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Research Article

A literature review: Effects of ohmic heating on inactivation of specific bacterial spores in various matrixes

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ABSTRACT

Ohmic heating is among the alternative processing technologies in order to inactivate microorganisms in food. With respect to conventional thermal processes, ohmic heating offers advantages such as; reduced process time, uniform heating, increased nutrient retention and energy efficiency. Due to the synergistic effect of rapid heating accompanied by unlethal temperature and electric field, some ohmic heating studies report significant log reductions achieved for spores as well as vegetative cells of microbial species in various matrixes. This study covers a comprehensive review of the selected publications regarding fundamental principles of ohmic heating, limiting factors, spore structure and resistance, thermal activation mechanisms of spores, heat and electric field effects on the spore's structure and responses, parameters that influence microbial inactivation via ohmic heating. Based on our review, it is concluded that each research on ohmic heating ought to be evaluated as a specific case and to be assessed by multiple perspective approach. Evaluation to be made considering internal and external factors as well as their synergistic interactions. Regarding microbial inactivation, unpredicted results may also be obtained as a consequence of additional chemical effects originated from the product content. In some cases, straightforward comparison data of relevant ohmic heating work may be misleading. Therefore, this review deliberately doesn't include comparison tables. Instead, each remark is supported with a specific relevant literature work as far as possible. It is suggested that more research on the application of ohmic heating on various matrixes to be done so that microbial inactivation via ohmic heating can be utilized in diverse industrial applications as well as food products.

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INTRODUCTION

Ohmic heating, also referred to as Joule heating is one of the alternative thermal processes in order to inactivate microorganisms generally in food products by passing alternative current within food which acts as a conductive medium. With respect to conventional thermal process technologies; rapid and uniform heating, less energy consumption, higher nutrient retention is obtained by ohmic heating. On the other side, some studies reveal that ohmic heating offers

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synergistic effect of rapid heating which is accompanied by both unlethal temperature and electric field, leading to inactivation of specific bacterial spores as well as their vegetative cells. Due to multiple layered protective structure, spores of some pathogenic species are highly resistant to a wide range of stress conditions such as; heat, chemical agents, ultraviolet (UV) radiation and pressure which would normally inactivate vegetative cells. Spores which are metabolically inactive with anhydrous structure can survive even for centuries. A spore which is in the dormant state, monitors its environment and can rapidly transform into vegetative cell once it comes into contact with nutrients in soil, food or human body, leading to food poisoning. Nutrient retention and process economy are factors limiting the use of some conventional processing technologies such as high temperature and long duration dry heat applications. Therefore, novel technologies which enable inactivation of both vegetative cells and spores in various matrixes with increased nutrient retention, energy efficiency and avoid damage to product components are interesting research subjects. In this study, a literature review was conducted regarding the basic principles of ohmic heating, bacterial spore's structure and specific stress responses, thermal activation mechanism of spores, synergistic effects of ohmic heating constituents on spore's structure, responses and inactivation mechanism, factors that influence microbial inactivation during ohmic heating. This study has gathered the related key information in the literature in one source for the readers and aims to contribute to new research studies by defining critical parameters and their interactions to focus on.

FUNDAMENTAL PRINCIPLES OF OHMIC HEATING

The basic principle of ohmic heating is the use of Ohm's Law and the heat energy produced by this method is based on Joule's Law. Ohm's Law, which is given in Equation 1 defines the relationship between voltage, current and resistance in an electrical circuit. In Equation 1; I is electrical current (A), V is voltage(V) and R is resistance (ohm).

$$I = \frac{V}{R} \quad (1)$$

Ohmic heating is based on the principle by passing alternating current between the electrodes. The product to be heated has conductive property and is directly in contact with the electrodes, serving as an electrical resistor. Required electric current from the supply to the product is transmitted by the use of the properly designed metal electrodes. According to Joule's law of heating, which is given in Equation 2, electrical energy is converted into heat energy when an electrical current is allowed to pass through a material with resistance [1]. In Equation 2; I is electrical current (A), Q is the amount of heat (Joule), R is the amount of electrical resistance in the conductor(ohm), t is the time (s).

$$Q = I^2RT \quad (2)$$

Ohmic heating which is generally acknowledged as a food processing technology, in fact can be applied to a various types of product that fulfill the required parameters for this operation. Conventional conductive heating systems utilizes contact with hot surface heat exchangers, some practices leading to overheating related problems as well as unstable thermal performances due to deposit formation on the heating medium. Owing to volumetric and direct resistance heating properties, ohmic heating enables rapid and homogenous heating with efficient performance. There are different ohmic heating technologies depending on technical parameters such as; batch or continuous process, tubular, plane or jet flow, electrode material and dimension. On the other side; electric supply parameters include; voltage gradient (V/cm); frequency (Hz), current density (A/m^2) and maximum power (kW). Product to be heated is generally a homogenous liquid, solid-liquid suspension or heterogenous foods such as fruits, meat mixtures, tomato particles [2]. A schematic of a simple laboratory scale ohmic heating set up is shown in Figure 1.

