Chapter II

Review of Literature

Chapter-II

REVIEW OF LITERATURE

This chapter includes a detailed review of recent research conducted on Ohmic heating and its application on various food materials. The emphasis is given on the effects of OH on important quality parameters of food, PPO and POD enzymes and microbes, further this chapter include some interesting findings while processing mango pulp with the application of conventional and novel food processing methods. Conventionally the mango products such as mango puree are processed by heat treatment or commonly known as thermal processing. Although thermal processing is still the most common and widely used processing method of mango puree, however due to many drawbacks of the thermal processing such as overheating, energy and time deficient opens the scope for development of novel processing methods to overcome such drawbacks. Ohmic heating is a novel thermal processing method, which heats food material rapidly, informally and utilizes maximum energy thus it is an energy efficient processing method.

2.1 Ohmic heating (OH) process

Ohmic heating (OH) is a food processing operation during which electric current is allowed to pass through the food material thus resulting in its volumetric heating due to Joules effect [35], it is also known as Joules heating or electric resistance heating. The main parts of OH assembly include heating chamber, electrodes, voltage and current measuring devise, temperature measuring and controlling devise, electric power supply, variac transformer and data (Temperature, current and voltage) acquisitioning system [93, 32, 47]. In solid liquid mixture type of foods, should the electrical properties essentially the electrical conductivity of particulate and liquid phase be similar, OH process enables solid particles to heat as fast as liquids. The rapid heating and similar heating of particulate and liquid phase makes possible to use OH process as High Temperature Short Time sterilization techniques on particulate foods [35]. The advantage of OH process is that it heats the food material rapidly and uniformly, without the presence of hot wall and temperature gradient therefore reduces the overheating and fouling. In OH process, heat gets generated unlike conventional thermal processing where heat is being transformed from heating medium to the food. Hence this process is time and energy efficient, and easy to control and monitor. Ohmic heating can be useful for producing high quality food with a shelf life comparable to that of canned, sterile and aseptically processed products [93].

Qihua et al. [73] designed an experimental setup using single phase power supply (220 V, 50 Hz) to study the performance of static and continuous OH process of 0.1 M NaCl solution and orange juice. The general performance of the OH system was found to be good. The static process could heat the food at a rate of 1 to 4 °C/s at electric field strength (EFS) of 40 V/cm. The heating was uniform for all practical purposes and led to the measurement of electrical conductivity of liquid as a function of temperature. At elevated temperatures the bubble generation was emphasized to give a serious consideration. Ghnimi et al. [27] designed and build an automated OH setup; the setup was consisted of three different sections, a pre-heater, heating zone and cooling zone. The thermal performances and the technical feasibility of this innovated automated ohmic heater were approved. The performance of OH assisted vacuum evaporation of pomegranate juice was evaluated by Cokgezme et al. [21]. The exergy efficiency increased with increasing the EFS at specific TSS, finally it was recommended that OH assisted vacuum evaporation used energy more efficiently than simple vacuum evaporation for the concentration purpose of pomegranate juice. Bozkurt and Icier [12] also applied the exergy approach for the performance evaluation of cooking process of cylindrical shaped beef samples containing different fat levels. The energy and exergy efficiency of OH cooking was reported to be in the range of 69-91% and 63.2-89.2% respectively.

2.2 Ohmic heating and electrical conductivity (EC)

There are many factors which affect the OH rate and behavior of the food material. The factors include EC of fluid and particles (if present), specific heat, particle size, shape and concentration as well as particle orientation in the electric field [101]. Electrical conductivity is the most critical parameter for heating behavior and heat generation during OH process of any food material [94].

The common method of calculating the EC during OH is mentioned in Eq. (2.1) [101, 56, 17, 39], it is determined by the geometry of the electrodes.

$$\mathrm{EC} = \frac{\mathrm{I}}{\mathrm{V}} \times \frac{\mathrm{L}}{\mathrm{A}} \qquad \text{Eq. (2.1)}$$

In conventional heating system, the thermal conductivity of a particle controls its heating rate, whereas in an OH, the EC is the controlling factor. The change in EC of food may be due to many reasons such as dissolution of cell wall components such as protopectin and hemicelluloses, loss of intercellular adhesion and rigidity of cell. The cell lysis due to breakage of cell wall components makes efflux of cytoplasmic components and influx of the surrounding fluid possible. Loss of non conductive gas bubbles may also occur from the structure of the food material. Thus EC may either increase or decrease depending upon the conductivity of internal and external fluids [33].

