

## A procedure for reproducible measurement of redox potential ( $E_h$ ) in dairy processes

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**Abstract** The oxidoreduction potential ( $E_h$ ) is of growing interest in dairy research and the dairy industry. Lactic acid bacteria are able to decrease the  $E_h$  to considerable reducing values, and it is an important feature of the technological characteristics of lactic starters and probiotics. Reducing  $E_h$  is also necessary for the development of the characteristic flavour of certain fermented dairy products such as cheeses. Moreover, data comparisons and interpretations are often difficult due to an absence of standardised expression of the results. The objective of this work was to propose a complete procedure for the use of combined and half-cell platinum (Pt) sensors to

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obtain stable, repeatable and reproducible  $E_h$  measurements and to standardise data. Three measurement systems, each made up of a sensor, cable and interface, were compared in tap water. A control chart was used to calculate a confidence interval (CI=20 mV) to verify whether the process was under control. This complete procedure was then applied to the measurement of  $E_h$  in milk samples to characterise both pure strains of lactic acid bacteria and complex mixed starters, to study the impact of a process parameter (oxygen) on starter activity, to follow  $E_h$  during cheese making and to measure  $E_h$  gradients in cheeses. This manuscript presents a procedure for the measurement of  $E_h$  as well as the feasibility and interest of this procedure in the dairy chain.

**Keywords** Redox potential ·  $E_h$  · Metrology · Lactic acid bacteria · Cheese making

## 1 Introduction

In the manufacture of fermented products, attention must be paid to all the parameters that may affect starter culture metabolism and product characteristics. The main physicochemical parameters used for quality control and measurement, as well as for the transformation of the raw material, are temperature, pH, water activity and pressure. The oxidoreduction potential, called  $E_h$  (by reference to a standard hydrogen electrode), is an additional parameter which is being increasingly studied. A measurement system is composed of a sensor, a cable and an interface. A conventional combined redox sensor is composed of a measuring electrode with an inert noble metal and a reference electrode. The measuring electrode is typically platinum, but gold is also used. Depending on the medium studied, the metal can be of different designs: point, plate, band, cap or disc.

Recent results have shown that such a measurement could be an important development in cheese making, improving its control and standardisation (Abraham 2007; Ledon and Ibarra 2006; Caldeo and McSweeney 2012).  $E_h$  influences both physicochemical and microbiological changes (Bourel et al. 2003; Kieronczyk et al. 2006; Monnet et al. 1994; Martin et al. 2009). In the same way as the transfer of hydrogen ions between chemical species determines pH, the transfer of electrons between chemical species determines  $E_h$ . A positive  $E_h$  indicates an environment that favours oxidation reactions, whilst a negative  $E_h$  indicates a reducing environment. There are many ways of modifying  $E_h$  in dairy applications, such as the reducing activity of lactic acid bacteria (Cachon et al. 2002; Brasca et al. 2007), the use of neutral or reducing gas (Abraham et al. 2007; Ledon and Ibarra 2006; Martin et al. 2009, 2010, 2011; Ebel et al. 2011), the electroreduction process (Bolduc et al. 2006; Schreyer et al. 2008) and the addition of oxidising/reducing agents (Dave and Shah 1997a, b). Moreover, comparisons and interpretations of data from the literature relating to redox potential are often difficult because in many cases the measurement methods are not detailed and the results are not standardised. Accordingly, the aim of this paper was to propose a procedure to measure  $E_h$  using a redox sensor to obtain repeatable, reproducible and stable values. This procedure has been applied to  $E_h$  measurement in milk, characterisation of lactic acid bacteria, monitoring of cheese manufacture and measurement of  $E_h$  gradients in cheeses.

## 2 Materials and methods

### 2.1 Measurement systems

For measurements in milk, during milk fermentation and in cheese processes, we used a combined redox sensor, Pt48-DPAS-SC-K8S/120 (the design of platinum was a band and the diameter of the sensor was 12 cm), and a combined pH sensor, 405-DPAS-SC-K8S/120 (Mettler Toledo, Paris, France).

For measurements in cheese, we used miniaturized sensors: a half-cell Pt redox electrode (the measuring and reference electrodes are separated) and a combined pH sensor (World Precision Instruments, Aston, Stevenage, UK); the tip diameters were 1–2  $\mu\text{m}$  and 1.7 mm, respectively (Abraham et al. 2007).

Shielded cables were used to prevent electrical interference (Mettler Toledo). Multichannel redox meter/pH meter interfaces (Absciss, Fixin, France; Bioblock, Illkirch, France; Almemo 4290-7, Ahlborn, Allemagne) were used to follow redox potential and pH simultaneously. A simulator (pH simulator 112, Mettler-Toledo SARL) was periodically used to control the electrical equipment.

### 2.2 Conversion of redox measurements into conventional $E_h$ and $E_{h7}$ values

Both measuring and reference electrodes are half-cells; the measurement corresponds to the potential difference between the two half-cells. An oxidoreduction potential ( $E_h$ ) is defined in relation to the normal hydrogen electrode. The latter is not used as the reference electrode for practical reasons; other reference electrodes are generally used, such as Ag/AgCl or the calomel electrode. The redox potential ( $E_m$ ) measured must therefore be corrected as follows:

$$E_h = E_m + E_r$$

Where  $E_h$  is the redox potential in relation to the normal hydrogen electrode (in millivolts),  $E_m$  is the redox potential measured with the redox sensor (in millivolts), and  $E_r$  is the redox potential of the reference electrode used for measurement (in millivolts).  $E_r$  depends on the type of reference electrode used for measurement, the nature and concentration of the filling solution, and the temperature of measurement (Galster 1991, 2000).