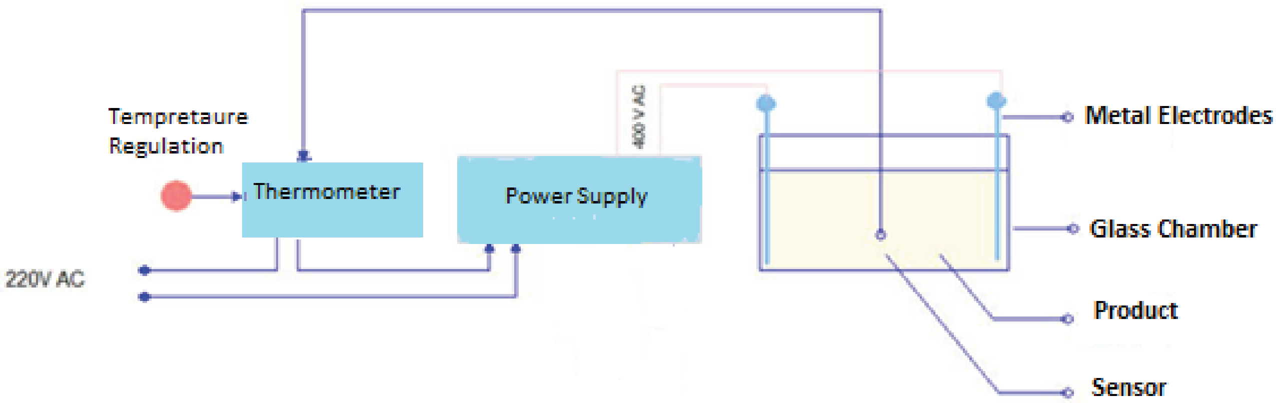

Figure 1. Simple laboratory scale ohmic heating set up.

A typical power supply is a low frequency (50-60 Hz) alternating current (AC) power coming from public utility. Voltage applied to the electrodes can be controlled by the use of variable transformer. High frequency alternating current can also be utilized in ohmic heating, by connecting electrodes to an insulated-gate bipolar transistor (IGBT) power supply. In terms of security measures, use of an isolation transformer is essential to prevent the operator to be exposed to possible shock hazards. By the use of voltmeter and ampermeter which are connected in accordance with the circuit, voltage applied across the product material and the current passing through it can be measured. Temperature of the product is measured by thermocouple which is connected to the thermometer device. Thermometer is connected in series with the circuit, having relay contact thus accordingly allowing either to close or open the relay, therefore connecting or disconnecting the circuit. Once the product reaches the target maximum temperature, signal produced by the thermocouple reaches the thermometer which opens the relay and the power supply unit is de-energized. Signals received from thermocouple, voltage and current transducers can be sent to a data logger to be recorded. Power supply output should be connected to an oscilloscope to monitor the applied and actual waveform and width. Materials with electrical conductivities in the range of 0.01-10 S/m are accepted as suitable for efficient ohmic heating process [3]. According to some studies related to electrochemical behavior of specific electrode materials, stainless steel has been found to be the most active whereas platinized- titanium to be relatively inert. Necessary precaution should be taken with the selection of proper electrode material and operation frequency in order to prevent undesirable electrochemical reactions [4]. Different design, configuration and operating types are possible for the ohmic heating systems. Batch operating systems will be the simplest type of the ohmic heaters. For instance; a rectangular-trough design is the basic configuration of a laboratory-scale batch type ohmic heating application. Appropriately sized electrodes mounted at the end of the heating container, to be supplied with power to heat the material in contact with. Heating container needs to be insulated with proper material and insulation thickness should be adequate in order to minimize heat and evaporation losses [5]. Another example for batch type ohmic heating system has been prepared as a glass T-tube. In this system, electrodes are mounted at the ends of the tube whereas thermocouple will be mounted at the top to monitor the temperature of the product. System offers to obtain sterilization temperatures for the product in case the tube cell is pressurized accordingly. A glass tube with four openings can be used as an example of a continuous ohmic heating system. Properly shaped cylindrical electrodes to be mounted at two ends of the tube with insulating spacers whereas the other two ends to be connected to insulating piping which allows to continuously fill and discharge the material [6].

STRUCTURE OF THE BACILLUS SEVERAL SPECIES (SPP) SPORES

Rod shaped gram positive bacteria species such as; *Bacillus subtilis (B.subtilis), B.amyloliquefaciens, B. cereus, B.licheniformis, B. pumilus,* and *B. thuringiensis* are pathogenic for humans and naturally be found in the structure of soil and plants. *B.cereus* and *B.anthrax* species are indicator bacteria for spore forming pathogens [7]. *B.cereus* contamination leads to intestinal or nonintestinal pathogenic cases which is closely related with the production of tissue-destructive exoenzymes [8]. Basic structure of a spore is shown in Figure 2.

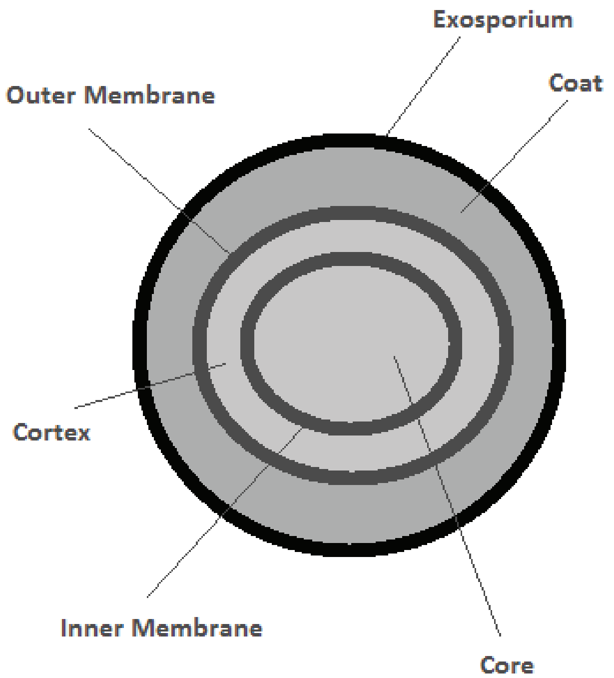

Figure 2. Spore structure.