The EC strongly depends on the temperature of the material [38], the EC was increasing linearly with the temperature during heating of pomegranate juice [22, 36]. However, Castro et al. [17] studied strawberry puree and reported that EC may increases with temperature, in every case, although it may not be a linear relation always. While studying the EC of apple and sour cherry concentrate, Icier and Ilicai [37] found that, in addition to the temperature and EFS the concentration also plays a critical role in determining the EC of a material [39]. Electrical conductivity decreases with solid content but the decrease is more significant for the bigger size particles tested. The heating time and overall EC of two phase food system was reported to get influenced by the concentration and particle size [101]. The addition of salt increased the conductivity thus decreased the OH times of meat batters and higher levels of added fat in the batters had a reducing effect on the conductivity [82]. Srivastav and Roy [86] reported that the rate of change of the EC of tomato juice with temperature at EFS of 70 V/cm was higher as compared to 50-60 V/cm. The EFS was statistically significant on EC and system performance coefficient. Another study found that the rate of temperature change was higher in apricot puree than that of peach puree, and for the design of ohmic heaters the EC and system performance coefficients was found to be important [38].

2.3 Effects of ohmic heating on quality characteristics of food

Rheological parameters of pomegranate juice were determined for OH and conventional heating at 90 °C. It was confirmed that the heating process and any interaction related with hating time did not affect the rheological properties of pomegranate [98]. Bozkurt and Icier [13] investigated the effects of OH and conventionally heating on consistency coefficient of whole liquid egg. The liquid egg exhibited the shear thinning behavior or the flow behavior index (n) values were less than unity. The consistency coefficient values were in the range of 0.0074–0.0132 and 0.0071–

0.0131 for OH and conventional heating respectively, and it was decreased up to 50 °C however due to structural changes of proteins the consistency coefficient increased sharply at 60 °C. However the apparent viscosity of ohmically heated liquid egg was less sensible to temperature.

An and King [7] found that low voltage (20V) OH caused higher gelatinization of four different starches than that of conventional heating. Therefore, OH could be used to produced starch and flours with different cooking characteristics and different stabilities to retrogradation. Further, An and King [8] reported that ohmic treatment may cause hydrolysis of starch resulting from electroporation. Thus, OH made commercial rice starch swell faster and resulted in different pasting characteristics for rice starch and rice flours than conventional heating. The electrical conductivity of starch solutions changes synchronously with gelatinization therefore, Wang and Sastry [91] noticed the potential of quantifying starch gelatinization by electrical conductivity changes, hence suggested the application of OH device as an on-line sensor to monitor gelatinization. Ohmic heating at low frequency is very effective technology to improve texture agricultural products. It causes electroporation and resulting reduction of impedance of Japanese white radish membrane [42]. Non-thermal permealization was observed by Sensoy and Sastry [81] during OH blanching of mushrooms, The permealization resulted in higher weight loss, however OH doesn’t require large amount of water for the blanching purpose of mushrooms. Shirsat et al. [82] cooked meat emulsion batter by OH and steaming methods to similar cooking value or end point temperatures. Texture profile parameters were found to be different between the two heating methods. Fish protein gel prepared with starch were investigated during OH by Chai and Park [18]. The properties of the gel were different for OH and conventional heating. The gels heated rapidly by OH were had greater gel strength than conventionally cooked gels.