Reference electrode	$E_r$ (25°C)	$E_r$ (35°C)
Ag/AgCl (3 mol.L <sup>-1</sup> KCl)	207 mV	199 mV
Calomel (saturated KCl)	244 mV	237 mV

For Ag/AgCl (3 mol.L<sup>-1</sup> KCl), a general equation can be proposed:

$$E_r(\text{mV}) = 207 + 0.8 \times (25 - T)$$

The variation in medium pH indirectly modifies  $E_h$  (Nernstian response of the redox sensor). Therefore, Leistner and Mirna (1959) proposed an equation

that takes into account a correlation factor in order to standardise the  $E_h$  value independently of the pH:

$$E_{h7} = E_h - [(7 - \text{pH}) \times \alpha]$$

Where  $E_{h7}$  is the redox potential at pH 7,  $E_h$  is the redox potential at the pH of the medium, and  $\alpha$  is the regression factor. According to this equation, a pH variation of one unit modifies  $E_h$  by approximately  $\alpha$  mV. This correlation factor must be determined experimentally for each medium (Jacob 1970). Consequently,  $E_h$  measurements should be accompanied by a statement of the pH value and temperature at which they were taken. The  $E_h$ -pH regression factor was determined in milk. Flasks, equipped with a combined pH sensor/redox sensor and 200 mL of milk, were placed in a water bath at 28 °C. The sensors were connected to a multichannel interface in order to monitor the pH and redox potential in the milk simultaneously. The pH was modified gradually (pH 8 to 4) by the addition of acid and base solutions and the variation in  $E_h$  was measured. The evolution of  $E_h$  depending on pH was determined in ten different milk samples and an identical  $E_h$  variation of  $\alpha=38$  mV/pH unit ( $\pm 1$  mV,  $n=30$ ) at 28 °C was calculated in all the milk samples. This result is in agreement with that of Cachon et al. (2002). Therefore, to compare the redox potential of milk samples with different pH values, the redox potential needs to be standardised as  $E_{h7}$ . As an example, a milk sample at pH 4.5 with an  $E_m$  of  $-350$  mV has an  $E_h$  of  $-145$  mV ( $E_r=205$  mV at 28 °C with the Ag/AgCl reference electrode (3 M KCl)) and  $E_{h7}$  values of  $-240$  mV (with  $\alpha=38$  mV/pH unit) and  $-293$  mV (with the theoretical Nernst correlation factor of 59 mV/pH unit). The difference between the latter two  $E_{h7}$  values is then 53 mV.

### 2.3 Metrology for redox measurements: control chart method

The control chart indicates the range of variability that is built into a system (known as common cause variation). It can thus be determined whether or not a process is operating consistently or whether something specific has occurred to change the process mean or variance (ISO 7870; Tscale and Aly 1996). The control chart means ( $\bar{X}$ ) were used. The mean was monitored with a statistical control chart, an  $\bar{X}$  chart. The  $\bar{X}$  chart controls the process mean. These charts consist of a central line (CL), which is the sample grand mean (mean of means), and the upper and lower control limits (UCL and LCL). The equations for the control limits of  $\bar{X}$  charts are

$$\begin{aligned} \text{CL} &= \mu \\ \text{UCL} &= \mu + k\sigma / \sqrt{n_i} \\ \text{LCL} &= \mu - k\sigma / \sqrt{n_i} \end{aligned}$$

where  $\mu$  is the process mean (here,  $\mu = \bar{X}$ ),  $\sigma$  the process standard deviation,  $n_i$  the sample size of the  $i$ th subgroup and  $k$  is the default limits with  $k=3$ , at a 99.8% confidence level (these are referred to as  $3\sigma$  limits).

As well as the individual points,  $X_i$ , the  $\bar{X}$  chart plots a central line at  $\mu$  ( $\mu$  is usually estimated by the mean of  $X_i$ —mean of measurements in the  $i$ th subgroup), and warning and control limits are calculated as confidence interval (CI). Analysis of

variance (ANOVA; at a 95% confidence level) was used to determine the stabilisation time of the redox value in tap water and in milk. The software Minitab® (version 13, 2004) was used to calculate the control limits, ANOVA and the control charts represented.

#### 2.4 Determination of CI in milk

Milk was reconstituted with sterile tap water and low-heat milk powder (Spray 0, Ingredia, Arras, France) supplemented with fat from milk (Elle-et-Vire 30%, Conde-Sur-Vire, France) and calcium ( $0.5 \text{ g.kg}^{-1}$ ) in order to obtain concentrations close to those in milk: 35% nitrogen compounds, 41% fat and 48% lactose.

