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Ohmic heating assisted inactivation of enzymes and microorganisms in foods: A review



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ABSTRACT

Background: Ohmic heating (OH) is a novel method of heating various food materials efficiently, instantly and volumetrically. The quick and volumetric heating during OH results in efficient enzyme and microbial inactivation. Thus, OH can be used as an efficient alternative to the conventional thermal processing method. *Scope and approach:* OH has been applied to various food materials, which include fruits and vegetable products, milk, meat, seafood etc. Inactivation of quality degrading enzymes and spoilage causing microbes to a desired safe level is the prime objective of thermal processing of food, which can be easily achieved by OH process. In addition to the thermal effects, OH also has some non-thermal effects on microbial and enzyme activities due to the presence of electric current during heating. However, these non-thermal effects of OH are possible only in enzymes containing prosthetic metallic groups such as, Cu in PPO, Fe in lipoxygenase, Zn and Mg in alkaline phosphatase.

Key findings and conclusions: Various models such as First order, Bi-phasic and Lumry–Eyring mechanism have been reported in literature for the enzyme inactivation by OH. Enzyme inactivation has been found to increase with increasing electric field strength (V/cm) during the OH process. The spoilage causing microorganisms can also be inactivated efficiently by OH as compared to conventional heating. Higher voltage gradients enhance the non-thermal effects of OH on microbial reduction by resulting in the higher electroporation. The frequency of the electric current also plays an important role in microbial reduction. Various components present in food such as fat, sugar and acid content affect the electrochemical properties of food material, thus affect the performance of OH in reducing the microbial load.

1. Introduction

Thermal processing is one of the oldest and widely used methods of food preservation, its first and foremost objective is to inactivate the spoilage or deterioration causing microbes and enzymes (Donnell, Tiwari, Bourke, & Cullen, 2010). Additionally, it may assist in quality improvement of the product by improving its color, flavor, texture etc. such as pigment retention and softening of fruits and vegetables during blanching (Agüero, Ansorena, Roura, & Del Valle, 2008). Enzymes are proteins that speed up selective chemical reactions, in other words, they are also considered as highly specific biological catalysts (Law, 2002). The control of enzymatic activity is required in many food processing steps, which are responsible for detrimental effects on food quality attributes such as production of off-flavors and off-tastes as well as changes in rheological properties and color (Vicente, Pereira, Penna, & Knirsch, 2010). Hence, inactivation of such enzymes is one of the prime goals of the food processing (thermal processing). The main cause for the inactivation of enzymes (proteins) is denaturation caused due to rearrangement and/or destruction of non-covalent bonds such as hydrogen bonds, hydrophobic interactions and ionic bonds of the tertiary protein structure (Castro, Macedo, Teixeira, & Vincente, 2004). Microbial spoilage also results in development of off-flavors and off-odors, which renders the food product unacceptable for human consumption. Almost all groups of microorganisms initially present in a food product can contribute to spoilage of foods, alteration in the nutrient composition and chemical as well as physical parameters (Gram et al., 2002).

The kinetic parameters such as decimal reduction times (D) or Dvalue, inactivation rate constant (k), z-values (z) and activation energy (E_a) describe the microbial and enzyme inactivation during thermal processing. The D-value is the time required for reducing the original

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Received 15 January 2019; Received in revised form 5 January 2020; Accepted 7 January 2020 Available online 15 January 2020 0924-2244/ © 2020 Elsevier Ltd. All rights reserved. microbial count or enzyme activity by 90% at a specific temperature (Eq. (1)) (Castro et al., 2004). As the temperature is increased the decimal reduction time (D) decreases, the dependency of the D-value on temperature is defined by z-value (Eq. (2)). Castro et al. (2004) defined the temperature dependence of rate (k-value) of an enzyme or microbial reduction during thermal processing in terms of activation energy (E_a) Eq. (3) (Li, Chen, Ren, Wang, & Wu, 2015).

$$\frac{\text{LogC}_{A} - \text{LogC}_{A_{0}}}{t} = \frac{1}{D}$$
(1)

$$\frac{\text{LogD}_2 - \text{LogD}_1}{\text{T}_2 - \text{T}_1} = \frac{1}{\text{z}}$$
(2)

$$K(t) = k_0 \exp\left[-\frac{E_a}{RT}\right]$$
(3)

where, C_A = activity (U), C_{A0} = initial activity (U), D = decimal reduction time (min), Z = temperature sensitivity indicator (°C), Ea = activation energy (kJmol-1), k = rate constant (s-1), and k_0 = pre-exponential factor (s-1).

In the conventional heating process, heat transfer mechanism consists of conduction, convection, and radiation, under both steady and unsteady state operations. Therefore, heterogeneous thermal distribution occurs due to the internal resistance by heat conduction within the different sections of the food material, this uneven distribution of heat causes the additional thermal deterioration to the quality of food product (Sengun, Kendirci, & Icier, 2013). Negative effects of conventional food processing methods and demand of higher quality products by consumers have inspired researchers and the food industry to investigate novel processing technologies to replace conventional processing methods (Donnell et al., 2010). In its response, the food industries have attracted renewed interest in a novel heating methods, during which heat is generated by directly passing electric current through the food product, this phenomenon is commonly known as Ohmic heating (OH), also called as Joule heating, electro-conductive heating, electro-heating, and direct electrical resistance heating (Sastry, 2009). It is proven to be considered as a potential food processing technology (Costa et al., 2018). As enzymes carry some amount of electrical charge hence they do respond when exposed to external electric fields during OH (Oey, 2010). Therefore z value can be expressed in terms of electric field strength (Z_v) during OH (Eq. (4)), in a similar manner as Liu, Hu, Zhao, and Zhang (2013) has expressed Z value in terms of pressure during high-pressure carbon dioxide processing of watermelon juice. Saxena, Makroo, and Srivastava (2016a) reported Z_v for PPO inactivation in sugarcane juice, Z_v can be defined as the change in voltage gradient or electric field strength required to achieve 1 log cycle decrease in D value of an enzyme inactivation process Eq. (4) (Saxena et al., 2016a).

$$\frac{LogD_2 - LogD_1}{[VG]_2 - [VG]_1} = \frac{1}{z_\nu}$$
(4)

Where, VG is voltage gradient (V/cm), z_v is z value in terms of voltage gradient or voltage sensitive parameter.

The effects of OH process on food material are believed to be mainly thermal in nature (Wang & Sastry, 2002). However, Castro et al. (2004) and Park and Kang (2013) have reported non-thermal effects in addition to the thermal effects of OH. Lebovka, Shynkaryk, & Vorobiev, 2007 reported two mechanisms upon applying an electrical current, to the food materials, namely, permeability enhancement (non-thermal effect) and thermal effect (due to Joule heating). The food material characteristics and processing conditions (temperature, frequency, and electric field strength) affect the extent of electric field's non-thermal effect and electrically induced damage to cells and the thermal effects is similar to the conventional thermal inactivation of microbes due to the thermal disruption of microbes structures (cell membranes and wall) (Gavahian, Chu, & Sastry, 2018). The presence of an electric field during the OH can affect biochemical reactions by changing molecular



Fig. 1. Schematic diagram of typical ohmic heating setup; (1) OH chamber (2) Thermocouple (3) Electrode (4) Current sensor (5) Personal computer.

spacing and increasing inter-chain reactions in enzymes. Whereas, in microbial cell membrane the permeability changes due to electroporation caused by applied electric field during OH. Besides, reporting the challenges and future prospects of the OH process, Tian, Yu, Wu, and Dai (2018b) reviewed the effect of OH on various microbes and comprehensively explained the intrinsic and extrinsic factors as well as mechanism of microbial inactivation during OH. In addition to spoilage causing microbes, food may contain different quality degrading enzymes that are equally important for food processing and preservation. Therefore, the aim of the review is to provide a combined and comprehensive compilation of literature on inactivation of the important enzymes and microbes during OH of food. The specific enzymes and microbes has been reviewed and presented individually along with their inactivation kinetics.

2. Ohmic heating and its application

The amount of heat generated due to the electrical resistance of a material to the flow of electric current induced due to the application of electric voltage is known as ohmic heating (OH) (Sastry, 2009). OH heats the material rapidly and uniformly with easy control. Fig. 1 indicates the essential parts of OH equipment, which includes power supply, heating chamber, electrodes, thermocouple, current sensor and data acquisition system (Li et al., 2015). Since the past few decades researchers have conducted a number of studies on OH of various food material (Tian et al., 2018b) such as orange juice, apple juice, cloudberry juice, watermelon juice, pomegranate juice, acerola pulp, meat, egg, carrot, potato, bottle guard juice, bovine milk. These studies were aimed at evaluating various aspects namely, heating behavior, change in electrical conductivity, modeling or and simulation, enzyme and microbial inactivation, changes in rheology, color and nutritional components (Bhat, Saini, & Sharma, 2017; Icier & Ilicali, 2005; Jakób et al., 2010; Makroo, Saxena, Rastogi, & Srivastava, 2017; Park & Kang, 2013; Pez, Damasceno, Marczak, & Domeneghini, 2016; Shim, Hyun, & Jun 2010; Yildiz, Bozkurt, & Icier, 2009). OH has been found to be a rapid and uniform method of thermal processing; besides, requiring less energy as compared to conventional heating (Demirdöven & Baysal, 2014; Makroo, Prabhakar, Rastogi, & Srivastava, 2019). OH has several advantages over the conventional heating process and it may prove to be suitable for many potential applications in food processing and related industries. Some of the important potential applications of OH are reported in Table 1.