The change in color of acerola pulp during OH was studied by Mercali et al. [60], they observed that using lower frequency leads to higher color changes in acerola pulp as compared to the higher frequency, possibly because of the electro-chemical reactions. Whereas, Yildiz et al. [99] reported that the color parameters of spinach puree were unaffected by the EFS at a constant temperature but changing the temperature at a specific EFS results in change of color parameters. Bhat et al. [10] reported that green color of bottle guard was retained by OH to greater extent than that of conventional heating. The OH and conventional heating was reported to cause browning of the orange juice [50]. The meat batter emulsion cooked rapidly by OH at higher EFS showed less changes in value of hue and ‘a’ as compared to that of cooked at slowly by steam or OH at lower EFS [82]. The effects of OH on anthocyanin, ascorbic acid, phenols and β-carotene were studied by Sarkis et al. [76], Mercali et al. [60], Yildiz et al. [98] and Yildiz et al. [99] in blue berry pulp, acerola pulp, pomegranate juice and spinach puree respectively. Higher anthocyanin content degradation was observed in blueberry pulp during OH treatment at higher EFS, when compared with OH at lower EFS and conventional treatment. The electrochemical changes at lower frequency resulted in higher ascorbic acid degradation in acerola pulp; the degradation can be reduced significantly by using frequency higher than 100 Hz. The reason for such degradation was reported to be the generation of oxygen in the medium by the hydrolysis of water present in pulp, and this oxygen catalyzes the degradation pathway [60]. In addition, Lima et al. [53] reported that ascorbic acid degradation in orange juice followed first order kinetics during both conventional and ohmic treatments. Electrolytic reactions appear to occur when using stainless steel electrodes, but are not evident with specially coated titanium electrodes. The OH and conventional heating of pomegranate juice resulted in the increase in the phenolic compound content; however the increase was insignificant among the method of heating [98]. Yildiz et al. [99] observed that the β-carotene increased during the OH of spinach when held for 10 min, the increase is believed to be due increasing of bioavailability of phenolic compounds. The increase was reported be higher while heating the puree at higher EFS, and this may be due to the higher cell wall rupture at higher EFS thus making greater amount of β-carotene available, and similar results were obtained for the chlorophyll. Aonla (*Emblica officinalis*) pulp was observed to retain higher quality characteristics (Vitamin C, tannins, color and acidity) when treated with OH at 90 °C for 1 min [83].

Soy milk contains nutritional inhibitors namely Trypsin and ChemoTrypsin, Lu et al. [58] reported that OH could accelerate inactivation of these inhibitors with lower electric energy consumption as compared to induction and conventional heating methods. ohmic heating had both electrochemical and thermal effects, OH had an inhibitory effect on SH (Sulphur-Hydrogen bond) loss, which might be due to its electrochemical reduction effect on SS bonds. Ohmic heating also slightly enhanced the formation of protein aggregates.

2.4 Various processes assisted by ohmic heating

In addition to the thermal processing of food material OH has been tested to assist many processes such as extraction [66, 57, 23, 74], juice expression [69], drying [48, 34, 102], hydro-distillation [80, 25], rice bran stabilization [46], diffusion [54], Lye peeling [32]. The OH treatment was found to be efficient, time efficient and environmental-friendly method for the extraction of pectin from passion fruit feel. The pectin extracted was having high degree of esterification and galacturonic acid content [23]. Saberian et al. [74] extracted the pectin from orange juice waste by the assistance of OH, significantly greater amount of pectin was extracted by OH than conventional heating due to more intensive cell wall rupturing. The OH at higher EFS was found to extract pectin with higher emulsifying activity. Under identical thermal profiles, as compared to conventional heating, OH assisted extraction yielded enhanced recovery of phyto-chemicals from colored potato. Even. The increase of extraction yields with application of OH support the hypothesis of non-thermal electroporation effect of vegetable cell wall, through electrical disturbances [66]. Ohmic heating assisted solvent extraction was found to be promising for the preparation of natural colorant powder from balck pigmented rice. Higher yield of colorant was obtained by OH as compared to steam-assisted solvent extraction methods [57]. Even at mild temperatures the juice recovery was enhanced by OH during juice expression of apple and potato tissue; however the OH process was observed to be energy consuming when compared with the pulse electric field assisted expression. Additionally it was found that that tissue disintegration depends on several treatment parameters (field intensity, temperature and time duration) and type of plant tissue [69]. Ohmic heating enhance the carrot juice recovery, the increment in juice recovery with OH was up to 13.76 %. The OH does not cause much change in the color, but may reduce the β-carotene content [70]. Wang and Sastry [92] found that lowering the frequency resulted greater moisture diffusion, which resulted in high juice yield from apple tissue. The OH pretreated samples also showed less mechanical resistance possibly due to breakdown of cell membrane and wall constituents. Another study on beet root showed that the volume of beet dye diffusing into solution was significantly enhanced during OH at 42 and 58°C however increasing the temperature to 72 °C didn’t cause any such increase [54].