#### 2.5 Reduction and acidification kinetics parameters

*Lactococcus lactis* subsp. *lactis* SL03 (ARILAIT, Paris, France) and *Lactobacillus brevis* BCRC 12945 (Bioresources Collection and Research Center, Hsin-Chu, Taiwan) were used to follow the reduction and acidification kinetics in pure cultures. Two commercial mixed starters (CS1 and CS2) composed of strains of *Lactococcus* sp. (92%) and *Leuconostoc* sp. (8%) were used to compare the effect of oxygen on the reduction and acidification kinetics. The material and culture conditions have been described previously by Cachon et al. (2002). In order to avoid interference with the redox measurement caused by atmospheric oxygen, the cultures were performed under static conditions. When the effect of oxygen was studied, the milk was gassed for 40 min with sterile air. The reduction and acidification kinetics of three different experiments were treated mathematically to calculate the acidification rate ( $V^a=(dpH/dt)$ , in pH unit per hour) and reduction rate ( $V^r=(dE_h/dt)$ , in millivolts per hour). These rates allow the maximum acidification rate ( $V_m^a$ , in pH unit per hour) and the maximum reduction rate ( $V_m^r$ , in millivolts per hour); the time at which these maximum rates occurred,  $T_m^a$  (in hours) and  $T_m^r$  (in hours) respectively; and the pH and  $E_h$  at which these maximum rates occurred,  $pH_m^a$  (pH unit),  $E_{h,m}^a$  (in millivolts) and  $pH_m^r$  (pH unit),  $E_{h,m}^r$  (in millivolts), respectively, to be determined.

#### 2.6 Monitoring of $E_h$ during cheese making

The cheeses were manufactured in a mini experimental cheese-making plant located at INRA in Poligny. The vats had a capacity of 12 L for making 1 kg cheeses. Reconstituted milk was used (see Section 2.4). The cheeses were uncooked pressed cheese (“Raclette-type” cheese) and the cheese-making process is described in Fig. 5. A lyophilized commercial starter MA11 consisting of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* was inoculated at 0.10 U/10 L (DuPont, Paris, France) and an active rennet chymosin ( $0.27 \text{ mL.kg}^{-1}$ ) from DuPont (Paris, France) was added to the milk vat.

#### 2.7 Measurement of $E_{h7}$ gradients in cheeses

Two different full fat soft cheeses differing by their manufacturing technology were purchased in a supermarket and immediately used to measure the  $E_{h7}$

gradients in cheeses. Cheese 1 corresponded to a Camembert cheese and cheese 2 to a washed-rind cheese. The experimental procedure has been detailed previously by Abraham et al. (2007).

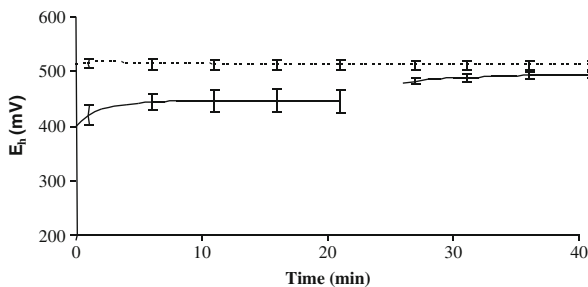
### 3 Results and discussion

#### 3.1 Cleaning procedures

Platinum has a high adsorption activity of substances such as organic substances and oxides; the latter may form a layer around the platinum that can lead to the misestimation of the potential measured. The rougher the surface, the more substances bind. For these reasons, a regular and systematic treatment of the platinum surface is required for a well-defined surface quality and high reproducibility. Various methods for cleaning have been proposed in the literature, involving chemical, electrolytic, heat or mechanical treatment (Jacob 1970; Galster 2000), mechanical cleaning being the most common. We recommend polishing the platinum surface with a slightly humidified fine alumina powder (diameter, 0.1  $\mu\text{m}$ ; ESCIL, Chassieu, France). The platinum surface must be polished systematically before use (typically 30 s). Figure 1 shows the differences between a polished and an unpolished redox sensor. These sensors were placed in tap water for verification (see Section 3.2). There was a significant difference of approximately 150 mV between these two sensors in favour of the polished sensor. Moreover, the stability of the measurement was higher for the polished sensor than for the unpolished sensor. After polishing the latter, this difference was neutralized.

#### 3.2 Metrology of redox measurements

Due to some measurement problems caused by the interface or the cables, the whole measurement system is taken into account for the redox measurement. An important step in potentiometric measurements is the calibration of the sensors (i.e. for pH sensors, the calibration correlates the voltage produced by the probe with the pH scale). Redox potential is measured and expressed in volts; consequently, a calibration process is not necessary. Nevertheless, to validate data obtained from the



**Fig. 1**  $E_h$  measurement in tap water using a polished (*dashed line*) and an unpolished redox sensor (*solid line*,  $n=3$ ). After 20 and 25 min, the unpolished sensor was polished. Each curve is the mean of three measurements; error bars represent standard deviations

