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Chapter 3

Redox Potential: Monitoring and Role in Development of Aroma Compounds, Rheological Properties and Survival of Oxygen Sensitive Strains During the Manufacture of Fermented Dairy Products

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Additional information is available at the end of the chapter

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1. Introduction

Lactic acid bacteria can be found in a diversity of ecosystems, which is consistent with their ability to adapt to highly variable environments. Among the various parameters that characterize these environments (temperature, pH, water activity), redox is relatively recent. It has however already been addressed indirectly in studies relating to the impact of oxidative stress on lactic acid bacteria. Indeed, the concept of oxidation has often been associated with the presence of oxygen; however, oxidoreductive effects on microorganisms must not be limited to oxygen.

A broader vision could be proposed concerning the adaptation of lactic acid bacteria to extracellular redox. The metabolism of lactic acid bacteria, chemosynthetic organisms, involves a series of dehydrogenation (oxidation) and hydrogenation (reduction) reactions. This metabolism follows the principle of conservation of energy and matter, and therefore requires the availability of a terminal electron acceptor. In lactic acid bacteria, a carbon metabolic intermediate is reduced (mainly pyruvate). In homofermentative lactic acid bacteria, redox coenzymes (NAD^+/NADH) enable coupling between oxidation and reduction reactions. During anaerobic glycolysis, glucose is oxidized to 2 moles of pyruvate with the formation of 2 moles of NADH, which then further reduces pyruvate to form lactic acid. Consequently, the typical equation of homolactic fermentation is: 1 glucose →2 lactate.

Such a perfect matching, theoretically consistent, must be qualified according to the environmental conditions, including the redox state of the extracellular medium. The

adaptation of lactic acid bacteria to extracellular redox depends on their ability to positively or negatively interfere with oxidants (electron acceptors) or reducing molecules (electron donors). Carbon and electron flow management by the cell will thus be highly dependent on the ability of the microorganisms to interact with the redox environment.

Potentially, all biochemical reactions in the cell, and therefore the enzymatic activity, may be influenced by the redox state of the environment. Dissolved oxygen is an oxidizer and can reach concentrations of 8 $mg.L^{-1}$ of medium (equilibrium with air). Despite the strict anaerobic metabolism of some lactic acid bacteria, the majority are aerotolerant and can react with dissolved oxygen at varying levels. Lactic acid bacteria provided with NADH oxidase can reduce oxygen to water (reduction reaction coupled with the re-oxidation of NADH). This process influences both the intracellular and extracellular redox environment, and will result in a change in the metabolism, cellular physiology and physico-chemical environment surrounding the microorganism.

Changes in the extracellular environment can be monitored by measuring the redox potential (E_h). This parameter plays a key role in the quality of fermented dairy products, but is still rarely taken into consideration or is completely ignored during the manufacturing process. The reasons for this lack of interest can be attributed to difficulties associated with its measurement and control. Over the past ten years, several studies advocate the monitoring and control of E_h in fermented products using lactic acid bacteria selected for their reducing ability, redox molecules, or heat treatment. In terms of food applications, the variation in E_h must involve compounds that do not alter the product characteristics. So, modifying the E_h using gas, which enables the product characteristics to be maintained, may be advantageously exploited in industry.

The aim of this chapter is to present the latest knowledge concerning the adaptation of lactic acid bacteria to their redox environment, and the interest of modifying E_h using gas for lactic acid bacteria applications in the food industry.

2. Redox potential

E_h, like pH, is a parameter of the state of biological media which indicates the capacity to either gain or lose electrons. During oxidation, electrons are transferred from an electron donor to an electron acceptor, which is reduced. Electrochemical measurement of E_h is not new but has attracted little attention as a parameter for controlling fermentation processes due to the sensitivity of its measurement. However, E_h is already indirectly taken into account in industry through oxygen, of which the inhibitory effect on lactic acid bacteria is well-known. Indeed, oxygen modifies the growth capacity of microorganisms and the formation of end products, and so may contribute to the quality of fermented products [1, 2].

2.1. Definition of E_h

Oxidation is a reaction in which a molecule, atom or ion, loses electrons.

Reduction is a reaction in which a molecule, atom or ion, gains electrons.

An **oxidant** (also known as an oxidizing agent, oxidizer or oxidiser) can be defined as a substance that removes electrons from another reactant in a redox reaction.

A **reductant** (also known as a reducing agent or reducer) can be defined as a substance that donates an electron to another species in a redox reaction.

In the same way pH defines acid-base characteristics of a solution, E_h defines the reducing and oxidizing characteristics.

Presented below is the reduction half-reaction of an oxidant (Ox) to its corresponding reduced species (Red):

$$Ox + ne^- \leftrightarrow Red \quad (1)$$

The Nernst equation gives the relationship between the redox potential and the activities of the oxidised and reduced species:

$$E_h = E_h^0 + 2.3 \times \left(\frac{RT}{nF}\right) \times \log\left(\frac{[Ox]}{[Red]}\right) \quad (2)$$

where:

E_h = redox potential (mV) (in relation to a normal hydrogen electrode).

E_h^0 = standard redox potential (mV) (in relation to a normal hydrogen electrode) at pH 0

F = Faraday constant (96500 $C.mol^{-1}$)

n = number of electrons exchanged

R = gas constant (8.31 $J.mol^{-1}.K^{-1}$)

T = temperature in K

$2.3 \times \frac{RT}{F} = 59$ mV (at 25 °C)

However, chemical reactions in aqueous media involve protons, and the following half-reaction:

$$Ox + mH^+ + ne^- \leftrightarrow Red + H_2O \quad (3)$$

From Equation (2) it can be written:

$$E_h = E_h^0 - 2.3 \times \left(\frac{mRT}{nF}\right) \times pH + 2.3 \times \left(\frac{RT}{nF}\right) \times \log\left(\frac{[Ox]}{[Red]}\right) \quad (4)$$

m = number of protons involved in the reaction

Equation (4) is used to determine $E_h^{0'}$ defined as the standard redox potential at pH 7, which is closer to biochemical and biological processes (Figure 1).

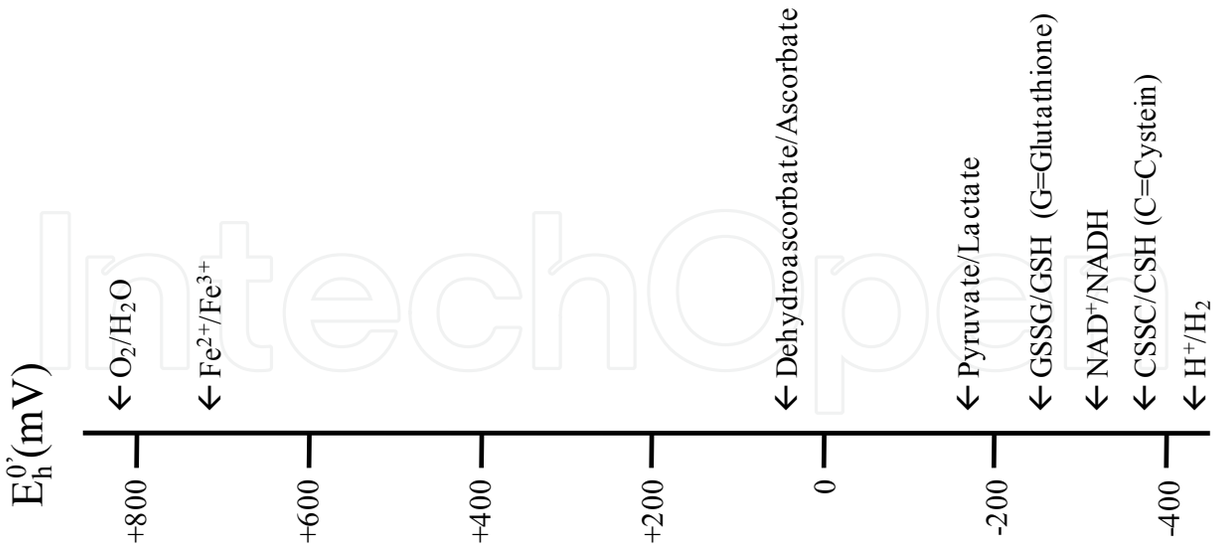

Figure 1. Standard reduction potential $E_h^{0'}$ (mV) of some important half-reactions involved in biological processes at 25 °C and pH 7.