Ohmically heated rice bran samples yielded 49–92% of total lipids through liquid extraction as compared to 53% of total lipids with untreated samples. Lowering the frequency of alternating current increased the extraction yields, possibly due to electroporation effects that may be more pronounced at lower frequencies due to cell wall charge build up [46]. OH assisted hydro-distillation increased the concentration of ethanol up to 84.3% v/v, as compared to 76% in conventional hydro-distillation in the same concentration time [25]. Similar reports were reported by Seidi Damyeh and Niakousari [80] during the extraction of *Pulicaria undulate* essential oil by OH assisted hydro-distillation. The extraction time was shortened and the yield was increased with lower energy consumption as compared to conventional hydro-distillation. On top of that the quality of the essential oil such as antioxidant and antimicrobial activity was more effective in oils extracted by OH assisted hydro-distillation.

Drying time of tomato paste was reduced to an extent of 80–97% by OH when compared with that of hot air drying. Minimum specific energy consumption (3.8 MJ/kg water) and maximum pre-drying efficiency (83.8 %) was obtained at 16 V/cm EFS levels [34]. Zhong and Lima [102] reported that OH resulted in reduction of 22–24% in vacuum drying time. In addition to the drying time Lebovka et al. [48] reported that OH pretreatment helps in lowering the drying temperature a for potato tissue approximately by 20 °C. While comparing microwave and OH pre-treatment for the electro thermal effects Wang and Sastry [94] found that ohmic pretreatment provided the greatest increasing in drying rate, especially at the high-temperature level, while for microwave pretreatment the effect was limited. Soghani et al. [84] studied the effects of OH blanching on the microwave drying of white mushrooms; it was found that the mass diffusivity increased with the EFS of OH treatment, the blanching duration and voltage had a significant effect on mass loss, heat transfer, variations of electrical conductivity, and energy consumption during blanching process.

Recently, Gupta and Sastry [34] studied the OH assisted lye peeling of pear, it was found that the presence of electric field resulted in enhanced peeling effect and lowers the concentration requirements of lye. The diffusion of NaOH was also reported to increase by OH, therefore peeling time of tomato was also reduced by the application of OH [96]. Sweet whey samples were subjected to OH and conventional heating by Costa et al. [21] and observed that lower electric field intensity during OH resulted in higher release of bioactive peptides. The OH caused least damage to the rheology and sensory parameters of the sweet whey.

2.5 Effects of ohmic heating on enzymes and microbes

Enzymes are proteins that speed up selective chemical reactions, therefore, in other words, they are also considered as highly specific biological catalysts [47]. 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, off-tastes development, changes in rheological properties and color [90]. 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 [16]. Many studies have been conducted on effects of OH on Polyphenol oxidase (PPO) and Peroxidase (POD) enzymes preset in various food materials as mentioned Table 2.1.

2.5.1 Effect of OH on Polyphenol oxidase (PPO)

Fruits and vegetables contain various phenols and at the same time they get 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 forces and the compounds come into contact with air (oxygen) [85]. Hence, it is expected that an increase in PPO activity occurs after peeling and cutting of fruits and vegetables. Thet thermal inactivation of PPO is one of the primary objectives during blanching process of fruits and vegetables. Lespinard et al. [52] and Brochier et al. [15] 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 [73].

PPOs are nuclear-encoded copper metal-o-proteins and their molecular mass is approximately 59 kDa [16]. Generally, an exposure to a temperature of 70–90 °C is optimum to destroy the catalytic activity of PPO [20]. Pham et al. [67] studied indirectly OH at 20, 30 and 40 V/cm voltage gradient, to inactivate PPO in ready to eat pineapple wedges packed in a plastic pouch. The PPO activity of untreated wedges was found 27.25 units, which was reduced to undetectable values by the indirect ohmic treatment of 80 °C at 20-40 V/cm or 70°C at 30-40 V/cm. Finally, it was recommended that the indirect OH at 30 V/cm, for 1 min at a temperature of 70 °C was optimum for treating ready to eat pineapple wedges packed in polypropylene pouches.