equipment, it is necessary to test the accuracy of the sensor. Many commercial chemically defined redox buffer solutions have been proposed for testing redox sensors. They are all oxidising with  $E_m$  values between +200 and +700 mV; there are no reducing solutions available due to problems with chemical stability. These solutions are strongly buffered and may mask a possible deviation of the measurement (data not shown). Therefore, they can only be recommended to control the reference electrode, as specified by some manufacturers (Metrohm, Villebon Courtaboeuf, France). Thus, if the measurement in redox buffer solutions is erroneous, only the following causes may be suspected: incorrect reference electrolyte, diaphragm clogged or dirty and problem with electrical contact inside the sensor. However, a method to control the accuracy and the reliability of sensors remains necessary, especially for media where only small changes in  $E_h$  values should be measured. The important point is to compare different measurement systems (as defined in Section 2.1) in order to detect a possible problem related to these systems. Therefore, instead of a calibration as used for pH sensors, we proposed a method of verification for a redox sensor. The procedure set up in our laboratory was to compare different measurement systems in tap water. It is a good indicator of deviation because, in contrast to commercial buffers, it contains few agents. All redox sensors were plunged into a unique container containing tap water at room temperature. Firstly, the stabilisation time in tap water was determined, corresponding to the time required to reach a stable redox value. As no significant difference was observed for the values at 5, 10 and 15 min, a stabilisation time of 5 min was retained. According to metrological studies, five different measurement systems were used to determine control limits using the control chart method. Five measurements were obtained for each measurement system in the same medium, and the control chart method allowed these control limits and, consequently, a confidence interval (Table 1 and Fig. 2a) to be calculated. The confidence limits were calculated and a CI of 20 mV ( $\pm 10$  mV around the mean) in tap water was determined at a 99.8% confidence level. The CI determined provided the accuracy of further measurements in tap water. Before each experiment, the number of measurement systems could be reduced to three (for practical reasons) to verify whether they were operating consistently in tap water. If the value of one sensor was not within this CI of 20 mV, the sensor was not under control. Therefore, the sensor had to be polished again and was eliminated from the batch if, after repeated cleaning cycles (approximately three), it was still outside the interval. Thus, with regard to all these elements, a diagram describing redox sensor maintenance processes is proposed (Fig. 3).

### 3.3 Applications to dairy processes

#### 3.3.1 Determination of the confidence interval in milk

The control chart can be used advantageously to discriminate different milk samples. The time to reach a stable  $E_h$  value is medium-dependent; in milk, the stabilisation time was  $\sim 10$  min. In Table 1, the redox values in milk observed after 10 min of stabilisation, measured with five redox sensors, are compared. The CI was calculated from a control chart at 15 mV at a 99.8% confidence level (Fig. 2b). For further experiments, the number of measurement systems could be reduced to three using the CI determined with five.

**Table 1** Means ( $n=5$ ) and SDs of redox measurements carried out using redox sensors in tap water and in reconstituted milk powder (fat, 0%; protein, 28%; pH 6.8)

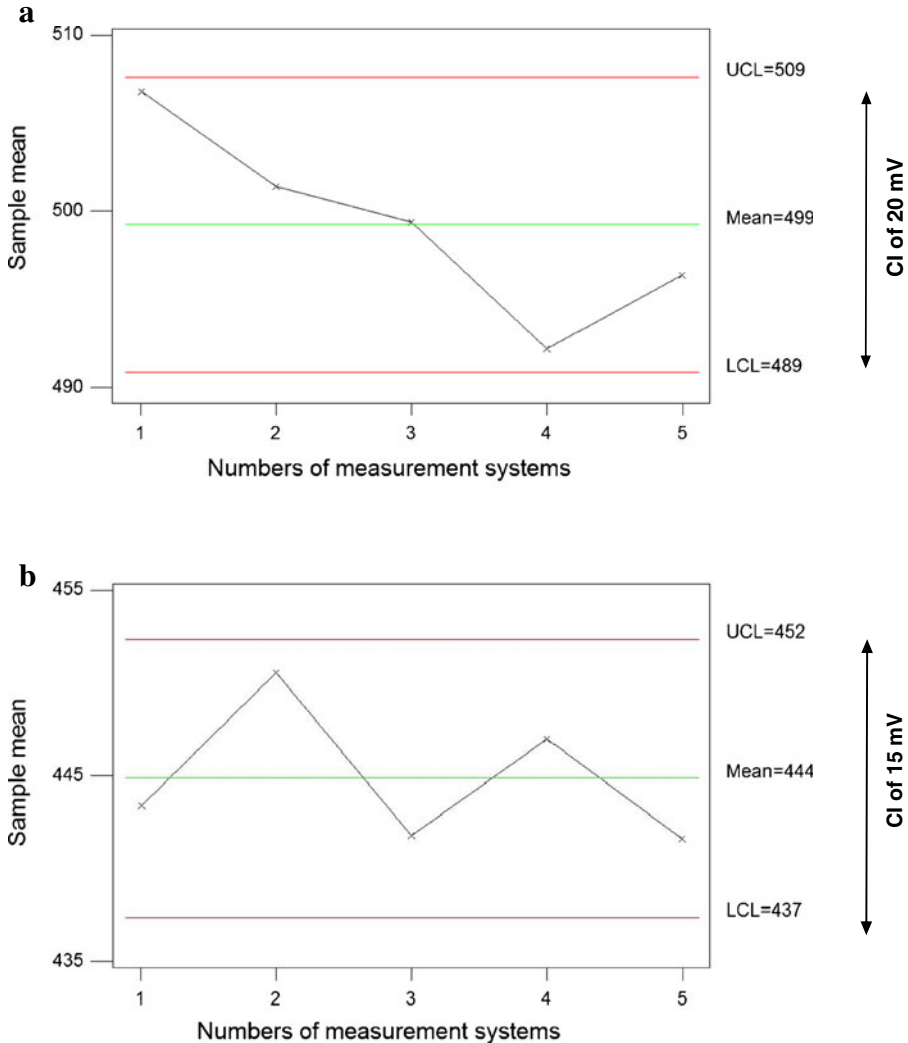
		$n=1$	2	3	4	5	Mean (mV)	SD (%)
$E_h$ values (mV) measured in tap water								
Redox electrodes	$E_1$	517	512	500	489	490	502	11
	$E_2$	510	503	500	495	503	502	5
	$E_3$	509	503	504	495	503	503	4
	$E_4$	494	492	500	490	494	494	3
	$E_5$	504	497	493	492	492	496	5
$E_{h7}$ values (mV) measured in milk								
Redox electrodes	$E_1$	445	456	441	454	442	448	7
	$E_2$	447	452	444	448	450	449	3
	$E_3$	428	442	440	446	437	439	7
	$E_4$	448	452	440	445	439	445	5
	$E_5$	449	451	444	442	440	446	5