2.2. Measurement of E_h

The first technique for measuring E_h is based on the use of coloured indicators (redox indicators), which are mostly indophenols or indigo derivatives with a reversible structure between oxidized (coloured) and reduced (colourless) state. However, the use of coloured indicators for measuring E_h, including biological media or food, is limited. Indeed, these molecules behave as electron donors and acceptors; they affect and can change the equilibrium. These compounds can also catalyse or inhibit biological reactions and may be toxic to microorganisms. Furthermore, in some cases it is difficult to appreciate a significant colour change and some E_h indicators also change colour with the pH of the medium. For these reasons, redox indicators are rarely used. They are more often used as indicators of redox thresholds, especially in the manufacture of strictly anaerobic culture media (resazurin) where maintaining a minimum level of reduction is essential for the growth of anaerobic microorganisms. Resazurin is also used to evaluate the reducing activity of starter cultures, for sterility testing and for the detection of microorganisms in dairy milk.

The second method commonly used in microbiology is a potentiometric technique which, contrary to redox indicators, is a direct method. The principle consists in measuring a potential difference determined between an inert electrode (usually made of platinum or gold) in contact with a redox couple in solution and a reference electrode. Electron exchange with the reduced and oxidised species takes place at the inert electrode. The inert electrode is made of stainless metals with a high enough standard potential to be electrochemically stable. These metals act as electron conductors between the measuring medium and the

reference electrode. The reference system is the standard hydrogen electrode, but in practice two other references are used: the calomel electrode and the silver / silver chloride (Ag/AgCl) electrode. The redox potential is expressed in volts or millivolts. Redox values should always be expressed in relation to the hydrogen electrode. Consequently, potential measurements (E_m) using other references must be adjusted according to the reference potential of the hydrogen electrode (E_r):

$$E_h = E_m + E_r \quad (5)$$

For example, E_r of the Ag/AgCl electrode is equal to 207 mV at 25 °C [3]. According to data from Galster [3], we propose the following equations linking E_r and temperature for the two reference electrodes:

$$\text{Ag / AgCl } (\text{KCl 3M}) \qquad E_r = 207 + 0.8 \times (25 - T) \quad (6)$$

$$\text{Calomel } (\text{Saturated KCl}) \qquad E_r = 244 + 0.7 \times (25 - T) \quad (7)$$

Before use, the redox electrodes must be polished with fine aluminium powder to restore the platinum surface, and controlled in tap water. Three measurements in tap water should be compared and need to be within the confidence interval around their mean value (calculated at 20 mV, 95% confidence level) to ensure correct measurement [4].

Equation (4) shows the dependence of E_h on pH. It is possible to overcome pH dependency by applying the Leistner and Mirna equation [5]:

$$E_{h7} = E_{h\beta} - \alpha \times (7 - \beta) \quad (8)$$

where:

E_{h7} = redox potential (mV) at pH 7
$E_{h\beta}$ = redox potential (mV) at pH β
β = pH of medium
α = Nernst E_h–pH correlation factor (mV/pH unit).

To calculate Eh7 in biological media, the Nernst factor (α) must be determined experimentally by measuring Eh variation as a function of pH using an acid or a base. This value may vary according to the nature of the oxido-reducing molecules in the media. For example, the Nernst factor is 40 mV/pH unit in milk [6].

2.3. Use of gas to modify E_h

Gas applications in the food industry are numerous: modified atmosphere packaging (MAP), beverage distribution, cooling, freezing or carbonation. The advantage of using gases such as hydrogen (H_2), nitrogen (N_2) or carbon dioxide (CO_2) to modify E_h is that they are not directly toxic to microorganisms. There are no safety issues for the product with these gases and they can be used sequentially. Finally, their use is authorized at European

level. Of the gases used in the food industry, in this chapter we will focus more particularly on nitrogen and hydrogen.

Nitrogen (N_2) is odourless, colourless, tasteless, non-toxic, and non-flammable. It is used to extend the life of packaged products (authorized additive E941). It is used to expel oxygen from the packaging before it is closed, which prevents oxidative phenomena involving pigmentation, flavours and fatty acids. It is also used for rapid freezing and refrigeration of food during transport.

Hydrogen (H_2) has major potential in food as it is colourless, odourless and has no known toxic effects. It is already used in the food industry for the hydrogenation of liquid oils and their transformation into solid products such as margarine or peanut butter. Hydrogen is a powerful reducer in solution, even at very low concentrations. It has been used to demonstrate the effect of E_h on the heat-resistance of bacteria [7]. Hydrogen is a special reducing agent: it imposes an E_h value on the medium associated with the introduction of the H^+/H_2 couple ($E_h^{0'}$ = -414 mV). This E_h value is highly dependent on the concentration of this couple that mainly influences the stability of the E_h imposed.

With the prospect of food use, hydrogen has the advantage over chemical reducing agents of not changing the product formulation, and therefore not altering the taste. Its industrial use has been rarely seen in this context because of its low flammability limit of 4% in air at 20 °C [8], this is why N_2-H_2 (96%-4%) is preferred to pure hydrogen. Its use in food technology is authorised at the European level (E949).

3. Effect of E_h on a fermented dairy product: Yoghurt

3.1. Reminder regarding the manufacture of yoghurt

We chose to focus on the key steps in the manufacture of yoghurt, which are:

- Delivery of milk: The raw material can be either fresh milk, reconstituted milk (from skim milk powder), or a mixture. In all cases, it is generally accepted that a quality product can be made from an extremely high quality raw material. With this in mind, it is essential that when the milk and other raw materials are received methods are established to detect any potential defects as early as possible. Two parameters must therefore be analysed as soon as the milk is received:
  - Its microbiology: to ensure consumer health, prevent the degradation of milk components that persist in the finished product and eliminate any possible competition between the starter culture and the endogenous flora that may involve bacteriophages.
  - Its chemistry: a rapid analysis of the chemical composition of the milk is necessary in order to identify any problems such as colostrum and late-lactation milk. Furthermore, these data concerning the chemistry of the milk can be useful in the standardization of the mixture.
- Standardization of the mixture: each component in milk plays a role. Fat has an effect on the smoothness and the feeling of softness in the mouth, lactose is the raw material used by

lactic acid bacteria for acidification, proteins act on the texture and minerals help stabilize the gel. These components vary in cow's milk according to race, diet, stage of lactation of the animal and season, which is why, during yoghurt manufacture, it is necessary to standardize the milk fat and protein content to meet the nutritional and organoleptic characteristics of the product and obtain consistent quality throughout the year.

- Homogenization: homogenization has two main effects on milk fat and proteins. The fragmentation of the fat globules prevents the separation of the lipid phase and the rest of the mixture, thus preventing the cream rising to the top during fermentation. Homogenization also stabilizes the proteins.
- Heat treatment: This eliminates most of the microbial flora originally present in the milk, including pathogenic or spoilage flora. It denatures the whey proteins, improves the consistency and viscosity of fermented milks and prevents whey separation. The risk of syneresis is reduced.

  Heat treatment of milk also has a positive effect on enzyme activity by providing a supportive environment. The environment becomes reductive through the elimination of a high proportion of oxygen. This medium is more conducive to fermentation that takes place under anaerobic conditions.
- Cooling: After heat treatment, the mixture must be cooled to temperatures approaching 43 °C for inoculation and incubation of the starter culture.
- Fermentation: "Yoghurt" refers to a product fermented by *Streptococcus thermophilus* (*S. thermophilus*) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (*Lb. bulgaricus*). In general, milk is fermented at 40-45 °C, the optimum growth temperature, with an incubation time of 2 and a half hours. However, a longer incubation period of 16-18 hours can be used at a temperature of 30 °C, or until the desired acidity is attained [9].

