic compartment. Consequently, the versatility of the electro-activation reactor can be easily exploited in different technological processes, including acidification of different extracts such as beet juice. This new technology has been succesfuly used to stabilize maple juice by decreasing its pH and increasing its redox potential as well as to enhance the antioxidant properties of whey.[13,14] Moreover, electro-activation was successfully used to sterilize canned pea and different vegetables.[15,16] Furthermore, electro-activation was also successfully used against different molds and pathogens responsible for foodborne diseases.[17,18] Thus, in the present study, it was hypothesized that electro-activation treatment can be successfully used as a nonthermal and efficient treatment to produce stabilized and high-quality beetroot juice.

The main aim of this work was to study the impact of electro-activation technology on the main physico-chemical properties of a red beet juice. Two different configurations of the electro-activation reactor were tested. For each configuration, the specific objectives were to investigate the influence of the experimental conditions (electric current intensity and reaction time) on the physico-chemical properties of the juice such as the pH of the juice, its proximate composition, and antioxidant potential of the electro-activated beet juice.

2. MATERIALS AND METHODS

2.1. Materials. The raw materials, red beets cultivated in Canada, were purchased from a local grocery and stored at 4 °C until they were transformed into juice. The NaCl solution at a molar concentration of 0.25 M was prepared by dissolving crystalline sodium chloride (NaCl) with distilled water. NaOH, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, gallic acid, Folin–Ciocalteu reagent, ABTS, and persulfate potassium were from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used in this study were of analytical grade.

2.2. Beet Juice Preparation. First, the beets were washed with abundant tap water to remove the dirt present on the surface. After, the beets were cut and reduced into slurry-containing juice. Then, the juice was extracted by using a juice extractor, which allowed producing 500 mL of juice from 1 kg of the crushed beets. After that, the juice was centrifuged at 10,000g during 15 min to remove the suspended particles[19] by using a Cole-Parmer 6-tube centrifuge, EW-83058-02 model. The juice was then stored at −18 °C until it was submitted to the subsequent electro-activation treatment.

2.3. Electro-Activation Reactor and Protocol of Juice Electro-Activation. In this study, a three-compartmental electro-activation reactor was used as shown in Figure 1. A 4 × 12 cm ruthenium-iridium ($RuO_2/IrO_2/TiO_2$)-coated titanium electrode was used as the anode, and an electrode made of food-grade stainless steel was used as the cathode. These electrodes were connected to a direct electric power source (CircuitSpecialists Co., Tempe, AZ, USA). The anodic compartment was separated from the central compartment by an anion exchange membrane (AEM) and from the cathodic compartment by a cation exchange membrane (CEM). The used ion exchange membranes were purchased from Membranes International Inc. (Ringwood, NJ, USA). The total membrane contact facing each electrode area was 30 cm². Each compartment was filled with 150 mL of the corresponding solution (juice or electrolyte). This config-uration of the electro-activation reactor is selected to exclude the influence of the hydroxyl ions (OH^-) generated in the cathodic compartment on the acidity of the treated juice in the cathodic side since the cation exchange membrane (CEM) is repulsive to the OH^- ions.

In this study, beet juices were electro-activated in two different reactor configurations. In Configuration A, the raw beet juice was filled into the anodic and central compartments, whereas the cathodic compartment was filled with a 0.25 M NaCl solution as the electric current-conducting electrolyte. In Configuration B, the raw beet juice was introduced in the central and cathodic compartments, whereas an aqueous solution of sodium propionate was placed in the anodic compartment. The electro-activation treatment was carried out at three different electric current intensities (100, 200, and 300 mA). These values were determined following some preliminary assays. The pH and redox potential (ORP) were measured during the electro-activation after 5 and 10 min with a combined pH-ORP meter (Ultrapen PT2 and PT3, Myron L Company).

Configuration A: beetroot juice/AEM/beetroot juice/CEM/NaCl solution.

Configuration B: $C_3H_5NaO_2$/AEM/beetroot juice/CEM/beetroot juice.

2.4. Physico-Chemical Analyses. *2.4.1. Color Measurements.* The color measurements were determined using a color reader (Minolta CR6300 Chroma Meter, Minolta data processor DP6300, Osaka, Japan). The results were expressed in the $L*a*b*$ scale: lightness ($L*$), greenness/redness ($a*$), and blueness/yellowness ($b*$). Numerical values of $a*$ and $b*$ were converted into color saturation (chroma index) as follows[20] (eq 1):

$$chroma = \sqrt{a^2 + b^2} \tag{1}$$

2.4.2. Determination of the Acidity of the Juice. The acidity of the juice was determined according to the AOAC

method 942.15. A solution of NaOH (0.1 M) was used to titrate the beet juice. This solution was added until the pH reached a value of 8.2.[21]

2.4.3. Total Soluble Solids. The total soluble solids of the juice were determined with a refractometer. One drop of juice was placed on the prism of the Sper Scientific digital refractometer, model 300034, and percent dissolved solids (%Brix) were determined automatically at 20 ± 1 °C. The moisture content of the juice was measured using the oven method. The sample was dried at 105 °C during 24 h.[22]

2.4.4. Quantification of Betalains. The quantification of betalains was carried out according to the method of Wruss et al. (2015) with slight adaptation.[23] The juice was diluted with distilled water (1:100, v/v), and the absorbance of the juice at wavelengths of 536, 485, and 650 nm was measured with an HP 8453 UV−Vis spectrophotometer (Agilent, Memphis, TN). The absorption at 536 nm quantifies the betacyanins and that at 485 nm quantifies the betaxanthins. The concentration of betalains was considered as the sum of the concentrations of betacyanins and betaxanthins. The following formula was used to calculate the concentrations (eq 2):

$$betacyanin\ (betaxanthin)\ content\ (mg/L)$$
$$= \frac{A \times DF \times MW \times 1000}{\varepsilon \times i} \tag{2}$$

where $A = A_{536nm} - A_{650nm}$ (betacyanins) or $A_{485nm} - A_{650nm}$ (betaxanthins). DF is the dilution factor. MW (molecular weight) = 550 g/mol (for betacyanins) or 339 g/mol (for betaxanthins). ε = 60,000 (molar extinction coefficient in L mol^{-1} cm^{-1} for betacyanins) or 48,000 (for betaxanthins). i is the path length (cm). All samples were measured in triplicate.