Sensors were polished between each measurement; stabilisation times were 5 min in tap water and 10 min in milk

### 3.3.2 Reduction and acidification kinetics during lactic acid fermentation in milk

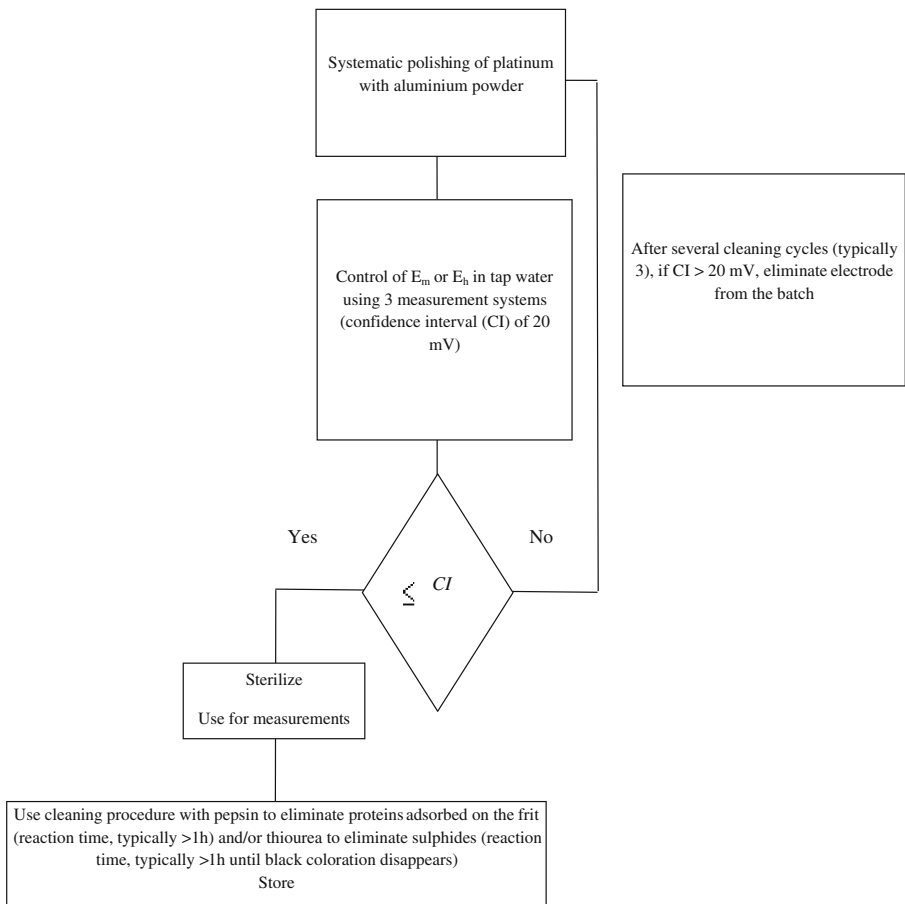
**Strain effect** In the dairy chain, the parameters  $V_m^r$ ,  $T_m^r$ ,  $\text{pH}_m^r$  and  $E_{h7}^r$ ,  $V_m^a$ ,  $T_m^a$ ,  $\text{pH}_m^a$  and  $E_{h7}^a$  have been proposed to characterise lactic starters in order to select adequate starters for different industrial dairy processes (Cachon et al. 2002; Spinnler and Corrieu 1989).  $E_h$  and pH in pure *L. lactis* subsp. *lactis* SL03 and *L. brevis* BCRC 12945 cultures were followed during lactic acid fermentation in milk. Figure 4 shows the kinetics obtained with *L. brevis*, whilst typical curves for *Lactococcus* sp. can be found in a previous study (Cachon et al. 2002). All the data are summarized in Table 2. The initial  $E_{h7}$  and pH values of milk were  $240 \pm 10$  mV and  $6.4 \pm 0.1$ , respectively (means of three repetitions). For *L. lactis* subsp. *Lactis*, the reduction preceded the acidification; the minimum  $E_{h7}$  value was reached before reaching the maximum acidification rate ( $T_m^r=2.3$  h,  $T_m^a=6.5$  h). For *L. brevis*, the maximum acidification and reduction rates were reached at a similar time ( $T_m^r=7.6$  h,  $T_m^a=7.8$  h). The variation in  $E_{h7}$  was 447–460 mV during fermentation. This variation was due to the activity of the strains, and it can be noted that whilst the kinetics were different between *L. lactis* and *L. brevis*, the final pH and  $E_{h7}$  were close. The mechanisms involved in reduction were the reduction of dissolved oxygen to water, the consumption of other oxidising agents (Fe and Cu) and the production of reducing agents (assumed to be exofacial thiols), as shown for *L. lactis* (Michelon et al. 2010; Tachon et al. 2009). The acidification modified the kinetics of the  $E_h$  drop during lactic acid fermentation in milk (see Section 2.2). Differences of approximately 106 and 121 mV were noted between the final  $E_h$  and  $E_{h7}$ , which corresponded to a decrease in pH of 2.3 pH units for *L. lactis* subsp. *lactis* and 2.6 pH units for *L. brevis*. Such a method has already been used to compare commercial lactic starters (Aubert et al. 2002), and it can also be used to study the effect of the process on the reduction and acidification activities of starters.