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 [24]. Therefore, Delfiya et al. [24] studied the effects of OH at the voltage gradients of 15 and 20 V/cm (heated up to 80, 90 and 100 °C at each voltage gradients) on the PPO activity in tender coconut water. The electrical conductivity of the tender coconut water was found in the range between 0.015 and 0.035 S/m. The PPO activity was decreased ($p \leq 0.01$) by increasing the EFS from 15 to 20 V/cm and holding time from 0 to 3 min. Tender coconut water heated for 3 min at 80 °C at a EFS of 20 V/cm was found to have minimum PPO activity, Abedelmaksoud et al. [1] also reported that due to higher PPO inactivation the apple juice quality can be retained to higher level by using OH (80 °C using 40 V/cm) as compared to conventional heating, both the temperature and EFS was found to have a combined effect on PPO inactivation. Saxena et al. [77] also studied PPO activity during OH of sugarcane juice, the PPO activity was found to get affected significantly ($p<0.01$) by temperature, holding time, EFS and their interaction. The maximum inactivation was observed at 32 V/cm at 90 °C held for 5 min. Overall, at 60 °C there was a decrease in residual PPO activity with increasing voltage gradient; at 70 and 80 °C, a significant increase in the residual PPO activity was observed from 24 to 32 V/cm following a decreased PPO activity at 48 V/cm and at 90 °C, the residual PPO activity observed an increase at 48 V/cm [77]. 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 [78]. For the PPO inactivation, the biphasic model (Eq.2.2) was found to be the best during OH treatment of sugarcane juice. According to this model, PPO of sugarcane juice consists of the labile and the stable fractions [77].

$$\frac{A_t}{A_o} = A_L e^{(-k_L t)} + A_S e^{(-k_S t)} \qquad \text{Eq. (2.2)}$$

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 the treatment time (min).

Moreno et al. [62] studied inactivation of PPO in apple (cv. *Granny Smith*) cubes by applying a combination of OH and osmotic dehydration with vacuum impregnation. Ohmic heating in combination with vacuum impregnation was found to reduce PPO activity more than 2 times greater than that of the conventional method. During storage of the apple cubes at 5 °C, lower recovery in PPO activity was observed by applying OH in combination vacuum impregnation than 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 [44]. The presence of electric field during OH reduced the time of PPO inactivation, therefore ohmic and conventional heating resulted in significantly different z values of PPO inactivation in apple (cv. *Golden delicious*) cubes [16]. As the similar level of PPO inactivation can be obtained with the lower thermal destruction of vitamins and pigments and fruit texture, thus increasing the final quality of the products as compared to conventional thermal processing. PPO inactivation was found to be rapid by the application of OH by Makroo et al. [59] in watermelon juice. However a slight increase in PPO activity in the later phase of OH observed, such behavior could arise due to the change of conformation of the enzyme which results in higher enzyme-substrate interaction and consequently, an optimal consumption of the substrate occurs.

Icier et al. [41] conducted a study on PPO inactivation during OH of grape juice. The study was conducted in two parts; firstly change in PPO activity was analyzed during heating from 20 °C to 60, 70, 80, and 90 °C at voltage gradients of 20, 30 and 40 V/cm. Whereas in the second part, the effect of holding time of 5, 10, 15, 20 and 25 min at 30 V/cm was evaluated. As the temperature was raised from 20 to 60 °C an increase in PPO activity was observed at all the voltage gradients. Maximum activity of PPO was found at 70 °C during heating at 20 and 30 V/cm, however; at 40 V/cm, it was observed to be at 60 °C. Initially, a slight increase in the activity was observed with holding time at 60 °C until deactivation started after 15 min. Saxena et al. [77] have also reported similar observations during PPO inactivation in sugarcane juice by OH. The effect of temperature, holding time and their interaction on the PPO activity in grape juice was significant at 95% confidence level.PPO activity was found to be significantly lower at 40 V/cm than at 20 or 30 V/cm at 70 and 80 °C and the critical temperature at which the inactivation starts was also found to be lower during heating at higher EFS when compared with treatment at lower voltage gradients [41]. Guida et al. [30] studied the comparison of OH and conventional heating, they reported that holding time of 6 min for OH (24 V/cm, 80±2 °C) and 480 s for conventional heating (100 °C, medium temperature) was required to achieve total inactivation of PPO in artichoke heads having initial PPO activity of 7.8 ± 0.69 U/mg. Additionally, it was found that the PPO Inactivation was relatively faster than that of POD.

Kinetic study of PPO inactivation was found to follow the first order (Eq.2.3) in which the enzyme deactivation is assumed to be a single step irreversible first-order reaction [16, 41, 59].

$$\mathrm{E} \xrightarrow{\mathrm{k_1}} \mathrm{D}$$

$$a = \frac{C}{C_0} = e^{-k_1 t} \qquad \text{Eq. (2.3)}$$

Where, C_0 and C are values of enzyme activity at initial or zero holding time and at any time (t), respectively. The coefficient k_1 represented the first-order rate constant for a one-step irreversible transition of the native enzyme (E) into an inactive form of enzyme (D).