2.4.5. Quantification of Total Polyphenols. The quantifi-cation of total polyphenols in the beet juice was carried out according to the method of Wootton-Beard et al. (2011).[24] Briefly, the samples were centrifuged at 10,000g for 10 min and the supernatant was used for total polyphenol quantitation. An aliquot volume of 0.2 mL of juice was mixed with 1.5 mL of Folin−Ciocalteu reagent (1:10, v/v, with water). The mixture was allowed to stand for 5 min at ambient temperature (22 ± 1 °C) followed by addition of 1.5 mL of sodium carbonate solution (60 g/L). After 90 min of incubation at ambient temperature in the dark, the absorbance was measured at 725 nm. The total polyphenol content was expressed as gallic acid equivalents in g/L sample. Each juice sample was measured in triplicate.

2.5. Determination of Antioxidant Capacity. *2.5.1. DPPH• Radical Scavenging Capacity.* The antioxidant capacity of the beet juice after electro-activation was analyzed by investigating its ability to scavenge the DPPH• free radical according to the method of Wootton-Beard et al. (2011).[24] The radical DPPH• has an intense violet color with a maximum absorbance at 517 nm. The radical became colorless when the unpaired electrons were scavenged by antioxidants. A mixture containing 0.1 mL of juice (diluted 1:5) and 3.9 mL of 50 μM DPPH• (prepared in ethanol) was incubated for 30 min in the dark at ambient temperature (22 ± 1 °C). Every 15 min, the absorbance of the mixture was measured at 517 nm with the HP 8453 UV−Vis spectrophotometer. The DPPH radical scavenging activity (%) was calculated against a control by using the following formula (eq 3):

$$\text{DPPH\% scavenging activity} = \frac{(A_C - A_S)}{A_C} \times 100 \quad (3)$$

where A_C is the absorbance of the control (only DPPH solution), and A_S is the absorbance of the sample.

2.5.2. ABTS$^{\bullet+}$ Radical Scavenging Capacity. The antioxidant capacity of the juice after electro-activation was also analyzed by investigating its ability to scavenge the ABTS$^{\bullet+}$ free radical using a methodology previously reported by Wootton-Beard et al. (2011).[24] The radical ABTS$^{\bullet+}$ was obtained by the reaction between a solution of ABTS (7 mM in 20 mM sodium acetate buffer, pH 4.5) and a solution of potassium persulfate (2.45 mM). This reaction permitted obtaining this blue-green radical after 12−16 h of incubation in the dark under the refrigerated condition at 4 °C. After incubation, the solution of ABTS$^{\bullet+}$ was diluted to obtain an absorbance of 0.7 ± 0.01 at 734 nm. The diluted solution forms the test reagent. For analyzing the juice, a dilution was necessary (1:5, v/v). After that, an aliquot volume of 20 μL of the analyzed sample and 3 mL of reagent were mixed and incubated for 30 min in the dark. An aliquot from each test was removed and placed into a cuvette before the absorbance of each sample was measured at 734 nm. The absorbance of the sample at 734 nm is supposed to decrease because the radical ABTS$^{\bullet+}$ reacts with unpaired electrons, which turn the solution colorless. The percentage of inhibition was calculated against a control (solution of ABTS$^{\bullet+}$ alone) by using the following formula (eq 4):

$$\text{ABTS\% scavenging activity} = \frac{(A_C - A_{S1})}{A_C} \times 100 \quad (4)$$

where A_C is the absorbance of the control (only ABTS solution), and A_{S1} is the absorbance of the sample.