3.2. Yoghurt strains: Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus thermophilus

The association of *S. thermophilus* and *Lb. bulgaricus* is called proto-cooperation. Each species produces one or more substances, initially absent from the culture medium, that stimulate the growth of the other species [10]. During the symbiosis observed in yoghurt, the growth phases of these two bacterial species are staggered. Initially, growth of *S. thermophilus* is observed which is then slowed by the inhibitory effect of the lactic acid produced; the growth rate of *Lb. bulgaricus* then increases [11].

S. thermophilus is a strain that often shows little proteolytic activity, due to general low activity or absence of a wall protease. Its growth is limited because the peptides and amino acids initially present in milk are insufficient to cover its needs. In contrast, *Lb. bulgaricus* membrane protease degrades milk caseins releasing small peptides and amino acids which can be used by *S. thermophilus* intracellular peptidases [12].

The cooperation between these two strains also involves the production by *S. thermophilus* of pyruvic acid, formic acid, and carbon dioxide (CO_2 obtained from the decarboxylation of milk urea by urease) which stimulates the growth of *Lb. bulgaricus* [9, 13]. However, formic acid is released late in fermentation and in small quantities. The two bacterial species also consume the formic acid resulting from the heat treatment of milk [14].

Some authors have also demonstrated that the association of *S. thermophilus* and *Lb. bulgaricus* affects the production of volatile compounds involved in flavour development in yoghurt [15]. *S. thermophilus* produces more acetaldehyde, acetoin and diacetyl than *Lb. bulgaricus*, contrary to the rest of the bibliography concerning acetaldehyde [9, 16, 17]. Quantities of these molecules and other carbonyl compounds are not crucial per se for yoghurt flavour, but there are relationships between them that give yoghurt its distinctive flavour.

Finally, Ebel *et al.* [18] showed that during the manufacture and storage of a fermented dairy product, the populations of *Lb. bulgaricus* and *S. thermophilus* are the same whatever the E_h of the milk.

3.3. Texture

3.3.1. A look at yoghurt texture

The transformation of milk into yoghurt is called acid gelation. This gelation is a phenomenon that results in a remarkable change in the physical state of the system which changes from a liquid to a system with the characteristics of a solid. Several phases in the formation of a gel can be distinguished:

- The "solution" phase, where the polymer forms a solution: the macromolecules are not held together;
- The "gel" phase occurs when enough chains have joined together to form a network or gel, with dominant elastic rheological behaviour;
- Sometimes, additional aggregation of associated areas is observed; the gel becomes increasingly rigid and syneresis may occur with time: the gel shrinks and exudes some of the liquid phase.

The slow acidification of milk is due to bacteria that metabolize lactose and produce lactic acid. While casein micelles are stable at normal milk pH and room temperature, this supramolecular structure becomes unstable and leads to the formation of a gel with the slow progressive acidification of milk.

- From pH 6.7 to pH 5.8, the casein micelles seem to retain their integrity, shape and size.
- From pH 5.8 to pH 5.5, the micelles get closer together due to the decrease in the potential ζ and begin to form groups of micelles.
- From pH 5.5 to pH 5.0, significant changes in shape and size take place: micelle aggregates appear and these particles partially fuse. This is the phase transition between the solution and the acid gel.
- When the pH reaches 5, the solubilisation of micellar calcium, which occurs steadily from pH 6.8, is complete. From pH 5 to pH 4.8, rearrangements of the aggregates take place. At pH 4.9, gelation is complete. At pH 4.6, the acid gel is definitively formed. Aggregation of casein micelles at pH 4.6 is irreversible. Hydrophobic interactions are facilitated at this pH due to reduced electrostatic repulsion, leading to micelle aggregation.

Rheology is used to characterize the texture of yoghurt that specifically targets the mechanical properties. The rheological characterization of a product involves the

application of a shear stress and measurement of the deformation, or application of a deformation (compression, stretching or shear) and measurement of its ability to withstand this distortion. Yoghurt can be defined as a viscoelastic fluid. It therefore has both the viscous properties of a liquid and the elastic properties of a solid.

3.3.2. Effect of E_h on a model acid skim milk gel

It has been shown that dairy products are affected by E_h [4, 19]. Delbeau *et al.* [19] showed that the use of gas to change the E_h of milk can modify the sensory properties of a fermented dairy product. However, we do not know if these modifications are due to the impact of E_h on physicochemical phenomena, lactic acid bacteria, or both. For this purpose, Martin *et al.* [20] wanted to determine to what extent chemical phenomena affect acid milk gelation under different E_h conditions. Glucono-δ-lactone (GDL) was used to acidify milk to avoid variations caused by microorganisms sensitive to E_h.

Martin *et al.* [20] studied the effects of E_h on model acidified skim milk gels obtained using GDL and prepared under different gaseous conditions. The milk prepared in air is an oxidizing medium; nitrogen, which is a neutral gas, can be used to remove oxygen from milk - even so the milk Eh remains oxidizing in these conditions - and hydrogen leads to a reducing E_h (below 0). Martin *et al.* [20] focused on the effect of gas bubbling on gel structure through viscoelastic properties and measurement of whey separation (Table 1).

Gaseous conditions applied to milk	pH		E_{h7} (mV)		η (Pa.s)	WS (g/100g of GDL-gel)
	At t=0	At t=3.5 hours	At t=0	At t=3.5 hours		
Air	6.80 ± 0.03	4.6^{a} ± 0.0	405 ± 22	414 ± 8	0.039^{a} ± 0.000	4.74^{a} ± 1.42
Air bubbling	6.70 ± 0.04	4.6^{a} ± 0.0	433 ± 6	430 ± 5	0.032^{c} ± 0.001	1.26^{b} ± 0.26
N_2 bubbling	6.8 ± 0.06	4.6^{a} ± 0.0	283 ± 13	288 ± 11	0.035^{b} ± 0.001	1.93^{b} ± 0.33
N_2 – H_2 bubbling	6.73 ± 0.04	4.6^{a} ± 0.0	- 349 ± 6	- 83 ± 18	0.032^{c} ± 0.001	0.59^{c} ± 0.12

$^{a\text{-}c}$: different letters indicate that groups were significantly different at an α risk of 5% (ANOVA test). Values in the same column should be compared.

Table 1. Characteristics of gel structure depending on the different E_h conditions (milk acidified using GDL):

- Apparent viscosity η at 500 1/s of GDL-gel at pH 4.6 and 4 °C. Measurements were carried out 24 hours after addition of GDL.
- Evolution of average whey separation (WS) over 28 days in GDL-gels.

Values are means from triplicate experiments (mean value ± standard deviation).

The apparent viscosity of each gel was characterized at pH 4.6, 4 °C, 24 hours after addition of GDL under the different E_h conditions (Table 1). For GDL-gels, apparent viscosity ranged from 0.032 to 0.039 Pa.s. GDL-gels produced in air had the highest apparent viscosity, whereas values obtained with air and N_2 – H_2 bubbling were similar and significantly lower than those obtained with N_2 bubbling. So, for GDL-gels, the viscosity was affected by bubbling. Martin *et al.* [20] showed that the type of gas used for bubbling has a significant influence but no clear trend can be deduced from these results in terms of the influence of an oxidizing or reducing environment.

The gel structure was then observed during storage for up to 28 days. The mean whey separation values of GDL-gels produced under different E_h conditions are presented in Table 1. For each gaseous condition, the authors observed that whey separation occurred from the very first day of storage and the volume of whey separation was relatively constant during the 28 days of storage [20]. Whey separation ranged from 0.59 to 4.74 g / 100 g of GDL-gels. The highest whey separation was obtained with air but this value was lower than values reported in the literature: 18.48% of GDL-gels in the work by Lucey *et al*. [21] and 10% in a study by Fiszman *et al.* [22]. One explanation for is that in the study by Lucey *et al.* [21] the method used to measure whey separation was to remove the gels from their flasks and thus whey separation could have been over-estimated. Whey separation obtained with gas bubbling was lower (1.26 g / 100 g with air bubbling, 1.93 g / 100 g with N2 bubbling and 0.59 g / 100 g with N_2 - H_2 bubbling). The lowest whey separation was observed with GDL-gels made under N_2 – H_2. Adjusting the E_h of milk to reducing conditions (under N_2 – H_2) could be a possible way of significantly decreasing the phenomenon of whey separation.

