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Green strategies to control redox potential in the fermented food industry.

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Abstract

Lactic acid bacteria (LAB) are important microorganisms in the food industry as functional starters for the manufacture of fermented food products and as probiotics. Redox potential (E_h) is a parameter of the physicochemical environment of foods that influences key oxidation-reduction reactions involved in process performances and product quality. E_h can be modified by different methods, using redox molecules, catalytic activity of enzymes or LAB themselves, technological treatments like electroreduction or heating, and finally gases. Nowadays new applications for food manufacture must undertake green process innovation. This paper presents the strategies for E_h modification in a sustainable manner for production of LAB biomass (starters, probiotics) and fermented food products (fermented milks, cheeses and others). While the use of chemical or enzymes may be subject to controversy, the use of gases offers new opportunities, in combination with LAB. Protection against food-borne microorganisms, an increasing growth and viability of LAB, and a positive impact on food flavour are expected.

Keywords. Lactic acid bacteria, oxidoreduction potential, sustainable innovation, gases, starters, probiotics, fermented milks, cheeses.

1. Introduction

Lactic acid bacteria (LAB) are Gram positive bacteria which belong to the Firmicutes phylum; these bacteria have GRAS (generally recognized as safe) status. LAB can be found in a diversity of environments highlighting their adaptive evolution. Amongst the different physicochemical parameters which characterise these environments, the redox state is seldom considered. It has however already been addressed indirectly in studies on the impact of oxidative stress on LAB (Sugimoto, Abdullah Al, & Sonomoto, 2008). Indeed, the notion of oxidation has often been associated with the presence of oxygen, and the amalgam oxygen/oxidation can narrow the view of the oxidoreduction process. A broader vision can be proposed regarding the adaptation of LAB to extracellular redox. The metabolism of these chemotrophic organisms involves a series of dehydrogenations (oxidations) / hydrogenations (reductions) and is governed by the requirement to balance reactions producing and consuming redox cofactors (NADH). In addition, the acidifying activity of LAB is associated with a reducing activity that can lead media to very reducing values (Brasca, Morandi, Lodi, & Tamburini, 2007; Cachon, Jeanson, Aldarf, & Divies, 2002). The reducing activity varies amongst species and sometimes strains, and we are only just beginning to discover the mechanisms involved (Michelon, et al., 2013; Tachon, et al., 2009; Morandi et al., 2016). Studies aimed at reducing the medium by injecting gases (Cachon, Capelle, Diviès, & Prost, 2001; Cachon, Diviès, Jeannot, & Prost, 2002), adding molecules (Dave & Shah, 1997a, 1997b; Grosch & Wieser, 1999; Moreton, 1998) or using technological treatments (Bolduc, Bazinet, Lessard, Chapuzet, & Vuillemard, 2006; Moreton, 1998; Riondet, 1999) have shown that it is possible to act on the metabolisms of interest such as the production of aroma compounds, bacterial growth and cell survival (Bolduc, Raymond, Fustier, Champagne, & Vuillemard, 2006; Dave & Shah, 1998; Ebel, Martin, Le, Gervais, & Cachon, 2011; Martin, et al., 2010). New avenues are being envisaged aimed at optimising food processes and controlling product quality in a sustainable manner. The results presented here are very promising and underline the consequential prospects.

2. Definition, measurement and manipulation of E_h

In the same way as pH value characterises the acid-base characteristics, E_h characterises the oxidoreductive properties of a medium. Using redox sensors as recommended by Clark and Hewitt in the middle of the last century, the redox state can be measured directly and monitored on line (Clark, 1960). The use of sensors to measure the pH of a medium is very easy to implement nowadays, however measuring E_h is more complex and requires a specific metrological approach (Abraham, 2007; Galster, 2000).

2.1. E_h Measurement

The principle of measuring the E_h of a medium consists in measuring the electromotive force between a metal electrode (platinum, gold) and a reference electrode immersed in the medium being measured. In practice, two references are used: the calomel electrode and the silver/silver chloride electrode (Ag/AgCl). The reference electrode and the measuring electrode are combined and the oxidoreduction potential (E_m) is measured in volts. Oxidoreduction potentials must always be reported relative to the standard hydrogen electrode, consequently the potentials measured (E_m) must be adjusted according to the reference value (E_r) in relation to the hydrogen electrode: $E_h = E_m + E_r$. For example, the reference Ag/AgCl is 207 mV at 25°C ($E_r = 207 + 0.8 (25 - T^\circ\text{C})$). Microelectrodes are preferred for heterogeneous systems, E_h gradients have been highlighted in cheese matrices such as Camembert (Abraham, Cachon, Colas, Feron, & DeConinck, 2007) and Cheddar (Topcu, McKinnon, & McSweeney, 2008). Thanks to sensor measurement, E_h measurement meets pH measurement and user expectations with regard to food technology measurement devices. Easy to use, the speed of the measurement, a lower cost per analysis, the fact that there is little or no sample preparation, and that the results of the measurements on site are consistent with the reference methods (laboratory).

2.2. Manipulation of E_h in food systems

There are four methods to manipulate E_h of foods that can be used in food industry, i.e. use of LAB, redox molecules, gases and heating or electroreduction.

2.2.1. Use of LAB

Traditionally, LABs are s selected for acidifying, texturizing, proteolytic and aromatic properties, resistance to phages and barrier effects regarding undesirable microflora. They must however be able to withstand unfavourable conditions met in industrial processes, including oxidizing environments. LAB do not usually have a complete respiratory chain but can survive in the presence of oxygen due to active mechanisms against oxidative stress (Condon, 1987). These latter have been more particularly studied in LAB of technological and probiotic interest (Higuchi, Yamamoto, & Kamio, 2000). The ability to reduce the medium contributes to the resistance of LAB to oxidative environments but also the sensory and microbiological quality of fermented products (Riondet, et al., 2000; Urbach, 1995; Vermeulen, Kretzer, Machalitz, Vogel, & Gänzle, 2006). The continuous monitoring of E_h during lactic fermentation, which can be coupled with pH measurement, provides qualitative and quantitative data for the comparison of LAB species or strains, different physiological states (impact of stress, method of cell preparation), and fermentation conditions (matrices, dissolved oxygen concentration, etc.). This will be presented in the following section 2.2.1.1 and 2.2.1.2.