2.6. Statistical Methodology. All experiments and measurements were carried out in three replications ($n = 3$). Data were presented as the mean value ± standard deviation (SD). The analysis of the variance (ANOVA) was performed by using SAS software (version 9.3, SAS Institute, Cary, NC, USA), and the significance level was set at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Configuration A: Study of the EA-Juice in the Anodic Compartment. *3.1.1. Evolution of pH during Electro-Activation.* During the electro-activation process, the pH of the beet juice in the anodic compartment was measured at 5 min intervals. The initial pH of the beet juice was 6.73 ± 0.1, and this value was in accordance with another study, which reported that the pH of beet juice was between 6 and 7.[19] The obtained results showed that during the electro-activation process, regardless of the used electric field intensity, the pH of the juice decreased as a function of treatment duration (Figure 2). The statistical analysis of the obtained data showed a highly significant effect of the electro-activation time ($p < 0.001$), whereas the effect of the applied electric current was intensity-dependent. Indeed, no significant difference ($p > 0.05$) was observed between 200 and 300 mA, whereas the effect at 100 mA was different from these values. By applying an electric current intensity of 100 mA and after 45 and 75 min of electro-activation, the pH decreased down to 5.05 ± 0.1 and 4.54 ± 0.09, respectively. As the duration of the electro-activation treatment was increased, the beet juice pH continuously decreased following a quasi-linear kinetics even if during the
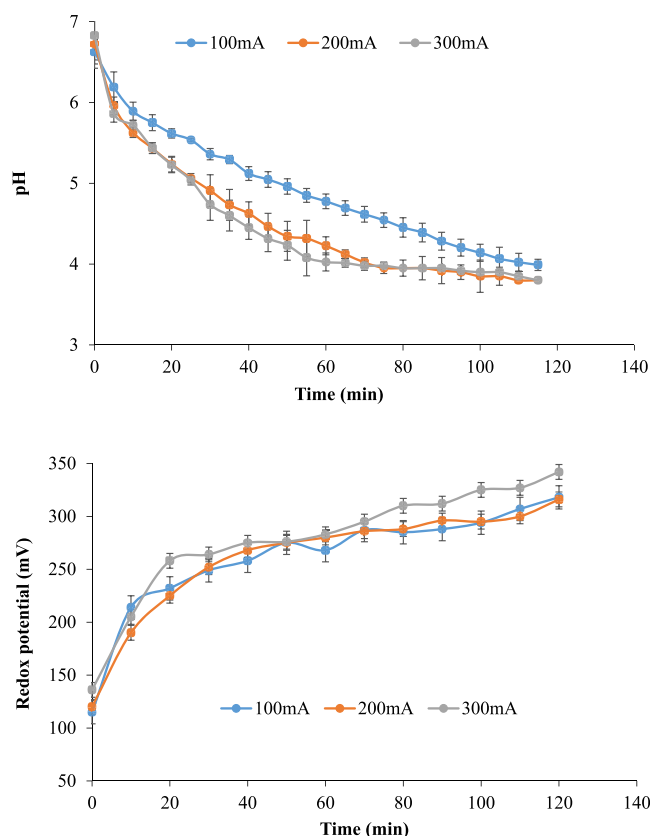

Figure 2. Evolution of the pH and redox potential of the electro-activated (EA-activated) beetroot juice as a function of the applied electric current intensity and electro-activation time.

first minutes of electro-activation, the pH decreased rapidly, which was probably related to the intense water electrolysis. The final pH value of the electro-activated beet juice under 100 mA was close to pH 4. By increasing the applied electric current intensity to 200 and 300 mA, the pH decrease of the electro-activated beet juice was different from what was observed at 100 mA with an inflection point that appeared after approximately 70 min of electro-activation. After that, the beet juice pH continued decreasing but with slower intensity to reach a final value of approximately 3.6 after 120 min of electro-activation. Such observation concerning the evolution of pH was also observed in another study on the electro-activation of different salts like sodium propionate, calcium lactate, and potassium acetate.[25]

This study showed that as the applied electric current intensity increased, the more the pH decreased rapidly. For example, a pH of 4 was reached after 110 min of electro-activation under 100 mA, and the same value was reached only after 70 min at 200 mA and 65 min at 300 mA. Thus, the higher the electric current, the more the targeted final pH value can be reached rapidly. The observed effect of the applied electric current during the electro-activation process of the beet juice agreed with the reported information in the scientific literature. In the study of El Jaam et al. (2017),[26] a solution of potassium acetate was electro-activated in a three-compartmental reactor under four different electric current intensities (100, 200, 300, and 400 mA). The initial pH of the solution was 8.49, and after 60 min of the electro-activation treatment, the pH was decreased down to 4.34, 3.77, 3.60, and 3.18 under 100, 200, 300, and 400 mA, respectively. Another study
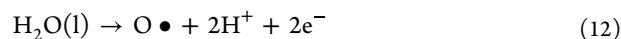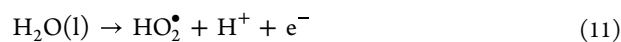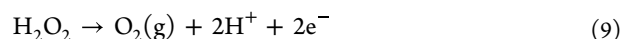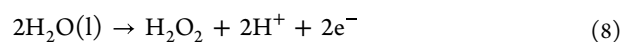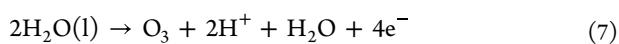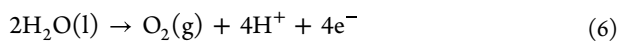
conducted on the electro-activation of a solution of NaCl showed the same results. After 60 min of electro-activation, the pH of the solution reached 2.20 ± 0.07 under 100 mA, 2.23 ± 0.16 under 150 mA, and 1.96 ± 0.02 under 200 mA.[27,28]

The decrease in pH during the electro-activation is due to the accumulation of H^{+} ions, which are intensively produced at the anode−solution interface following water molecule electrolysis during the electro-activation process. This phenomenon is electric current intensity-dependent and is followed by oxygen generation, leading to the formation of a highly oxidative medium (eq 5):[29]

$$2H_2O(l) \rightarrow O_2(g) + 4H^+ + 4e^- (E^\circ = +810 \text{ mV}) \quad (5)$$
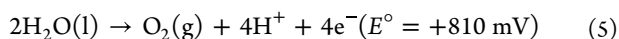
In the case of the beet juice, this reaction was possible because the beet juice is rich in water (100 g of raw beet contains 87.6 g of water). The reached low pH of the juice after electro-activation will contribute to its microbiological stability because the minimal pH for the growth of micro-organisms is around 4.5. Moreover, electro-activation is also favorable for a generation of different oxidizing agents that are able of reach the cytoplasm of the bacterial cell.[30]