3.3.3. Effect of E_h on a non-fat yoghurt

In a second step, the authors proposed studying the extent to which lactic acid bacteria affect acid milk gelation under different E_h conditions [23]. Indeed, oxygen modifies the growth capacity of bacteria and the formation of end products. So, E_h may contribute to the quality of fermented products [2, 24, 25]. Martin *et al.* [23] wanted to determine the effects of E_h on yoghurts made under various gaseous conditions. In this study they focused on exopolysaccharide production and gel structure (Table 2). The same gaseous conditions as in the study on the effect of E_h on model acid skim milk gels were chosen.

Lb. bulgaricus and *S. thermophilus* produce exopolysaccharides (EPS) which can contribute to improving the texture and viscosity of fermented dairy products [26]. In standard yoghurts (produced in air) the concentration of EPS was 63.60 $mg.L^{-1}$, in accordance with the literature (50 to 350 $mg.L^{-1}$) [27, 28]. The concentration was lower in yoghurts produced with air bubbling (15.22 $mg.L^{-1}$) than in yoghurts produced with N_2 bubbling, which was lower than those made with N_2 – H_2 bubbling. The EPS concentration of yoghurts made in Air and with N_2 – H_2 bubbling were similar. In reducing E_h conditions, lactic acid bacteria produced the same amount of EPS as in ambient air. This result has already been observed in the literature. Indeed, *Lactobacillus sake* 0-1 was reported to have optimal EPS production in anaerobic conditions [29], while higher EPS yields were correlated with a lower oxygen tension [30].

Gaseous conditions applied to milk	pH		E_{h7} (mV)		C_{EPS} (mg/L)	η (Pa.s)	WS (g/100g of yoghurt)
	At t=0	At t=3.5 hours	At t=0	At t=3.5 hours			
Air	$6.80^{a} \pm 0.0$	$4.6^{a} \pm 0.0$	$425^{a} \pm 20$	$171^{a} \pm 2$	$63.60^{b} \pm 3.72$	$0.046^{a} \pm 0.00$	$1.98^{a} \pm 0.54$
Air bubbling	$6.80^{a} \pm 0.0$	$4.6^{a} \pm 0.0$	$435^{a} \pm 3$	$241^{a} \pm 8$	$15.22^{a} \pm 0.74$	$0.046^{a} \pm 0.00$	$1.76^{a} \pm 0.31$
N_2 bubbling	$6.81^{a} \pm 0.0$	$4.6^{a} \pm 0.0$	$285^{b} \pm 11$	$139^{b} \pm 5$	$25.29^{c} \pm 0.40$	$0.035^{b} \pm 0.00$	$1.03^{ab} \pm 0.27$
$N_2 - H_2$ bubbling	$6.81^{a} \pm 0.0$	$4.6^{a} \pm 0.0$	$-345^{c} \pm 4$	$-309^{c} \pm 10$	$62.70^{b} \pm 0.75$	$0.021^{c} \pm 0.01$	$0.59^{b} \pm 0.12$

[a, b, c]: different letters indicate that groups were significantly different at an α risk of 5% (ANOVA test). Values in the same column should be compared.

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Table 2. Characteristics of gel structure depending on the different E_h conditions (milk acidified using lactic starters):

- Concentrations of exopolysaccharides (C_{EPS}) in yoghurts after one day of storage.
- Apparent viscosity η at 500 1/s of yoghurt at pH 4.6 and 4 °C. Measurements were made 24 hours after addition of starter culture.
- Evolution of average whey separation over 28 days (WS) in yoghurts.

Values are means from triplicate experiments.

The apparent viscosity of each yoghurt was characterized at pH 4.6 and 4 °C, 24 hours after addition of bacteria under the different Eh conditions (Table 2). The apparent viscosity ranged from 0.021 to 0.046 Pa.s. Yoghurts produced in air and with air bubbling had the highest apparent viscosity. The apparent viscosity of yoghurts made with N_2 bubbling was lower (0.035 Pa.s) than other oxidizing conditions (0.046 Pa.s), and values obtained with $N_2 - H_2$ bubbling were the lowest (0.021 Pa.s). Apparent viscosity is clearly affected by the gas type. A reducing environment reduces the apparent viscosity of yoghurt.

Apparent viscosity depends on the solid fraction in the gel as well as the relationships between the different solid elements. In yoghurt, solid particles include milk proteins, lactic acid bacteria and their EPS. Indeed, the gel of yoghurts produced under $N_2 - H_2$ conditions is weaker despite greater EPS production [23]. It is a common assumption that EPS produced by bacteria contribute to the rheological properties of yoghurt [31-33] but, as reported by Hassan *et al.* [34], van Marle [35] and Martin *et al.* [23], no correlation between the viscosity of yoghurt and EPS concentrations was found.

Whey separation of yoghurts produced under different E_h conditions over 28 days of storage was then studied [23] (Table 2). Concerning GDL-gels, whey separation of yoghurts occurred from the very first day of storage and the volume of whey separation was relatively constant over the 28 days of storage. Whey separation ranged from 0.59 to

1.98 g/100 g of yoghurt. The highest whey separation was obtained with air and air bubbling and these values are in accordance with the literature [22]. Whey separation obtained with N_2 bubbling (1.03 g / 100 g) and N_2 - H_2 bubbling (0.59 g / 100 g) was lower. So, the more reducing the environment, the lower the whey separation. Adjusting the E_h of milk to reducing conditions (under N_2 – H_2) could be a possible way of significantly decreasing the phenomenon of whey separation.

3.4. Aroma compounds

3.4.1. A look at yoghurt aroma compounds

The typical flavours of fermented milk are mainly due to a blend of the following compounds: lactic acid, carbon compounds such as acetaldehyde, acetone, acetate and diacetyl, non-volatile acids such as pyruvic, oxalic and succinic acids, volatile acids such as acetic, propionic and formic acids and products from the thermal degradation of proteins, lipids or lactose.

Ott *et al.* [36] identified 91 aroma compounds (GC-olfactometry) in yoghurt among which 21 were detected more frequently and would thus have a major impact on flavour. Acetaldehyde is found in significant quantities and is responsible for the characteristic smell of yoghurt. Diacetyl, pentane-2,3-dione, and dimethyl sulphide also have a major impact on yoghurt flavour [36, 37].

Acetaldehyde was firstly reported by Pette *et al.* [38] as the main aromatic compound in yoghurt. During manufacture, production of this compound is only highlighted when a certain level of acidification is reached (pH 5.0). Concentrations found in the final product are 0.7 to 15.9 $mg.kg^{-1}$. The maximum amount is obtained at pH 4.2 and stabilizes at pH 4.0. The production of acetaldehyde and other flavour compounds by *S. thermophilus* and *Lb. bulgaricus* occurs during yoghurt fermentation and the final amount is dependent on specific enzymes which are able to catalyse the formation of carbon compounds from the various milk constituents.

Three metabolic pathways producing acetaldehyde were identified and some pathways may take place simultaneously [39]:

- From glucose in the glycolytic pathway,
- From the degradation of DNA,
- From L-threonine with threonine aldolase.

However, 90% of acetaldehyde produced by *Lb. bulgaricus* comes from glucose and 100% in the case of *S. thermophilus* [39].

Diacetyl and pentane-2,3-dione also have a significant impact on the final aroma of yoghurt: 1 mg of diacetyl and 0.1 mg of pentane-2, 3-dione per kg of yoghurt are produced by lactic acid bacteria during fermentation. These diketones are produced by decarboxylation of their precursors, 2-acetolactate and 2-aceto-hydroxybutyrate [39]. These compounds are thermally unstable and in the presence of oxygen are converted into their corresponding

diketones [40, 41]. Moreover, during storage at 4 °C, the concentration of the two diketones increases slightly [41] due to the basal metabolic activity of the bacteria.