Exosporium Layer

Some species such as; *Bacillus anthracis, Bacillus cereus, Bacillus megaterium, Clostridium sporogenes* and *Clostridium difficile* have an outer surface layer which is called the exosporium [9]. Typically, exosporium is a loosely placed, balloon-like and multiple layered structure. Exosporium surrounds all the spore including coat, however is not attached to spore or coat region. In fact, it is supposed that there is a gap between the spore and exosporium and it is not certain whether there is a material in this gap or not. Exosporium structure is known to be consist of protein and lipid, also including exosporium-specific glycoproteins [10]. It is foreseen that, exosporium of some pathogenic species as *B.cereus*, has the function of establishing boundary between the spore and the host cell. Besides, some pathogenic species are able to adhere to surfaces such as stainless steel and polymer via exosporium. Adhesion strength is related with the size and strong hydrophobicity of the exosporium [11].

Spore Coat

Due to multi-layered structure, the spore coat is able to protect bacterial genome against stress conditions [12]. The basic components of the coat layers constitute approximately 30% of the total protein in the spore structure [13]. Fluorescence microscopy images supported by high resolution image analysis reveal that spore coat consists of at least 4 separate layers, as shown in Figure 3 [14].

Spore coat which consists mainly of protein and glycoprotein, is a molecular sieve which allows the small molecules such as specific germination agents to pass through whereas the passage of large molecules such as lysosomes is not allowed [15]. According to some research on the dormant spore structure, the location of the germination specific lytic enzymes which are responsible for the degradation of spore cortex has still not been clarified yet [16]. Nevertheless, another study indicates that the coat contains some enzymes that has a direct role in the germination process including cortex-lytic enzymes. The spore coat provides significant protection against both chemical agents and external lytic enzymes that can degrade the spore cortex [17]. The coat is reported to promote the spore's moist heat resistance [18]. A study focusing on the *B.subtilis* spore's resistance to hydrogen peroxide clarifies that some proteins that are found in the spore coat are likely react with and detoxify chemical agents.

Cortex

Spore cortex has a thick peptidoglycan, loosely cross linked and electronegative structure. The cortex surrounds the nucleus and protects it from the effects of organic solvents [19]. Spore inner membrane acts as a molecular sieve, not allowing small hydrophilic molecules to enter into nucleus. A study on *Bacillus spp* spores indicates that, peptidoglycan cortex structure protects the spore inner membrane, thus also protecting nucleus indirectly.

Cortex matrix which contains peptidoglycan, glycopeptide and calcium ion (Ca^{2+}), ensure spore's heat and UV resistance [20]. Spore cortex plays an important role for maintaining spore's dormant state, core dehydration and spore resistance. Vegetative cell also has peptidoglycan, nonetheless spore cortex peptidoglycan has a modified structure. The amount of peptidoglycan in the spore structure has significant effect on the spore resistance. One research investigating the relationship between peptidoglycan amount and spore resistance deliberately reduced the peptidoglycan accumulation during spore formation. As a result, obtained spore displayed increased hydration along with decreased resistance to heat and organic chemicals [21]. Loosely cross linked structure of the cortex is associated with the peptidoglycan layers to comply with spore core's volume changes which occur during humidity change periods in spore's natural environment. Due to flexible structure of cortex, spore is able to retain core dehydration and dormancy during such periods [22].

Outer Membrane

A study concerning *B.cereus* spores indicates that the outer membrane can be distinguished during the early stages of the spore formation [23].

According to some publishing, it is suggested that outer membrane to have a role only during spore formation [24]. The findings regarding permeability of the outer membrane are controversial. In one study, the authors concluded that the outer membrane was quite permeable even to large molecules [25]. Later, the authors changed their view and indicated that the outer membrane to be defined as the main permeability barrier of the spore [26]. Another study concerning *B.subtilis* and *B.cereus* spores revealed that the outer membrane works as a barrier to the diffusion of large ions [27]. Thus, the permeability of outer membrane of the bacterial spores may be an interesting topic for future research work.

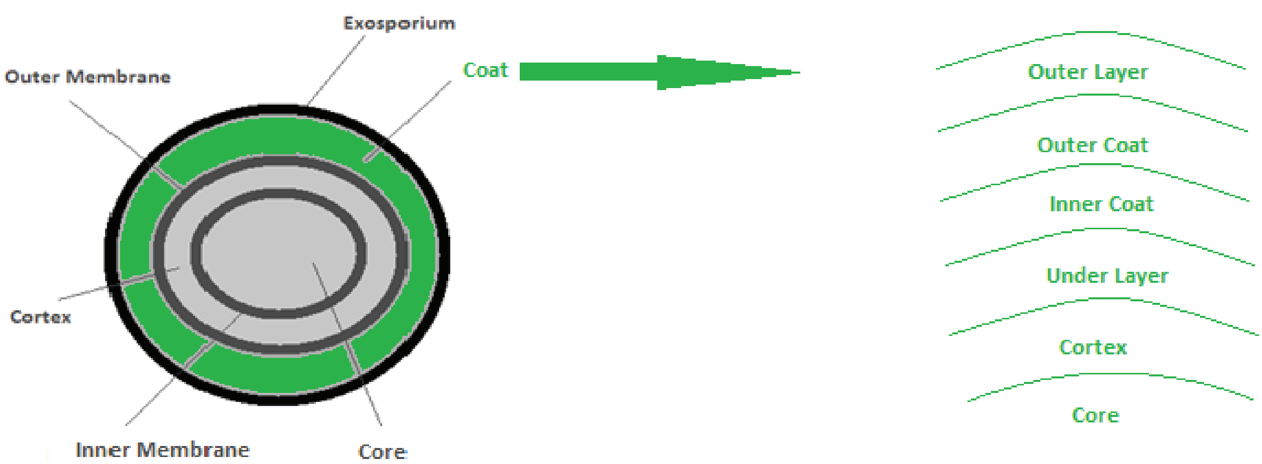

Figure 3. Spore coat.