3.1.2. Evolution of ORP during the Electro-Activation. The redox potential (ORP) was measured during the electro-activation of the beet juice. The redox potential of the control juice without electro-activation was +124 ± 11 mV, indicating that the initial raw juice has a weak oxidative character even if this value is very low to have any effect on an eventual microbial growth. After the electro-activation, for all the treatments, the ORP was increased, as shown in Figure 2. The obtained results showed that the duration of the electro-activation treatment influenced the redox potential value of the beet juice, showing that as the duration increased, the ORP value also increased in the anodic compartment of the electro-activation reactor. By applying an electric current intensity of 100 mA, and after 40 and 110 min of treatment, the ORP value reached values of +258 ± 11 and +307 ± 8 mV, respectively, indicating that the oxidative character of the electro-activated juice significantly increased. However, even if an increase in ORP was observed after the electro-activation treatment, the redox (ORP) potential of the juice after electro-activation treatment was in the same range as that of tap water (+200 to +600 mV) and that of bottled water with an ORP average value of +400 mV. The observation made in the present study with the beet juice agreed with the obtained results with the electro-activated maple juice.[13,14] The increase in redox potential contributes to the protection of the juice against bacterial growth because it induces a modification of the redox environment of the cell, making the environment unfavorable for bacterial growth because this modification of the environment increases the porosity of the cell.[31] The increase in redox potential is due to the production of some reactive compounds during water electrolysis in the anodic compartment of the electro-activation reactor.[29] Indeed, during water electrolysis, several oxidizing radicals such as HO_2^- (peroxide anion), $OH^\bullet$ (hydroxyl radical), $^1O_2$ (singlet molecular oxygen), O_3 (ozone), $O^\bullet$ (atomic oxygen), H_2O_2 (hydrogen peroxide), and O_2^- (superoxide anion) can potentially be formed at the interface electrode/electrolyte in accordance with the illustrated formulas in eqs 6−12.[32]

$$2H_2O(l) \rightarrow O_2(g) + 4H^+ + 4e^- \quad (6)$$

$$2H_2O(l) \rightarrow O_3 + 2H^+ + H_2O + 4e^- \quad (7)$$

$$2H_2O(l) \rightarrow H_2O_2 + 2H^+ + 2e^- \quad (8)$$

$$H_2O_2 \rightarrow O_2(g) + 2H^+ + 2e^- \quad (9)$$

$$H_2O_2 \rightarrow HO_2^\bullet + H^+ + e^- \quad (10)$$

$$H_2O(l) \rightarrow HO_2^\bullet + H^+ + e^- \quad (11)$$

$$H_2O(l) \rightarrow O\bullet + 2H^+ + 2e^- \quad (12)$$

Concerning the effect of the applied electric current intensity, the obtained data showed that there is no significant difference ($p > 0.05$) between 100 and 200 mA, whereas the treatment at 300 mA was different from the others. Indeed, after 100 min of treatment, the ORP values were +307 ± 7 mV at 100 mA, +300 ± 11 mV at 200 mA, and +327 ± 6 mV at 300 mA. The increase in current intensity permitted reaching a specific value more rapidly. The combination of the obtained results concerning the evolution of the beet juice pH and its redox potential during the electro-activation shows that for all the studied electric current intensities (100, 200, and 300 mA), the pH decreased, whereas the ORP decreased, making the electro-activated beet juice acidic with oxidative character. This intrinsic combination can be successfully exploited to ensure juice stability against spoilage bacterial growth.[33]

3.1.3. Titratable Acidity of the Juice. The titratable acidity permits quantifying the total acid content with the neutralization of ionized or non-ionized H^{+} by a NaOH solution. In the present study, the titratable acidity of the initial raw beet juice was 10 meq/L because the beetroot contains some organic acids but in a small amount.[34] In beet juice, malic, citric, oxalic, shikimic, and fumaric acids have been found but are present at levels of few mg/g dry weight basis.[23,34] For oxalic acid, the content was between 0.3 and 0.5 g/L juice.[23] After the electro-activation, the beet juice titratable acidity was increased after all the treatments (Figure 3).
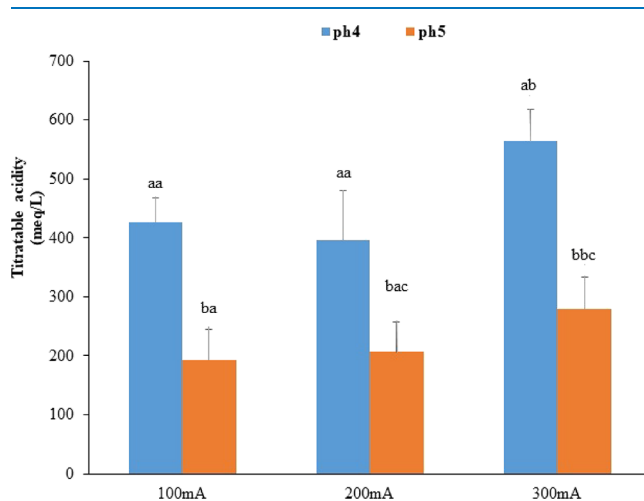
Figure 3. Titratable acidity of the electro-activated beetroot juice.