3. Effect of ohmic heating on various enzymes

The effects of OH on various enzymes present in foods are summarized in Table 2. The detailed observations on inactivation of

Table 1

Potential applications of OH in food processing.

Applications	Salient findings	References			
Pasteurization and sterilization					
	Quick and rapid heating method saves time and energy; uniformly and rapidly heating of viscous food materials; maintains quality attributes as compared to conventional thermal processing e.g. retention of higher xanthophylls in citrus juices and no harmful effects on fatty acids of milk	Pereira, Martins, and Vicente (2008); Ghnimi, Flach-Malaspina, Dresch, Delaplace, and Maingonnat (2008); Bozkurt and Icier (2010a, 2010b); Achir et al. (2016); Cho, Yi, and Chung (2016)			
Blanching					
	OH enhances heat and mass transfer in fruit tissue and retains higher solids content; uniform heating results in higher overall acceptability; requires less water as compared to conventional blanching	Sensoy and Sastry (2004); Allali, Marchal, and Vorobiev (2010)			
Cooking and b	aking				
	Firm texture, lower volume changes and lower losses as compared to conventional cooking. Besides, cooking time of turkey meat was reduced by 8–15 times. Lower temperature during dough proofing enhanced quality.	Zell, Lyng, Cronin, and Morgan (2009); Bozkurt and Icier (2010a, 2010b); Gally et al. (2017)			
Food for space and military					
	Potential to produce self-stable food product involving convenient and easy process control. Retort pouches with pulsed OH can be used for reheating and sterilization technology for space missions.	Jun and Sastry (2005); Jun, Sastry, and Samaranayake (2007); Somavat, Kamonpatana, et al. (2012) and Somavat, Mohamed, et al. (2012)			
Microbial and	enzyme inactivation				
	Higher microbial inactivation with less decimal reduction time. Electroporation aides the additional antimicrobial effect as compared to thermal effects. Enzymes (PPO, LPO, ALP) containing metallic prosthetic group showed higher rate of inactivation as compared to conventional heat treatment. Additional non-thermal effects were probably due to the interaction between electric current and the metallic prosthetic group.	Icier et al. (2006, 2008); Jakób et al. (2010); Lee et al. (2012, 2013, 2015); Kim and Kang (2015a, 2015b, 2017a, Kim and Kang, 2017b; Bozkurt and Icier (2010a, 2010b); Brochier et al. (2016); Makroo et al. (2016); Saxena et al. (2016a); Park et al. (2017) Gomes et al. (2018); Tian et al. (2018b); Rodrigues et al. (2018)			
Thawing	and the metallic productic group.				
Ū	Uniform temperature distribution and lower thawing losses helped in maintaining the texture and histological properties of the product.	Icier, Izzetoglu, Bozkurt, and Ober (2010); Bozkurt and Icier (2012)			
Extraction and	Distillation				
	Ecofriendly and higher yield rendered it more suitable than conventional process. OH assisted hydro-distillation was energy efficient and quick with better process control. It didn't cause negative effect on the quality of the final product. Extracted essential oils had higher antioxidant and antibacterial activities.	Gavahian, Farahnaky, Shavezipur, and Sastry (2016); Hashemi et al. (2017); Gavahian and Farahnaky (2018)			
Peeling and tissue softening					
	OH assisted peeling of fruits required less time and lower lye solution. Peel yield was higher compared to conventional or microwave heating.	Gupta and Sastry (2018); Wongsa-Ngasri and Sastry (2016a; 2016b);			

individual enzymes are presented in the following sections.

3.1. Polyphenol oxidase (PPO)

Fruits and vegetables contain various phenols, which are used as substrates for PPO, therefore, the PPO enzyme is mainly responsible for browning in most of the fruits and vegetables. Enzymatic browning is observed due to the formation of melanins and benzoquinone from natural phenols in fruits and vegetables when the tissues are damaged by mechanical force and the released compounds come into contact with air (oxygen) (Somogyi, Ramaswamy, & Hui, 1996). Hence, it is expected that an increase in PPO activity occur after peeling and cutting of fruits and vegetables. The thermal inactivation of PPO is one of the primary objectives during blanching. Brochier, Mercali, and Marczak (2016) have reported a slight increase in PPO activity during thermal treatment of sugarcane juice. In many cases, wounds or injuries also encourages the synthesis of some enzymes responsible for browning reactions and substrate biosynthesis (Rocha & Morais, 2002).

PPOs are nuclear-encoded copper metal-o-proteins and their molecular mass is approximately 59 kDa (Castro et al., 2004). Generally, an exposure to a temperature of 70–90 °C destroys the catalytic activity of PPO (Queiroz, Lopes, Fialho, & Valente-mesquita, 2008). Pham, Jittanit, and Sajjaanantakul (2014) showed that the PPO activity of untreated pineapple wedges was reduced to undetectable values by the indirect OH treatment. The indirect OH at 30 V/cm for 1 min for 70 °C was found to be optimum.

The yellow, brown or even pink color development due to PPO activity is the most critical problem associated with the shelf life enhancement of tender coconut water. Therefore, Delfiya and Thangavel (2016) demonstrated that PPO activity was decreased ($p \le 0.01$) by OH treatment. Tender coconut water heated for 3 min at 80 °C at a voltage

gradient of 20 V/cm was found to have minimum PPO activity. Abedelmaksoud, Mohsen, Duedahl-Olesen, Elnikeety, and Feyissa (2018) also reported that due to higher PPO inactivation the apple juice quality can be retained to higher level by OH treatment as compared to conventional heating. The temperature and voltage gradient both were found to have a combined effect on PPO.

PPO activity showed a significant increase (p < 0.01) initially at 24 and 32 V/cm; however, it gradually decreased with increasing the treatment time. Biochemical reactions that may occur due to changes in the molecular spacing that accelerated the inter-chain reactions could be the possible reason for such behavior (Saxena, Makroo, & Srivastava, 2016b). The PPO inactivation was observed to follow the biphasic model (Eq. (5)) during OH treatment of sugarcane juice. According to this model, PPO of sugarcane juice consists of two fractions, namely thermo-labile fraction and thermo-stable fraction (Saxena et al., 2016a).

$$\frac{A_t}{A_o} = A_L e^{(-k_L t)} + A_S e^{(-k_S t)}$$
(5)

Where " A_L " is the fraction of the thermo-labile enzyme and " A_s " is the fraction of the thermo-stable enzyme; " k_L " and " k_s " are the inactivation rate constants of labile fraction (min⁻¹) and stable fraction (min⁻¹) respectively; and "t" is time (min).

Moreno et al. (2013) showed that OH in combination with vacuum impregnation reduced the PPO activity more than 2 times as compared to that of the conventional method. At the same time, during storage of the OH treated apple cubes at 5 °C, lower recovery in PPO activity was observed as compared to conventional heating. The destabilization effect of OH may modify the enzyme surface charge and/or the enzyme environment by ionizing components of the solution and distribute these ions in the electric field (Jakób et al., 2010). The presence of