Inner Membrane (Cytoplasmic Membrane)

Inner membrane or cytoplasmic membrane has a sensitive, semi-permeable structure. It has an important role to protect the core against chemical agents [28]. The inner membrane has extremely low permeability to some small molecules including water [29]. Similar to vegetative cell's cytoplasmic membrane, spore inner membrane has a lipid structure. One study indicates that, extremely low permeability of the inner membrane is associated with the fluidity of the lipids on the spore inner membrane [30]. Possible damage to the inner membrane is of critical importance to the spore. The cytoplasmic membrane can be damaged due to membrane-active agents such as cationic biocides and physical applications such as high temperature and freezing processes [31]. Inner membrane is also known to host most of the proteins which take part in the spore germination [32]. Inner membrane has channels which are made up of proteins. During spore germination, germinant receptors respond to germinant signals. As a consequence, induced germinant receptors transmit a signal to these channels. The channels respond to this signal and the cations from the spore core are released [33].

Spore Core

Spore core has an unique structure which mainly includes ribosomes, divalent cations, inert enzymes as well as small acid soluble proteins (SASP's)[32]. In collaboration with the other constituents of the spore structure, the core substantially contributes to spore's extreme resistance to diversified stress factors such as heat and chemical agents [34]. Spore's deoxyribonucleic acid (DNA) is saturated with SASP's which has an important role to protect DNA in dormant spores against heat, UV and enzymatic degradation [35]. Although, spore core and the nucleus of vegetative cell have functional similarities, the spore core content which includes SASP's, dipicolinic acid (DPA), calcium (Ca), magnesium (Mg) and manganese (Mn) at high concentrations differs from the nucleus of the vegetative cell.

Owing to this specific content, spores are able to maintain strong resistance to heat and UV. The spore core has high level of DPA which is able to bind divalent cations, mainly Ca^{2+} This chelate formation leads to dehydration and mineralization of the core, thus ensuring high heat resistance [36].

MATRIX, THERMAL ACTIVATION, HEAT AND ELECTRIC FIELD EFFECTS ON THE SPORE'S STRUCTURE AND RESPONSES

Matrix can be defined as an environment that ensure shelter to bacterial spores. Spores can exist in diversified matrixes. Various food to be an example of complex matrix structures for spores. On the other side, spores can be suspended in simple structures such as clay mineral and bentonite which is a solid matrix. Regarding our experience on ohmic heating, biogas digestate is another example of a complex matrix structure for *Bacillus cereus* and *Clostridium spp* spores. Matrix structure with its chemical, nutritional, physical and physicochemical properties, all together with their synergistic interactions, have significant impact on the microbial inactivation success of the ohmic heating. Heat treatment at sublethal temperatures can activate the dormant spores and trigger their transition to the vegetative cell form. The thermal resistance of the activated spores decreases, therefore allowing inactivation at lower temperatures than their dormant forms [37]. Some literature study covering germination of *Bacillus spp* spores in various matrixes indicates that spores are generally activated by heating at 70°C for an average of 15 minutes before being exposure to amino acid and nucleoside derived germination agents such as l-alanine, inosine etc [38]. Dormant spores are metabolically inactive. Unless they are activated, dormant spores can't start germination even if they are exposed to triggering agents. During activation, spore undergoes a reversible transformation process while retaining its DPA content. Some recent literature research indicates that activation process mainly includes changes in the configurations of macro molecules rather than metabolic events. On the other side, the results of one study indicates that, thermal activation activates enzymes in the dormant spores, triggering changes in the morphology and permeability as well as increasing the rate of germination [39]. A study on *B.subtilis* spores suggests that as a result of thermal activation of the spore suspension at 60°C-15 minutes, the activity of the protease enzyme is increased. Another study predicts that germination enzymes which are attached to the nucleus with weak electrostatic bonds, are released during activation process [40]. A study on *B.cereus* spores declares that release of DPA which is observed during thermal activation could occur due to the change in permeability of the spore coat and break down of the chemical bonds between DPA and enzymes in the spore [41].

Activation kinetics have been found to be similar with the melting curves of the macromolecules which are present in the spore's structure. In this respect, physicochemical effects are thought to be dominant during the activation process. General view indicates that transition from dormant to active state includes changes in the tertiary structure of some macromolecules. Besides, a change occurs in the tertiary structure of a protein which is responsible for the dormancy of the spore, this can be interpreted as the reversible denaturation of the protein. According to a hypothesis, cystine rich spore coat protein sustains the dormancy of the spore. This macromolecule is known to exist in a sulfur-sulfur bond structure. Reversible reduction of these bonds via activation by heat, reducing agents, low pH etc is expected to result either in a change in the permeability of the spore structure that is responsible of the dormancy or unblocking of the enzyme system. Spore structure before and after activation is shown in Figure 4.