Agitating a mixed culture of *Lactococcus* and *Leuconostoc* promotes diacetyl production by allowing oxidative decarboxylation of 2-acetolactate [42, 43]. In unstirred cultures, the redox potential of the medium decreases rapidly at the start of fermentation. Only acetoin and 2-acetolactate are produced. The authors also showed that controlled oxygenation of the *Lactococcus lactis* ssp. *lactis* culture medium favoured diacetyl production by increasing the activity of diacetyl synthase [44].

Neijssel *et al.* [40] showed that the distribution of carbon flux from pyruvate depended on the NADH / NAD^+ ratio, intracellular redox potential or the concentration of metabolites and particularly that of pyruvate. Finally, the authors suggested adding air or oxygen to milk in order to increase the amount of diacetyl in cheese [45].

References [36] and [37] are the only articles that mention dimethyl sulphide as a compound having a significant impact on the flavour of yoghurt. The metabolic pathways involved in the synthesis of sulphur compounds are not well-known in yoghurt. However, the literature mentions these synthetic pathways in the development of cheese flavour.

In general, the majority of sulphur aromatic compounds come from methionine [46]. Methanethiol is easily oxidized to dimethyl disulphide and dimethyl trisulphide [47]. The appearance of these compounds is the direct result of the methanethiol content and is modulated by the low redox potential in Cheddar. Dimethyl sulphide is produced by a metabolic pathway that does not involve methanethiol, but that is different to that of dimethyl sulphide and trimethyl disulphide from methionine [48].

Studies have also shown that when the redox potential decreases, methanethiol and hydrogen sulphide concentrations increase [45]. Moreover, the cheeses to which reducing compounds (dithiothreitol or glutathione) were added contained higher amounts of sulphur compounds and had better qualitative and quantitative flavour performances [45]. It therefore seems that a reducing environment is essential for the production of aroma compounds by bacteria. If a cheese is exposed to air, the redox increases and this leads to the oxidation of sulphur compounds, resulting in lower quality aromatics.

3.4.2. Impact on aroma biosynthesis by lactic acid bacteria

Studies on aroma biosynthesis by LAB usually take into account environmental factors such as pH and temperature. However, the E_h of the medium has not yet been considered, although it is supposed to affect bacterial metabolism [49, 50]. Martin *et al.* [51] determined to what extent E_h can affect the metabolic pathways involved in the production of aroma compounds in *Lb. bulgaricus* and *S. thermophilus.* Four aroma compounds (acetaldehyde, dimethyl sulphide, diacetyl and pentane-2,3-dione) were chosen as metabolic tracers of lactic acid bacteria metabolism. The same gaseous conditions as in the study of the effect of E_h on model acid skim milk gels and non-fat yoghurt were chosen. The amounts of each of the four aroma compounds extracted using a headspace solid-phase micro-extraction

technique (HS-SPME) and analysed using gas chromatography coupled with mass spectrometry (GC-MS) during 28 days of storage are reported in Table 3.

Firstly, the authors focused on the impact of these different E_h conditions on the biosynthesis of these four aromas by bacteria after one day of storage [51]. In the standard yoghurt (made in ambient air), diacetyl was observed in the highest concentrations, and acetaldehyde the lowest. This result is contrary to the literature where the lowest concentrations were reported for dimethyl sulphide (0.013-0.070 $mg.kg^{-1}$; measured using dynamic and trapped headspace GC [37, 41]). In the same way, published concentrations were generally higher for acetaldehyde (0.7-15.9 $mg.kg^{-1}$) than in our standard yoghurt (0.18 $mg.kg^{-1}$). In the literature, the concentrations of diacetyl (0.31-17.3 $mg.kg^{-1}$) and 2,3-pentanedione (0.02-4.5 $mg.kg^{-1}$) were lower than in our standard yoghurt (162 $mg.kg^{-1}$ and 115 $mg.kg^{-1}$ respectively). An explanation for these differences can be put forward: the quantification technique used by Ott *et al.* [41] and Imhof *et al.* [37] was dynamic and trapped headspace GC. This technique requires Tenax® traps which may be saturated, as we showed in a preliminary experiment. Furthermore, in our study, to enable a more complete extraction of the aroma compounds, a saturated solution of NaCl was added to the yoghurt. Finally, we did not use the same species of LAB as Ott and Imhof, which may have resulted in different quantities of the various aroma compounds.

Yoghurts made with air bubbling had significantly higher concentrations of acetaldehyde and diacetyl compared to standard yoghurts. The concentration of dimethyl sulphide was significantly lower and that of pentane-2,3-dione was the same.

With N_2 bubbling, the concentration of acetaldehyde was similar to that in yoghurts made with air bubbling, whereas the concentration of dimethyl sulphide was lower. The concentration of diacetyl was the same as in standard yoghurts and the concentration of pentane-2,3-dione was not significantly different from that in yoghurts made in air (bubbling or not).

The authors also demonstrated that oxidative E_h conditions clearly increased the production of aroma compounds [51]. These results are consistent with the bibliography. Oxidative conditions stimulated the production of volatile sulphur compounds such as dimethyl sulphide, and aldehydes such as acetaldehyde [49]. In the presence of oxygen, the oxidative decarboxylation of 2-acetolactate and 2-aceto-hydroxybutyrate to diacetyl and pentane-2,3-dione respectively was also favoured [40, 42, 44, 52]. For diacetyl, our result can be explained by the fact that in anaerobic conditions lactic acid bacteria dehydrogenate the NADH produced during glycolysis via lactate dehydrogenase (LDH) activity. Boumerdassi *et al.* [44] confirmed that oxygen increases NADH oxidase activity [53], which causes NADH re-oxidation at the expense of LDH, butanediol dehydrogenase and acetoin dehydrogenase activity [54]. Then, excess pyruvate is partially eliminated through acetolactate production, which increases diacetyl production [44].

Finally, bubbling with $N_2 – H_2$ (reducing conditions), the concentration of acetaldehyde and pentane-2,3-dione was the same as in standard yoghurts. The concentration of dimethyl sulphide was the same as in yoghurts made without oxygen and the concentration of diacetyl was significantly lower than under the other three E_h conditions.

Then, [51] kept the yoghurts in Hungate tubes at 4 °C for 28 days in order to prevent exposure of the contents to oxygen, and the gaseous conditions applied to the milk are thus assumed to be constant during storage.