Table 2

Effect of OH on various enzymes.					
Enzymes/Materials	Salient findings	References			
Polyphenol oxidase (1.14.18.1)					
Pineapple	Ready-to-eat pineapple slices packed in PP pouches was produced by indirect OH (30 V/cm, 70 °C, 1 min)	Pham et al. (2014)			
Tender coconut water	Increase in 5 V/cm voltage gradient significantly decreased the PPO activity of tender coconut water and did	Delfiya and Thangavel (2016)			
Artichoke heads	Higher PPO and POD deactivation was achieved, which led to control of enzymatic browning of the artichoke heads as compared to conventional heating.	Guida et al. (2013)			
Sugarcane juice	Conventional heating (80 °C, 10 min) resulted in a residual PPO activity of 6.47 \pm 0.25%, whereas during OH (32 V/cm. 80 °C) it was reduced to 10.07 \pm 0.32% within 1 min.	Saxena et al. (2016b)			
	OH (70 °C, 3 min) was found to be the one of the best treatments considering inactivation of PPO, change in color and microbial inactivation.	Abilasha and Pal (2018)			
Apple	Complete inactivation of PPO in apples was found in OH assisted osmotic dehydration and OH assisted vacuum impregnation. The treatments also resulted in minimizing color change during storage.	Moreno et al. (2013)			
Watermelon juice	OH was found to be more effective enzyme inactivation method as compared to conventional treatment. OH inactivated PPO in watermelon juice rapidly as compared to conventional heating. However, slight	Castro et al. (2004) Makroo et al. (2017)			
Grape juice	increase in PPO was observed at initial stages of treatment. Critical deactivation temperature of PPO at 40 V/cm was observed to be lower at 20 V/cm. At constant voltage gradient and 60 °C minimum 15 min were required deactivation in PPO	Icier et al. (2008)			
Urease (3.5.1.5)	······				
Soymilk	Soymilk urease was found to contain thermostable and thermolabile isoenzymes. Complete inactivation of thermostable enzyme was neither possible by OH nor by conventional heating.	Li et al. (2015)			
Peroxidase (1.1.1.1)					
Pea puree	OH blanching of pea puree was found to be more effective than hot water blanching. The color change during OH blanching was found to be satisfactory.	Icier et al. (2006)			
Broccoli, potato and carrot	POD deactivation showed a distinct behavior during OH. Increase in voltage gradient caused an increase in the enzyme activity near optimum temperature (60 °C). At 70–75 °C only thermal inactivation was observed.	Jakób et al. (2010)			
Sugarcane juice	POD showed variation in inactivation behavior at different temperatures. Also, no non-thermal effect was reported during OH treatment	Brochier et al. (2016)			
	OH of sugar cane juice at 75 °C using voltage gradients below 20.5 V/cm with different forms of oscillation and frequencies ranging between 10 and 105 Hz did not influence the inactivation causing biochemical reactions associated with POD.	Brochier et al. (2018)			
Pumpkin	Mathematical model (Weibull's distribution) predicted that the OH causes higher POD inactivation as compared to that of conventional heating	Gomes et al. (2018)			
Artichoke heads	OH blanching reduced the POD in shorter duration as compared to the conventional heating. Additionally, OH processing also helped in higher retention of protein and polyphenols	Guida et al. (2013)			
Polygalacturonase/Pectinase (3.2	.1.15)				
Tomato	Thermal treatment lead to higher release of phyto-chemicals (lycopene content) from the matrix. Polygalacturonase inactivation was achieved in 5 times less treatment time in OH as compared to	Makroo et al. (2016)			
	Model was developed for enzyme inactivation under the applied electric field. Lower voltage gradient and the constant temperature had a significant effect on enzyme activity.	Samaranayake and Sastry (2016)			
Commercial (Enzyme)	Non-thermal effects of OH were not evident for pectinase.	Castro et al. (2004)			
Pectin methylesterase or Pectines	sterase (3.1.1.1)				
Orange juice	OH had no non-thermal effects on inactivation pectinesterase.	Leizerson and Shimoni (2005a)			
Tomato paste	Combination of Ori and inforowave enhanced shell-life of orange juice compared to conventional nearing.	Vildiz and Baysal (2006)			
Tolliato paste	Higher retention of color was achieved in case of OH as compared to conventional heating.	Makroo et al. (2016)			
	During OH treatment, frequency of electric field had a significant effect on the enzyme inactivation.	Samaranayake and Sastry (2016)			
Apple juice & cloudberry jam	OH and conventional heating had the same mechanism for enzyme inactivation.	Jakób et al. (2010)			
Lipoxygenase (1.13.11) Soybean	Lipoxygenase inactivation followed first-order kinetics during OH and conventional heating, However,	Castro et al. (2004)			
Alkaline phosphatase (3.1.3.1)	v · · · · · · · · · · · · · · · · · · ·				
Bovine and caprine milk	Safety of the processed milk using OH can also be assessed by considering alkaline phosphatase as an indicator for process effectiveness.	Jakób et al. (2010)			
Raw milk	No visible difference was observed between OH and conventional heating for alkaline phosphatase inactivation at higher temperature; however, variation in D-value was observed at lower temperature due to prolonged exposure.	Castro et al. (2004)			
Beta-glucosidase					
Fermentation broth	No additional effect of electric field during OH was observed on beta-glucosidase enzyme.	Castro et al. (2004)			

*Enzyme Commission number.

electric field during OH reduced the time of PPO inactivation in case of apple, therefore OH and conventional heating resulted in significantly different z values of PPO inactivation in apple cubes (Castro et al., 2004). As the similar level of PPO inactivation can be obtained with the lower thermal destruction of vitamins and pigments and fruit texture, thus OH may increase the final quality of the products as compared to conventional thermal processing.

Makroo et al. (2017) found that PPO inactivation in watermelon

juice was 1.5 times faster during OH as compared to hot water treatment. However, in the later phase of OH a slight increase in PPO activity was observed due to the change of conformation of the enzyme that resulted in higher enzyme-substrate interaction. Icier, Yildiz & Baysal (2008) showed that the rise in temperature resulted in an increase in PPO activity for all the voltage gradients (Fig. 2), however, once the target temperature was achieved the PPO activity decreased with holding time. Similar observations in PPO inactivation of



Fig. 2. Change in PPO activity during heating time from 20 to 60 °C in grape juice at different voltage gradients (Filiz Icier et al., 2008).

sugarcane juice during OH (Saxena et al., 2016a). Guida et al. (2013) found that 20 °C less temperature was required for the complete inactivation of PPO in case of artichoke head during OH as compared to conventional heating. Additionally, it was found that the PPO inactivation was relatively faster than that of POD.

Kinetic of PPO inactivation was found to follow the first order (Eq. (6)) in which the enzyme deactivation is assumed to be a single step irreversible first-order reaction (Castro et al., 2004; Icier, Yildiz, & Baysal, 2008; Makroo et al., 2017)

$$E \xrightarrow{K_1} D$$

Where 'D' is the irreversible and complete denatured form of enzyme 'E' and ' k_1 ' is the rate constant.

$$a = \frac{C}{C_0} = e^{-k_1 t} \tag{6}$$

Where 'a' is the relative enzyme activity, which was the ratio of the current (C) and initial (C₀; at zero holding time) values of enzyme activity. The coefficient k_1 represented the first-order rate constant for a one-step irreversible transition of the native enzyme into an inactive form and t is the holding time.

3.2. Peroxidases (POD)

Peroxidase (POD) is a heme containing enzyme, which catalyzes a large number of reactions in which it is reduced while an electron donor is oxidized (Jakób et al., 2010; Połata, Wilińska, Bryjak, & Polakovič, 2009). POD's having maximum resistance to the heat than any other enzyme therefore, their inactivation by blanching or any other processing method indicates the inactivation of quality degrading enzymes or used as biological indicator of the blanching process (Guida et al., 2013). Icier, Yildiz, and Baysal (2006) investigated critical time of POD inactivation during OH of pea puree at different voltage gradients at a fixed frequency of 50 Hz and the results were compared with the water blanching. The details OH treatment and critical time of POD inactivation obtained are presented as Fig. 3. Peroxidase inactivation time was significantly affected by voltage gradient. The critical inactivation times during OH blanching were statistically different from water blanching, which indicated the contribution of the non-thermal effects of OH on POD inactivation (Icier et al., 2006). The non-thermal effects of OH can be linked with the alteration of enzyme surface charge and/or enzyme environment due to the ionization of solution components and distribution of their ions in an applied electric field during OH (Jakób et al., 2010). The severity of the OH treatment on POD may be different for different food materials. Jakób et al. (2010) reported the susceptibility of POD in carrot was different from that of POD in broccoli and potato due to higher thermal resistance of POD in carrot. The POD inactivation was described by Lumry-Eyring mechanism, although no difference was observed among conventional and OH in POD



Fig. 3. Heating time to reach 100 °C and critical inactivation time of POD in pea puree during OH at different voltage gradients (Icier et al., 2006).

inactivation during the first phase, however OH treatment showed faster inactivation in the second phase.

Sugarcane juice (pH 5.36 ± 0.05; electrical conductivity 2.022 mS/cm at 20 °C) with an initial POD activity of 4631 \pm 469 UEA/min/g was heated by OH and conventional heating by Brochier et al. (2016) showed that at 60 °C POD activity was observed higher in OH when compared to the conventional heating, whereas heating from room temperature up to 80 °C reduced POD activity by 27%. During OH at a specific temperature, activation and inactivation of the POD in sugarcane juice was influenced by low-intensity electric field. Such nonthermal effects of OH or mild electric field treatment were reported by Samaranayake and Sastry (2016). Brochier, Mercali, and Marczak (2018) could not find any non-thermal effect of OH on the POD inactivation; however, different waveforms and change in frequency influenced the enzyme inactivation. Therefore, these effects of OH are still not fully understood and are commendable for further investigation. Fig. 4 indicates the effects of heating method (OH and conventional heating), voltage gradient, frequency and type of wave on the POD inactivation in sugarcane juice (Brochier et al., 2018).

Guida et al. (2013) showed that OH required only three fourth time of conventional blanching to achieve similar inactivation of POD due to electric effects of OH. Gomes, Sarkis, and Marczak (2018) also suggested that the higher POD reduction occurred during OH as compared to that of conventional heating of pumpkin.