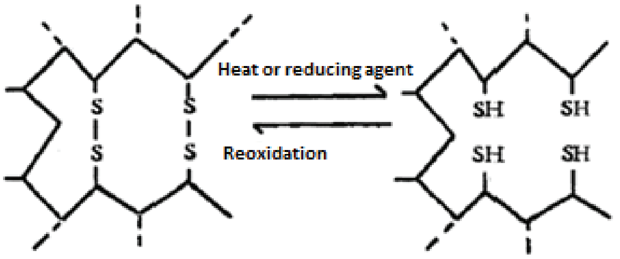

Figure 4. Spore structure before and after activation.

Moist heat application can be an effective way for obtaining spore activation. Such application processes can cause rupture in the spore structure, promoting water to reach the critical parts of the spore, thus providing some hydration. Temperature and pH are among the important factors that determine the success of the activation by moist heat application. Studies focusing on *B.cereus* spores indicate that activation of the spores regresses in the low pH environment [42]. In case heavy metals such as iron (Fe) and copper (Cu) are present in the medium, activation of B.cereus spores have been reported to be inhibited [43]. An ohmic heating study has been conducted on the spores of highly heat resistant *B.cereus* strain which have been found in a fermented soybean product. *B.cereus* spores in such a viscous matrix is assumed to be protected against heat, thus being highly heat resistant. Ohmic heating application with 26.7 V/cm electric field, 25 kHz frequency and 105°C- 1 minute process parameters has ensured *B.cereus* count reduction which meet Korean National Standards. In comparison with the conventional heating practice which has been applied under the same conditions, ohmic heating is reported to obtain remarkably higher spore reduction.