2.5.2 Effect of ohmic heating on Peroxidases (POD)

Peroxidase (POD) is a heme containingenzyme, which catalyzes a large number of reactions in which it is reduced while an electron donor is oxidized [44]. POD’s having maximum resistance to the heat than any other enzyme they are generally used as a biological indicator of the blanching process in vegetables. Therefore their inactivation by blanching or any other processing method indicates the inactivation of quality degrading enzymes [30]. Icier et al [40] investigated critical time of POD inactivation during OH of pea puree at different voltage gradients (20, 30, 40 and 50 V/cm) at a fixed frequency of 50 Hz, and the results were compared with the water blanching. Pea puree was heated from 30 to 100 °C during both the treatments. Peroxidase inactivation time was significantly affected by EFS and critical inactivation times during ohmic blanching were statistically different from water blanching, which implies the non-thermal effects of OH on POD inactivation [40].

Comparison of POD inactivation between conventional and OH of broccoli, potato and carrot were studied by Jakob et al. [44]. During conventional heating, the kinetics of POD in broccoli is described by a simple isozyme mechanism whereas in carrot and potato inactivation of one isozyme is described by the Lumry–Eyring mechanism [68]. The heat treatment of 58-74 °C was applied to broccoli and potato whereas due to the higher thermal stability of POD in carrot, it was treated at 62-78 °C. In the first phase of POD inactivation no major changes were observed between the OH and conventional method. However, in the second phase, OH showed faster POD inactivation. Jakob et al. [44] observed that the rate constants at the reference temperature were different while as the activation energies of the irreversible POD inactivation was essentially same for OH and conventional heating method. The rate constants (k) during OH, k_{30}, for potato POD was twice larger, k_{20} for broccoli POD was 3 times larger, and k_{30} for carrot POD was 10 times larger than the respective conventional heating.

Sugarcane juice (pH, 5.36±0.05 and electrical conductivity 2.022 $mScm^{-1}$, at 20 °C) with an initial POD activity of 4631 ± 469 UEA min^{-1} $g^{-1}$was heated by ohmic and conventional heating (60, 70, 75 and 80 °C) by Brochier et al. [15]. At 60 °C, POD showed significant increase in its activity, probably because the temperature of 60 °C falls in the optimum temperature range for the functionality of most of the enzymes, this increase in 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 gets influenced by low-intensity electric field [15]. Such non-thermal effects of OH or mild electric field treatment have also been reported by Samaranayake and Sastry [75]. As Brochier et al. [14] didn’t find any non-thermal effect on the POD inactivation in sugarcane juice during OH, however, different wave forms and change in frequency influenced the enzyme inactivation. The effects of OH were not fully understood thus recommended for further investigation, since food matrix significantly influences the electric field effect during OH. Guida et al. [30] studied the POD inactivation by conventional and ohmic blanching of artichoke heads. Due to the higher thermal resistance, the rate of POD inactivation was relatively lower as that of PPO by either of the heating methods in artichoke heads. Possibly due to electric effects of OH it requires only 6 min to achieve similar inactivation achieved by conventional blanching in 8 min, Gomes et al. [26] also suggested that the higher POD reduction occurs during OH as compared to that of conventional heating of *Tetsukabuto* pumpkin.

2.5.3 Effect of ohmic heating on Microbes

Spoilage causing microbial growth develops off-flavors and off-odors, which renders the food product unacceptable for human consumption. Almost all groups of microorganisms initially present on a food product can contribute to spoilage of foods, on nutrient composition and chemical as well as physical parameters [27]. 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, seafood etc [62,50,51,88,9,3]. Moreno et al. [62] 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 ohmically heated to 30, 40 and 50 °C for 90 min by using EFS of 13 V/cm at 60 Hz frequency. All the treatment conditions were found sufficient to restrict the number of mesophilic aerobic bacteria below the permissible limit of 105 CFU/g [61] during the storage at 5 and 10 °C for 37 days [62]. Blue mussels were blanched at 50, 70 and 90°C for 4 min by ohmic and conventional heating method. 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 EFS of 9.15 V/cm. Ohmic and conventional blanching resulted in a similar reduction in aerobic mesophiles at during both blanching methods. However, conventional heating was found 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 [3]. No significant difference was found in reducing the mesophilic aerobic bacteria at the same blanching temperature between the OH and conventional blanching [9]. Leizerson and Shimoni [50] reported similar findings during comparison of ohmic 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 EFS applied [65,92]. Probably for the same reasons Leizerson and Shimoni [51] didn’t find any extra effects of OH on microbial quality of ohmically treated orange juice during storage study of 105 days as compared to that of conventionally treated. After evaluating microbial quality of many ohmically heated stew type foods, Yang et al. [97] concluded that OH has a potential to maintain the quality of food to maximum limits after processing and during storage.

