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Ohmic heating for the dairy industry: a potential technology to develop probiotic dairy foods in association with modifications of whey protein structure

Ricardo Nuno Pereira[1], José António Teixeira[1], António Augusto Vicente[1], Leandro Pereira Cappato[2], Marcus Vinicius da Silva Ferreira[2], Ramon da Silva Rocha[3] and Adriano Gomes da Cruz[3]

The use of whey in dairy probiotics is a topic of great interest to the scientific community and the food industries. However, few studies address the effect of ohmic heating (OH) on cell metabolism and growth parameters of probiotic microorganisms. Despite of this, OH under sub-lethal conditions presents promising results regarding the enhancement of growth rate and bacteriocin activity, leading to considerable improvements in the fermentation process. Thus, this review highlights the main findings and advances on the effect of OH on probiotic metabolism, while addressing the modification of whey protein structure as potential carrier of probiotic entities, aiming at stimulating interest and encouraging the development of functional products using OH.

Addresses
[1] CEB-Centre of Biological Engineering, University of Minho, Braga, Portugal
[2] University Federal Rural do Rio de Janeiro, Instituto de Tecnologia, Seropédica, Brazil
[3] Instituto Federal de Educação, Ciência e Tecnologia (IFRJ), Departamento de Alimentos, Rio de Janeiro, Brazil

Corresponding authors: Pereira, Ricardo Nuno (rpereira@deb.uminho.pt), da Cruz, Adriano Gomes (adriano.cruz@ifrj.edu.br)

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Introduction

Ohmic heating (OH) is an outstanding example of a successful emergent technology in food processing. From the beginning of the XX century until now OH has survived by taking advantage of technological evolution and gathering fundamental and applied knowledge during decades. In fact, OH together with novel technologies such as pulsed electric fields and high-pressure processing is in the frontline of the 'emerging high-potential technologies for tomorrow' [1]. OH is based on the simple principle that the passage of electricity through a semi-conductive material will allow generation of internal heat (Joule effect). Knowing that the great majority of foodstuffs composition is rich in water, salts and organic acids, application of OH is straightforward.

The very first application of OH occurred in 1920 through electric pasteurization of milk that became known as 'electro-pure process of treating milk' [2]. Coincidently, or not, OH is now being strongly associated with many aspects of dairy processing, which include safety, quality and development of novel and healthy dairy products [3•,4,5] that can met the demands of a new and more active consumer.

This 'new' consumer behavior is actually shifting the paradigm of food processing that is now much more focused in developing new functional and healthier foods products. One outstanding example is the use of probiotics in dairy products that is increasingly being expanded in food industry [6••,7•]. OH has been bringing new insights toward important structural and functional aspects of important macromolecules and biological entities of food products. This review provides a comprehensive summary about the potential use of OH in the development of probiotic dairy products based on the latest advances of the effects of this technology in biomacromolecules and dairy ingredients.

Probiotic dairy foods

Probiotics are defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' [8]. Recent studies show that the intake of probiotic products promotes several health benefits, as they help to maintain a good balance of the intestinal flora, as well as, increase the resistance against the invasion of pathogens [9,10]. The *Lactobacillus* and *Bifidobacterium* are the most common probiotic genera; from the *Lactobacillus* species the strains more commonly used are *L. acidophilus*, *L. crispatus*, *L. amylovarus*, *L. gallinarum*, *L. gasseri*, *L. john-sonii*, *L. helveticus*, *L. delbrueckii* subsp. *bulgaricus*, *L. sali-varius* subsp. *salivarius*, *L. casei*, *L. paracasei* subsp. *paracasei*, *L. paracasei* subsp. *tolerans*, *L. plantarum*, *L. rhamnosus*, *L. fermentum*, *L. reuteri*; while from *Bifidobacterium spp.* are the *B. bifidum*, *B. longum*, *B. infanti*, *B. breve*, *B. adolescentis*, *B. animalis* [6••,11].

Dairy food products such as yogurts, dairy based drinks and fermented milks are among the top probiotic foods consumed in the world. However, food must contain an adequate number of probiotics (i.e. 10^8 to 10^9 cells per gram of products) when consumed to have a beneficial health effect [12,13]. In addition, probiotics must be able withstand the harsh environmental conditions of the human gastrointestinal tract. Thus, one of the major challenges in the industry is to maintain these characteristics, because several factors during processing and storage affect the viability of probiotics.

The use of new technologies in food processing, such as OH has been prominent in the development of new products, and is often reported as a promising technology for the dairy industry [14•]. However, information about the non-thermal effects of OH on the development of fermented dairy products, as well as, the effects on the metabolic activity of probiotics and the viability of the crops is still scarce, thus being a promising area for studies for future research in the dairy sector.