Trials revealed that inactivation success would improve with increasing temperature and application time. The study also provides the reader with scanning electron micrographs images of the treated and untreated spores. Images demonstrate that the initial structure of the spores to be smooth whereas the treated ones to be shrunken with disrupted coat [44]. A study investigated the effects of ohmic and conventional heating on *B.subtilis* spores in a sodium chloride (NaCl) solution. Treatments were performed at 88-99 °C through a single or double stage treatment which utilized tyndallization effect. In respect to the decimal reduction values and obtained survival curves, ohmic heating enabled considerably higher inactivation than conventional heating. Depending on the results, the authors concluded that ohmic heating ensures the inactivation of spores as a consequence of the thermal effect mainly but electric field also has an synergistic effect on the inactivation [45]. Another research which has been conducted on the inactivation kinetics of *Alicyclobacillus acidoterrestris* spores in orange juice, provides results regarding comparison between ohmic and conventional heating of the sample with corresponding temperature histories. Effects of voltage gradient, holding temperature and time on the ohmic heating process has been studied. For identical temperature data, the possible differences from conventional heating is to be related with the additional effect of electric field. The study uses inactivation kinetics constants D and z to evaluate the resistance of the spores in orange juice during ohmic and conventional heating. D values for ohmic heating were found to be considerably lower than conventional heating whereas z values for the two treatments were not significantly different for *A. acidoterrestris* DSM 3922 spores in orange juice. In this study, for *A. acidoterrestris* DSM 3922 spores in orange juice, ohmic heating at 30V/cm, 90°C-30 min is reported to assure 5 log reduction in viable spores. On the other side, at the same voltage gradient and temperature, conventional heating is reported to provide only 3.5 log reduction. In order to meet the relevant regulatory criteria, treatments need to ensure 5 log reduction of any bacteria related with foodborne diseases [46]. A study which is performed on the inactivation of *Geobacillus stearothermophilus* spores (ATCC 7953) in inoculated tomato soup samples, compared the results of ohmic heating and conventional heating that are performed under the same temperature ranges and application times.10 kHz ohmic heating ensured higher inactivation at all temperatures compared to conventional heating. As the processing temperature rised, 60 Hz ohmic heating enabled increased inactivation with respect to conventional heating. For 10 kHz ohmic treatment at 121°C, D value is 0.88 min whereas for 60 Hz ohmic heating 1.17 min. and for 60 Hz conventional heating 2.53 min. At 121°C application,10 kHz frequency resulted in apparently low D values. At 125°C application, D value is calculated as 0.64 min for conventional heating, whereas 0.43 min at 10 kHz,0.34 min at 60 Hz, suggesting that at this temperature ohmic heating enabled higher inactivation in comparison to conventional heating. At 130°C application, ohmic heating still ensured more inactivation than conventional heating while D values were not apparently different. Also considering the obtained z values for 60 Hz-10 kHz ohmic heating and conventional heating experiments, it can be concluded that ohmic heating is capable of expediting the inactivation of the thermophilic spores with respect to conventional heating. Limited information have been reported on the responses of thermophilic bacterial spores which are exposed to low frequency (50-60 Hz) electric field [47]. Dipicolinic acid (DPA) constitutes approximately 10-15% of the total dry weight of the dormant spores [48]. Ca-DPA chelate in bacterial spores are accepted to have significant importance on physical and chemical resistance of bacterial spores as well as core dehydration which promotes a decreased electrical conductivity of bacterial spores [49]. A study performed on the electrical conductivity of *B.cereus* spores reported significantly low electrical conductivities of dormant spores by dielectric measurements performed at 50 MHz [50]. It is assumed that during activation, germination and outgrowth phases, the mobile ions within the core and its surrounding coat to be substantially increased. Moderate heat activation of spores to be responsible for the reversible breakage of hydrogen and sulfide bonds in the tertiary structure of some spore proteins which is followed by the release of the related spore coat proteins and DPA [51]. A pulsed electric field study at moderate temperatures showed DPA release which might be expected for a polar molecule to respond to an electric field. On the other side, PEF has not been effective for spore inactivation [52]. Thus, the success of ohmic heating on spore inactivation can be explained with the synergistic effect of heat activation, oscillating electric fields and current. Ohmic heating causes heat activation of the spores to some degree which includes the oscillation and release of some ionic molecules such as Ca chelated DPA, components of denatured proteins from the core and coat as a consequence of the unlethal heat accompanied by electric current and fields. [53]. Released ionic molecules interact with the electric fields that occur during the ohmic heating. As a consequence, electrical conductivity of the spore is enhanced, also enabling the spore to be prone to non-thermal effects as well. Hereby, spore inactivation is considerably stimulated. Dipole components are known to respond to electric fields. However, the spore consists of complex molecular units and the respond mechanisms of these complex structures to electric fields and the effect of frequency are not fully identified yet [54]. From another perspective, at some point synergistic effect of heat and electric field may trigger some spore germination mechanisms which cause the spore to lose its dormant and resistant state structure, being susceptible to stress conditions. Ca^{2+} divalent cation originated from Ca-DPA leakage due to the synergistic effect of heat and electric field, may activate cortex hydrolysis in a similar manner as germination triggered by divalent cations. Besides, heat activation results in change in the tertiary structure of spore coat, may also lead to activation of cortex lytic enzymes some of which are assumed to be located on the spore coat. Regarding *B.subtilis* spores, cortex lytic enzyme cwlj is mainly activated by Ca-DPA either released from spore coat or added as a germination trigger agent. Other cortex lytic enzyme for *B.subtilis* spores is sleß which is activated when the cortex is deformed. Activated cwlj and sleß enzymes induce degradation of peptidoglycan structure of the cortex [47]. As the cortex peptidoglycan is hydrolysed enzymically, enough gap is created so that the spore core is able rehydrate to an extent along with the decrease in the moist heat resistance of the spore. Besides, Ca released from the core in the Ca-DPA chelate form leads to the cortex shrinkage, contributing to the expanding rehydrated core's pushing out effect on the cortex [55].A study has demonstrated that the peptidoglycan structure of the cortex competes with DPA to bind Ca^{2+}. Accordingly, in case CaDPA is available in the medium, the volume of the peptidoglycan is expected to be reduced as a result of the competition between DPA and the peptidoglycan. [56]. Thus, it can also be proposed that ohmic heating can trigger some germination related mechanisms, leading the resistant structure of the spore to be distorted, allowing the rehydration of the core. In our opinion, even the inactivation mechanisms of vegetative cells during ohmic heating still remains as a controversial subject in literature. Some review articles suggest that ohmic heating induced microbial inactivation is mainly due to thermal effects accompanied by electroporation process occurring in the living cells. In brief, if the critical electric field strength of the cell is exceeded, pores will be formed in the membrane of the bacterial cell. In contrary, owing to the dissimilar structure of the spore which is accompanied by rather stationary ions, electroporation effect is not expected to occur during the ohmic heating of the spores. A research on the Bacillus subtilis spores was performed to determine the spore structure constituent which interacts with the electric field during ohmic heating. Consequently, wild type spores and the isogenic mutants were exposed to identical conventional and ohmic heating applications. Each mutant type was deliberately arranged to be deficient of some components which were thought to play role in the spore's heat resistance. Those components are ; SASP's (small acid soluble proteins), the coat and the germination enzyme SleB. The experiments were based on the prediction that the mutant lacking the component which is estimated to protect the spore from the electric field will encounter increased inactivation. DPA release from the core was also monitored and evaluated for each trial. Besides, trials revealed that obtaining a minimum initial temperature range 70-100°C to be vital for ensuring spore inactivation due to electric fields. Ohmic heating and conventional heating were both found to have similar DPA release trend. Hence, it was concluded that ohmic heating doesn't have a special effect on the permeability of the inner membrane of the spore. The mutants lacking the spore coat encountered increased inactivation with respect to the wild type of the spores which suggested that the coat may provide mild protection against the electric field. Germination enzyme SleB had not been affected by ohmic heating, indicating that SleB was not affected directly by the applied electric fields. On the other side, the results comparing both ohmic and conventional heating of the wild type and SASP's deficient mutant spores indicate that SASP's could be the target of electric field for the wild type spores. Thus, the study implies that the main component interacting with the electric fields during ohmic heating to be the spore's core, most probably the SASP's -DNA complexes. The authors of the study estimates that inactivation effect on the spores occurs as a result of the combination of electric field and heat, electric field ensuring the related spore core constituents to become more sensitive to heat. Last but not least, it is declared that the inactivation effect on the spores is not based on the electroporation [57].

Another review declares that focus ought to be on the thermal effect rather than nonthermal effects of ohmic heating to inactivate pathogens. Based on our observations, nonthermal factors and their synergistic interactions with thermal effects are incontrovertible parts of microbial inactivation concept.

FACTORS THAT AFFECT BACTERIAL SPORES INACTIVATION BY OHMIC HEATING

Factors that affect microbial inactivation during ohmic heating can be classified as external and internal factors. Parameters such as; electric current value, electric field intensity, frequency, temperature and application time, heating rate are within the scope of external factors. Product composition, moisture content, physicochemical properties, matrix structure, electrical conductivity, ion, salt and fat content, sporulation medium, type, genetics and growth stages of microorganisms, pH and water activity are among the internal factors [47, 58,59]. An ohmic heating study on the inactivation of *B.cereus* spores in fermented soybean product expected the spores to be protected against thermal effects and heat transfer through conduction mechanism to be weak owing to the thick matrix structure of the product. Thus, *B.cereus* spores in the doenjang sauce is predicted to have high thermal resistance [60]. In another study comparing the thermal resistance of *B.cereus* spores in the buffer solution and chicken meat, it was determined that due to the thick matrix structure, the thermal resistance in chicken meat to be higher [43]. A study investigating the effect of water activity, it was reported that as the water activity of the environment in which *B.cereus* spores are located decreases, the thermal resistance of the spores increases.