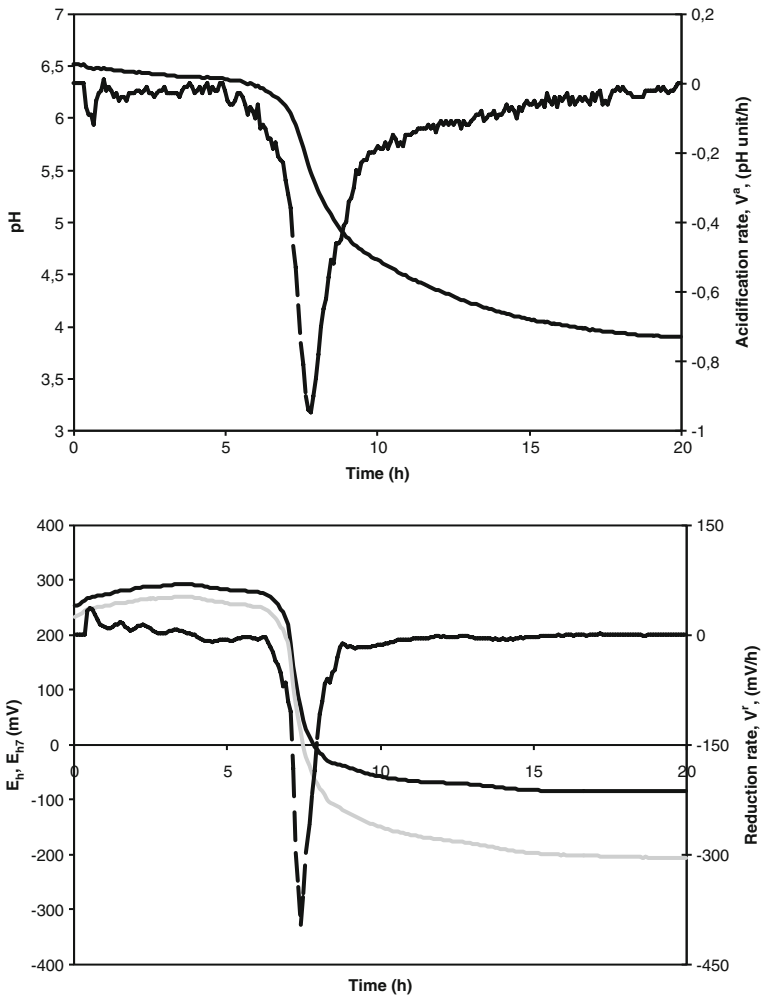
**Fig. 2** Control charts for five  $E_h$  measurement systems in tap water (a) and in milk (b) ( $n=5$ ). *UCL* upper control limit, *LCL* lower control limit, *CI* confidence interval

*Oxygen effect* An oxygen effect exists due to the incorporation of oxygen during dairy processes. Oxygen can be blended with milk in different ways, for example, when the milk is poured into the pipelines or into the vats and when it is mixed or stirred (see Section 3.3.3). Oxygen is a powerful oxidising agent ( $E'_0=816$  mV), which may delay acidification by lactic acid bacteria (Abraham 2007; Aubert et al. 2002; Jeanson et al. 2009; Tachon et al. 2009). Oxygen modifies the redox measurement in milk;  $E_h$  varies from 50 to 60 mV per log dissolved oxygen concentration (Abraham 2007; Jeanson et al. 2009; Tango and Ghaly 1999; Tengerdy 1961). CS1 and CS2 are commercial mixed starters from the same supplier, assumed to have the same composition, but from two



**Fig. 3** Diagram of the maintenance process for sterilizable redox sensors

different batches. In a dairy plant, a different milk acidification in vats has been observed, whereas at laboratory scale, CS1 and CS2 have the same acidification kinetics. A difference in sensitivity to oxygen has been assumed. At laboratory scale, the milk used to monitor the acidification kinetics of CS1 and CS2 was reconstituted from milk powder and autoclaved. It contained low levels of dissolved oxygen, whilst in the vats the milk was poured in from the top and stirred and contained high concentrations of dissolved oxygen. Table 3 shows the selected kinetic parameters to compare the two commercial mixed starters (CS1 and CS2) in milk with low (nitrogen-bubbled) or high (air-bubbled) oxygen levels at the beginning of the culture. In milk low in dissolved oxygen, CS1 had a significantly higher  $V_m^r$  than CS2 ( $p < 0.05$ ). No significant differences ( $p < 0.05$ ) were observed between  $T_m^r$ ,  $T_m^a$  and  $V_m^a$  of the two starters. The starters reached  $T_m^r$  first, then acidified and reached  $T_m^a$  about 2 h later. In milk samples with high levels of dissolved oxygen, oxygen delayed the reduction and acidification kinetics, but the main observation is that CS1 had lower  $T_m^r$  and  $T_m^a$  values than CS2. Such differences in starter



**Fig. 4** Kinetics of  $E_h$ ,  $E_{h7}$ , reduction rate ( $V^r$ ), pH and acidification rate ( $V^a$ ) of *L. brevis* BCRC 12945 at 37 °C in milk. Each curve is the mean of three measurements. Black lines, pH and  $E_h$ ; grey line,  $E_{h7}$ ; black dashed lines,  $V^r$  and  $V^a$

sensitivity to the inhibitory effects of oxygen, which cannot be identified in milk low in oxygen, were clearly underlined in milk containing high levels of oxygen. Consequently, the determination of such kinetic parameters may be a suitable procedure to evaluate the technological properties of starters in conditions taking into account the oxidative stress encountered in dairy plants.