OH is being triggering research and discussion about the effects of electrical variables (such as frequency and electric fields) on biological cells and bio-macromolecules (i.e. proteins). Studies about effects of OH on microorganisms (either vegetative cells or spores) and enzymes have been thoroughly reviewed [15•,16,17••]. Overall, it is unanimous that together with a promise of a direct, volumetric and fast heating, which *per se* bring significant changes on quality and functional aspects of proteinaceous dairy products, OH brings also possibility of modulate activation and inactivation of species of biological origin — i.e. microorganism and enzymes.

Fermentation of dairy products with probiotic microorganisms often involves production of enzymes. Likewise as case of microorganisms, it is generally recognized that OH can accelerate inactivation of certain food enzymes, especially at high temperatures. This mechanism of inactivation under alternating electric fields is not clear but is particularly evident in enzymes containing metallic prosthetic groups, such as lipoxygenase and polyphenoloxidase [18]. Equally interesting has been some latest findings about the effects of controlling frequency and electric field intensity on pectin methylesterase activity, for example. It was evidenced that treatments at temperatures ranging from 65°C to 90°C, using low frequencies (<60 Hz) and high electric fields intensity (>10 V/cm) may enhance enzyme inactivation, while these additional effects are considered to be negligible at the opposite conditions (i.e. high electric frequency and low electric field) [19,20]. Latter authors also pointed out that depending on the treatment temperature, enzyme activation can also happen under OH eventually due to changes in electrophoretic motion and better interaction between enzymes and substrate. These novel insights turn it clear that OH operational variables can be used to tune enzymatic activity, which can bring a crucial advantage over several food biotechnological processes, such as fermentation and enzymatic hydrolysis.

Several authors have already evidenced enhanced inactivation of microorganisms when compared with conventional pasteurization protocols [21–25]. This additional inactivation can be a result of thermal permeabilization of cell membrane due to Joule heating effect (internal heat), resulting in the reduction of the thermal resistance (lower D values). This effect allowed to perform treatments with lower thermal intensity without affecting food safety (same F_0 values) [15•]. But electro-permeabilization of cell membranes — commonly known as electroporation — should not be overlooked once enhanced inactivation under moderate electric fields (MEF) it is also reported to occur at sub-lethal temperatures [4,26].

In sub-lethal conditions, the electric field can promote an increase in cellular permeability and the diffusion of ions and molecules through the cell membrane, resulting in a better absorption of nutrients and, consequently, a faster cellular growth [27•]. Thus, at sub-lethal conditions, OH can affect the metabolic processes, being an interesting alternative in biotechnological processes. Despite the few studies relating the effect of the electric field on the metabolism of probiotics in fermentative processes, the published results demonstrate promising prospects for the development of novel probiotic products through OH.

Recent research reports that the effect of pulsed electric field (PEF) on biotechnological processes results in improvements in kinetic parameters of growth and cellular metabolism of probiotics [28–31]. However, in relation to OH, few studies have been found in the literature. Cho, Yousef [32] reported for the first time an activation effect on metabolic response *Lactobacillus acidophilus* OSU 133, an important probiotic microorganism, under ohmic heating fermentation applying MEF. Table 1 shows the results of the MEF effect under different process conditions (electric field and frequencies) on the kinetic parameters of growth and bacteriocin activity of *Lactobacillus acidophilus* OSU 133.

The results presented in Table 1 show that bacteriocin activity and growth parameters during the stages of growth (lag, exponential or stationary) are affected by the electric field strength and of the frequency of MEF treatment applied during fermentation. MEF applied in the initial stages resulted in increased bacteriocin activity, due to the stress caused by the electric field [33]. According to Serrazanetti, Guerzoni [36], stresses in the

Table 1

Effects of ohmic heating and its electrical variables on growth kinetics of *Lactobacillus acidophilus*.