This data confirms the hypothesis that inactivation of spores in solid food is more difficult than spores found in liquid food [61].

A research on the effect of slow and fast wet heating on the inactivation of *B.cereus* spores isolated from food samples was conducted. The results indicate that rapid heating has significantly higher inactivation success, emphasizing the importance of heating rate on the inactivation effect [62].

There are studies which confirm that the wet heat resistance of the spores to be affected by the sporulation medium. For instance; for the *Alicyclobacillus acidoterrestris* DSM 3922 species detected in fruit juices, it was determined that the spores obtained in mineral rich environment had high thermal resistance along with the highest DPA content [63].

An ohmic heating study performed on the raw milk discussed the correlation between the fat content of the milk and the electrical conductivity as well as the temperature increase rate. Additionally, potential preventive impact of the milk fat on the inactivation of microorganisms was also investigated. Electric current tends to bypass the insulating zones within a high electrical conductivity region. This phenomenon is also found to be observed during ohmic heating of the milk. Accordingly, fat globules in the milk content is expected to receive slower heating in comparison to its surrounding with high electrical conductivity. Consequently, pathogens found in this fat globules are predicted to be exposed to less thermal effect with respect to the remaining parts of the product [64]. During the conventional heating process, heat transfer occurs through conduction and convection mechanisms so that the fat content of the product doesn't have considerable impact on the microbial inactivation. In contrary, fat content of the product has a significant effect on the microbial inactivation capacity of the ohmic heating. Besides, it is reported that type of the microorganisms also determines the degree of this effect [65].

An ohmic heating application in food industry was performed on fruit juice and puree. Being evident at high temperatures and concentrations, fruit puree displayed lower electrical conductivity in comparison to the fruit juice. This situation is explained by the surplus of solids and particle size in the puree structure. For the apple juice application, as the amount of sugar increases, the electrical conductivity and heating rate are reported to decrease at all applied electric field values. Low electrolyte concentration in the product enables a nearly steady heating rate whereas the presence of high electrolyte concentration results in increased heating rate accompanied by an increased conductivity. During a ohmic heating application in meat processing, the heating rate of the product with salt and phosphate additives was reported to increase [66]. On the other side, the product having an inhomogeneous distribution of the ions, will be prone to encounter hot and cold spots formation during ohmic heating.

Unless homogenous distribution within the product is obtained, high electrolyte concentration will not absolutely ensure rapid and uniform heating [67]. The success of the microbial inactivation of the ohmic heating process is also related to the pH of the product. An ohmic heating study performed on the *E. coli* O157:H7, *Salmonella Typhimurium*, and *L. Monocytogenes* in orange juice samples, demonstrate that the inactivation rates of the pathogens increased in accordance with the decreasing pH values during the experiments [68]. Another study performed on *Bacillus licheniformis* spores in carrot juice extract suggests that pH influences microbial inactivation significantly at low operating temperatures. At high operating temperatures, thermal effect dominates the inactivation, thus leaving pH effect more recessive [69]. It is well known that the spores of the bacteria, also depending on their types, are significantly more resistant to stress conditions than the vegetative cells. In literature, several studies have demonstrated that the combined applications of more than one stress factors could enhance the inactivation of bacterial spores [70]. Prior to the ohmic heating, controlled acidification can be applied specifically depending on the type of the bacterial spore which is targeted to be destroyed. Being compatible with the nutritional product content as well as the bacterial flora, weak organic acids such as; citric acid, acetic acid, maleic acid etc can be utilized to lower the pH of the product to the optimum target level, also acting as an agent to inhibit the germination and outgrowth of some spores in specific matrixes [71]. Required acid consumption in order to decrease pH of the product to the target level will be the key parameter in terms of applicability and process economy. Acidification of food and agricultural products demand choice of organic acids rather than strong mineral acids. Usage of strong acids such as sulfuric acid will reduce acid consumption significantly with respect to weak organic acids. Among the weak organic acids, those having antimicrobial properties, availability and average costs should be preferred. Foremost, the buffering capacity of the product determines the acid consumption, in some circumstances regardless of the initial pH. Based on our studies on the acidification of biogas digestate originated from chicken manure, high buffering capacity of the product leads to excessive amount of acid comsumption to reduce the pH to the target value, thus making the acidification process infeasible. On the other side, appropriate acidification will provide benefits for retaining the ammonium content of the relevant product during heating applications. Products such as biogas digestate contain unionized ammonia in equilibrium with ammonium ion. In case the temperature and pH of the product rises, the equilibrium shifts to the advantage of free ammonia, leading the loss of nutrient content as well as occurence of ammonia emissions during the heating process. Acidification prior to the ohmic heating of such products can enable reaching higher temperatures without nutrient loss and ammonia emissions. However, the buffering capacity of the product to be calculated to foresee the required acid amount and process feasibility. Microbial species, genetics and stage of growth also have effect on the efficiency of microbial reduction during ohmic heating process. A ohmic heating research results on orange juice and tomato juice indicate that *E. coli* O157:H7 strain to be more resistant than *Salmonella Typhimurium* and *Listeria Monocytogenes* strains [72]. A study indicates that *Lactobacillus acidophilus* vegetative cells resistance to electric current and moderate electric fields differs in accordance with the growth phase of the cells [73]. On the other side, literature work on the ohmic heating of bacterial spores clearly indicates that the obtained results have been dedicated to a specific strain of a bacterial spore. Literature work is mainly investigating the effect of voltage gradient and frequency parameters of ohmic heating on the specific bacterial spores existing in matrixes. Typical power supply utilizes sinusoidal waveform AC power coming from the public utility. Based on our experience gained during the ohmic heating of biogas digestate, application of different waveforms and even pulses have resulted in either inactivation or even germination and outgrowth of some species of bacterial spores. Under identical application parameters, different species and strains may develop divergent responses. Germination and inactivation is an alternative method to deal with the bacterial spores. Germination triggering agents are deliberately added to the matrix to obtain the spores to germinate and lose their resistant structure. Mild stress treatments applied after this germination may result in complete inactivation of the spores. Germinant selection to be made depending on the bacterial spore type. Last but not least, germinant dosage and incubation period are critical spore type specific parameters for this strategy. Our studies on calcium chloride added ohmic heating of biogas digestate confirms that these parameters should be carefully monitored in order to prevent outgrowth of the germinated cells so that the number of vegetative cells are also ensured to be under control. Some research reveals that ohmic heating may provide additional chemical inactivation effect. This hypothesis is explained by the effect of free oxygen and hydrogen, chloride, hydroxyl and hydroperoxide radicals as well as metal ions which are formed during ohmic heating. An ohmic heating research on *E.coli* B vegetative cells in the phosphate buffer solution, showed that cells were inactivated primarily due to the toxicity of hydrogen peroxide formed in the solution [74]. During ohmic heating, some structures as free chloride may be formed in the environment, having a synergistic effect in terms of toxicity on the inactivation of microorganisms. However, the toxic structures are expected to vanish rapidly once the ohmic heating process is over [75]. Due to reacting and detoxifying proteins which are found on the coat, bacterial spores are known to be much more resistant to toxic substances like peroxides. However, bacterial spore which undergoes some transformations during ohmic heating, loses its rigid structure and may become more prone to the toxic effects of such toxic substances.