### 3.3.3 Monitoring $E_{h7}$ during cheese making

Figure 5 shows the evolution of  $E_{h7}$  at every step of the process. During the early stages in the vat, the  $E_{h7}$  only decreased by 60 mV, which is associated with the dissolved oxygen levels measured in the vat between 20% and 60% due to the different operations that facilitate its incorporation (i.e. stirring). At  $t=2$  h, pressing

**Table 2** Mean values of the kinetic parameters calculated from the reduction and acidification curves for *L. lactis* subsp. *lactis* SL03 ( $T=28\text{ }^{\circ}\text{C}$ ) and *L. brevis* BCRC 12945 ( $T=37\text{ }^{\circ}\text{C}$ )

	Final $E_h$ (mV)	Final $E_{h7}$ (mV)	$V_m^r$ (mV.h <sup>-1</sup> )	$T_m^r$ (h)	pH <sup>r</sup> <sub>m</sub>	$E_{h,m}^r$ (mV)	Final pH	$V_m^a$ (pH unit.h <sup>-1</sup> )	$T_m^a$ (h)	pH <sup>a</sup> <sub>m</sub>	$E_{h,m}^a$ (mV)
<i>Laetococcus lactis</i> ssp. <i>lactis</i> SL03 (SD) <sup>a</sup>	-114 (6)	-220 (6)	-378 (22)	2.3 (0.1)	6.3 (0.0)	-52 (21)	4.2 (0.1)	-0.45 (0.00)	6.5 (0.1)	5.1 (0.1)	-152 (2)
<i>Lactobacillus brevis</i> BCRC 12945 (SD) <sup>a</sup>	-85 (4)	-206 (3)	-442 (166)	7.6 (0.4)	5.7 (0.4)	28 (21)	3.9 (0.1)	-0.95 (0.1)	7.8 (0.0)	5.5 (0.2)	0 (106)

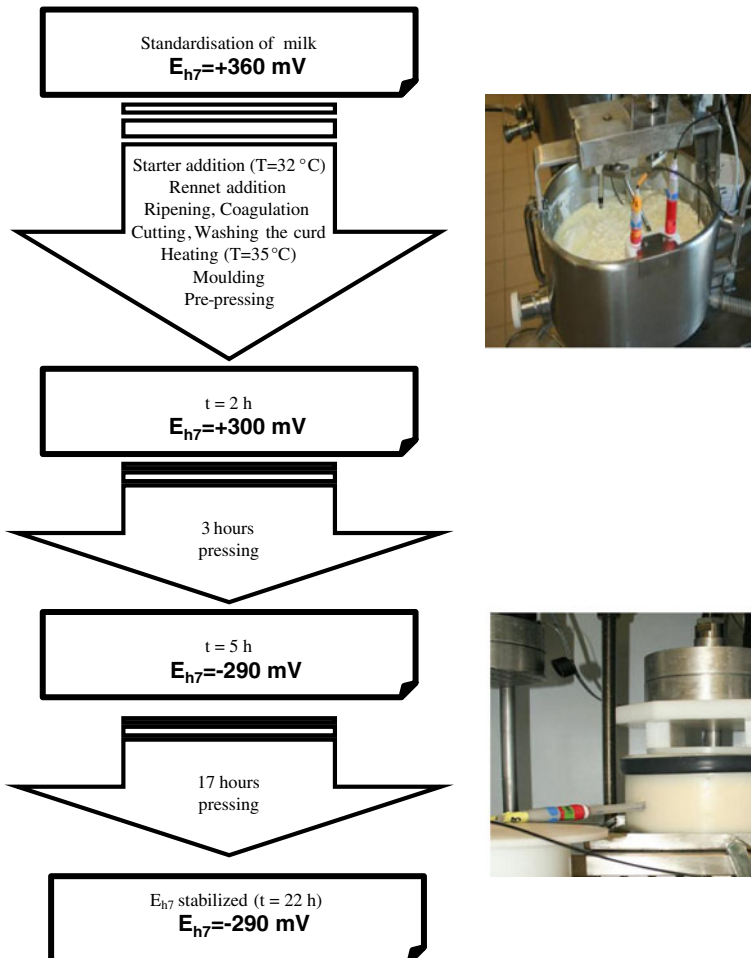
<sup>a</sup>  $n=3$

**Table 3** Mean values of the kinetic parameters calculated from the reduction and acidification curves for commercial starters CS1 and CS2 ( $T=30\text{ }^\circ\text{C}$ )