3.3. Pectinesterase or pectin methylesterase (PE)

Pectinesterase (PE) is generally present in every plant tissue and in various bacteria and fungi. Thermal treatment is applied to inactivate PE because it catalyzes the de-esterification of pectin in food material (Jakób et al., 2010). It is more thermally stable than many of the vegetative microorganisms, therefore, the design of the thermal processing of PE containing food such as orange juice is based on thermal destruction characteristics of PE (Leizerson & Shimoni, 2005a). The residual activity of PE in orange juice was found to be 2 and 5% by OH and conventional pasteurization, respectively. Leizerson and Shimoni (2005a) studied the effect of a combination of various temperatures and flow rate during thermal processing of orange juice. Except for the temperature (p < 0.001), no significant difference was observed between different flow rates or the interaction between temperature and flow rate (p > 0.05) for OH treatments. Also, no significant difference was found in between type of the heat treatment (OH or conventional heating) on PE inactivation. Jakób et al. (2010) reported same observation while studying inactivation of PE by OH and conventional



Fig. 4. Experimental and predicted POD residual activity values for sugarcane juice during conventional and OH (Brochier et al., 2018).

heating of apple juice and cloudberry jam.

Yıldız and Baysal (2006) demonstrated that the PE activity was increasing at 48 V/cm heated for 5 s and at 36 V/cm up to 30 s, whereas no such increase was observed at 108 V/cm. Thus, lower voltage gradients activate the PE probably by causing greater conversion of bound to soluble fraction of PE than the enzyme denaturation. Differing to the conclusion of Leizerson and Shimoni (2005a,b) and Jakób et al. (2010), Yıldız and Baysal (2006) suggested that the inactivation of PE by OH process includes both thermal and electrical effects, these disagreements could be due to the differences in applied voltage gradient and electrical properties (electric conductivity) of the food material analyzed.

Pectinesterase inactivation in apple juice and cloudberry jams followed the first-order kinetics (Eq. (6)) whereas the differences between the activation energy values for OH and conventional heating were within the error of estimation. Pre-exponential factors in the Arrhenius equation (Eq. (7)) (Castro et al., 2004) were always higher by about 30–40% for OH than conventional heating. It was concluded that due to changes in enzyme surface charge or enzyme environment by ionization of solution components only a very small contribution of non-thermal effects during OH was observed. Whereas the complete loss of PE activity with OH is caused by the Joule heating effect (Jakób et al., 2010).

$$k_i = k_{i0} e^{\frac{E_{ai}}{RT_0} \left[1 - \frac{T_0}{T} \right]}$$
(7)

where k_i represents a rate constant, k_{i0} its value at the reference temperature of $T_0,\,E_{ai}$ the activation energy, and R is the universal gas constant (i = 1–4)

Demirdöven and Baysal (2014) have reported that more than 95% PE inactivation was achieved at moderate temperature range (69–75 °C) by microwave and OH applications in orange juice, residual

PE activity of 92.6 and 90.6% were obtained after OH and conventional heating treatment, respectively. OH in combination with electroplasmolysis was found to be an efficient method of PE inactivation. The combination of electroplasmolysis with microwave heating had comparatively lower effect. It resulted in complete inactivation of heat labile fraction of the enzyme; however, the remaining activity was due to heat stable fraction.

Freshly extracted tomato juice was heated at 90 °C by OH and conventional heating for 60 and 5 min, respectively by applying a voltage gradient of 24 V/cm, whereas hot water method was used for conventional heating. PE activity was reduced to 6.42 ± 1.09 and $5.95 \pm 0.82\%$ by the OH and conventional heating, respectively (Makroo, Rastogi, & Srivastava, 2016). Therefore, it was suggested that faster PE inactivation could be achieved in tomato juice by the application of OH. Samaranayake and Sastry (2016) evaluated the effect of frequency of moderate electric field on PE activity of tomato homogenate. The frequency of less than 60 Hz with a very low electric field (0.4 V/cm) was observed to have a statistically significant effect on PE activity; however, the level of PE remained unchanged by applying frequency beyond 100 Hz.

3.4. Pectinase and polygalacturonase

Pectin is a polysaccharide found in the cell wall of plant material, it is made up of small building block units known as galacturonic acid. The linkages, which held these molecular units of pectin together, are hydrolyzed by the pectinase enzyme. The important uses of pectinase are clarification of wines and juices, reduction in viscosity of plant materials, acceleration of filtration process, inhibit gelling and color improvement (Somogyi et al., 1996). Castro et al. (2004) studied the thermal degradation of pectinase by applying OH and conventional heating. The D values of conventional and OH at 60, 65, and 70 °C were found to be 14.0, 3.41, 0.75 min and 15.0, 3.57, 0.83 min, respectively, while activation energy (E_a) calculated were 0.78 \times 10⁻³ and 0.81 \times 10⁻³ J/mol during conventional and OH, respectively. Therefore, from the results, it was suggested that there is no difference in the type of heating method on the pectinase enzyme, similar D and z values were obtained for both the heating methods. As pectinase doesn't have any prosthetic group, no additional effect except thermal effect was observed during its inactivation by OH, therefore these results are in agreement with the hypothesis which states that electric field of OH has influence on the enzymes with metallic prosthetic group such as copper in poly-phenoloxidase, iron in lipoxygenase, zinc and magnesium in alkaline phosphatase (Castro et al., 2004).

Principally all the substances consisted of electrically charged subatomic particles, therefore when these are exposed to an external electric field, these charged particles respond individually. When enzymes are in an aqueous environment, due to their protein nature they possess net charge and dipole moment, therefore application of external electric field causes some moment (Pereira, Teixeira, & Vicente, 2011). Samaranayake and Sastry (2016) showed that pectinase or polygalacturonase exists as two isozymes, PG1 and PG2 both of them have different thermal resistance. PG1 is more heat resistant and can be inactivated at 85 °C while as PG2 being relatively lower thermal resistant therefore can be inactivated at 65 °C (Anthon, Sekine, Watanabe, & Barrett, 2002). PG₂ was affected by the voltage gradient; however, there were no non-thermal effects of electric field reported on PG1. Further, the study on the role of the frequency on enzyme activity suggested that the non-oscillating voltage gradient (or DC or when the frequency was zero) has no effect on activity; however the electric field of alternating current with different frequencies (oscillating) was affecting the PG₂ activity (Samaranayake & Sastry, 2016). Hot break treatment of freshly squeezed tomato juice was applied by OH and hot water methods (Makroo et al., 2016). PG activity in fresh tomato juice was found to be 13.9 U/mL, which was reduced to 31.51 and 31.08% of fresh juice in 1 and 5 min of OH and hot water treatment, respectively. These observations were in agreement with Samaranayake and Sastry (2016) suggesting that electric field present in OH has an additional effect on the PG₂ fraction of PG. The inactivation of PG was observed to be follow first order kinetics (Eq. (6)) during both OH and hot water thermal treatment. D value at 90 °C (D₉₀) for PG in tomato juice was found to be 24.5 and 287.7 s for OH and hot water treatments, respectively. The pre-treatment of fresh tomato juice by using OH resulted in better color and consistency of the tomato paste (the finished product) and the nutritional characteristics were found to be comparable with that of hot water treatment.

3.5. Urease and lipoxigenase

Li et al. (2015) conducted a study on effects of frequency (50-10 kHz) and voltage (160-220 V) on urease enzyme during OH of soymilk. The results showed that the urease activity was higher in the samples treated with the conventional heating than that of treated with OH. During OH, the frequency was found to have a positive effect on the urease inactivation. The urease activity of the sample treated with the OH at 5 kHz was significantly lower than that at 0.05 kHz (P < 0.05). Because urease is such type of protein, which is characteristics of the polarity and is a nickel-dependent metallo-enzyme (Li et al., 2015). Therefore, the alternating electric field present in OH may remove the metallic prosthetic groups of metallo-enzymes, hence leads to higher inactivation with even similar thermal history as that of conventional heating (Li et al., 2015). Bi-phasic model (Eq. (5)) was studied and it was found that the rate constant (k_l) of the thermo-labile fraction of urease gradually decreased and changed irregularly by increasing the frequency and voltage respectively, whereas its D-value (D_L) increased and decreased generally with the increase in the frequency and voltage during the holding temperature stage, respectively.

However, the inactivation rate constant of the thermo-stable fraction (k_s) remained zero at all the frequencies and voltages. Therefore, Li et al. (2015) concluded that the frequency and voltage of the power supplied have no effect on the inactivation of the thermo-stable but could significantly inactivate the thermo-labile fraction of urease in soymilk.

Lipoxygenases are iron-containing oxidoreductase enzymes that catalyze the deoxygenation of polyunsaturated fatty acids containing a cis, cis-1,4-pentadiene-conjugated double bond system such as linoleate and linolenate to yield hydroperoxides. Therefore, linoleic acid was used as the substrate for evaluating the effects of OH on the activity of lipoxigenase extracted from soya beans (Castro et al., 2004). Decimal reduction time (D value) of lipoxigenase was significantly lower in OH than that of conventional heating. The z value was found to be 11.10 and 15.04 °C, whereas activation energy (E_a) was 0.757 and 0.969 J mol⁻¹ for conventional and OH, respectively. SDS-PAGE showed that there was no molecular breakdown or subunit separation of enzyme molecules due to either of the treatments (conventional or OH). Therefore, further investigations are required to establish the exact changes in enzyme structure during OH.