OH conditions	OH parameters (electric field and frequency)	Temperature profiles	Main results	Reference
Conventional	*E* = 0 V/cm	30°C/50 h 35°C/25 h 40°C/[a]	– OH resulted in the reduction of the lag time at 30°C compared to the conventional one, about 94% and 76% for the MEF at 1.1 and 2.9 V/cm respectively. – No significant differences ($p > 0.05$) were observed in the other kinetic parameters (minimum generation time (h), and maximum growth ($\log_{10}$ CFU/mL)). – OH resulted in lower bacteriocin production (Lacidin A) and lower activity. – The fermentation monitoring can be done through the current at a constant voltage.	[32]
MEF	*E* = 1.1 or 2.9 V/cm — 60 Hz	30°C/50 h 35°C/25 h 40°C/[a]		
Conventional	*E* = 0 V/cm	30°C/40 h 37°C/24 h	– OH did not have a significant effect ($p > 0.05$) on the microbial growth kinetics (lag time, maximum specific growth rate, biomass production) and pH change under the treatments conditions. – OH in combination with conventional heating resulted in a increase of bacteriocin (Lacidin A) activity, being the maximum activity observed for Early MEF at 30°C.	[33]
MEF	*E* = 1 V/cm — 60 Hz	30°C/24 h		
Early MEF	*E* = 1 V/cm — 60 Hz for 5 min; followed by *E* = 0 for 35 h	30°C/24 h		
Discrete MEF	*E* = 1 V/cm — 60 Hz for 2 min on/off	30°C/24 h 37°C/40 h		
Conventional	*E* = 0 V/cm	30°C/40 h 37°C/24 h	– OH at 30°C at all frequencies (exception for 60 Hz) for MEF and Early MEF processes, result a shorter lag phase ($p < 0.05$) than conventional fermentation. – Compared to conventional, lower frequency (45 Hz) resulted in higher reductions, about 60 and 65% in the MEF and Early MEF respectively. While at high frequencies (90 Hz) this reduction was 38% for MEF and 37% for Early MEF. – No significant difference ($p > 0.05$) was observed in the other kinetic growth parameters – Maximum activity of bacteriocin was observed in Early MEF at 60 Hz, followed by conventional fermentation, both at 30°C.	[34]
MEF	*E* = 1 V/cm — (45, 60 and 90 Hz)	30°C/40 h		
Early MEF	*E* = 1 V/cm — (45, 60 and 90 Hz) for 5 min; followed by *E* = 0 V/cm for 35 h	30°C/40 h		
Conventional	*E* = 0 V/cm	30°C/20 h	– Early MEF process at 45 Hz presented the highest cell permeabilization, followed by Early MEF at 60 Hz. – Greater susceptibility to permeabilization was observed in the cells during the lag phase, followed by the cells in the exponential phase. – The effect of electroporation on cells in the stationary phase was not observed.	[35•]
SD–MEF[b]	*E* = 2 V/cm — (45, 60, 1000, 10000 Hz)	30°C/2 h		
MD–MEF[b]	*E* = 2 V/cm — 45 Hz	30°C/6 h		
LD–MEF[b]	*E* = 2 V/cm — 45 Hz	30°C/20 h		

[a] No access to data.
[b] SD–MEF: short duration of MEF applied continuously during the course of 2 h (Lag phase); MD–MEF: mild duration of MEF applied continuously during the course of 6 h (Exponential phase); LD–MEF: long duration of MEF applied continuously during the course of 20 h (Stationary phase).

microorganism can affect metabolic processes, resulting in improvements in bioprocesses and in bioproducts.

In relation to growth parameters, the MEF applied at low frequencies (45 Hz) and in the initial stages resulted in the reduction of lag phase time, due to the temporary increase in cellular permeability. According to the authors, the application of electric fields may have promoted physical disturbances on cell walls helping to dislodge adhered polar antimicrobials or other molecules, thus improving absorption of nutrients during lag phase of fermentation. Temporary permeability has been proven by Loghavi, Sastry [35•] using dye staining techniques in the cells, where intact and permeabilized cells could be identified through microscopic observations.

Thus, OH applied and its MEF may be potentially useful in biotechnological processes, being an alternative technology in the development of fermented probiotic products. However, studies involving other probiotic strains under different parameters (electric field, frequency) should be performed, aiming at the development and optimization of the process conditions. Furthermore, the effect of OH on the viability and metabolic activity of probiotics are important characteristics to be evaluated, as well as studies in food matrices instead of culture media, bringing a more realistic scenario.

The metabolism and viability of probiotics are affected by different factors, such as physical-chemical characteristics of foods (such as pH, titratable acidity), process parameters (temperature profiles, cooling rate, storage environment). Thus, the understanding of the effect of OH on the properties of dairy products, as in the conformational structure, aggregation, unfolding and denaturation of whey proteins, bioactive compounds (like bioactive peptides), fatty acids profiles and in physical properties (e.g. rheological parameters), presents as one of the great challenges for the elaboration of probiotic dairy products [37,38•,39]. However, no information was found in this search field.

Whey protein

The use of whey, a residue of the cheese industry, shows great interest in the elaboration of functional beverages in recent years. Besides that, whey ingredients despite their recognized biological value (e.g. anti-tumoral activity, essential amino acids and immune system modulation [40••], and technological properties (e.g. water holding capacity and gelation and emulsifying abilities) [41], they can also improve probiotic viability, rheological and sensorial characteristics of probiotic dairy products, even can also have positive effects on modulation of gut microbiota [42].

In opposition to casein fraction, whey proteins are very susceptible to thermal denaturation under pasteurization conditions. Whey proteins, for example can have positive effects on the viability of probiotics strains during processing and refrigerated storage, due the higher buffering capacity that delays the post-acidification during storage. In addition, the heat treatment may lead to the release of sulfur amino acids resulting in the reduction of the redox potential aiding in the fermentation process and in the survival of the probiotic [7•,43].