2.2.1.1. Variability of LABs reducing activity

Table 1 shows a broad overview of the reducing capacity of LAB. Species such as *Streptococcus thermophilus* are very weak reductants, contrary to *Lactococcus* sp. or *Lactiplantibacillus plantarum*. The reducing capacity of the latter two species actively contributes to the reducing E_h found in the center of some cheeses (Abraham, 2007; Aubert, et al., 2002; Topcu, et al., 2008). Cachon *et al.* (2002) proposed to characterise the reducing capacity in the same way as the acidifying capacity. Characteristic kinetic parameters such as maximal rate (V_m^r , V_m^a), the time required to reach maximal rate (T_m^r , T_m^a) and pH in the same time (pH_m^r , pH_m^a), are calculated from the reduction and acidification kinetics (the superscript r and a referring to reduction and acidification, respectively). Interspecies differences can thus be characterised better. Similar procedures using different activity parameters have been proposed by Brasca's team (Brasca et al.,

2007, Morandi et al., 2016) in liquid medium, and Michelon et al. (2010) for screening LAB in solid medium. Commercial cocktails used for manufacturing fresh cheese, soft cheese, blue cheese and for milk maturation have shown T_m varying from 1 to 8 hours (Aubert, et al., 2002). An industrial strategy would be to define the mixed cultures used in manufacturing by combining strains with different reducing capacities and by offering innovative fermented products. The mechanisms involved in LAB reducing activity will be detailed in section 3.

2.2.2. Use of redox molecules and oxidoreductases

While the sensory quality, shelf-life and functionality of food products involve several oxidoreduction mechanisms, few redox molecules are authorised for use in the food industry. In dough proofing, ascorbic acid is added to act on the equilibrium between the sulphhydryl / disulphide forms of cysteine residues in gluten proteins, increasing loaf volume and improving crumb structure (Grosch & Wieser, 1999). Dave and Shah (1997a, 1997b) and Soto et al. (2019) used ascorbic acid, cysteine or tocopherol to lower the E_h during milk fermentation. Use of oxidoreductases is a solution to modify redox environment. In food applications, the glucose oxidase (GOD) / catalase (CAT) system is used to remove oxygen or glucose from foods, GOD catalyzes glucose oxidation to gluconic acid, using oxygen as hydrogen acceptor, and producing hydrogen peroxide, CAT catalyzes the breakdown of hydrogen peroxide into oxygen and water. The Food and Agriculture Organization of the United Nations (FAO) authorizes the activation of the lactoperoxidase / thiocyanate / hydrogen peroxide system (LPS), a chemical method to preserve raw milk, mainly applied in remote regions lacking refrigeration (developing regions). This method uses chemical addition of a pre-packaged activator containing thiocyanate and a source of hydrogen peroxide such as sodium percarbonate (FAO, 1991; IDF, 2013). LPS is based on oxidation of thiocyanate to hypothiocyanite ions, these ones oxidize the thiol groups of amino acid residues in microbial proteins, leading to impaired function and death of the microorganism. In US, LPS is generally recognized as safe (GRAS) as a processing aid (together with glucose oxidase and catalase), in fresh cheese including mozzarella and cottage cheeses, frozen dairy desserts, fermented milk, flavoured

milk drinks, and yogurt. Consumers view dairy products as 'natural', and IDF has concerns about some chemical activator breakdown products such as cyanide, which at high levels or consumed at low levels on a continuous basis can have a negative effect on consumer health (IDF, 2013). Apart from LPS, hydrogen peroxide can also be used in some types of cheeses like Swiss in state of pasteurization, together with catalase which is used to remove residual hydrogen peroxide (Abada, 2019). In a context of a consumers demand for 'clean label' foods, which refer to what is seen as little processed and 'natural' or 'free from' negatively associated ingredients (Aschemann-Witzel, Varela & Peschel, 2019), the use of chemical additives is of course not sound. About enzymes authorized as processing aid (Eroli-Cura et al., 2015), their use is considered as safe, but the increase in regulations (positive list for French regulation) and consumers awareness highlight the need for innovation with alternative solutions.

2.2.3. Use of nitrogen and hydrogen

Dissolved oxygen content can traditionally be decreased using vacuum; pure nitrogen or a gas mixture (nitrogen/hydrogen) can also be injected into the medium or the product headspace. The major advantage of these gases is they are odorless, colourless, tasteless, and nontoxic, and that they are authorised as processing aids in the food industry, thus being convenient for a so-called 'clean label'. In addition, it is possible to work with pure gases or mixtures, they can be used sequentially in a process, and state of the art for monitoring of aerated bioreactors can be transferred to anaerobic bioreactors (Cachon et al., 2019). Cachon *et al.* (2001; 2002) proposed a method of reducing E_h using a flow of a mixture of nitrogen and hydrogen (96%/4%). An E_h of -286 mV was thus attained in milk, compared with +220 mV obtained with pure nitrogen. Gases can be introduced and retained in dairy products by whipping, pressurisation, sparging, fermentation, chemical addition and using gas hydrate (Bhaskar et al., 2018). A gas-liquid exchanger has been designed by Jallais & Ibarra (2009) to prevent foaming when gas was sparged in milk.

2.2.4. Heating and electroreduction

Autoclave sterilisation decreases E_h by nearly 150 mV in media like TSB and is essentially due to the production of antioxidant compounds resulting from the Maillard reaction (Riondet, 1999). Heat treatments also promote the elimination of dissolved oxygen (of which the solubility decreases as the temperature increases) and the formation of reducing sulphur groups (Greenbank & Wright, 1951; Moreton, 1998). In a green process perspective, the equipment and method need to be sustainable, concerns pertaining to costs and the environment have demanded reductions in both energy-consuming technology and CO₂ emissions. In this perspective, heating processes are not the most appropriate to modify E_h .