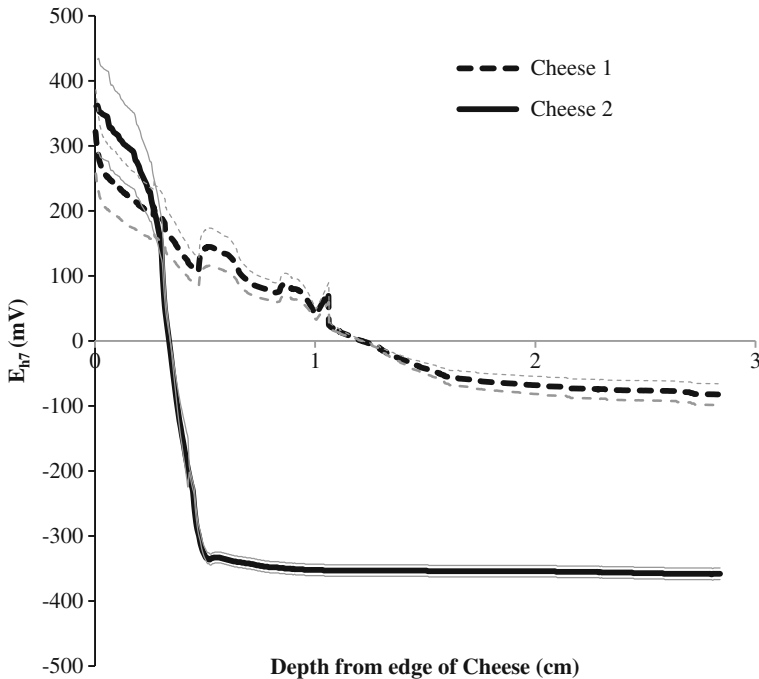
Initial O <sub>2</sub> content	$V_m$ (mV.h <sup>-1</sup> )		$T_m$ (h)		$V_m^a$ (pH unit.h <sup>-1</sup> )		$T_m^a$ (h)	
	CS1	CS2	CS1	CS2	CS1	CS2	CS1	CS2
0%	-593±13 <sup>c</sup>	-491±27 <sup>d</sup>	4.92±0.01 <sup>a</sup>	4.51±0.81 <sup>a</sup>	-0.24±0.01 <sup>a</sup>	-0.29±0.03 <sup>ab</sup>	7.13±0.11 <sup>d</sup>	7.13±0.18 <sup>d</sup>
100%	-798±3 <sup>e</sup>	-720±33 <sup>f</sup>	8.02±0.58 <sup>e</sup>	9.06±0.18 <sup>b,c</sup>	-0.25±0.01 <sup>a</sup>	-0.34±0.01 <sup>b</sup>	9.18±0.23 <sup>e</sup>	10.43±0.12 <sup>f</sup>

Superscript letters are the results of Newman–Keuls’ test at  $p<0.05$ . For the same kinetic parameter, values with the same letter are not significantly different

began, and after 3 h of pressing the reduction was already significant with a 590 mV  $E_{h7}$  decrease. During pressing, the incorporation of oxygen in the curd was limited, allowing microorganisms to express their reduction activities and to decrease the  $E_{h7}$ .



**Fig. 5** Monitoring of  $E_{h7}$  during cheese making



**Fig. 6** Measurement of  $E_{h7}$  gradients in cheese. Cheeses 1 and 2 are different types of soft cheese. 0 cm corresponds to the surface and 3 cm to the centre of the cheese

Consequently, both the process and starters were involved in  $E_{h7}$  change, and monitoring  $E_{h7}$  throughout cheese making could allow better control and standardisation of the process (Caldeo and McSweeney 2012).

### 3.3.4 Measurement of $E_h$ gradients in cheeses

The procedure to prepare combined redox sensors was applied to measurements using miniaturized sensors. The simultaneous measurement of  $E_h$  and pH allowed  $E_{h7}$  to be calculated and redox gradients to be compared independently of the differences in pH. Microsensors were used to compare two cheeses; cheese 1 corresponded to a Camembert cheese and cheese 2 to a washed-rind cheese. Figure 6 clearly shows the interest of microsensors to measure  $E_h$  gradients; cheeses 1 and 2 were oxidising near the surface and reducing in the centre. However, cheese 2 was more reducing in the centre and consequently exhibited a larger redox gradient than cheese 1. As stated in Section 3.3.3, the redox status of cheeses is modified by the technology used for their manufacture, and the measurement of  $E_{h7}$  gradients might help provide better standardisation in cheese making and enable cheese quality to be monitored at the different stages of ripening.

## 4 Conclusion

From the results obtained in this study, it can be concluded that sensors with a Pt electrode vs. a reference electrode, used in a procedure allowing stable, repeatable

and reproducible results, is convenient for measuring redox status in food systems. The CIs obtained were 20 mV in tap water and 15 mV in milk. Moreover, this paper provides important recommendations for obtaining precise redox values and for standardising data from which interpretations and comparisons can be easily made. The recommendations can be summarized as follows: (1) systematic polishing of the Pt surface before each measurement is essential; (2) the reference electrode used must be specified and the variation in its potential value as a function of temperature must be taken into account; (3) the redox values expressed as  $E_m$  must be corrected to  $E_h$ ; (4) the pH must be measured simultaneously and the  $E_h$ -pH correlation factor must be calculated experimentally so the values can be expressed as  $E_{h7}$ ; (5) dissolved oxygen concentrations can be monitored for better interpretation of  $E_h$  variations; and (6) the procedure for preparing the conventional 12-mm redox sensors is transferable to redox microsensors 0.61 mm in diameter. Thanks to these recommendations, the validity of the procedure has been illustrated by experiments performed in milk, during lactic acid fermentation, during cheese making and in cheeses. The interest of using such a preparation procedure for measuring the redox status of raw materials and variations in dairy processes has been clearly shown. In view of the increasing need for better control and standardisation in food processing in the dairy industry,  $E_h$  seems to be an innovative control parameter that can be easily used. This is the more complete methodological study to give a reliable, standardised procedure for monitoring the redox potential that will be of increasing interest for the scientific community.

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