EFFECT OF LOW ANODE/CATHODE VOLTAGE DIFFERENCE APPLICATION ON REDOX POTENTIAL MODULATION DURING MILK ELECTROREDUCTION AND STORAGE

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ABSTRACT Milk degradation during processing and storage is mainly due to oxidation-reduction reactions. Recently, electroreduction was applied to modulate the redox potential of milk but on a very large range of anode/cathode voltage (2, 4, 6, 8 and 10 V). The suggested value based on process effectiveness was 4 V. Our objectives in the present work were to 1) investigate the effect of low anode/cathode voltage differences on milk redox potential modulation during electroreduction, 2) optimize the process and 3) compare storage of a low-voltage electroreduced milk with a non electroreduced milk. It appeared from these results that electroreduction at anode/cathode voltage difference of 3 V was sufficient to ensure a significant decrease in ORP and DO. The application of a 3V treatment instead of the 4 V value would allow an energy saving of 79%. It appeared also that oxygen is an important parameter to consider during storage of electroreduced milk.

Keywords: Milk, electroreduction, anode/cathode voltage difference, storage.

INTRODUCTION Many modifications of milk composition appear during processing and storage. Milk degradation is mainly due to oxidation-reduction reactions. These reactions result in the alteration of sensitive compounds (unsaturated fat (Jensen, 1991), flavor substances (van Boekel, 1998)), in change in microbiological flora (Hewitt, 1950; Brown & Emberger, 1980) and thermal stability of milk (Moreton, 1998). Oxidation-reduction reactions have an important effect on the quality of dairy products but manufacturers have few resources available to control them. Methods to control the redox potential were evaluated in food industries (addition of cystein or vitamin C) (Dave et Shah, 1997a,b) but they have the disadvantage of using chemical products.

Electrolysis which is an electrochemical process based on electrode redox reactions has already been used to reduce oxygen in fruit juice (Hékal, 1983), to coagulate proteins (Janson and Lewis, 1994) and to reduce disulfide bonds in proteins (Bazinet et al., 1997).
The electrolysis cell operates with only one membrane that separates two solutions circulating in each electrode compartment. In electrolysis, an external anode/cathode voltage difference is applied to the cell and chemical reactions occur at the electrode-solution interface. The anode induces oxidations, and reductions occur at the cathode (Klein et al., 1987). The electrode can act only as a source (for reduction) or a sink (for oxidation) of electrons transferred to or from species in solution and this transfer always occurs at the electrode surface (Bard and Faulkner, 1983).

Since electroreduction is an electromembrane process, energy consumption is a major concern. Hence, in this present work, our objectives were to investigate the effect of low anode/cathode voltage differences on milk redox potential modulation during electroreduction, to optimize the process energy consumption and to compare the storage of a low-voltage electroreduced milk with a non electroreduced milk.

MATERIAL AND METHODS

Material.

*Milk*: The electroreduction treatments were carried-out on pasteurized skim milk of the brand ULTRAMILK, commercially available and manufactured by Natrel (Quebec, Canada).

*Electroreduction System*: The system used was a Microflow type electrodialysis cell with a membrane electrolysis configuration (ElectroCell AB, Karlskoga, Sweden). The cell was separated in two different compartments by a cationic membrane (CMX-SB type, Tokuyama Soda Inc., Japan). On one side of the membrane, the milk was in contact with a food-grade stainless steel cathode and on the other side of the membrane, the electrolyte (0.1 M H$_2$SO$_4$) was in contact with a dimensionally stable electrode (DSA-O$_2$) both supplied with the cell. Each cell compartment was connected to its own external tank (300mL) to allow a continuous circulation during each treatment. The DC current between the two electrodes was supplied from an electrical power supply.

Protocols.

*Treatments*: Five anode/cathode voltage differences applied between the electrodes were tested; 1.5, 2, 2.5, 3, 3.5 volts. During each treatment of 45 min., the oxidation-reduction potential (ORP) and the dissolved oxygen (DO) of milk were recorded.

*Storage*: After each treatment, a sample of electroreduced milk was rapidly poured in three 75 mL-polypropylene jars. The head-space in each jar was minimized by overfilling the containers. The first jar was opened each day during 8 days, while the second and the third jars were opened only at the fourth and ninth day of storage respectively. Redox potential and dissolved oxygen were recorded on each sample during storage.