Electroreduction is a physical treatment that consists in reducing an aqueous solution (milk, water...) using electrolysis (Figure 2). The electrolytic cell is made up of two compartments separated by an ion exchange membrane. The liquid being treated circulates in the compartment containing the cathode and the reactive compounds are converted into their reduced forms by electroreduction (Bazinet, Lamarche, Boulet, & Amiot, 1997; Bolduc, Raymond, et al., 2006). Electroreduction decreases E_h and the concentration of dissolved oxygen in milk (Bolduc, Bazinet, et al., 2006), but the process requires optimization because the reducing E_h obtained by electroreduction is not stable over time. After 4 days of conservation, E_h is again positive (Schreyer, Britten, Chapuzet, Lessard, & Bazinet, 2008).

3. Physiological adaptation of LAB to external E_h

The bacterial cell exchanges electrons with the external medium via redox mechanisms or redox compounds, so any variation in extracellular E_h is likely to affect the intracellular redox equilibrium and consequently the related metabolic reactions (Hansen, 2018; Liu et al., 2019; Soghomonyan et al., 2011).

3.1. Mechanisms involved in LAB reducing activity

The mechanisms involved in the reducing activity of *Lactococcus lactis* subsp. *lactis* have been proposed (Figure 1) which include reduction mechanisms of oxidizing compounds in the medium,

such as oxygen, and mechanisms responsible for the stabilization of the reducing E_h in an anaerobic environment (Michelon, et al., 2010; Michelon, et al., 2013; Tachon, et al., 2009). Menaquinones coupled to NADH dehydrogenases, NoxA and NoxB, and the NADH oxidase, NoxE, are involved in the reduction of an aerobic medium (Tachon, et al., 2009). Proteins in the cell envelope exposing exofacial thiol groups could be the cause of the reducing E_h measured in anaerobic medium condition (Michelon, et al., 2010).

3.2. Physiological adaptation to oxidative media

LAB can reduce the oxygen in the medium to water using NADH oxidase activity, thus eliminating this inhibitory molecule; this reaction consumes NADH and modifies the redox equilibrium by decreasing the availability of NADH for other re-oxidation pathways. A reorientation of the metabolic fluxes is then observed in homofermentative LAB such as *Lactococci* with a shift toward mixed acid fermentation (Neves, Pool, Kok, Kuipers, & Santos, 2005), and in heterofermentative LAB such as *Leuconostocs* (Plihon, Taillandier & Strehaiano, 1995). The production of carbonyl compounds from citrates, of which diacetyl (characteristic aroma of certain fermented milk products), requires an oxidizing medium. Alpha-acetolactate, coming from pyruvic acid condensation, is converted into diacetyl by oxidative decarboxylation (Jönsson & Pettersson, 1977; Monnet, Schmitt, & Divies, 1994). Kieronczyk et al. (2006) have shown a clear influence of E_h on amino acid catabolism (phenylalanine, leucine, methionine) in *Lactococcus lactis* subsp. *lactis*. The production of aldehydes and volatile sulphur compounds is favoured by oxidizing conditions and is mainly the result of chemical oxidation of the α -keto acids produced by amino acid transamination. Conversely, carboxylic acid synthesis (phenylacetic, methylthiopropionic and isovaleric acids) is promoted by reducing conditions. Afzal et al. (2013) demonstrated the effect of different dissolved oxygen concentrations on the intracellular biosynthesis of 3-methylbutanal from leucine catabolism in *Carnobacterium maltaromaticum*, a LAB retrieved from meat and cheeses. This was attributed to the simultaneous activation and stimulation of both the α -ketoacid decarboxylase and the α -ketoacid dehydrogenase pathways. At membrane level, an oxidizing environment can lead to lipid

peroxidation. This chain reaction results in the formation of compounds such as aldehydes, unsaturated fatty acids, alkanes, ketones and epoxides, with shorter carbon chains thus increasing membrane fluidity (Hauville, Remita, Therond, Jore, & Gardes-Albert, 2001). This fluidification can affect membrane transport by increasing permeability to H^+ ions inducing a decrease in internal pH thus destroying the ΔpH and therefore the proton motive force and associated ATPase activities (Miyoshi, et al., 2003). Another possible mechanism is the oxidation of thiols on membrane Na^+ , K^+ -ATPase, resulting in increased permeability (Zhang, et al., 2010).

3.3. Physiological adaptation to reducing media

Reducing conditions affect membrane fluidity and surface properties of bacteria. Ouvry et al. (2002) highlighted in *Lactiplantibacillus plantarum* an attenuation of the redox effect on the growth and activation of ATPase activity at 10°C in comparison with 37°C. At 37°C, a stiffening of the cell membrane and a difference in growth are observed in reducing conditions. Furthermore, this stiffness is maintained throughout growth in reducing conditions. This indicates that the E_h of the medium can influence the synthesis of fatty acids and/or membrane proteins. If the E_h leads to an increase in membrane proteins, the membrane protein / lipid ratio can increase and maintain membrane rigidity. Moreover, *Lactiplantibacillus plantarum* is a weaker electron donor and a stronger electron acceptor at -400 mV than at +250 mV and in stationary phase; the cells are clearly more hydrophilic in reducing conditions. The surface properties are directly linked to the composition of the cell envelope. The presence of groups such as $-COO^-$ could therefore be the cause of this electron donor characteristic and modifications in the teichoic acid content could affect the hydrophobicity. In *Escherichia coli* (Riondet, Cachon, Waché, Alcaraz, & Diviès, 1999) or *Saccharomyces carlsbergensis* (Petrov, Smirnova, & Okorokov, 1992), the decrease in E_h could modify the oxidoreductive equilibrium of the thiol functions of membrane proteins, leading to the permeabilization of the membrane to protons. However, Waché et al. (2002) did not observe any modification in the internal pH and the ΔpH in a strain of *Lactococcus lactis* subsp. *lactis* grown in

acidic medium with dithiothreitol. These initial results underline the microbial biodiversity regarding redox.