CONCLUSION

Owing to the multiple layered protective structure, spores of some pathogenic species are highly resistant to a wide range of stress conditions which could normally inactivate vegetative cells of microorganisms. In favorable conditions, the spores may germinate into vegetative cells which are capable of producing toxins, resulting in food spoilage or poisoning. Consequently, emerging technologies which ensure inactivation of both living cells and spores with energy efficiency accompanied by increased nutrient retention are interesting research topics. Ohmic heating is among the alternative thermal technologies to inactivate microorganisms generally in food products. Until recently, ohmic heating was acknowledged to inactivate microorganisms through the effect of rapid and uniform heat generation. Gradually, there are more evidence which suggest that non thermal inactivation effects may occur during ohmic heating. However, literature on the non-thermal effects on bacterial spores is still insufficient. In this study; basic principles of ohmic heating, structure of spores, synergistic effects of heat and electric field on spore's structure, responses, inactivation mechanisms and factors which influence microbial inactivation during ohmic heating are presented as a literature review. As a result of this research, it has been concluded that the effect of ohmic heating on the inactivation of spores should be evaluated by a multiple perspective approach. Both external and internal factors can play role solely and/or in a synergistic concept during the inactivation of a spore during ohmic heating. In particular, obtained inactivation success depends on the product matrix and its properties, microorganism type, genetic and applied process parameters. Therefore, each research on this topic should not only provide insight into future study but also be carefully evaluated as a specific case. Each case assessment needs to be performed by considering both internal and external factors as well as their synergistic interactions with each other. Besides, more interdisciplinary research is necessary to explain the exact mechanisms of the thermal and non-thermal effects on the microbial inactivation during ohmic heating. Research to be made on the preparation of inactivation kinetics of the specified spores, corresponding internal and external factors, changes in the spore structure during ohmic heating to be clearly defined for each application. Genetics, mutant or wild type of the spores also has significant effect on the responses to ohmic heating conditions. Consequently, well qualified microbiological laboratory will be essential to interpret the results. In order to enhance process efficiency as well as inactivation degree, uniform heating must also be ensured. Therefore, further research and improvement is required regarding topics such as; design, modelling, configuration and optimization of the ohmic heating systems. To sustain product quality and not to misinterpret the obtained results, use of an electrode material which has optimum cost and minimum interaction with the matrix is an important issue. For this reason, future research of material science in collaboration with electrical engineering is required. Electrical circuit design of the system equipment of the ohmic heating set up is important in terms of process continuity, reliability and efficiency. Research on electrical engineering perspective of the ohmic heating system will contribute to new application designs as well as ensuring process continuity, reliability and efficiency factors. Moreover, ohmic heating is an effective alternative thermal microbial inactivation method which can be applied not only on food products but also on various matrixes that meet the required parameters for this treatment. To obtain the expansion of sectors where ohmic heating to be applied, trials should be performed on various types of product, the results need to be published and evaluated.

NOMENCLATURE

I Current, A
V Voltage, V
R Resistance, *ohm*
Q The amount of heat, Joule
T Time, s

AUTHORSHIP CONTRIBUTIONS

Authors equally contributed to this work.

DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

STATEMENT ON THE USE OF ARTIFICIAL INTELLIGENCE

Artificial intelligence was not used in the preparation of the article.

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