Gaseous conditions and storage period (days)	Aroma compound (mg.kg)			
	ACH	DMS	DY	PTD
Ambient air				
1	$0.18^{a} \pm 0.02$	$10.16^{a} \pm 0.59$	$162.08^{a} \pm 13.49$	$115.25^{a} \pm 33.70$
7	$0.13^{ab} \pm 0.02$	$10.16^{a} \pm 0.99$	$115.55^{cb} \pm 5.13$	$84.33^{ab} \pm 1.86$
14	$0.10^{b} \pm 0.00$	$9.52^{a} \pm 0.42$	$91.63^{c} \pm 8.02$	$71.56^{ab} \pm 4.49$
21	$0.18^{a} \pm 0.00$	$13.06^{b} \pm 1.21$	$141.29^{ab} \pm 11.77$	$65.19^{b} \pm 5.49$
28	$0.16^{ab} \pm 0.01$	$11.82^{ab} \pm 0.48$	$112.66^{c} \pm 7.56$	$51.79^{b} \pm 2.95$
Air bubbling				
1	$0.28^{a} \pm 0.00$	$5.27^{a} \pm 0.53$	$299.90^{a} \pm 18.37$	$123.47^{a} \pm 3.23$
7	$0.14^{b} \pm 0.01$	$6.34^{ab} \pm 0.30$	$127.58^{bc} \pm 3.93$	$83.98^{b} \pm 2.20$
14	$0.11^{b} \pm 0.01$	$6.85^{b} \pm 0.32$	$104.23^{b} \pm 4.66$	$78.10^{b} \pm 3.15$
21	$0.24^{a} \pm 0.02$	$9.33^{c} \pm 0.55$	$143.84^{c} \pm 6.69$	$67.89^{c} \pm 0.80$
28	$0.13^{b} \pm 0.02$	$6.78^{b} \pm 0.30$	$101.91^{b} \pm 3.03$	$54.36^{d} \pm 4.19$
N_2 bubbling				
1	$0.35^{a} \pm 0.04$	$3.72^{a} \pm 0.22$	$147.79^{a} \pm 9.91$	$110.87^{a} \pm 4 .49$
7	$0.22^{bc} \pm 0.02$	$5.68^{ab} \pm 0.34$	$103.13^{bc} \pm 6.50$	$75.67^{b} \pm 1.98$
14	$0.18^{c} \pm 0.01$	$6.16^{b} \pm 0.17$	$78.80^{c} \pm 4.60$	$58.16^{c} \pm 0.48$
21	$0.28^{ab} \pm 0.04$	$10.34^{c} \pm 1.37$	$122.27^{ab} \pm 8.94$	$51.95^{c} \pm 2.29$
28	$0.18^{c} \pm 0.00$	$7.16^{b} \pm 0.77$	$84.76^{c} \pm 9.92$	$38.72^{d} \pm 3.03$
$N_2 - H_2$ bubbling				
1	$0.17^{ab} \pm 0.00$	$2.71^{a} \pm 0.57$	$102.73^{a} \pm 9.10$	$76.99^{a} \pm 5.90$
7	$0.13^{bc} \pm 0.02$	$5.49^{b} \pm 0.53$	$89.61^{ab} \pm 7.70$	$66.10^{ac} \pm 2.29$
14	$0.11^{c} \pm 0.01$	$6.07^{bc} \pm 0.43$	$74.35^{b} \pm 5.42$	$57.60^{bc} \pm 0.86$
21	$0.19^{a} \pm 0.01$	$7.48^{c} \pm 0.53$	$112.09^{a} \pm 7.82$	$52.10^{bd} \pm 0.59$
28	$0.12^{c} \pm 0.01$	$7.22^{c} \pm 0.63$	$78.36^{b} \pm 9.30$	$40.60^{d} \pm 7.93$

$^{a, b, c, d}$: different letters indicate that groups were significantly different at an α risk of 5% (ANOVA test).

Table 3. Evolution of average amounts of aroma compounds ($mg.kg^{-1}$) quantified in headspace of yoghurts made under different E_h conditions (ambient air, bubbling with air, bubbling with N_2 and bubbling with $N_2 - H_2$) during 28 days of storage. ACH: Acetaldehyde (A); DMS: Dimethyl sulphide (B); DY: Diacetyl (C); PTD: pentane-2,3-dione (D). Values are means of experiments carried out in triplicate. Values in the same column should be compared.

During the 28 days of storage, for the standard yoghurt, the quantities of acetaldehyde and dimethyl sulphide produced were relatively stable, while diketone concentrations significantly decreased.

For yoghurts made with air bubbling, the aroma profiles remained almost constant. During storage, the concentration of acetaldehyde decreased slightly whereas that of dimethyl sulphide increased slightly. The diketone concentration significantly decreased.

For yoghurts made without oxygen (bubbling with N_2), the quantities of acetaldehyde, diacetyl and pentane-2,3-dione decreased during storage while that of dimethyl sulphide increased.

For yoghurts produced under reducing conditions (bubbling with N_2 – H_2), the aroma profiles during storage were the same as those made without oxygen. The concentration of acetaldehyde, diacetyl and pentane-2,3-dione decreased while that of dimethyl sulphide increased.

Furthermore, during storage, different profiles were observed for the four aromas depending on the E_h conditions [51]. Under oxidizing conditions (+170 to +245 mV), the concentration of acetaldehyde was relatively stable during storage, which is in accordance with the literature [9, 55, 56] and the concentration of dimethyl sulphide was also stable. On the contrary, under reducing conditions (-300 to -349 mV), the concentration of acetaldehyde decreased and that of dimethyl sulphide increased. The metabolic pathways involved in the biosynthesis of sulphur compounds are still unclear. Under reducing conditions, it seems that another pathway promotes the production of dimethyl sulphide and that acetaldehyde may be reduced to ethanol. For diketones, whatever the E_h conditions, the concentration decreased during storage. Diacetyl and pentane-2,3-dione can be reduced respectively to acetoin and pentane-2,3-diol [57].

4. Impact of E_h on other dairy products

4.1. Probiotic dairy products

The use of gas to modify E_h seems to be an interesting way of varying the organoleptic properties of dairy products as well as improving the survival of oxygen sensitive strains during storage in fermented dairy products containing probiotics. Indeed, these microorganisms are mainly anaerobes. Oxygen, which is a powerful oxidant, has a drastic effect on E_h values and the viability of probiotic bacteria during manufacturing and storage [58-60]. So, many studies modify the redox potential to protect probiotics from oxygen toxicity in dairy products [1, 61-65]. However, these techniques sometimes have deleterious effects on the organoleptic properties of fermented milk. An alternative to these methods could be the use of gases. Indeed, Ebel *et al.* [18] showed that fermented dairy products made from milk gassed with N_2, and more particularly those made from milk gassed with N_2 – H_2, were characterized by a significant increase in *Bifidobacterium bifidum* survival during storage (Figure 2).

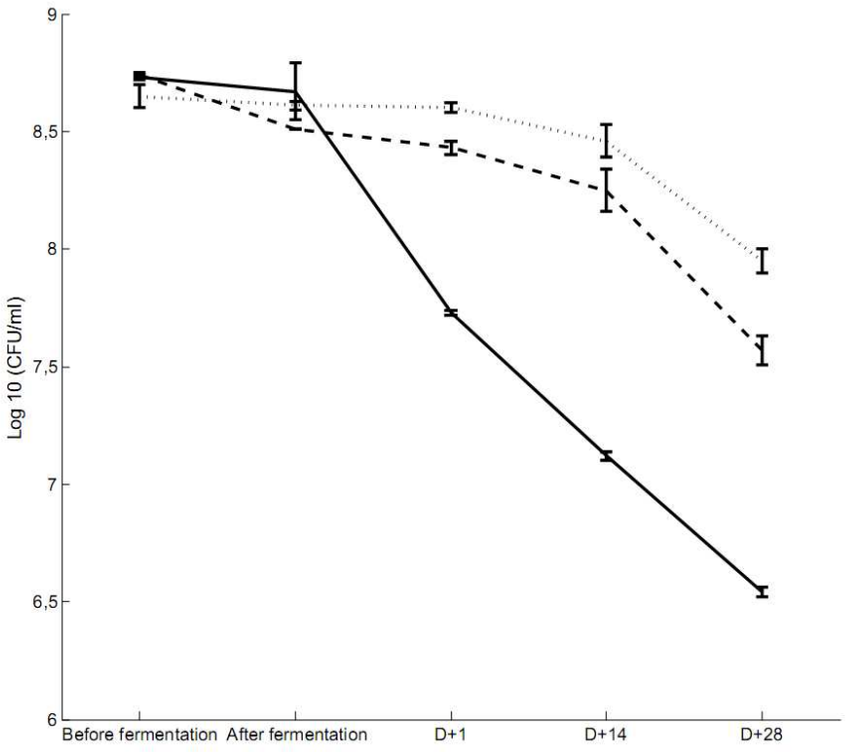

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Figure 2. Evolution of a population of *Bifidobacterium bifidum* during fermentation and storage. Different gaseous conditions were applied to the milk: control (solid line), gassed with N_2 (dashed line), or gassed with $N_2 – H_2$ (dotted line).

After 28 days of storage, a difference in bacterial counts of 1.2 log and 1.5 log was observed between the control milk and after bubbling with N_2 or $N_2 – H_2$ respectively. No differences were highlighted during the fermentation process. It is interesting to note that this technique was set up without affecting the fermentation kinetics and survival of *S. thermophilus* and *Lb. bulgaricus*. The use of gas is a possible way of improving probiotic survival during storage without affecting acidification properties of yoghurt strains and consequently organoleptic properties.