4. Applications in food processes

4.1. Growth and viability of LAB

The sensitivity of LAB strains (technological starters or probiotics) to oxidative stress mainly generated by oxygen incorporation is a major problem (Yamamoto, Gaudu, & Gruss, 2011; Larsen et al., 2015). Altering the expression of genes coding key proteins involved in intracellular redox is a solution to improve antioxidative properties of LAB (Zhang & Li, 2012). However, this causes ethic problem because altering genome makes strain a genetically modified organism. In contrast, external E_h can be controlled or modified using chemical additives, but these are negatively perceived by consumers and remain strictly regulated. The addition of whey proteins (which are rich in sulfur-containing aminoacids), cysteine (redox-active amino-acid), tocopherol (radical scavenger) and ascorbic acid (electron-donor antioxidant with a low reduction potential), have been used to decrease E_h ; the fermentation time was significantly reduced and the viability of LAB and probiotics after refrigerated storage was improved (Dave & Shah, 1997a, 1998; Soto et al., 2019). However, only the use of cysteine enables a negative redox potential to be conserved during product storage (Dave & Shah, 1997a) as ascorbic acid is rapidly oxidized in the containers used (Dave & Shah, 1997b). In addition, the use of cysteine or ascorbic acid for making starters has a negative impact on the organoleptic properties of fermented milk (Talwalkar & Kailasapathy, 2004). Electroreduction is another avenue to be envisaged which enables E_h to be decreased without affecting the organoleptic and nutritional qualities of milk, and this with no addition of chemicals (Schreyer, 2007). A positive impact on the viability/survival of probiotic strains has also been observed; electrochemical reduction of milk, as well as deaeration or addition of reducing agents could be applied to enhance the survival of bifidobacteria during extended storage at 7 °C in milk. (Bolduc, Raymond, et al., 2006). Cruz et al. (2012, 2013) proposed the use of a food grade glucose

oxidase coupled with low oxygen permeability packaging systems to minimize oxidative stress in fermented milk containing probiotics. Finally, to increase the viability/survival of probiotics in products, an alternative method to those described previously consists in using gases. Bubbling with nitrogen or a mixture of nitrogen and hydrogen has a positive effect on the survival of *Bifidobacterium bifidum* in fermented milk during storage (Ebel, et al., 2011). Furthermore, the use of gas has no impact on the manufacturing time or the viability and survival of the starters. The improvement in the survival of LAB by modification of E_h can also be considered in dry products. Indeed, Cachon et al. (2006) have shown that reducing culture conditions obtained using a reducing gas (N_2-H_2) favour the viability of freeze-dried *Lactocaseibacillus rhamnosus* bacteria from industrial source (Table 2). Rodrigues et al. (2011) have shown the beneficial role of cysteine on the survival of atomised probiotics during storage. The latter result is however strain-specific; of the three strains tested only the survival of the *Lactobacillus acidophilus* strain was improved by the presence of cysteine.

~~Lactic microflora can also spoil food leading to various changes (acidification, production of off-flavour, gas production, slime formation) in desserts, retail food products, juices and sauces. For products packaged under modified atmosphere, the partial or complete exclusion of oxygen inhibits the proliferation of Gram negative bacteria whilst favouring the growth of Gram positive bacteria such as LAB. Ouvry et al. (2002) showed the effect of a reducing E_h obtained using hydrogen gas combined with common inhibitory factors (NaCl, acetic acid and temperature) on the slowdown of growth and acidification. The combination of E_h and heat stress has also been studied for slowing the growth of microorganisms (Riondet, et al., 2000), and has been applied in the field of pasteurized orange juice (Alwazeer, Cachon, & Divies, 2002; Alwazeer, Delbeau, Divies, & Cachon, 2003). In both cases, the contaminant was *Lactobacillus plantarum*.~~

4.2. Manufacture of fermented milk products

Milk E_h is generally above 150 mV at pH 6.5-6.7 (table 1). In raw milk, E_h is governed by the quantity of dissolved oxygen, but also by molecules naturally present in milk such ascorbic acid,

riboflavin, iron, copper, thiol groups and phenolic compounds (Abraham, 2007; Walstra, Wouters, & Geurts, 2006). If heat treatment is applied to milk before cheese making, milk E_h decreases (McSweeney, Caldeo, Topcu, & Cooke, 2010). The E_h is much lower in cheese than in milk; values reported in the literature are from -259 mV to -360 mV for Camembert (Abraham, et al., 2007; Aubert, et al., 2002), from -118 mV to -270 mV for Cheddar (Green & Manning, 1982; Topcu, et al., 2008), -156 mV for Comté (Aubert, et al., 2002), and from -270 mV to -300 mV for Emmental (Langeveld & Galesloot, 1971) and -213 mV for UF white cheese (Bulat and Topcu, 2019). All these cheeses are manufactured with mixed culture of LAB. Caldeo and McSweeney (2012) studied changes in redox potential (E_h) during the simulated cheesemaking of Cheddar, Gouda, Emmental and Camembert cheeses; each obtained from 4 L of milk. At the end of manufacturing process (beginning of ripening), they measured E_h values of -117 mV, -118mV, +230mV and -115mV for Cheddar-type, Gouda-type, Emmental-type and Camembert-type, respectively. Green and Manning (1982) observed that the decrease in redox in cheese was due to lactic starters. A Cheddar-type cheese manufactured with only δ -glucono-lactone to acidify had an E_h of +315 mV whereas in Cheddar made with LAB (*Lactococci*) it is normally -150 mV to -200 mV. In the case of Cheddar, the stabilisation of the redox measurement is long, approximately 48 hours, contrary to Camembert that is between 10 minutes and 1 hour (Abraham, et al., 2007; Topcu, et al., 2008). However, according to McSweeney et al. (2010), this measurement is more rapid in Cheddar by introducing miniature electrodes in the cheese during moulding and pressing. The evolution of the redox potential depends on the cheese manufacturing process implemented and the lactic starters used. In the manufacturing of a semi-hard cheese, Abraham (2007) has shown that during renneting, mixing, coagulum cutting and lactose removal phases, the medium remains oxidizing due to persistent levels of dissolved oxygen between 20% and 60%, then progressively becomes reducing during pressing when no oxygen is incorporated thus allowing the LAB to express their reducing activity. It thus seems possible to modulate E_h during cheese manufacturing via the action of LAB. Gases or reducing molecules (cysteine and ascorbic acid) can also be used, like those used in the