Analysis methods

*Redox potential measurement (ORP)*: The ORP was measured using a VWR Symphony platinum electrode (Pt/Ag/AgCl, VWR Scientific, Mississauga, ON, Canada) filled with a solution of KCl 3M. This electrode was connected to a VWR Symphony portable SP20 pH/ISE meter.
**Dissolved oxygen:** The DO was measured using a VWR Symphony electrode (VWR Scientific) mounted with the specified membrane and filled with the supplied DO electrolyte solution. The electrode was connected to a VWR Symphony SP50D portable DO meter.

**Energy consumption:** The energy consumption was calculated according to the following equation (Bazinet et al., 1999): \( E = UIt \) (Equation 1) where \( U \) the voltage (volt), \( I \) the current (ampere), \( t \) the time of treatment (second) and \( E \) the energy consumption (joule).

**Statistical Analyses:** The data from the redox potential and dissolved oxygen of milk as a function of time were subjected to repeated measure analysis of variance. The data from the evolution of redox potential and dissolved oxygen during storage with and without opening were also submitted to repeated measure analysis of variance.

**RESULTS AND DISCUSSION**

**Electroreduction treatments of skim milk**

**Redox potential (ORP):** All treatments performed on milk samples resulted in a significant exponential decrease of the ORP value as can be seen on Figure 2. In this experiment, the mean initial ORP value for the pasteurized skim milk used was +130 mV. Generally speaking, raw milk has an ORP between +200 and +300 mV under aerobic conditions. During electroreduction, the general trend was that the redox potential decreased drastically in the first minutes of the treatment, to then stabilize at a constant value corresponding to a plateau (Figure 1).

![Figure 2. Evolution of redox potential (ORP) during electroreduction treatments of milk at different low voltages.](image)

However, according to the anode/voltage difference the value of the final plateau was different; after 45 min of treatment, by applying an anode/cathode voltage difference of 1.5 volts, it was possible to decrease the ORP value at 13 mV. Similarly, voltage differences of 2, 2.5, 3.0 and 3.5 volts, decreased the ORP value to -137 mV, -222 mV, -331 mV and -350 mV respectively. This decrease in the plateau value was in a quite
linear fashion between 1.5 and 3 V to stabilize thereafter. Between 3V and 3.5V the final value of redox potential were similar at -331 mV and -350 mV respectively.

It appears from these results that treatments with anode/cathode voltage difference under 3.0 V does not provide enough energy to reduce all reducible species present in the milk. The different values of plateau for the redox potential would be explained by the different working electrode potential; the greater the electrode potential the faster the electron transfer rate (Tallec, 1985). From 3.0 V and over the maximum reduction seems to be reached, and no more species would be reduced. These results were in accordance with those of Bolduc et al (2006) who observed, for a same electrolysis system in the same conditions of electrolyte, that a 2 V-treatment was not enough to reach the maximum ORP value. However, the plateau values observed by these authors were more negative than the one observed in the present work (-490 mV vs -350 mV). Furthermore they observed a plateau value of -259 mV at 2 V while in the present work an averaged value of – 137 mV was obtained. These differences in the final redox potential would be due to the milk composition variation (Vahcic et al., 1992).

**Dissolved oxygen (DO)**: The DO decreased during the treatment as shown in Figure 2. This decrease in dissolved oxygen was proportional to the increase in voltage difference applied between the working electrodes. The DO concentration was brought down from an averaged initial value of 9.5 mg/L to 6, 6.8, 6, 4.2 and 3.2 mg/L during the course of the electroreduction treatments at 1.5 V, 2V, 2.5V, 3V and 3.5V respectively. The final level of DO observed for anode/cathode voltage differences of 1.5, 2 and 2.5 V were quite similar, while the levels for 3 and 3.5 V where different from one another and from the levels observed at lower voltage differences.

These results were in accordance with those reported for pasteurized skim milk by Bolduc et al. (2006) and Schreyer et al. (2008). The decrease in the concentration of oxygen is directly related to the reduction reactions taking place at the cathode; \( \frac{1}{2} O_2 + 2 H^+ + 2 e^- \rightarrow H_2O \) (Tallec, 1985). Electrons are transferred from the electric circuit to the cathode and then to milk in which they are accepted by active species, one of them being oxygen. The protons necessary to this reaction would be provided by sulphuric acid from the anodic compartment. The protons of the dissociated acid would migrate through the cationic membrane to the milk cathodic compartment. These migrated protons would be consumed by dissolved oxygen to form water or they would simply be reduced in dihydrogen according to the following equation, \( 2 H_2O + 2 e^- \rightarrow H_2 + 2 OH^- \) (Tallec, 1985). As demonstrated by Schreyer (2007) during electroreduction, in a static electrolysis cell, of different milk fractions, oxygen is an electrochemically active compounds, and its reduction contributes to the decrease in the redox potential value.