In reason of this, during the last few years, the effects of OH on whey proteins have been comprehensively investigated (see Table 2). Whey protein nano and microstructures, as well as gel-systems can be tailored with application of OH using different thermal loads and varying electric field intensities [44]. The presence of MEF seems to disturb the conventional pathways of protein denaturation aggregation by changing not only the size but also the morphologies of produced aggregates [38•]. This has shown to be mostly relevant in concentrated protein systems, where viscoelastic behavior of protein solutions is changed under the influence of electric fields [45]. By controlling physical-chemical environment (i.e. pH, ionic strength, type of protein, protein concentration and electrical conductivity), in combination with certain OH operational conditions (i.e. electrical field intensity, temperature and time of treatment) it is possible to design protein gel networks for incorporation of functional compounds or nutrients [46]. OH can be used as pre-treatment tool to 'functionalize' globular whey proteins, for development of hydrogels or cold gel-like emulsions [47,48]. Latest reviews about the state-of-art of OH highlight the importance of evaluating the effects of this technology on allergenicity aspects (such as allergy sensitizing or eliciting potential) of food proteins [3•,15•]. Whey proteins are considered one the major cow's milk

Table 2

Effects of ohmic heating and its electrical variables on denaturation and gelation of whey proteins.

Whey proteins	OH parameters (electric field and frequency)	Heating conditions	Main results over conventional heating	Reference
β-Lactoglobulin A	4–8 V/cm at 25 kHz	0.5 min at 85°C	– Higher contents of native protein detected by RP-HPLC	[45]
β-Lactoglobulin B	4–8 V/cm at 25 kHz	0.5 min at 85°C	– No significant differences	[45]
α-Lactalbumin	4–8 V/cm at 25 kHz	0.5 min at 85°C	– Higher contents of native protein detected by RP-HPLC	[45]
Whey protein isolate	4–8 V/cm at 25 kHz	30 mn at 85°C	– Gel with weaker viscoelastic structure (decreases in both G′ and G″) – Progel state	[45]
	4–8 V/cm at 50 Hz	30 s to 30 min/75 to 90°C	– Less protein denaturation particularly at fast come-up times – >30% of soluble protein – Denaturation kinetically traduced by lower values of reaction order (*n*) and rate constant (*k*)	[44]
	6–12 V/cm at 25 kHz	5 min at 90°C	– Protein networks characterized by the presence of small fibrils – Ability to reduce protein aggregation levels	[38•]
	10 V/cm at 25 kHz	10 min at 90°C	– Protein hydrogels with ability to incorporate higher amounts of Fe^{2+} through a cold gelation process	[48]
Lactoferrin	6–20 V/cm at 25 kHz	30 min at 90°C	– Development of cold gel-like emulsions with distinct gel structure	[47]

allergens [49]. OH either by reducing thermal load, changing whey protein aggregation pathways or altering interactions with other molecules (e.g. Maillard reactions), may be influencing allergenicity responses of these proteins, and this needs to be addressed.

Besides the effects on milk characteristics, the addition of fruit juices and pulps, a technique widely used for the elaboration of whey beverages, can affect the viability and metabolic mechanisms of probiotic strains, due to the modification of the intrinsic characteristics, such as acidity and the presence of antimicrobials [7•]. Another important factor in the elaboration of probiotic products by OH is due to corrosion of electrodes, which may result in the migration of metal ions to the food and may adversely affect the microbial metabolism. Thus, corrosion is a major concern in the design and development of biotechnological processes in OH. The use of inert electrodes, such as stainless steel and titanium, can prevent such a problem [50].

Perspectives

Studies involving the use of OH under sub-lethal conditions in growth rates and bacteriocin activity are scarce, however previous results demonstrate beneficial effects on fermentation, suggesting the need of determining the best OH parameters. Future studies should be performed to observe the respective effects on the metabolism of different probiotics and their viability, aiming to assist in the development of probiotic dairy products by this emerging technology.

Recent findings also point out that whey protein networks can be tailored by OH treatments and encompass an interesting synergetic potential to be used as a matrix for the incorporation of probiotic entities. Whey systems, with all biological and nutritional benefits associated with it, can be designed as vehicles to transport, protect and deliver probiotic during gastrointestinal digestion. Nevertheless, more fundamental knowledge is still required to understand interactions between electric fields and whey protein structures

In this sense, OH can open news perspectives and strategies for development of fermented probiotic products that are worthy of further research. Although, some questions arises: can electric field be used to control or tune biological responses? What are the best combinations of electric field and frequency to be applied? Are the metabolism of different probiotics affected in the same way by the electric field and what is the effect on the viability of probiotics in the products? In fact, the interaction between alternating electric fields and microorganism is far from being understood.

Conflict of interest

None declared.

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