2.6 Mango pulp/puree and its characteristics

Mango (*Mangifera indica L.*) fruit belongs to the family of *Anacardiaceae* in the order of *Sapindales*. It is mostly grown in the tropical countries and more than 1000 varieties of mangoes are available worldwide [43]. Around 87 countries produce the mangoes however the major producers are India, China, Thailand, Indonesia, Philippines, Pakistan, and Mexico [64]. Mango can be considered a good source of dietary antioxidants, it is one of the highly accepted fruit worldwide due to its exotic taste, flavor and excellent nutritional value in terms of bioactive compounds such as bioactive compounds such as the carotenes, vitamin C and phenolic compounds [63,72]. As it contains both carotenes as well as polyphenols it is considered as an essential source of natural antioxidants [79]. Mango is processed into various products such as mango puree, slices in syrup, nectar, leather, pickles, canned slices and chutney are the main industrial products obtained from mango fruits. Puree is one of the important intermediate mango products, which is thermally processed to enhance its shelf life and stored for the manufacture of beverage [5]. Mango pulp or puree are the most popular mango products and are generally preserved as a canned product by subjecting them to thermal processing to extend their year-round availability [31]. Physical properties of mango pulp such as density, thermal conductivity and heat capacity were strongly depending on temperature and have a multivariable relationship with each other [11]. Due to its highly viscous nature, many studies have been performed on its rheology [35]. The flow behavior of mango puree is best described by the Herschel-Bulkley model, and it depends upon its soluble solids content [28].

Table 2.1 Studies conducted on effects of ohmic heating on PPO and POD

Enzyme	Food Material	Reference	Interesting Observations/Findings
Polyphenol oxidase (1.14.18.1)	Pineapple	Pham et al. [67]	Ready to eat pineapple packed in PP pouches, can be processed by indirect OH at 30 V/cm at 70 °C for and 1 min holding
	Tender coconut water	Delfiya and Thangavel [24]	OH didn't lead to any significant change in pH, acidity and color of the tender coconut water and an increase of 5 V/cm EFS significantly decreased the PPO activity
	Artichoke heads	Guida et al. [30]	Higher PPO and POD deactivation can be accomplished by OH. This color change in artichoke heads due to enzymatic browning can be controlled in a superior way by OH as compared to conventional heating
	Sugarcane juice	Saxena et al. [78]	In sugarcane juice, 10 min at 80 °C of conventional heating resulted in a residual PPO activity of 6.47±0.25 %, whereas PPO activity was reduced to 10.07±0.32 % in ten times shorter time during OH at 32 V/cm at the same temperature.
		Abhilasha and Pal [3]	Ohmic heating for 3min at 70 °C was reported to be best when considering inactivation of PPO, change in color and destruction of microbial load.
	Apple (cv. Grany Smith)	Moreno et al. [62]	OH was found helpful in complete inactivation of PPO in apples treated with OH assisted osmotic dehydration and OH assisted vacuum impregnation. Also, OH assisted treatments were proved to help in reducing color change during storage
	Apple (cv. Golden delicious)	Castro et al. [16]	The electric field of OH process reduces the D value of PPO enzyme. Hence, OH was found to be more effective enzyme inactivation method as compared to conventional one.
	Watermelon juice	Makroo et al. [59]	OH method inactivated PPO in watermelon juice rapidly as compared to conventional heating, however, slight increase was observed at initial stages in OH due to the presence of electric field
	Grape juice	Icier et al. [41]	Critical deactivation temperature of PPO was studied in grape juice. The critical temperature at 40 V/cm was observed to be lower than at 20 and 20 V/cm. At constant EFS and 60 °C minimum 15 minutes were required to cause the deactivation in PPO of grape juice
Peroxidase (1.11.1)	Pea puree	Icier et al. [40]	Ohmic blanching was more effective than hot water blanching of pea puree. The color change during ohmic blanching was satisfactorily described by first-order reaction kinetics
	Broccoli, potato and carrot	Jakób et al. [44]	POD deactivation in sugarcane juice showed a distinct behavior during OH. EFS caused an increase in the enzyme activity near optimum temperature (60 °C). At 70-75 °C only thermal inactivation was observed however at 80 °C EFS showed its additional (non-thermal) effects on the POD inactivation
	Sugarcane juice	Brochier et al. [15]	POD showed variation in inactivation behavior at different temperatures. Also, no non-thermal effects were observed in PPO inactivation during OH treatment
		Brochier et al. [14]	Ohmic heating 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 to105 Hz did not influence the inactivation causing biochemical reactions associated with POD in sugarcane juice
	Tetsukabuto pumpkin	Gomes et al. [26]	Mathematical model (Weibull distribution) of experimental data predicted the OH causes higher POD inactivation as compare to that of conventional heating