3.6. Alkaline phosphatase (ALP)

Alkaline phosphatase (ALP) is extensively distributed in animal tissues and microbes. It is a membrane-bound glycoprotein. For the highest enzyme activity it requires two metals namely, zinc and magnesium. (Castro, Swanson, Barbosa-Cánovas, & Meyer, 2001). Phenol is liberated when ALP acts upon its substrate disodium phenyl phosphate at controlled temperature and pH. Hence, the activity of ALP can be determined by this phenomenon calorimetrically at 620 nm by letting the generated phenol molecules react with 2,6-dichloroquinone-4chloroimide to form indo-phenol blue (Wilinska, Bryjak, Illeova, & Polakovic, 2007). Wilinska et al. (2007) have found that the thermal inactivation of milk ALP during conventional heating followed the first order model (Eq. (6)); however, the thermal inactivation kinetics of ALP in bovine milk was based on Lumry-Eyring mechanism, which is a twostep inactivation model (Eq. (8)). In general, the rate constant (k_x) and activation energy (E_a) of ALP inactivation during OH were found higher by 45% and 18%, respectively than that of conventional heating. Specifically for ALP in bovine milk, the value of k₁ was higher than that of k₂ and k₃, during conventional heating. However during OH, k₃ was observed to be higher than that of k_1 and k_2 (Jakób et al., 2010).

$$E \xrightarrow[k_2]{k_1} D \xrightarrow[k_3]{} I \xrightarrow[k_2]{} (8)$$

Where 'D' and 'I' are reversibly intermediate and irreversible complete denatured form of enzyme (E) respectively, and k_1 , k_2 and k_3 are respective rate constants.

Castro et al. (2004) also studied the effect of OH on ALP of milk by heating milk using an electric supply of 50 Hz the electric field was varied as per the requirement to exactly follow the same heating profile as that of conventional treatment. A similar mechanism for inactivation was observed for OH and conventional heating method. At lower treatment temperatures (55–60 °C), the D-value of ALP inactivation was quite different for the two heating methods. However, as the treatment temperature was raised (65–70 °C) this difference was narrowed down indicating that the electric field applied for longer duration (to maintain lower treatment temperature) had some additional effects on the enzyme inactivation, whereas when the treatment temperature was higher, lesser was the time of applied electric field and hence similar Dvalues were observed.

3.7. Beta-glucosidase

Beta-glucosidase enzyme liberated by the fermentation of lactose was studied by Castro et al. (2004) for the comparison of the OH and

Table 3

Studies conducted on effects of OH on different microorganisms.

Microorganisms/Materials	Salient findings	References			
Total plate count/Yeats and Molds					
Apple cubes	OH in combination with osmotic dehydration or vacuum impregnation was shown to have a	Moreno et al. (2013)			
	synergistic effect on the microbial reduction resulting in enhanced the shelf-life.				
Orange juice	Spore inactivation during OH was primarily due to thermal effect; however, additional non-	Leizerson and Shimoni (2005a)			
Different stew type foods	No post process contamination was observed in stew type products processed by OH, the shelf	Yang et al. (1996)			
Blue mussel	At lower electric field strength (9.1 V/cm), no significant non-thermal effect on microbial	Bastías et al. (2015)			
	reduction was observed.				
Escherichia coli O157:H7; Salmone	Ella typhimurium; Listeria monocytogenes Effectiveness of OH in reducing the pathogens was dependent on electric field strength time	Lee et al. (2012)			
orange juice & toniato juice	temperature and electrical conductivity. Increase in electric field reduced number of microbes. Tomato juice required shorter treatment time to reduce the population of <i>Listeria</i>	Ice (1 m. (2012)			
Orange juice	Combination of low pH and OH has a synergistic effect on the reduction of pathogens. However different pathogens showed different tendency towards such effect	Lee et al. (2015)			
Peptone water and apple	Electric field had a strong relationship with the microbial reduction: however, frequency and	Park and Kang (2013)			
juice	electric conductivity need to be optimized for OH process. Due to the formation of pores in cell				
	membrane during OH, it resulted in 2–3 fold higher reduction in pathogens as compared to conventional heating.				
Apple juice	Concentration was found to have a significant effect on the reduction of pathogen reduction	Park et al. (2017)			
	during OH at 30 and 60 V/cm for different treatment duration (0–72 s). Three times more time				
	was required to reduce the population by 5-log cycle at 30 V/cm in sample having 1SS of 36° Brix than that at 60 V/cm for Brix range of $0_{-}60^{\circ}$ Brix				
Phosphate buffer	OH at 5 and 10 V/cm caused a comparable but sub-lethal injury to the <i>E coli</i> cells in phosphate	Tian et al. (2018a)			
	buffer.				
Salsa	Lower frequency (500 Hz) required more time to reduce <i>E. coli</i> below the detection limit than	Lee et al. (2013)			
	at a frequency above 1000 Hz. Reduction in S. typhimurum was 3.85 log CFU/ml and > 6.47 -log or below the detection limit after 50 and 54 s of OH treatment respectively.				
	Synergistic effect of carvacrol and OH was observed against the reduction of bacteria in salsa.	Kim and Kang (2017a)			
Salsa with oil components	Reduction in E coli 0157:H7, Salmonella typhimurium; Listeria monocytogenes was observed to be	Kim and Kang (2017b)			
(flavorings)	5.0, 5.4 and 5.4 log CFU/mL, respectively. Due to the presence of phenolic compounds in				
	thymol and citral, it was observed that the combination of these essential oils with OH had a synergistic effect against hacterial degradation				
Skim milk and cream	Time duration of 140 s and 240 s were required to reduce the <i>E. coli</i> below the detection limit	Kim and Kang (2015)			
	in OH and conventional heating, respectively. <i>S. typhinurium</i> was reduced more effectively by OH at 60–65 °C than by conventional heating.				
	Listeria monocytogenes was also reduced to below detection level three times faster during OH				
Mills fot in huffored nontone	treatment (60 s) as compared to conventional heating (180 s).	View and Vana (2015)			
water	Fat content (0–10%) was found to have protective effect on the pathogenic bacteria during OH.	Kini and Kang (2015)			
Infant formula	Non-thermal effects were observed at lower (57 $^\circ C$) temperature, which were not evident at	Rodrigues et al. (2018)			
	higher temperature (65 °C)				
Leuconostoc mesenteroides	Shelf-life of sugarcane juice stored at refrigeration temperature was found to increase by OH	Savena et al. (2016b)			
bugureune jurce	treatment as compared to conventional heating.	buxenti et ili. (2010b)			
Enterobacteriaceae					
Chilean blue mussel	OH process at an electric field of 9.15 V/cm was not found to be efficient to show non-thermal effect (electroporation) on Enterobacteriaceae population.	Bastías et al. (2015)			
Aspergillus niger	man and the state of the state				
Tomato homogenate	Time required to initiate the deduction in population (critical time T_c) of Aspergillus niger decreased with increasing the electric field strength during OH.	Yildiz and Baysal (2006)			
Clostridium sporogenes (spore) Chicken alginate particle	Z-value of <i>Clostridium sporogenes</i> was found to be same as that of <i>C. hotulinum</i> , which is 10 °C	Kamonpatana et al. (2013)			
	therefore It could be considered suitable marker organism.				
MS-2 bacteriophage					
Salsa Salsa with oil components	<i>MS-2 bacteriophage</i> was reduced synergistically by OH and carvacrol treatment.	Kim and Kang (2017a)			
(flavorings)	The reductions of MS-2 bacteriophage during OH combined with citral and thymol in peptone	Killi aliu Kalig (2017b)			
	water was 3.30 and 3.36 log CFU/mL, whereas in salsa the reduction was 2.39 and 2.53 log				
	CFU/mL respectively.				
Alicyclobacillus acidoterrestris (S	pore) log reduction in Alicyclobacillus acidaterrestris was attained in 30 min at 90 °C using 30 V/cm.	Baysal and Icier (2010)			
orange julee	electric field during OH whereas only 3.5 log reduction was achieved by conventional heating				
	for same time-temperature histories				
Geobacillus stearothermophilus (spore)				
Spore suspension	D-value for Geodacillus stearothermophilus at 121 °C during OH at 10 kHz, 60 Hz and conventional heating was observed to be 0.88 1.17 and 2.53 min, respectively	Somavat, Kamonpatana, et al. (2012) and Somavat Mohamed et al. (2012)			
Streptococcus thermophilus	conventional nearing was observed to be 0.00, 1.17 and 2.00 mm, respectively.	contavat, monanica, et al. (2012)			
Milk	Non-thermal injury caused by electric current during OH resulted in significant differences in	Sun et al. (2008)			
o 1	the killing of Streptococcus thermophilus during OH and conventional (hot water) heating.				
Saccharomyces cerevisiae					

(continued on next page)

Table 3 (continued)

Microorganisms/Materials	Salient findings	References
Suspension in phosphate buffer	No H_2O_2 was produced during the supply of electric current (DC, 0.1–1.0 A) for 3 h at 20 °C and as the electric current supply was stopped, the antimicrobial effect also stopped that indicated that the reduction in microbial population was encouraged by electrolysis rather due to toxic substances.	Guillou and El Murr (2002)
Staphylococcus aureus Infant formula	D-values of OH and conventional heating were significantly different at the same temperature. Non-thermal effects were observed from 57 to 65 $^\circ$ C.	Rodrigues et al. (2018)

conventional thermal treatment. However, no difference was observed in OH and conventional thermal inactivation due to absence of no metallic prosthetic group present in beta-glucosidase.