Moreover, no correlation between the pH and the used electric current intensity was observed even if the pH of the juice had a significant impact on the electro-activated juice titratable acidity ($p \leq 0.05$). This result was probably due to the fact that at the end of the electro-activation treatment, which was set at 120 min, the juice final pH values were dependent on the electric current intensity value. Thus, for each used electric current intensity, the titratable acidity was higher when the pH

Table 1. Analysis of the Color of the Electro-Activated and Control Beetroot Juice ($L*$, $a*$, $b*$, and Chroma Index)[a]

	100 mA		200 mA		300 mA		
	pH 4	pH 5	pH 4	pH 5	pH 4	pH 5	control
$L*$	27.18 ± 1.50^a	25.32 ± 0.18^a	26.38 ± 2.31^a	27.35 ± 0.21^a	25.10 ± 0.58^a	26.08 ± 2.11^a	26.79 ± 0.38^a
$a*$	0.37 ± 0.15^d	0.68 ± 0.04^c	1.16 ± 0.12^a	0.53 ± 0.04^c	0.82 ± 0.16^{ab}	0.97 ± 0.06^{ab}	0.16 ± 0.03^e
$b*$	-0.27 ± 0.48^b	-0.08 ± 0.05^d	0.24 ± 0.49^b	-0.17 ± 0.04^c	-0.45 ± 0.20^a	-0.18 ± 0.19^a	-0.40 ± 0.45^a
chroma	0.95 ± 0.06^a	0.68 ± 0.04^b	1.22 ± 0.08^a	0.55 ± 0.05^b	1.05 ± 0.04^a	1.00 ± 0.13^a	0.47 ± 0.07^a

[a]The superscript letters indicate that there is no significant difference at a 95% confidence level for the treatments with same superscript letters.

reached a value of 4 than the pH of 5. For example, by using an electric current intensity of 100 mA, the titratable acidity values of the juice were 426.7 ± 40.4 meq/L at pH 4 and 193.3 ± 51.3 meq/L at pH 5, indicating the significant effect of the juice pH. Thus, as the electro-activated beet juice pH decreased, its titratable acidity correspondingly increased. The effect of the applied electric current intensity significantly influenced the juice titratable acidity ($p \leq 0.05$), a fact that can be summarized as follows: at pH 4, the titratable acidity values were 426.7 ± 40.4 meq/L at 100 mA, 396.7 ± 83.9 meq/L at 200 mA, and 563.3 ± 55.1 meq/L at 300 mA. However, no significant difference was observed between 100 and 200 mA, but a significant difference existed between 100 and 300 mA and between 200 and 300 mA in the case of pH 4 of the electro-activated beet juice. When electro-activated beet juice samples were collected at pH 5, the treatment under 100 mA was different from that at 300 mA, but it was not different from the one conducted under 200 mA, indicating a positive correlation between the used electric current intensity, the pH of the electro-activated beet juice, and the titratable acidity. Indeed, the increase in acidity is also due to the accumulation of a high amount of H^+, which is generated following water molecule hydrolysis in the anodic compartment of the electro-activation reactor. Moreover, the dissociation of the organic acids and their electromigration through the anionic exchange membrane, which was placed between the central and anodic compartments, also partly contributed to the increase in electro-activated beet juice titratable acidity.[35]

3.1.4. Brix Degree and Total Dry Matter Content. The total dry soluble matter of the beet juice, expressed as °Brix, had been measured before (initial raw beet juice) and after each electro-activation treatment. The obtained results showed that the Brix degrees of the raw juice and the juice treated by electro-activation were not significantly different, indicating that the electro-activation treatment only affected the physico-chemical property but not the total soluble dry matter of the juice. In the study, the total dry matter of different beet juice samples ranged between 9 and 12 °Brix, which is in adequation with the data reported in other studies that showed Brix degrees between 14 and 15[36] and between 11 and 14.[37] The measurement of the Brix degree also showed that the sucrose content was not affected by the electro-activation treatment. Moreover, the acidification of the juice, which occurred in the anodic compartment of the used reactor, did not induce any hydrolysis of sucrose into glucose and fructose since the analytical analysis showed that sucrose remained the major sugar present in the beet juice. The sucrose content of beets was near 930.40 ± 13.65 mg/g on a dry weight basis.[34] Nevertheless, the analysis of the sugar profile showed that glucose and fructose were present in small amounts in the treated juice. In the study of Koffi et al. (2014),[38] the electro-activation of the maple juice with an acidification of the solution also showed the maintenance of the value of Brix

degree. This result indicates that the beet juice Brix degree is mostly dependent on the harvesting period and the storage conditions of the beet.[36]

3.1.5. Color Measurement. The color of the beet juice was analyzed by using a colorimeter in the CIE Lab system in which the $L*$ value represents the lightness, $a*$ represents the redness (when positive), and $b*$ represents the yellowness (when positive) and blueness (if $b*$ is negative), which is the case in the present study since all the measured $L*$ and $a*$ color parameters were positive, whereas the $b*$ value was negative. The obtained results showed that for all the samples, the value of lightness ($L*$) was low ($L* < 30$) (Table 1). These results were expected since the beet juice is dark red. Moreover, no significant difference was found between the sample $L*$ values ($p \leq 0.05$), indicating that the electro-activation treatment did not affect the lightness of the juice. For the $a*$ parameter, positive values were obtained for all the beet juice samples and the values of this parameter ranged between 0.4 and 1, which is in accordance with the visual appearance of the beet juice, which is characterized by a pronounced red color mainly attributed to the presence of betalain compounds. The statistical analysis of the obtained data showed significant differences ($p < 0.05$) between the treatments regarding the juice redness ($a*$ value). Moreover, the beet juice is tending more to blue than yellow because the $b*$ values were negative, and significant differences were observed between the treatments.

The chroma was obtained with the following formula (eq 13):

$$\text{chroma} = (a^2 + b^2)^{1/2} \quad (13)$$

The chroma index corresponds to the saturation of the color, and significant differences ($p < 0.05$) were also observed between the juice samples. For the electro-activated beet juice as well as for the control sample, the values of the chroma index varied from 0.47 ± 0.07 to 1.22 ± 0.08, indicating the significant effect of the treatment and the related parameters such as the juice pH, which in turn was influenced by the applied electric current intensity and the electro-activation duration.