manufacturing of fermented milk/yoghurt and cheese (Dave & Shah, 1997a, 1997b; Ebel, et al., 2011; Ledon & Ibarra, 2006; Martin, et al., 2011; Martin, et al., 2010). Martin et al. (2009, 2010, 2011) studied the effects of E_h on yoghurts made under various gaseous conditions. The milk prepared under air was an oxidizing medium. Nitrogen, which is a neutral gas, can be used to remove oxygen from milk (even so the milk E_h remained oxidizing in these conditions) and hydrogen gave a reducing E_h (below 0). These studies focused on the effect of gas bubbling on gel structures through viscoelastic properties, the measurement of whey separation and production of aroma compounds by *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (Table 3). Yoghurts produced under air and under bubbled air have the highest apparent viscosity. The apparent viscosity of yoghurt made under bubbled N_2 was lower (0.035 Pa.s) than other oxidizing conditions (0.046 Pa.s), whereas values obtained under bubbled $N_2 - H_2$ were the lowest (0.021 Pa.s). Apparent viscosity of GDL-gels was also clearly affected by the gas type. A reducing environment reduces apparent viscosity of yoghurt. Whey separation of yoghurts occurred from the very first day of storage and the volume of whey separation was almost constant during the 28 days of storage. The highest whey separation was obtained for yoghurt made under air and bubbled air. Whey separation was lower for yoghurts prepared under bubbled N_2 (1.03 g / 100 g) and $N_2 - H_2$ (0.59 g / 100 g). So, the more reducing the environment, the lower the whey separation. Adjusting the E_h of milk under reducing conditions (under $N_2 - H_2$) could be a possible way of significantly decreasing the phenomenon of whey separation. The impact of these different E_h on the biosynthesis of acetaldehyde, dimethyl sulphide, diacetyl and pentane-2,3-dione by bacteria in yoghurts was studied. The analyses of the amount of the four aroma compounds produced during 28 days of storage showed that acetaldehyde and diacetyl concentrations were significantly higher in yoghurts made under bubbled air compared to standard yoghurts. The concentration of dimethyl sulphide was significantly lower and that of pentane-2,3-dione was the same. Under bubbled N_2 the acetaldehyde concentration was similar to that of yoghurts made under bubbled air 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 of yoghurts made under air (bubbled or not). So, oxidative E_h conditions clearly increased the production of these four aroma compounds in yoghurts. These results are consistent with the literature. Indeed, (Kieronczyk, et al., 2006) showed that oxidative conditions stimulated the production of volatile sulphur compounds such as dimethyl sulphide and aldehydes such as acetaldehyde. In the presence of oxygen, oxidative decarboxylation of 2-acetolactate and 2-aceto-hydroxybutyrate to diacetyl and pentane-2,3-dione respectively was also favoured (Boumerdassi, Desmazeaud, Monnet, Boquien, & Corrieu, 1996; Hugenholtz & Kleerebezem, 1999; Monnet, et al., 1994; Neijssel, Snoep, & Teixeira de Mattos, 1997). The result obtained for diacetyl can be explained by the fact that in anaerobic conditions LAB dehydrogenate the NADH produced during glycolysis via lactate dehydrogenase (LDH) activity. Boumerdassi et al. (1996) showed that oxygen increases NADH oxidase activity (Condon, 1987), which causes NADH re-oxidation to the detriment of LDH, butanediol dehydrogenase and acetoin dehydrogenase activity (Bassit, Boquien, Picque, & Corrieu, 1993). Then, excess pyruvate is partially eliminated through acetolactate production, which increases diacetyl production (Boumerdassi, et al., 1996). Finally, for yoghurts made under bubbled N_2-H_2 (reducing conditions), acetaldehyde and pentane-2,3-dione concentrations were the same as in yoghurts prepared under ambient air. The concentration of dimethyl sulphide was the same as in yoghurts made under N_2 and the concentration of diacetyl was significantly lower than under the other three E_h conditions. So, controlling the redox in cheese is primordial as the aromatic characteristics are closely linked to this parameter. Green & Manning (1982) and Kristoffersen (1985) have shown that a negative redox is essential for the good development of the characteristic flavour of certain fermented milk products such as cheese, notably *via* the production of thiol compounds. In addition, Ledon & Ibarra (2006) improved the sensory properties, notably through the increased production of volatile fatty acids, by ripening the cheese under a reducing atmosphere. More recently, Caldeo et al (2016) and Bulat & Topcu (2020) showed that redox potential influenced development of volatile flavour compounds in Cheddar cheese and

UF white cheese, respectively, and confirmed that a reducing redox potential is essential for development of sulphur compounds. Moreover, nutritional quality of foods is an important factor for consumers nowadays and E_h , like pH, has become a significant parameter for the dairy industry to improve or create new dairy products. Giroux et al. (2008) studied the combined effect of dissolved gas composition (Air, N_2 , N_2-H_2) and heat treatment on the oxidative degradation of a new dairy beverage enriched with 2% linseed oil. Saturation with N_2 or N_2-H_2 significantly reduced oxidative degradation of polyunsaturated fatty acids and provided some protection against color changes during storage. Alwazeer et al. (2020) showed that fortification of yoghurt with plant extracts modified the acidification and reducing activities of starters, thus changing the fermentation time and quality attributes of the product. Finally, according to Beresford et al. (2001), redox acts as a selective agent for bacterial growth; the heart of the cheese is considered as an anaerobic medium (which varies according to the size of the cheese) promoting the growth of strict or facultative anaerobic bacteria. Munsch-Alatossava et al. (2016) showed that N_2 gas flushing, could constitute an alternative to chemical preservatives (H_2O_2 and thiocyanate) used for activation of LPS to control bacterial growth in raw milk. Recently, it has been proved that E_h from LAB was able to downregulate genes of virulence from pathogens and therefore prevents enterotoxin production (Nogueira Viçosa et al., 2019; Nouaille et al., 2014).