4. Effect of ohmic heating on various food borne microbes

OH process has an advantage of quick and uniform heating as it raises the temperature of food material volumetrically. Therefore, to obtain safe and shelf-stable food, OH can be a potential substitute for time and energy consuming conventional thermal processing methods. Table 3 presents the effect of OH on various important specific microorganisms present or inoculated in different food materials. The detailed review on effect of OH the microbes is presented in following sections.

4.1. Escherichia coli O157:H7

E. coli O157:H7 is one of the major pathogen responsible for foodborne outbreaks such as diarrhea and haemolytic-uraemic syndrome etc. Cody et al. (1999) reported that E. coli infection in cider in the United States in 1996 led a child to death and at least 70 people fell sick. Orange juice and tomato juice were treated with OH at varying temperatures at 25-40 V/cm voltage gradient by Lee, Sagong, Ryu, and Kang (2012). Tomato juice was heated with higher rates as compared to orange juice at all the voltage gradients applied due to higher electrical conductivity of tomato juice than that of orange juice. Voltage gradient was found to be in positive relationship with the reduction of the number of survivals in orange juice. E. coli was reduced by 1.14 and 6.1 log CFU/mL; however, in tomato juice the number was reduced to undetectable limits (< 1 CFU/mL). As discussed earlier tomato juice was heating rapidly, hence higher amount of electric current would have flown and ultimately lead to higher damage to the E. coli cells in tomato juice. Transmission electron microscopy images (Fig. 5) revealed that although both the thermal treatments caused aggregation of cytoplasm, relatively higher aggression was resulted by OH as compared to conventional heating (Lee et al., 2012).

Park and Kang (2013) studied effect of OH on three stains of *E. coli* O157:H7 viz, ATCC 35150, ATCC 43889 and ATCC 43890 in buffered

peptone water (BPW) and pasteurized apple juice (11.8 °Brix) having pH 7.2 and 3.5, respectively. In BPW, heat treatment for 30 s at 58 and 60 °C caused a reduction of 4.0 and 5.6 log cycles by OH, respectively. Whereas, conventional heating treatments resulted in 1.6 and 2.3 log cycle reduction, which showed the non-thermal effects of OH on E. coli population. Transmission electron microscopy (TEM) results of Park and Kang (2013) were found to be in agreement with Lee et al. (2012), which proved that OH had some additional effects (electroporation caused damage of cell) on the morphology and structure of E. coli O157:H7 as compared to that of same degree of conventional heating (Fig. 6). The cell membrane was intact in untreated cells (Fig. 6A, B), and while shrinkage of intracellular substances or slight membrane damage was found in conventionally treated cells (Fig. 6C, D). However, OH caused relatively higher damage or released higher intracellular substances (Fig. 6E, F) than conventional heating. Electroporation in the cell membrane occurs due to the stresses set up by the electric field. As the electric field (voltage gradient) reaches a critical point or above, the membrane conductance increase dramatically allowing leakage of the cellular material. The membrane breakage due to electric field also was found to have strong inverse dependence on temperature (Coster & Zimmermann, 1975).

The concentration or total soluble solids (TSS) also played an important role in microbial reduction during OH process such as OH for 60 s using 30 V/cm of apple juice having TSS of 72, 48, 36, 24 and 18°Brix caused 0.95, 2.59, 6.78, 5.21, and 2.71-log cycle reduction, respectively (Park, Ha, & Kang, 2017).

Carvacrol is a monoterpenoid phenol, which is found in greater quantities in the number of essential oils. It increased the permeability of the bacterial cytoplasmic membrane and caused oxidative damage to DNA. It also targets capsids and subsequent RNA in viruses, therefore it possessed a potential antimicrobial property, which can be used for the application of food processing and preservation (Chueca, Pagian, & García-Gonzalo, 2014). Due to these properties of carvacrol, Kim and Kang (2017a) conducted the experiment to evaluate the synergistic effect of carvacrol and OH on the various pathogens in salsa, the OH treatment was applied using voltage gradient and frequency of 12.1 V/ cm and 60 Hz, respectively. Non-thermal effect of OH and carvacrol damaged cell membranes of bacteria, hence a synergistic effect on *E*.



Fig. 5. TEM images of *E coli* O157:H7 in orange juice (a) untreated (b) conventionally heated for 180 s (c) continuous ohmic heating 180 s at 30 V/cm (Lee et al., 2012).



Fig. 6. TEM micrographs of untreated, conventional heat and OH treated E. coli O157:H7 cells Park and Kang (2013).



Fig. 7. Inactivation of *E. coli* and *S. aureus* during OH and conventional heating treatments at 62.5 °C (Rodrigues et al., 2018).

coli was observed after the treatment of 30 and 40 s on selective and resuscitation media, respectively. The synergistic effect of oil compounds and OH treatment was also observed during the OH treatment of salsa and peptone water added with thymol and citral (Kim & Kang, 2017b). Saxena et al. (2016b) demonstrated that no growth appeared during one-month storage of OH treated sugarcane juice at refrigerated conditions. The possible reason could be due to the combined effect of OH and naturally present antimicrobial components in sugarcane juice such as ascorbic acid. Kim and Kang (2015) heated skim milk and cream by OH and conventional heating, the fat content significantly affected the electrical conductivity thus the heating rate was different at the different level of fat content during OH process. Holding the skim milk and cream for 1 min at 55, 60, 65 and 70 °C during OH reduced the

E. coli from 6.73 to 6.26, 5.47, 1.77 and $< 1 \log \text{CFU/mL}$ in skim milk and from 6.72 to 6.50, 6.08, 5.24 and 4.36 log CFU/mL in cream, respectively. Effects of OH on E. coli O157:H7 in skim milk were significantly different from conventional heating, whereas no difference was observed in OH and conventional heating for the reduction of E. coli in cream. Further, the authors reported that OH treatment at 60 °C for 40 and 50 s using frequency 20 kHz and voltage gradient of 19.2 (heating) and 9.6 V/cm (holding) were sufficient to reduce E. coli population below the detection level in 0 and 10% fat containing peptone buffer water. Lee, Ryu, and Kang (2013) subjected salsa sample to OH treatment at 90 °C at a fixed voltage gradient of 12.5 V/cm at different levels of AC frequencies to study the impact of frequency on various parameters including microbial reduction and other quality parameters. The waveform was observed to affect the heating rate at lower frequency (0.06 kHz); however, no major difference was found in the heating rate while using frequency \geq 0.5 kHz among different waveforms. OH treatment for 50 s using 0.5 kHz frequency reduced E. coli population by 3.5 log CFU/mL, whereas no growth was observed by using frequency of 1-20 kHz for the same period. OH of orange juice (13°Brix) was conducted and effect of different levels of pH on the microbial reduction was investigated by Lee, Kim, and Kang (2015). Lower pH caused higher reduction than at higher pH, during OH of 20 s more than 5.72 log reductions was found at pH 2.5, whereas at pH of 3.5 and 4.5 the log reduction achieved in 60 s was 1.84 and 0.89, respectively. Voltage gradient of 5 and 10 V/cm caused sub-lethal damage to the E. coli cells in phosphate buffer (Tian, Yu, Shao, Li, & Dai, 2018a). D-value of E. coli in infant formula was significantly different between OH and conventional heating when the treatment temperature was maintained between 57 and 65 °C (Fig. 7, Rodrigues et al., 2018).

4.2. Salmonella typhimurium

Consumption of food contaminated with Salmonella bacteria is still one of the most common and widely cause of foodborne disease worldwide (Ma, Zhang, Bhandari, & Gao, 2017). Annually more than 1.4 and 0.16 million cases of salmonellosis have been reported in the US and the European Union, respectively, whereas in China 40-60% of foodborne outbreaks are reported to be of salmonellosis (Jianchang, Rui, Lianxia, Jinfeng, & Jing, 2016). Studies on the effects of OH on S. typhimurium has been reported for orange and tomato juice (Lee et al., 2012) and apple juice (Park et al., 2017; Park & Kang, 2013) and carvacrol in salsa Kim and Kang (2017a). Continuous OH of orange juice for 60 and 90 s resulted in the reduction by 1.32 log CFU/mL and more than 6.52 log CFU/mL respectively, and treatment of 180 s at 30 V/cm caused the reduction to an undetectable limit (< 1 CFU/mL) (Lee et al., 2012). However, in tomato juice OH treatment time for 55 s caused the reduction below detection level (< 1 CFU/mL). Based on these results, it is very clear that the processing time or time to achieve a desirable reduction of a specific microorganism may vary within different food materials being processed. Additionally, it was observed that the applied electric field during OH has an additional non-thermal effect on the inactivation of microorganisms. In addition to the treatment method, such as OH and conventional heating, Park and Kang (2013) found that pH of the food material is also an important parameter for reducing the population of S. typhimurium. In apple juice, the reduction of 6.45 and 4.7 logs were achieved by treatment of 30 s at 50 °C during OH and conventional heating, respectively. Treatment time for the complete inactivation of S. typhimurium was found much lower for OH than the conventional heating in apple juice. Kim and Kang (2015b) also reported that the pH plays important role during inactivation of S. typhimurium in orange juice during OH. It was observed that at higher pH (4.5), thermal effect was pronounced, whereas the effect of OH was more dominant at lower pH (2.1). Park et al. (2017) found that OH treatment (1 min, 30 V/cm) of apple juice having 18, 24, 36, 48, and 72°Brix resulted in reduction of 3.27, 6.70, 6.71, 2.88 and 1.40 log CFU/mL, respectively. OH was reported to cause no significant additional effects on the essential quality parameters of apple juice as compared to conventional heating.