3.1.6. Quantification of Betalains. Beetroots contain high quantities of betalains (betaxanthins and betacyanins), which are pigments responsible for the specific color of the derived juice. The betaxanthins are yellow pigments, whereas betacyanins are violet pigments and are dominant in the beet juice.[8] Among the betacyanins, the betanins are the dominant compound followed by the prebetanins, isobetanins, and neobetanins.[39] For the betaxanthins in the beet, vulgaxanthin I is dominant followed by vulgaxanthin II and indicaxanthin.[39] These two groups of pigments are responsible for the characteristic color of the beet and possess high antioxidant potential.[2] In the present study, the content on betalains was

Table 2. Pigment Content of Juice Treated with Electro-Activation and Untreated One (Control)[a]

electric current (mA)	pH	pigment content (mg/L)			BC/BX ratio
		betacyanin (BC)	betaxanthin (BX)	total betalain	
100	4	626.91 ± 7.52[a]	388.68 ± 13.23[a]	1015.59 ± 7.79[a]	1.61
	5	579.02 ± 175.10[a]	362.30 ± 95.06[a]	941.32 ± 269.97[a]	1.60
200	4	469.74 ± 184.63[a]	282.26 ± 107.73[a]	752.00 ± 292.35[a]	1.66
	5	654.22 ± 16.76[a]	566.01 ± 8.86[a]	1420.23 ± 23.89[a]	1.51
300	4	642.47 ± 214.96[a]	404.59 ± 152.03[a]	1047.06 ± 366.54[a]	1.59
	5	756.02 ± 138.96[a]	482.66 ± 86.57[a]	1238.67 ± 223.55[a]	1.57
control	6, 5	819.18 ± 43.67[a]	563.81 ± 43.67[a]	1382.99 ± 80.64[a]	1.61

[a]The superscript letters indicate that there is no significant difference at a 95% confidence level for the treatments with same superscript letters (a: within a column, different letters indicate significant differences ($p \leq 0.05$)).

determined for juice before and after the electro-activation process, and the obtained results are summarized in Table 2. In the initial raw juice (untreated), the contents of betacyanins were 819.18 ± 43.67 and 563.81 ± 43.67 mg/L for betaxanthins. The ratio between these two pigments was 1.61, indicating that the ratio obtained was similar to what was previously reported in another study showing a ratio ranging between 1 and 1.75.[23] In the study of Czapski et al. (2009),[19] the contents of yellow pigments were 1.8 times lower than those of red pigments. Moreover, a correlation between red and yellow pigments has been observed. Regardless of the variety, the ratio is always the same, but the total content can change.[23] The content of these pigments is influenced by numerous factors such as the variety of the beetroot, the size of the root, the part of the root, and the cultivation conditions (water stress, organic or conventional culture, and the use of specific fertilizers).[36,37,40]

After electro-activation, the content of betaxanthins and betacyanins was kept the same regardless of the pH of the juice or the condition of electro-activation. The statistical analysis of the obtained data did not show a significant difference between the juice treated by electro-activation and the untreated one ($p > 0.05$). The ratio was kept at approximately 1.6, and the contents of betacyanins and betaxanthins were 654.73 ± 134.86 and 414.42 ± 98.57 mg/L, respectively. The values obtained are similar to those reported in the study of Czapski et al. (2009).[19] The content of betacyanins was between 300 and 2200 mg/L, and for the betaxanthins, it was between 200 and 1400 mg/L.

In the present study, the pH of the juice did not influence the content of betalains. In fact, the betalains are generally stable between pH 3 and pH 7.[41] More precisely, the betanins, the most abundant betacyanins in beet juice, are stable between pH 4 and pH 6. Degradation is only observed when the pH is below 3 and above 9. An acidification permitted the recondensation of glutamic acid with an amine group (betaxanthins) or cyclo-dopa-5-O-β-glucoside (betacyanins). Indeed, it has been reported that mostly in the alkaline condition, the imine bond hydrolysis can occur but not in an acidic medium, which is the case in the present study since the anodic electro-activation process allowed beet juice acidification.[42] So, only under alkaline conditions, the betalains are degraded and the juice color shifts to more yellowish. At low pH, the mechanism of degradation is not clearly understood, but C15 isomerization of betanin and betanidin into isobetanin and isobetanidin occurs.[8,43] The broad-spectrum stability of betalains permits using them in many food products, which have neutral or acidic pH. Other than the pH, the stability of betalains is also influenced by the temperature, the presence or

absence of oxygen, refrigeration, the light that is responsible for the photo-oxidation, the moisture content, and the initial pigment content.[8] The electro-activation of the juice with this configuration does not affect the content of the coloring pigments of the beet juice, which kept its properties. Thus, the electro-activated beet juice remained with good betalain content, which eventually will contribute to the juice's natural good antioxidant capacity.[2]

3.1.7. Total Polyphenol Content. The beet juice is also a source of different phenolic compounds, but their contents are small. The phenolic compounds include the flavonoids and phenolic acids. In beets, different phenolic acids have already been identified. The beet contains ferulic, *p*-coumaric, vanillic, caffeic, chlorogenic, cinnamic, 4-hydroxy benzoic, and anisic acids.[1] In the raw beet, the content of ferulic acid is between 0.01 and 0.04 mg/g.[19] For the flavonoids, rutin and epicatechin were found in beets.[2] The flavonoids and phenolic acids are known as antioxidants.[2] In the present study, the raw juice polyphenol content was 0.364 g/L GAE (gallic acid equivalent). This value is low compared with the results obtained in other studies. For example, in the study of Kujala et al. (2000),[39] the value was reported to be 4 mg/g for the flesh and 15.5 mg/g GAE for the peel. Indeed, the phenolic content varies depending on the variety, cultivation condition, the part of the root, and the method of extraction of the derived juice.[37,39]

After electro-activation, regardless of the pH and the used electric current intensity, the polyphenolic content was not significantly different from the raw juice (Table 3) ($p > 0.05$).