5. Conclusion

Modifying the E_h of a food product can involve the reducing activity of starters, the injection of gases as processing aids (nitrogen, hydrogen), the product formulation with the addition of molecules (cysteine, ascorbic acid), the use of enzymes as processing aids, or a technological treatment (heat treatment, electroreduction). The diversity of reducing activities between LAB and use of gases have both a significant impact on the E_h of the medium and, in our opinion, are the most suitable to improve food processes and innovation in a sustainable manner. In addition, the increased knowledge of the mechanisms involved in the reducing activity of LAB enables new

applications to be envisaged for controlling E_h in food matrices. Gases act directly on the physico-chemical properties of the medium interacting also with lactic starters with an impact on the microbiological, nutritional and sensory properties of food products.

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Figure captions

Figure 1. Summary diagram of the cell functions involved in the reducing activity of *Lactococcus lactis* subsp. *lactis*. P-SH, proteins-thiols; P-SSP, oxidated proteins-thiols; Trx-TrxR, thioredoxin-thioredoxin reductase system; NoxAB, NADH membrane dehydrogenases NoxA and NoxB; NoxE, NADH oxydase; AhpR, alkyl hydroperoxide reductase complex; Ox, oxidized compound; Red, reduced compound; MK, menaquinone; MKH₂, menaquinol; ?, hypothesis (Michelon, et al., 2010; Tachon, et al., 2009).

Figure 2. Simplified diagram of an electrolytic cell.

Table 1. Acidifying and reducing capacities of different LAB used in the food industries.

LAB	Food origin	Culture medium	Starting pH, E_h (mV), E_{h7} (mV) ^a	Final pH, E_h (mV), E_{h7} (mV) ^a	References
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	Probiotic food	FS-Milk	6.5 , 372, 352	5.8 , 30, -16	
<i>Bifidobacterium bifidum</i>	Probiotic food	FS-Milk	6.5 , 405, 380	5.6 , 15, -20	
<i>Enterococcus durans</i>	Cheese	RS-Milk	NS , 200 to 250, <i>NS</i>	NS , -120 (n=10) ^b , <i>NS</i>	(Brasca, Morandi, Lodi, & Tamburini, 2007)
<i>Enterococcus faecalis</i>	Cheese	RS-Milk	NS , 200 to 250, <i>NS</i>	NS , -221 (n=10) ^b , <i>NS</i>	(Brasca, et al., 2007)
<i>Enterococcus faecium</i>	Cheese	RS-Milk	NS , 200 to 250, <i>NS</i>	NS , -138 (n=10) ^b , <i>NS</i>	(Brasca, et al., 2007)
<i>Levilactobacillus brevis</i>	Probiotic food	S-Milk	6.5 , 252, 233	3.9 , -85, -207	
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	Yoghurt Cheese	S-Milk RS-Milk	6.5 , 233, 215 NS , 200 to 250, <i>NS</i>	3.2 , 30, -122 NS , 54 (n=9) ^b , <i>NS</i>	(Brasca, et al., 2007)
<i>Lactobacillus helveticus</i>	Cheese Cheese	S-Milk RS-Milk	6.5 , 233, 215 NS , 200 to 250, <i>NS</i>	3.4 , 83, -60 (n=5) ^b NS , 54 (n=8) ^b , <i>NS</i>	(Brasca, et al., 2007)
<i>Lactiplantibacillus plantarum</i>	Cheese Delicatessen Bakery product	RS-Milk MRS* Buckwheat dough	NS , 200 to 250, <i>NS</i> 6.0 , 220, 190 6.5 , 372, 345	NS , -154 (n=5) ^b , <i>NS</i> 5.1 , -60, -117 3.9 , 256, 89	(Brasca, et al., 2007) (Capuani, Behr, & Vogel, 2012)
<i>Lactocaseibacillus rhamnosus</i>	Probiotic food Probiotic food	RS-Milk MRS	6.5 , 320, 300 6.5 , 190, 175	4.0 , -80, -200 4.7 , -110, -202	
<i>Latilactobacillus sakei</i> subsp. <i>sakei</i>	Bakery product Delicatessen	Buckwheat dough MRS*	6.5 , 313, 286 6.0 , 220, 190	4.0 , 267, 105 5.1 , -53, -110	(Capuani, et al., 2012)
<i>Lactocaseibacillus paracasei</i> subsp. <i>paracasei</i>	Cheese	RS-Milk	NS , 200 to 250, <i>NS</i>	NS , -169 (n=10) ^b , <i>NS</i>	(Brasca, et al., 2007)
<i>Lactococcus</i> sp.	Cheese Cheese Cheese	S-Milk S-Milk MRS	6.5 , 233, 215 6.5 , 233, 215 6.5 , 198, 183	4.4 , -114, -213 4.4 , -117, -216 (n=9) ^b 4.5 , -138, -213 (n=3) ^b	(Cachon, Jeanson, Aldarf, & Divies, 2002) (Michelon, et al., 2010)
<i>Lactococcus</i> sp. protease ⁻	Cheese	RS-Milk	NS , 200 to 250, <i>NS</i>	NS , -162 (n=10) ^b , <i>NS</i>	(Brasca, et al., 2007)
Commercial mixed starter	Cheese Cheese	S-Milk S-Milk	6.5 , 233, 215 6.4 , 315, 293	5.4 , -144, -207 (n=8) ^b 4.5 , -133, -229	(Cachon, et al., 2002)
<i>Leuconostoc</i> sp.	Cheese	S-Milk	6.5 , 233, 215	5.8 , 120, 72 (n=13) ^b	
<i>Pediococcus pentosaceus</i>	Cheese Bakery product Delicatessen	RS-Milk Buckwheat dough MRS*	NS , 200 to 250, <i>NS</i> 6.6 , 331, 310 6.0 , 220, 190	NS , 59 (n=6) ^b , <i>NS</i> 4.5 , 317, 182 5.4 , -20, -68	(Brasca, et al., 2007) (Capuani, et al., 2012)
<i>Staphylococcus xylosum</i>	Delicatessen	MRS*	6.0 , 220, 190	5.9 , -40, -73	
<i>Staphylococcus carnosus</i>	Delicatessen	MRS*	6.0 , 220, 190	5.8 , -21, -57	
<i>Streptococcus thermophilus</i>	Cheese, Yoghurt	S-Milk RS-Milk	6.5 , 233, 215 200 to 250	4.7 , 0, -93 (n=6) ^b NS , 8 (n=10) ^b , <i>NS</i>	(Brasca, et al., 2007)
<i>Weissella cibaria</i>	Bakery product	Buckwheat dough	6.5 , 359, 332	4.1 , 187, 30	(Capuani, et al., 2012)