**Energy consumption**: It appeared from these results that electroreduction at anode/cathode voltage difference of 3 V was sufficient to ensure a significant decrease in ORP and DO. In addition, to confirm the interest of performing electroreduction at 3 V instead of the 4 V value suggested by Bolduc et al. (2006) the energy consumption were compared.

Hence, based on a 35 min-treatment calculation, and with the data obtained for the averaged current at 3 V (27 mA in the present work, with an averaged initial conductivity of 4484 µS/cm) and at 4 V (100 mA, according to Bolduc et al (2006) with an averaged
initial conductivity of 4484 µS/cm and the same electrolysis cell), the respective energy consumption calculated were 170 and 840 J. The application of a 3V treatment instead of the 4 V value would allow an energy saving of 79%. For 3.5 V, the energy consumption calculated was 478 J and the energy saving for a 0.5 V decrease in the anode/cathode voltage difference applied would be of 43%.

Figure 2. Evolution of dissolved oxygen (DO) during electroreduction treatments of milk at different low voltages.

Storage of electroreduced milk. After treating the milk by electroreduction, the objectives were to verify if the ORP value, the dissolved oxygen values and the pH remained stable during the storage of milk at refrigerated temperature and to compare their evolution with a non-electroreduced milk. Furthermore, these three parameters were measured in two conditions, with a daily measurement of the same jar (with opening) during 8 days and with one jar for one measurement (without opening) at day 1, 4 and 9, in order to limit the incorporation of oxygen and to study its impact.

Redox potential (ORP). With and without a daily opening, the non-electroreduced control milk presented the same evolution of ORP during its storage, with a quite linear increase of redox potential from 124 mV to 225 mV (Figures 3a and b). Milk electroreduced at 1.5, 2.0 and 2.5 V showed also similar evolution of their ORP with and without opening during all the storage with increase of their respective ORP of approximately 135, 367 and 358 mV, but with some difference in their evolution. Samples that were treated at 1.5 V had always a positive ORP value, with a starting value lower than the one of control milk (42 vs 124 mV), but after only one day of storage, they reached the values of control milk. Milk treated at 2.0 and 2.5 V, with initial ORP values of -131 and -203 mV reached a positive ORP after 2 days of storage, and thereafter presented similar values as control milk. Milk treated at an anode/cathode voltage differences of 3.0 and 3.5 V showed similar evolution but this ORP evolution was different with and without daily opening of the jar. With opening, these samples started with similar negative values of -317 and -315 mV respectively at 3.0 and 3.5 Volts, and
after 3 days of storage presented positive values of 48 and 8 mV respectively, and thereafter reached a plateau at approximately 63 and 116 mV. Without opening, they presented negative values for a longer period since after 4 days of storage they presented negative values of -146 and -156 mV respectively for 3.0 and 3.5 V-treatments. However at the end of the 9 day-storage their values were positive at 126 and 124 mV.

Figure 3. Changes in oxidoreduction potential (ORP) of pasteurized milk treated at different low voltages during its refrigerated storage a) with daily opening and b) without opening.

These experiments confirm the fact that the ORP modulation by electroreduction treatment has a major impact on the starting ORP value during storage, and that reincrease in ORP would be dependent on the oxygen exposition. This exposition certainly had an impact on the stability of the ORP values measured during storage since oxygen could diffuse in the milk medium easily (Moyssiadi et al., 2004). However, it appeared from these results, that an electroreduction treatment followed by storage
limiting contact with air would have a great impact on redox potential increase during storage. According to the extrapolation of curves obtained for 3 and 3.5 V treatments, milk would have stay under negative redox potential values up to 7 days, and would have contribute to limit oxidation reaction in milk during this period.