**Table 3. Phenolic Content of Juice after Electro-Activation[a]**

current, mA	pH	phenolic content (GAE g/L)			mean value	standard deviation
100	4	0.498	0.172	0.337	0.336[a]	0.163
	5	0.447	0.180	0.282	0.303[a]	0.134
200	4	0.302	0.119	0.217	0.213[a]	0.091
	5	0.504	0.365	0.266	0.378[a]	0.120
300	4	0.271	0.535	0.510	0.439[a]	0.146
	5	0.498	0.394	0.217	0.370[a]	0.142

[a]a: within a column, different letters indicate significant differences ($p \leq 0.05$).

The polyphenolic content was 0.340 ± 0.070 GAE g/L. The decrease in pH did not also influence the polyphenolic content of the juice. Thus, the obtained results showed that the electro-activation treatment allowed the decrease in the pH of the juice but without significantly affecting the composition of betalain and polyphenolic contents, showing that electro-

activation is a suitable treatment to ensure beet juice stability without affecting its biological active value.

3.1.8. Antioxidant Activity. The beetroot is a vegetable rich in bioactive compounds such as betalains, polyphenolic compounds, nitrate, and ascorbic acid. These compounds endow the juice with many active properties like antioxidant or anti-inflammatory activity.[2] The antioxidant activity of the juice before and after electro-activation was determined by two different methods: the DPPH and ABTS assays. For the DPPH assay, the measure of the absorbance was performed every 15 min for each sample. The obtained results showed that for each given sample, the more the time of incubation was increased, the more the scavenging ability was also increased because the radical DPPH$^\bullet$ reacts with the antioxidants (proton donors) present in the juice. This result was confirmed by the fact that the mixture loses its violet color with a subsequent absorbance decrease (eq 14).

$$DPPH^\bullet + AH \rightarrow DPPHH + A^\bullet \tag{14}$$
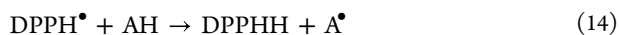
In the present study, the speed of the radical scavenging ability was the same regardless of the used treatment. The data summarized in Table 4 show that all the electro-activated beet

Table 4. Comparison between ABTS and DPPH Assay

| current, mA	pH	DPPH scavenging ability (%)	ABTS scavenging ability (%)
100 mA	4	32.63 ± 2.40	44.37 ± 4.55
	5	31.66 ± 6.33	36.71 ± 3.03
200 mA	4	35.49 ± 2.33	29.98 ± 1.86
	5	34.02 ± 1.96	49.72 ± 7.69
300 mA	4	36.51 ± 5.24	36.09 ± 4.20
	5	32.68 ± 2.90	42.24 ± 6.27
control juice		20.26 ± 3.96	30.17 ± 2.19

juice samples had quite similar DPPH scavenging activity, whereas the control sample corresponding to the raw juice was different from the other juice with a significantly lower DPPH scavenging capacity of 20.26 ± 3.96%. Also, the obtained results showed that the applied electric current intensity as well the subsequent corresponding pH of the juice did not influence the antioxidant activity of the juice after being electro-activated.

The antioxidant activity of the raw beet juice had been largely studied, and its antioxidant activity is mainly attributed (ensured) to the betalains and the different polyphenolic compounds. Indeed, the positive correlation between the betacyanin content and the antioxidant activity of raw beet juice has been already reported.[19,40] The betanins are able to donate electrons, which permits stabilizing free radicals.[2] This antiradical activity was higher for betacyanins than betaxanthins.[25] A correlation between antioxidant activity and total polyphenol content has also been reported,[37] showing that the raw beet juice is characterized by a high antioxidant capacity, making it a suitable functional food.[24] The difference between the treated or untreated juice in this study is not due to betalain or phenolic compound content. Indeed, electro-activation does not influence the polyphenolic and betalain contents in the juice. Other factors are responsible for this difference concerning the antioxidant activity. The pH, but also the organic acid like citric acid and the presence of reactive compounds, may contribute to improving the antioxidant activity of the electro-activated juice.

Concerning the ABTS assay, the same tendency was observed as in the case with the DPPH assay. The juice treated by electro-activation possessed a higher antioxidant activity, expressed as the ABTS scavenging ability, than the raw beet juice. These two methods permitted obtaining the same results and the same conclusion that the electro-activation treatment is technologically suitable and efficient to stabilize the beet juice with a subsequent keeping of its high antioxidant capacity, making it a suitable candidate as functional beverage.