^a $E_{h7} = E_h$ at pH 7 calculated by applying the Leistner and Mirna equation (Leistner & Mirna, 1959), E_{h7} calculation allows to overcome pH dependency (Cachon, et al., 2002).

^b average values obtained with n different strains.

FS-Milk=Fortified Skimmed Milk; RS-Milk=Reconstituted Skimmed Milk; S-Milk=Skimmed Milk; MRS=de Man Rogosa and Sharpe medium (De Man, Rogosa, & Sharpe, 1960); MRS*=modified MRS medium whose salts have been replaced by 4.1 % NaCl, 23 % tryptone, and 0,6 % KNO₃. NS = value Not Specified in the bibliographic reference.

In the References column, rows without references are personal data

Table 2. Effect of gaseous conditions during culture on the survival of a lyophilized probiotic lactic acid bacterium, *Lacticaseibacillus rhamnosus*, conserved at room temperature (Cachon et al., 2006).

Culture conditions	VPC after 1 day	VPC after 2 months	VPC after 7 months	VPC after 19 months
Standard - $E_{hi}=+320$ mV	$8.83^a \pm 0.07$	$7.08^a \pm 0.13$	$6.76^a \pm 0.05$	$6.04^a \pm 0.14$
N ₂ (100 %) - $E_{hi}=+220$ mV	$8.93^b \pm 0.02$	$7.34^b \pm 0.08$	$6.95^b \pm 0.24$	$6.30^b \pm 0.14$
N ₂ (96%) / H ₂ (4%) - $E_{hi}=-286$ mV	$9.00^c \pm 0.08$	$7.60^c \pm 0.07$	$7.28^c \pm 0.04$	$6.63^c \pm 0.11$

$E_{hi}=E_h$ at the beginning of the culture.

VPC = Viable Plate Counts (log CFU/g lyophilized powder), n=3.

Same superscript letters within a column indicate that groups were not significantly different at 5% α risk (ANOVA).

Table 3. Characteristics of the yoghurt gel structure depending on the different E_h conditions (Martin et al. 2009, 2010, 2011). Apparent viscosity η at 500 1/s of yoghurts at pH = 4.6 and 4°C (measurements were made 24 hours after addition of starter culture). Evolution of the average whey separation (WS) over 28 days in yoghurts.