Kim and Kang (2017a) demonstrated that OH treatment combined with carvacrol could be applied effectively to process salsa with reduced treatment time as compared to that of conventional processing method. The authors also found synergistic effect of oil compounds (thymol and citral) and OH treatment during inactivation of S. typhimurium in peptone water and salsa. Skim milk and cream containing 6.69 and 6.55 log CFU/mL were treated by OH and conventional heating by Kim and Kang (2015). In skim milk, during OH at 65 °C the level of S. typhimurium was reduced to undetectable level whereas in same conventional treatment brought the count to 1.2 log CFU/mL. However, reduction up to undetectable levels (< 1 CFU/mL) was observed in both types of heat treatments at 70 °C for 1 min. On the other hand, the study of cream witnessed no difference among the type of treatment in the reduction of S. typhimurium (Kim & Kang, 2015). Peptone buffer water containing different level of milk fat was studied for the effects of OH on inactivation of S. typhimurium by Kim and Kang (2015), OH at (heating at 19.2 V/cm and holding at 9.6 V/cm) at 60 °C was applied for different time periods. The OH treatment (35 s) was found to be sufficient to cause the reduction below the detection limit. In a study conducted on salsa, it was found that the frequency of alternating current during OH has a direct effect on the reduction of S. typhimurium (Lee et al., 2013). During OH (12.5 V/cm) at 90 °C, it took almost 83 and 50 s to reduce the S. typhimurium in salsa from 7.5 log CFU/mL to undetectable levels by using 0.06 and 20 kHz frequency, respectively. Therefore, the reduction had a direct relationship with the frequency of alternating current applied during OH. S. typhimurium was reduced by 5.15 logs when orange juice was OH treated at 50 °C for 60 s using voltage gradient of 16 V/cm and frequency of 20 kHz. However,

under same OH conditions when the pH of orange juice was increased from 2.5 to 3.5 or 4.5, which resulted in less than 1 log cycle (Lee et al., 2015). Hence, it was concluded that during OH, pH of the food material greatly influences the microbial inactivation.

4.3. Listeria monocytogenes

L. monocytogenes can grow over a large range of temperature, even at refrigeration temperature. There are quite good chances of its contamination after processing of the food material. Usually, it is found in ready to eat food products. It has a fatality rate of 20–40% and pregnant or elderly individuals are usually more vulnerable to its contamination (Balay, Dangeti, Kaur, & McMullen, 2017). During thermal processing of food, time and temperature have a direct relationship with the reduction in its population (Park & Kang, 2013). L. monocytogenes was found to be more heat resistant than S. typhimurium and E. coli. Apple juice heated for 30 s at 55 °C resulted in the reduction of population by 3.6 and 1.2 log cycle using OH (at 60 V/cm) and conventional heating, respectively. It was also observed that level of L. monocytogenes in apple juice could be reduced to an undetectable level by OH within 30 s at 58 °C However, conventional heating method was not sufficient to reduce the number below detectable limits at the same temperature. Park et al. (2017) showed that TSS was found to play an important role in OH profile of apple juice. Among all the TSS levels, TSS of 36°Brix showed the maximum reduction in population at 30 V/cm for 1 min. However, at 60 V/cm, the comparatively dramatic reduction was noticed in TSS range of 18-48 °Brix. OH treatment of the juice samples having TSS of 18, 24, 36 and 48°Brix caused the reduction of 5.93, 5.82, 5.70 and 5.71 logs, respectively. Park et al. (2017) suggested that heating apple juice of 48°Brix at 60 V/cm would be more efficient than heating 36°Brix at 30 V/cm. Inactivation of L. monocytogenes exhibited 1.23 and 5.1 log reduction when orange juice was OH treated at 35 V/ cm for 1 and 2.5 min, respectively. However, treatment at 25 V/cm showed no significant differences when compared with commercially pasteurized orange juice. The survival population in tomato juice was reduced to below detection level (<1 CFU/mL) by continuous OH treatment. The higher level of L. monocytogene destruction was witnessed in tomato juice as compared to that in orange juice (Lee et al., 2012). OH treatment and addition of carvacol in salsa showed a synergetic effect in reducing the L. monocytogene and the synergistic effect was increasing with the OH treatment time (Kim & Kang, 2017a). The phenolic compounds present in essential oil (thymol and citral) were reported to be responsible for the synergistic effect of oil compounds and OH during the inactivation (Kim & Kang, 2017b).

In skim milk, the OH treatment of 1 min was found to be sufficient to reduce the population of L. monocytogene from 5.6 log CFU/mL to undetectable limit, whereas similar conventional heat treatment caused reduction of only 3.65 CFU/mL (Kim & Kang, 2015a). Kim and Kang (2015b) studied buffer peptone water containing milk fat of 0-10% for OH and its effects on L. monocytogene, no additional or non-thermal effects were observed by OH in the reduction of L. monocytogene when compared to that of conventional heating (Fig. 8). The fat has been found to have a protective effect against the inactivation; therefore, the higher fat content of cream could be the possible reason for the lower reduction in cream than in skim milk (Kim & Kang, 2015a). The OH was generated using 20 kHz frequency and voltage gradient of 19.2 V/cm during heating up to 60 °C and 9.6 V/cm during holding, heating time of 40 s for 0% and 50 s for 10% fat containing samples was sufficient to cause the reduction below the detection limit (< 1 log CFU/mL). Orange juice (13°Brix) of pH of 2.5, 3.5 and 4.5 was OH treated at 50-60 °C by using 16 V/cm voltage gradient and 20 kHz frequency by Lee et al. (2015). The reduction of L. monocytogene by heating at 55 and 60 °C for 1 min was found 2.15 and 3.37 logs, respectively in juice at pH 2.5. On the other hand at pH 3.5 and 4.5, the reduction at 55 $^\circ C$ was 0.78 and 0.42, at 60 °C was 1.88 and 0.87 logs, respectively. Kim & Kang (2015) observed similar reduction during OH in case of orange



Fig. 8. Survival curves of *L. monocytogenes* corresponding to temperature OH (a) Conventional heating (b) of peptone water with 0% (\bullet), 3% (\bigcirc), 7% (\bigtriangledown) and 10% (Δ) fat content (Kim & Kang, 2015b).

juice; however, pH of the juice also had a significant role on the inactivation of *L. monocytogene* due to presence of acid.

4.4. Total bacterial, yeasts and molds count

Total bacterial count (TBC) is one of the fundamental and most commonly used methods to evaluate the microbial stability of a food material. Various studies have been conducted to find effects of OH on TBC in fruit juices, milk and seafood (Moreno et al., 2013; Leizerson & Shimoni, 2005a, 2005b; Sun et al., 2008; Bastías et al., 2015; Abilasha & Pal, 2018). Moreno et al. (2013) investigated the combined effect of OH, osmotic dehydration and vacuum on the microbial stability of apple cubes. Apple cubes contained in 63°Brix sucrose solution were OH treated to 30, 40 and 50 °C for 90 min by using voltage gradient of 13 V/cm at 60 Hz. All the treatment conditions were found to reduce mesophilic aerobic bacteria below the permissible limit of 10⁵ CFU/g (Minsal, 2008) during the storage at 5 and 10 °C for 37 days (Moreno et al., 2013).

Blue mussels were blanched at 50, 70 and 90 °C for 4 min by OH and conventional heating. A saline solution of 0.03% at a ratio of 9:1 (solution: blue mussel) was used as a heating medium, and OH was generated by applying voltage gradient of 9.15 V/cm. OH and conventional blanching resulted in a similar reduction in aerobic mesophiles during both the blanching methods. However, conventional heating was found to be more effective in reducing the number of mesophilic bacteria at 70 and 90 °C as compared to OH. Blanching at 90 °C reduced the number of mesophiles from 3.8 to 2.6 and 1.7 log CFU/g with OH and conventional heating, respectively.

The total plate count decreased from 6.3 to 3.47 log CFU/mL in sugarcane juice heated by OH for15 min at 90 °C, in this way OH was found be effective in shorter time as compared to conventional method (Abilasha & Pal, 2018). No significant difference was found in reducing the mesophilic aerobic bacteria at the same blanching temperature between the OH and conventional blanching (Bastías et al., 2015).