3.2. Configuration B: Study of the Electro-Activated Juice in the Cathodic Compartment. In this part, the configuration of the electro-activated reactor was set as follows: [Configuration B: anode-C$_3$H$_5$NaO$_2$/AEM/beetroot juice/CEM/beetroot juice-cathode]. Contrary to the previous reactor configuration in which the beet juice was electro-activated in the anodic compartment, under the present configuration, the impact of the cathodic electro-activation on the beet juice is the target of this study. Following the electro-activation, water molecules are intensively electrolyzed at the cathode−solution interface, leading to the formation of hydroxyl (OH$^-$) ions and the production of hydrogen gas (H$_2$), which is also intensively dissolved in the solution. The gaseous hydrogen is known as a strong reducing agent, which is partly responsible for the negative (reducing) oxidation−reduction potential (ORP) of the cathodic electro-activated solution.[44,45]

The pH and ORP of the juice in the cathodic compartment during the electro-activation were measured, and the obtained results showed that during the electro-activation process, the juice pH increased to reach a final pH of 8.86 ± 0.02. By using this configuration of the electro-activation reactor, a pH close to 9 was reached, but a foam was also formed in the juice. Like in the reactor Configuration A (Figure 1), the duration of electro-activation treatment and the applied electric current intensity have significantly influenced the increase in pH ($p < 0.05$). The more the current intensity increased, the more the pH was rapidly raised. At 100 and 300 mA, 25 and 10 min were enough to obtain a pH of 8.85. Then, the more the duration increased, the more the pH increased, but this observation was limited by the fact that the juice foamed and overflowed from the corresponding compartment of the used reactor. The increase in pH is due to the accumulation of hydroxide ions (OH$^-$) produced during the hydrolysis of water, and this decomposition is also associated with the release of dihydrogen (H$_2$) (eqs 15 and 16).

$$2H_2O(l) + 2e^- \rightarrow H_2(g) + 2OH^- \tag{15}$$

$$2H^+(aq) + 2e^- \rightarrow H_2(g) \tag{16}$$
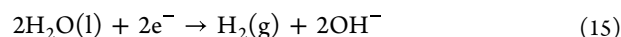
For the redox potential of the cathodic electro-activated beet juice, a decrease was observed, showing that the juice acquired a reducing character. Moreover, as the applied electric current intensity increased, the juice redox potential rapidly decreased. After 25 and 10 min of electro-activation under 100 and 300 mA, respectively, the ORP value of the juice was −697 ± 1 mV, indicating the highly significant effect ($p < 0.01$) of the electric current intensity. The obtained juice possessed reducing properties, which contribute to the protection of the juice against microbial growth. This negative redox potential is due to the high amount of different active reducing molecules such as excess of molecular hydrogen (H$_2$), which is intensively formed following the cathodic water electrolysis.

Concerning the physico-chemical analysis of the different beet juice samples, no differences between the juice electro-activated at 100 mA and 300 mA were observed. The current intensity did not influence the properties of the juice, but compared with the initial raw juice, the electro-activation treatment in the cathodic compartment modified the properties of the juice. The Brix degree and total soluble dry matter content were the same for the juice treated or not treated by electro-activation. The values were 11.73 ± 2.44 °Brix and $10.63 \pm 0.52\%$, respectively. However, differences concerning the color, betalain content, polyphenolic content, and antioxidant activity were observed.

The ratio between betacyanins and betaxanthins has also changed. It was not near 1.6 but close to 1.02. The betacyanin content was lower in the juice treated by electro-activation than in the initial raw juice. The betacyanin content in the electro-activated juice was 484.70 ± 76.75 mg/L compared to 819.18 ± 43.67 mg/L for the raw juice, indicating that some betacyanins were degraded during the electro-activation treatment. This degradation is induced by the alkalization of the juice following the water electrolysis at the cathode−solution interface. In fact, at high pH, mainly above pH 9, the betacyanins are unstable and are easily hydrolyzed. The bond between glutamic acid and cyclo-dopa-5-O-β-glucoside is broken.[8,43] Thus, as a consequence of this degradation of betacyanins, the cathodic electro-activated beet juice became more yellowish rather than reddish.

For the total polyphenolic compounds, a significant ($p < 0.05$) loss of the polyphenolic compounds was observed since their content in the electro-activated juice was 0.102 ± 0.031 g/L GAE, whereas for the raw juice, it was 0.364 g/L GAE. This loss is due to the degradation of the polyphenolic compounds in the cathodic compartment, which could be caused by the $OH^\bullet$ and $H^\bullet$ radicals. This degradation of the total polyphenols is also influenced by the juice pH since an alkaline pH is known to promote this degradation. Moreover, some studies reported that the degradation of some polyphenols can be influenced by the type of electrode material, the applied electric current intensity, and the initial content of the polyphenols.[46] In the present study, only a food-grade stainless steel electrode was used as a cathode; thus, it is not possible to validate the effect of the material.

The loss of betacyanins and polyphenolic compounds is responsible for the decrease in the antioxidant capacity of the cathodic electro-activated beet juice since the initial raw juice possessed a higher antioxidant capacity than the electro-activated juice. The scavenging ability of the raw juice was $20.26 \pm 3.96\%$, but for the electro-activated juice, it was decreased down to $15.58 \pm 1.71\%$. With the reactor Configuration B, the beet juice had an alkaline pH, reducing (negative) redox potential, dry matter, and Brix degree similar to the raw juice, but the content of betacyanins, total polyphenolic compounds, and antioxidant activity were lower. Compared with the reactor Configuration A in which the targeted juice was electro-activated in the anodic compartment of the electro-activation reactor, the impact on the juice reactor Configuration B was used since a more important decrease in its antioxidant properties occurred.

Finally, in this study, the beet juice was successfully electro-activated by using two different reactor configurations. The first configuration (reactor Configuration A) permitted acidifying the juice, modifying its redox potential, increasing its titratable acidity, and enhancing its antioxidant capacity

without affecting its color, total dry matter, sucrose content, and total polyphenolic content. However, with the second reactor configuration reactor (Configuration B), the juice content of betalains and polyphenolic compounds as well as its antioxidant capacity decreased after cathodic electro-activation, which was characterized by relatively high alkaline pH. The increase in the antioxidant capacity of the anodic electro-activated beet juice can be potentially exploited by the food industry regarding the application of this juice as a food additive to prevent the oxidation of different foods and to produce powders that can be used as antioxidant additives in different products.

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