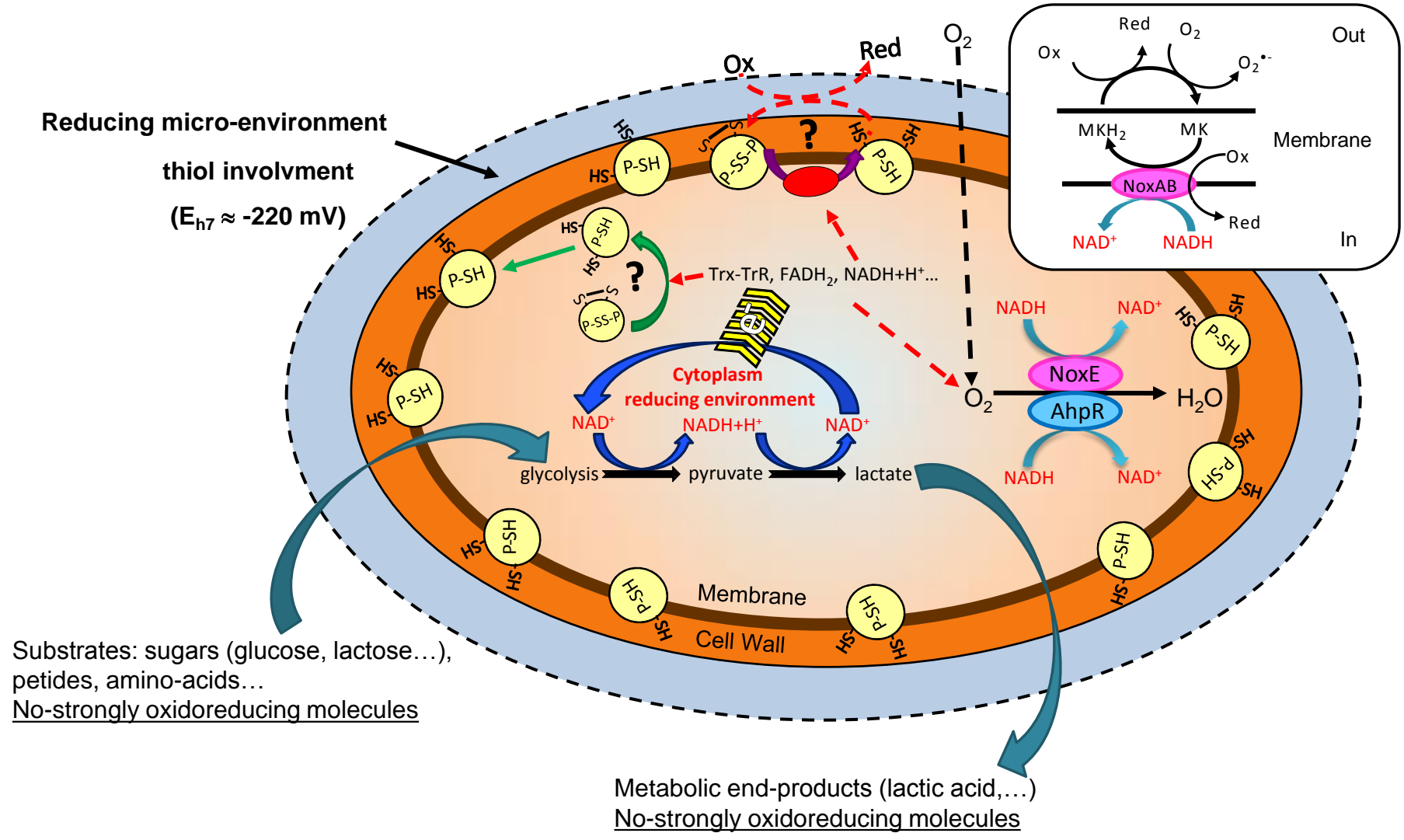
Gaseous conditions applied to milk	pH		E_{h7} (mV)		η (Pa.s)	WS (g/100g of yoghurt)
	At t=0 hours	At t=3.5 hours	At t=0 hours	At t=3.5 hours		
Air	6.80 ^a ± 0.0	4.6 ^a ± 0.0	425 ^a ± 20	171 ^a ± 2	0.046 ^a ± 0.00	1.98 ^a ± 0.54
Bubbled air	6.80 ^a ± 0.0	4.6 ^a ± 0.0	435 ^a ± 3	241 ^a ± 8	0.046 ^a ± 0.00	1.76 ^a ± 0.31
Bubbled N ₂	6.81 ^a ± 0.0	4.6 ^a ± 0.0	285 ^b ± 11	139 ^b ± 5	0.035 ^b ± 0.00	1.03 ^{ab} ± 0.27
Bubbled N ₂ – H ₂	6.81 ^a ± 0.0	4.6 ^a ± 0.0	-345 ^c ± 4	-309 ^c ± 10	0.021 ^c ± 0.01	0.59 ^b ± 0.12

Values are means from triplicate experiments.

Same superscript letters within a column indicate that groups were not significantly different at 5% α risk (ANOVA).

Reduction of external oxidants

Reducing micro-environment
thiol involvement
($E_{h7} \approx -220$ mV)



Substrates: sugars (glucose, lactose...),
peptides, amino-acids...
No-strongly oxidoreducing molecules

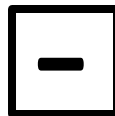
Metabolic end-products (lactic acid,...)
No-strongly oxidoreducing molecules

Cationic
membrane

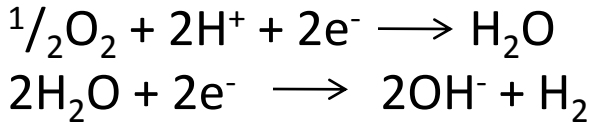
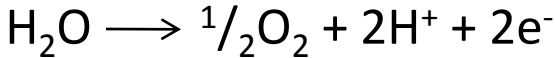
Anode



Cathode

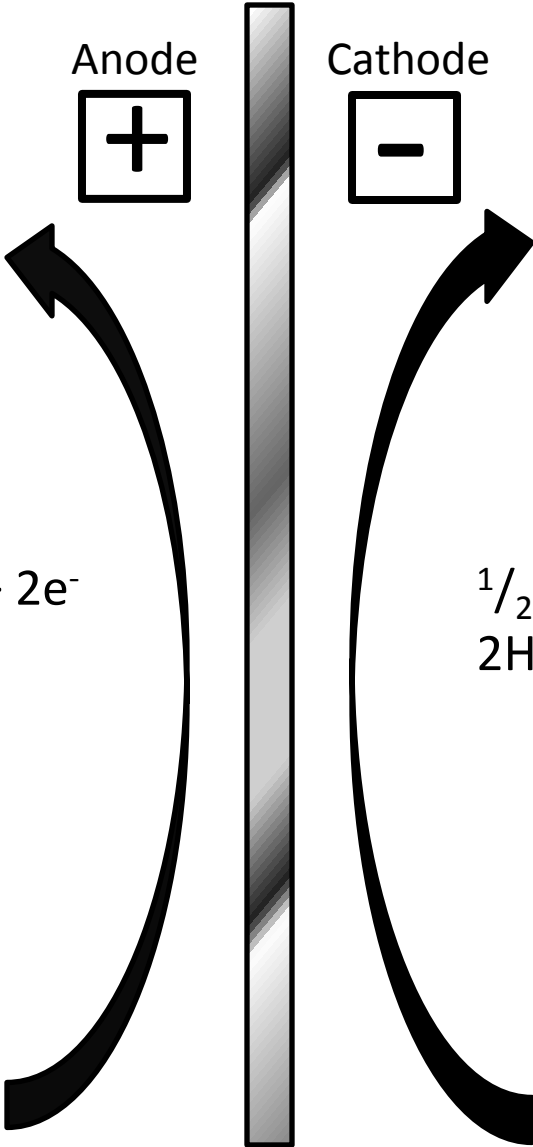


Reduced milk



Electrolyte

Milk



Various ways to modify the E_h in the food industry