Leizerson and Shimoni (2005a) reported similar findings during comparison of OH and conventional heating of orange juice. These results indicate that OH showed only thermal effects, whereas could not produce the non-thermal effects (electroporation of bacterial cells), probably due to the lower voltage gradient applied (Wang & Sastry, 2002). Probably for the same reasons Leizerson and Shimoni (2005b) didn't observe any additional effects of OH on microbial quality of OH treated orange juice during storage study of 105 days as compared to that of conventionally treated. After evaluating microbial quality of many OH heated stew type foods. Yang, Cohen, Kluter, Tempest, and Blackmore (1996) concluded that OH has a potential to maintain the quality of food to maximum limits after processing and during storage. Yeats and molds can grow in low pH and higher sugar content containing foods such as fruits or their products, additionally the presence of vitamins help in their growth and the maximal limits recommended for the YAM in fruits was $1 \times 10^3 \log$ CFU/g (Minsal, 2008). Leizerson and Shimoni (2005a) and Leizerson and Shimoni (2005b) studied yeast and mold in orange juice treated with OH and conventional heating, due to lower voltage gradient of OH, the non-thermal effects were not witnessed on veast and mold inactivation after the treatment as well as during the storage for 105 days. Moreno et al. (2013) treated apple cubes with combined treatment of OH and osmotic dehydration. Yeast and molds were completely inhibited by OH at 13 V/cm; however, under similar conditions at 50 °C during conventional heating, Finally OH treatment in combination with osmotic dehydration and storage at 5 °C was found to be the best treatment for apple cubes.

4.5. Other microorganisms

Saxena et al. (2016b) conducted storage study of sugarcane juice thermally treated at 80 °C by OH (at 32 V/cm) and conventional heating for 1 and 10 min, respectively. It was found that the growth of *Leuconostoc mesenteroides* did appear in 5–10 days when stored at room temperature, however, when the samples were stored at refrigeration temperature there was no growth until the 20 and 15 days in OH and conventionally treated samples, respectively.

Rodrigues et al. (2018) compared the effect of OH and conventional heating on *Staphylococcus aureus* in infant formula (Fig. 7). It was found that the D-values reduced with the increase in temperature of conventional and OH treatment; however, a significant difference was found in D-values of the OH (0.53 min) and conventional (1.42 min) heating at 65 °C. This also suggested that in addition to heat, electric field may also have influenced *S. aureus* inactivation.

Somavat, Kamonpatana, et al. (2012) and Somavat, Mohamed, et al. (2012), Baysal and Icier (2010) and Kamonpatana et al. (2013) investigated the effects of OH on spores of *Geobacillus stearothermophilus, Alicyclobacillus acidoterrestris,* and *Clostridium sporogenes.* OH caused a higher destruction of *Geobacillus stearothermophilus* in comparison to the conventional heating; however, frequency also had some effects on the spore destruction as shown in Fig. 9. Somavat, Kamonpatana, et al. (2012) and Somavat, Mohamed, et al. (2012) hypothesized that vibration and release of dipicolinic acid from the spore core and coat proteins at elevated temperatures backed the higher lethality of OH treatment.

A. acidoterrestris is a spoilage causing, rod-shaped, motile and sporeforming microorganism. Its D-value during OH heating was found to be considerably lower in comparison to conventional heating. On the other hand the z value (°C) decreased with increasing voltage gradient from 30 to 40 V/cm, however, very less difference was observed in the z value (°C) of 40 and 50 V/cm treatments (Baysal & Icier, 2010).

The fat content of the milk was found to have a negative relationship with the electrical conductivity (Sun et al., 2008). Electrical conductivity of whole and low-fat milk was found to be 0.39 and 0.42 S/m, respectively. The reason could be the electrically neutral nature of the fat or oil molecules. During OH by using an electric current of 20 kHz, the set temperature of 70 $^{\circ}$ C was achieved in 5 min at the center of



Fig. 9. Inactivation of *G. stearothermophilus* spores under OH (10 kHz and 60 Hz) and conventional heating at 121, 125 and 130 °C with holding times 0–120, 0–90 and 0–10 s respectively (Somavat et al., 2012).

250 ml milk sample in 30 \times 100 \times 85 mm acryl plastic vessel. The microbial reduction started during heating period and continued during the holding time. The higher microbial killing was observed with OH than conventional heating of milk. The D-value of S. thermophilus at 70, 75 and 80 °C were reported to be 7.54 \pm 0.37, 3.30 \pm 0.42 and 0.20 \pm 0.03 min during conventional and 6.59 \pm 0.35, 3.09 \pm 0.55 and 0.16 \pm 0.03 min during OH treatment, respectively (Sun et al., 2008). These results indicate the comparative difference in the effectiveness of destruction of S. thermophilus between the two methods of heating. Yıldız and Baysal (2006) indicated that complete inactivation of Aspergillus niger in tomato blend could be achieved in treatment time of 30 to 6 s by OH at voltage gradient of 36-108 V/cm, respectively. The rate of change of temperature is the function of applied voltage gradient, therefore the temperatures at which the inactivation started to occur at 36 and 108 V/cm were found to be 19 and 40 °C, respectively and resulted in rapid inactivation at higher voltage gradient.

Chilean blue mussels were treated for 4 min in 0.03 saline solutions at 50, 70 and 90 °C by OH and conventional heating. OH was conducted by applying voltage gradient of 9.15 V/cm using the direct current of 60 Hz frequency. The count of Enterobacteriaceae in untreated sample was 2.7 log CFU/g, it was reduced to 1.6 and 1.0 log CFU/g by OH and

conventional treatment (70 °C for 4 min), respectively. However, at 90 °C the number of Enterobacteriaceae was reduced to an undetectable level (less than $1.0 \log CFU/g$) by both the treatments. Due to the lower voltage gradient (9.15 V/cm) applied, non-thermal effects on the destruction of Enterobacteriaceae were not observed (Bastías et al., 2015). A MS-2 bacteriophage is single-stranded RNA virus, which infects the Ecoli and other members of the Enterobacteriaceae. Kim and Kang (2017a) examined the combined effect of pulsed OH and carvacrol on MS-2 bacteriophage in salsa. Pulsed OH at 12.1 V/cm and 60 Hz was applied to carvacrol containing salsa and log reduction of 6.20 and 4.23 were achieved after 100 s of OH with and without carvacrol, respectively. However, no significant role of carvacrol was observed in the reduction of MS-2 bacteriophage until the heating time of 90 s. In another study, Kim and Kang (2017b) evaluated the effect of oil components in combination with OH on MS-2 bacteriophage and it was found that the citral and thymol (oil components) in combination with OH had a synergistic virucidal against MS-2 bacteriophage.

5. Future scope and challenges associated with OH

The OH process has shown a potential for industrial application solely and or in combination with other techniques. The application may not be restricted to novel thermal processing method of food but also OH assisted extraction, concentration, baking and fermentation. However, there are many challenges to be understood and resolve while considering its application at industrial level. Scale up of the process in itself is a challenge in addition to the corrosive reaction of the electrodes with the material to be heated. Therefore, selection of electrode material is a very important factor while considering industrial application of OH. The electrode can be designed for specific OH conditions only, as the electro-chemical interactions of food and electrode may depend on the electric field strength, frequency and type of wave. The composition of food material is also a challenge for food processors while considering OH, because food products may contain different components with different properties e.g. electrical conductivity. OH behavior of the food material in such cases becomes complex, and may lead to under heating of component with low electrical conductivity or vice versa. The partial inactivation of enzymes and microbes during OH need to be studied in detail in future, Because enzymes and microbes may activate again once get the favorable conditions probably during storage and may put the safety at risk hence further studies need to be conducted to evaluate the partially injured microbes and enzymes during OH.

6. Conclusion

Ohmic heating heats the food material rapidly and uniformly by following the Joule's law of heating and it is possible to heat the material volumetrically and efficiently. OH behavior depends upon various properties of food material such as the amount of charged ions present, viscosity, TSS, pH, fat content, particle size etc. The present review concludes that the inactivation of undesirable enzymes and microbes of different food material can be achieved in shorter time during OH as compared to that of conventional heating. The enzyme inactivation during OH is mainly due to the thermal effects of OH treatment. However, enzymes (such as PPO, Lipoxigenase, Phospatase) which contain metallic prosthetic group undergo some non-thermal (electrical) effects during OH causing higher degradation as compared to that of conventional heating. In addition to thermal injuries, some non thermal effects such as chemical changes and electroporation of cell membrane are also responsible for microbial killings during OH. The frequency and voltage gradient has a direct relationship with the reduction of microorganism. Higher microbial destruction can be achieved at lower pH by OH. Fat content restricts the rate of OH, therefore shows negative effects on the microbial inactivation during OH. Very less or negligible non-thermal effects appear on microbial

destruction while using OH at lower voltage gradient. Therefore, because of numerous advantages such as quick and volumetric heating, high energy efficiency, easy to control, rapid microbial and enzyme inactivation etc. ohmic heating process has a great scope as a potential alternative to the conventional methods of thermal processing of food.

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