**Dissolved oxygen**: Control milk presented a different evolution of its DO values during its storage with and without opening (Figure 4a and b). During storage with daily measurement in the same jar, the level of DO was maintained constant during the first 4 days of storage and then decreased drastically at a value close to 0 after day 6. During storage without contact with ambient air, the DO concentration decreased in a linear fashion during the 9 day-storage from 8.5 to 3.3 mg/L. Electroreduced milks presented similar evolution as control milk but reached a DO values close to 0 faster than control milk (Figure 4a and b). Furthermore, the higher was the anode/cathode voltage difference applied, the faster was the decrease in DO. Hence, during storage with opening, the DO concentration of samples treated by electroreduction was quite constant during 4 days at 1.5 V, 3 days at 2.0, 2.5 and 3.0 V and only 2 days at 3.5 V. Thereafter the samples reached a concentration close to 0. During storage without opening, the DO concentration for samples treated at 2.5, 3.0 and 3.5 V reached values close to 0, after 4 days, while at 1.5 and 2.0 V, the DO concentrations were 2.2 and 0.5 mg/L for the same laps of time. However, the DO concentrations for samples electroreduced at 1.5 and 2.0 V were lower than those observed with opening of the jar ; 5.0 vs 2.2 mg/L and 2.0 vs 0.5 mg/L respectively.

In the present study the rise of the concentration of oxygen during the first two days was not observed or was not as clear as the one reported by Bolduc et al. (2006). However, these authors mentioned that this rise was not observed for samples treated at 2V. Furthermore, the fact that the DO concentration decreased during all along storage without opening while it was constant during 4-5 days confirmed the hypothesis of Bolduc et al. (2006) concerning the fact that the initial rise in the concentration of oxygen can be mainly attributed to the contact of milk samples with ambient air during measurements. However, concerning the hypothesis of Bolduc et al. (2006) on DO concentration decrease observed after or not the DO concentration plateau which would involve growth of aerobic microorganisms from the pasteurized milk, *Pseudomonas* spp., would be in contradiction with the present results. It appeared that the contamination of the control milk was very low, and no change in DO concentration was observed during 4 days of storage at 4°C; the critical biomass of aerobic microorganisms would be reached only after 4 days of storage. Consequently, the decrease in DO concentration observed during the 4 first days for electroreduced milk would not be due to the initial microflora of milk. Two hypotheses could be postulated, 1) either the contamination was due to the growth of aerobic microorganisms from the interior surface of the electroreduction system and electrodes surfaces or 2) other milk components were involved in this DO concentration drop in the first 4 days of storage. This other specie could not be lipids, which by the way of autooxidation could have consume oxygen, since in skim milk, residual lipids were at low concentration of about 0.1%. In fact, although the main residual fat molecules in skim milk are phospholipids, which are very sensitive to oxidation by oxygen, their low concentration could not explain the decrease in DO up to 5 ppm. The DO concentration decrease could be due to whey proteins. In fact, Fukuzawa et al. (2005) demonstrated the antioxidant effect of bovine serum albumin which is due amongst others to the trapping of active oxygen molecules. Consequently, proteins
probably also interfere on the modulation and stability of DO concentration as well as on the ORP during storage.

Figure 4. Changes in dissolved oxygen (DO) of milk treated at different voltages during its refrigerated storage a) with daily opening and b) without opening.

**CONCLUSION** It appeared from these results that a 3.0 V treatment is sufficient to reduce the redox potential of milk to very negative values. Furthermore, the values of redox potential reached by treatment of 3 V and over are effective to maintain negative or reductive conditions in milk during its storage and up to 7 days. It appeared also that oxygen is an important parameter to consider during storage of electroreduced milk. In fact, during electroreduction up to 70% of the dissolved oxygen was consumed. However, according to the conditions of storage and the presence or not of oxygen, its concentration can decrease constantly or stay stable during part of the storage before decreasing. Hence, with opening daily during storage, samples started with negative values and presented
positive values after only 3 days of storage, while without opening, they presented negative values for a longer period. After 4 days of storage samples without opening presented negative values close to -150 mV respectively for 3.0 and 3.5 V-treatments. Milk samples opening daily for taking measurements, simulate the habit of the majority of consumers. This practice allowed oxygen to be incorporated to the milk and this could be responsible for the re-oxidation of the milk electroreduced species. This demonstrated the importance of controlling the oxygen concentration during storage to keep the solution in reductive or protective conditions. The rise in ORP during storage confirms that the changes in redox state of some milk species (mainly proteins) caused by the electroreduction treatments would be reversible and that the storage container material would be an important factor to consider when storing electroreduced milk to diminish or limit reincorporation of oxygen due to a potential diffusion through the wall of the container (Schroder et al., 1985; Rysstad et al., 1998).

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