4.2. Cheese

Controlling E_h in cheese seems essential in governing aroma characteristics. Indeed, a reducing E_h is necessary for the development of the characteristic flavour of certain fermented dairy products such as cheeses, notably through the production of thiol compounds [45, 66]. It has also been reported that Cheddar has a reducing E_h and is an indicator of the establishment of the conditions required for the formation of aroma compounds [67]. As shown previously, E_h can modify the metabolic pathways of aroma production by lactic bacteria [51]. Kieronczyk *et al.* [49] demonstrated that reducing E_h conditions can stimulate carboxylic acid production in cheese, while oxidative E_h conditions

improve the production of volatile sulphur compounds and aldehydes. By ripening cheese under reducing E_h conditions, the production of volatile fatty acids increased [68]. Adjusting the E_h of the milk before cheese ripening could be a possible way of modifying the metabolism of lactic bacteria.

5. Conclusion

Pasteur defined fermentation as "life without air". In lactic acid bacteria, some exogenous electron acceptors may interfere significantly with the fermentative metabolism by acting on different cellular activities. A better understanding of the adaptive mechanisms to extracellular redox is still lacking, but the results in the literature show that lactic acid bacteria may use passive or active mechanisms. A remarkable feature in lactic acid bacteria is their ability to reduce the redox environment to low E_h values.

With the prospect of food applications, changing E_h using pure or a mixture of gases has the advantage of maintaining product safety as opposed to the use of oxidizing or reducing molecules. This chapter demonstrates the importance of E_h both on the physico-chemistry of milk gels and bacterial metabolism and viability. The use of gas to modify E_h seems to be an interesting way of varying the organoleptic properties of dairy products.

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6. References

[1] Dave RI, Shah NP (1997) Effectiveness of ascorbic acid as an oxygen scavenger in improving viability of probiotic bacteria in yoghurts made with commercial starter cultures. Int. dairy j. 7(6-7): 435-443.

[2] Rödel W, Scheuer R (2000) Redox potential of meat and meat produits. IV. Recording criteria of quality in meat and meat products by measuring the redox potential. Fleischwirtschaft. 2: 46-48.

[3] Galster H (1991) pH Measurement: fundamentals, methods, applications, instrumentation. New York: VCH Publishers.

[4] Abraham S, Cachon R, Colas B, Feron G, De Coninck J (2007) E_h and pH gradients in camembert cheese during ripening: Measurements using microelectrodes and correlations with texture. Int. dairy j. 178: 954-960.

[5] Leistner L, Mirna A (1959) Das redoxpotential von pökelladen. Fleischwirtschaft. 8: 659-666.

[6] Cachon R, Jeanson S, Aldarf M, Divies C (2002) Characterisation of lactic starters based on acidification and reduction activities. Lait. 823: 281-288.

[7] George SM, Richardson LCC, Pol IE, Peck MW (1998) Effect of oxygen concentration and redox potential on recovery of sublethally heat-damaged cells of *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes*. J. appl. microbiol. 84: 903-909.

[8] Medard (2002) Encyclopédie des gaz de l'air liquide 3rd ed. Amsterdam, The Netherlands: Lavoisier.

[9] Tamine AY, Robinson RK (1999) Yoghurt: science and technology. Washington DC: CRC Press.

[10] Fredrickon AG (1977) Behavior of mixed cultured of microorganisms. Annu. rev. microbiol. 31: 63-87.

[11] Rasic JL, Kurmann JA. Fermented fresh milk products (1978) In: Rasic JL, Kurmann JA, editors. Yoghurt scientific grounds, technology, manufacture and preparations. Copenhangen, Denmark: Technical Dairy Publishing House.

[12] Moreira M, Abraham A, De Antoni G (1999) Technological properties of milks fermented with thermophilic acid bacteria at suboptimal temperature. J. dairy sci. 83: 395-400.

[13] Zourari A, Accolas JP, Desmazeaud M (1992) Metabolism and biochemical characteristics of yoghurt bacteria: a review. Lait. 72: 1-34.

[14] Loones A. Laits fermentés par les bactéries lactiques (1994) In: De Roissart H, Luquet FM, editors. Bactéries lactiques. Paris, France: Lorica, Uriage. p. 133-154.

[15] Courtin P, Rul F (2003) Interactions between microorganisms in a simple ecosystem: yogurt bacteria as a study model. Lait. 84: 125-134.

[16] El-Soda MA, Abou-Donia SA, El-Shafy HK, Mashaly R, Ismail AA (1986) Metabolic activities and symbiosis in zabady isolated cultures. Egypt. j. dairy sci. 14: 1-10.

[17] Xanthopoulos V, Petridis D, Tzanetakis N (2001) Characterisation and classification of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* strains isolated from traditional Greek yogurts. J. food sci. 66: 747-752.

[18] Ebel B, Martin F, Le LDT, Gervais P, Cachon R (2011) Use of gases to improve survival of *Bifidobacterium bifidum* by modifying redox potential in fermented milk. J. dairy sci. 945: 2185-2191.

[19] Cachon R, Feron G, Delbeau C, Ibarra D, Ledon H (2006) Process for modifying the sensory characteristics of a fermented milk product and its maturation during conservation of said product. EP1649755. L'Air Liquide, France.

[20] Martin F, Cayot N, Marin A, Journaux L, Cayot P, Gervais P, Cachon R (2009) Effect of oxidoreduction potential and of gas bubbling on rheological properties and microstructure of acid skim milk gels acidified with glucono-δ-lactone. J.dairy sci. 92: 5898-5906.

[21] Lucey JA, Tamehana M, Singh H, Munro PA (1998) A comparison of the formation, rheological properties and microstructure of acid skim milk gels made with a bacterial culture or glucono-δ-lactone. Food res. int. 312: 147-155.

[22] Fiszman SM, Lluch MA, Salvador A (1999) Effect of addition of gelatin on microstructure of acidic milk gels and yoghurt and on their rheological properties. Int. dairy j. 912: 895-901.

[23] Martin F, Cayot N, Vergoignan C, Journaux L, Gervais P, Cachon R (2010) Impact of oxidoreduction potential and of gas bubbling on rheological properties of non-fat yoghurt. Food res. int. 431: 218-223.
[24] Dave RI, Shah NP (1997) Effectiveness of ascorbic acid as an oxygen scavenger in improving viability of probiotic bacteria in yoghurts made with commercial starter cultures. Int. dairy j. 76-7: 435-443.
[25] van Dijk C, Ebbenhorst-Selles T, Ruisch H, Stolle-Smits T, Schijvens E, van Deelen W, Boeriu C (2000) Product and Redox Potential Analysis of Sauerkraut Fermentation. J. agr. food chem. 482: 132-139.
[26] Ruas-Madiedo P, Hugenholtz J, Zoon P (2002) An overview of the functionality of exopolysaccharides produced by lactic acid bacteria. Int. dairy j. 12(2-3): 163-171.
[27] Cerning J, Bouillanne C, Desmazeaud MJ, Landon M (1986) Isolation and characterization of exocellular polysaccharide produced *Lactobacillus bulgaricus*. Biotechnol. letters. 89: 625-628.
[28] Cerning J, Bouillanne C, Desmazeaud MJ, Landon M (1988) Exocellular polysaccharide production by *Streptococcus thermophilus*. Biotechnol. letters. 104: 255-260.
[29] van den Berg D, Robijn GW, Janssen AC, Giuseppin M, Vreeker R, Kamerling JP, Vliegenthart J, Ledeboer AM, Verrips CT (1995) Production of a novel extracellular polysaccharide by *Lactobacillus sake* 0-1 and characterization of the polysaccharide. Appl. environ. microb. 618: 2840-4.
[30] De Vuyst L, Vanderveken F, van de Ven S, Degeest B (1998) Production by and isolation of exopolysaccharides from *Streptococcus thermophilus* grown in a milk medium and evidence for their growth-associated biosynthesis. J. appl. microbiol. 846: 1059-1068.
[31] Hess SJ, Roberts RF, Ziegler GR (1997) Rheological properties of nonfat yogurt stabilized using *Lactobacillus delbrueckii* ssp. *bulgaricus* producing exopolysaccharide or using commercial stabilizer systems. J. dairy sci. 802: 252-263.
[32] van Marle ME, van den Ende D, de Kruif CG, Mellema J (1999) Steady shear viscosity of stirred yoghurts with varying ropiness. J. rheol. 43(6): 1643-1663.
[33] Rawson HL, Marshall VM (1997) Effect of 'ropy' strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* on rheology of stirred yogurt. Int. j. food sci. technol. 323: 213-220.
[34] Hassan AN, Frank JF, Farmer MA, Schmidt KA, Shalabi SI (1995) Formation of yogurt microstructure and three-dimensional visualization as determined by confocal scanning laser microscopy. J. dairy sci. 7812: 2629-2636.
[35] van Marle ME. (1998) Structure and rheological properties of yoghurt gels and stirred yoghurts. Enschede, the Netherlands: Twente.
[36] Ott A, Fay LB, Chaintreau A (1997) Determination and origin of the aroma impact compounds of yogurt flavor. J. agric. food chem. 453: 850-858.
[37] Imhof R, Glättli H, Bosset JO (1994) Volatile organic aroma compounds produced by thermophilic and mesophilic mixed strain dairy starter cultures. LWT - Food sci. technol. 275: 442-449.
[38] Pette J, Lolkema H (1950) Yoghurt, III: Acid production and aroma formation in yoghurt. Neth. milk dairy j. 4: 261-273.

[39] Biavati B, Vescovo M, Torriani S, Bottazzi V (2000) Bifidobacteria: history, ecology, physiology and applications. Ann. microbiol. 502: 117-131.
[40] Neijssel OM, Snoep JL, Teixeira de Mattos MJ (1997) Regulation of energy source metabolism in streptococci. J. appl. microbiol. 83S1: 12S-19S.
[41] Ott A, Germond JE, Baumgartner M, Chaintreau A (1999) Aroma comparisons of traditional and mild yogurts: headspace gas chromatography quantification of volatiles and origin of α-diketones. J. agric. food chem. 476: 2379-2385.
[42] Monnet C, Schmitt P, Divies C (1994) Method for assaying volatile compounds by headspace gas chromatography and application to growing starter cultures. J. dairy sci. 777: 1809-1815.
[43] Starrenburg M, Hugenholtz J (1991) Citrate fermentation by *Lactococcus* and *Leuconostoc* spp. Appl. environ. microb. 57: 3535-3540.
[44] Boumerdassi H, Desmazeaud M, Monnet C, Boquien CY, Corrieu G (1996) Improvement of diacetyl production by *Lactococcus lactis* ssp. *lactis* CNRZ 483 through oxygen control. J. dairy sci. 795: 775-781.
[45] Green ML, Manning DJ (1982) Development of texture and flavour in cheese and other fermented products. J. dairy res. 4904: 737-748.
[46] Tachon S, Michelon D, Chambellon E, Cantonnet M, Mezange C, Henno L, Cachon R, Yvon M (2009) Experimental conditions affect the site of tetrazolium violet reduction in the electron transport chain of *Lactococcus lactis*. Microbiology+. 1559: 2941-2948.
[47] Singh H, Drake MA, Cadwallader KR (2003) Flavor of cheddar cheese: a chemical and sensory perspective. Compr. rev. food sci. f. 2: 139-162.
[48] Bonnarme P, Arfy K, Dury C, Helinck S, Yvon M, Spinnler HE (2001) Sulfur compound production by *Geotrichum candidum* from L-methionine: importance of the transamination step. FEMS microbiol. lett. 205: 247-252.
[49] Kieronczyk A, Cachon R, Feron G, Yvon M (2006) Addition of oxidizing or reducing agents to the reaction medium influences amino acid conversion to aroma compounds by *Lactococcus lactis*. J. appl. microbiol. 1015: 1114-1122.
[50] Riondet C, Cachon R, Waché Y, Alcaraz G, Diviès C (1999) Changes in the proton-motive force in *Escherichia coli* in response to external oxidoreduction potential. Eur. j. biochem. 2622: 595-599.
[51] Martin F, Cachon R, Pernin K, De Coninck J, Gervais P, Guichard E, Cayot N (2011) Effect of oxidoreduction potential on aroma biosynthesis by lactic acid bacteria in nonfat yogurt. J. dairy sci. 942: 614-622.
[52] Hugenholtz J, Kleerebezem M (1999) Metabolic engineering of lactic acid bacteria: overview of the approaches and results of pathway rerouting involved in food fermentations. Curr. opin. biotech. 105: 492-497.
[53] Condon S (1987) Responses of lactic acid bacteria to oxygen. FEMS microbiol. lett. 463: 269-280.
[54] Bassit N, Boquien CY, Picque D, Corrieu G (1993) Effect of Initial Oxygen Concentration on Diacetyl and Acetoin Production by *Lactococcus lactis* subsp. *lactis* biovar diacetylactis. Appl. environ. microb. 596: 1893-1897.

[55] Gardini F, Lanciotti R, Elisabetta Guerzoni M, Torriani S (1999) Evaluation of aroma production and survival of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus acidophilus* in fermented milks. Int. dairy j. 92: 125-134.

[56] Imhof R, Bosset JO (1994) Quantitative GC-MS analysis of volatile flavour compounds in pasteurized milk and fermented milk products applying a standard addition method. LWT - Food sci technol. 273: 265-269.

[57] Ott A, Germond JE, Chaintreau A (2000) Vicinal diketone formation in yogurt: 13C precursors and effect of branched-chain amino acids. J. agr food chem. 483: 724-731.

[58] Storz G, Imlay JA (1999) Oxidative stress. Curr. opin. microbiol. 22: 188-94.

[59] Talwalkar A, Kailasapathy K (2004) A review of oxygen toxicity in probiotic yogurts: Influence on the survival of probiotic bacteria and protective techniques. Compr. rev. food sci. f. 33: 117-124.

[60] Talwalkar A, Kailasapathy K (2004) The role of oxygen in the viability of probiotic bacteria with reference to *L. acidophilus* and *Bifidobacterium* spp. Curr. issues intest. microbiol. 51: 1-8.

[61] Bolduc MP, Bazinet L, Lessard J, Chapuzet JM, Vuillemard JC (2006) Electrochemical modification of the redox potential of pasteurized milk and its evolution during storage. J agric. food chem. 5413: 4651-4657.

[62] Bolduc MP, Raymond Y, Fustier P, Champagne CP, Vuillemard JC (2006) Sensitivity of bifidobacteria to oxygen and redox potential in non-fermented pasteurized milk. Int. dairy j. 169: 1038-1048.

[63] Dave RI, Shah NP (1997) Effect of cysteine on the viability of yoghurt and probiotic bacteria in yoghurts made with commercial starter cultures. Int. dairy j. 78-9: 537-545.

[64] Lourens-Hattingh A, Viljoen BC (2001) Yogurt as probiotic carrier food. Int. dairy j. 111-2: 1-17.

[65] Mortazavian AM, Ehsani MR, Mousavi SM, Reinheimer JA, Emamdjomeh Z, Sohrabvandi S, Rezaei K (2006) Preliminary investigation of the combined effect of heat treatment and incubation temperature on the viability of the probiotic micro-organisms in freshly made yogurt. Int. j. dairy technol. 59(1): 8-11.

[66] Kristoffersen T (1985) Development of flavor in cheese. Milchwissenschaft. 40(4): 197-199.

[67] Urbach G (1995) Contribution of lactic acid bacteria to flavour compound formation in dairy products. Int. dairy j. 58: 877-903.

[68] Ledon H, Ibarra D (2006) Method for modifying hygienic, physico-chemical and sensory properties of cheese by controlling the redox potential. US 2009/0214705 A1. L'Air Liquide, France.