The major reason for the quality deterioration and spoilage of mango products is microbial and enzymatic degradation. Therefore, the main objective of mango products processing is to reduce the activity of spoilage causing microbes and enzymes [64]. PPO and POD are the two major enzymes present in mango pulp responsible for its deterioration, POD is used an indicator of thermal processing for enzyme inactivation, as it has the thermal resistance. Residual POD activity of 0.78 to 0.043 was achieved in mango puree by thermal treatment at 65-85 °C using shell and tube and plate heat exchanger [87]. In addition to the microbial or enzymatic spoilage there may occur many reactions causing undesirable quality changes in mango products during storage such as acid- catalysed reactions (e.g., terpene rearrangement, hydrolysis of esters and hydrolysis of sugars), ascorbic acid degradation and oxidation reactions [95].

2.7 Mango processing

Thermal processing is the most common and old method of mango products processing, the conventional thermal processing has many drawbacks such as quality deterioration, time and energy deficient. Thermal treatment for 4 min at 90 °C caused complete inactivation of PPO in mango puree [29]. The thermal treatment enhanced caramelized, cooked, and vegetable flavors and eliminated throat irritation and tongue burn. Thermal processing results in significant decrease in identity flavor and causes changes in the texture of the mango puree [49]. It was reported to cause *tans-cis* isomerization of β-carotenes. However, 93 % total β-carotenes and 84.5% vitamin A of was retained by the time nearly 5 % POD activity was achieved. Thermal treatment caused browning of the puree as the value of color parameter ‘b’ was found to decrease with the treatment time [5]. Thermal treatment also results in disintegration of mango matrix [89]. Therefore in addition to thermal processing many researchers worked on to find alternative to conventional thermal processing method to overcome various drawbacks of thermal processing [71]. Akhtar et al. [6] studied the chemically preserved mango pulp and found that the amount of protein and fats decreased however the mineral content and TSS increased during the storage. The microbial population and acidity significantly increased with storage time. Pulsed light treatment was used to maintain nutritional quality of fresh cut mango slices by Charles et al. [19]. The color, firmness and carotenoid content were maintained by the treatment, however the PPO activity was found to increase after 3 days during storage. For the nutritional aspect, pulsed light maintained phenol and total ascorbic acid contents. PPO was completely inactivated in mango juice with the help of ohmic heating. The heating caused reduction and slight increase in the ascorbic acid and phenols of the juice respectively [1]. The effect of high pressure treatment on the rheological behavior and color of the mango pulp were analyzed by Ahmed et al. [4]. No significant change was witnessed in the color of mango pulp due to HPP treatment. However fresh and canned pulp showed opposite trend in the change of viscosity, behavior index, and storage and loss moduli with increasing the pressure. Kaushik et al. [45] found that during thermally assisted HPP (TAHPP) of mango puree the temperature had the dominant effect on the quality of mango pulp followed by level of pressure and holding time. Color, flavonoids, enzyme and microbial activity was significantly affected by the TAHPP process. HPP and HTST treatment were given to mango nectars by Liu et al. [55], the HTST treatment increased the viscosity whereas it remained unchanged after the HPP treatment. HPP and HTST treatments resulted in significant decrease of total sugar, glucose and fructose and increase in HMF of mango nectars. During the storage acidity, HMF, glucose and fructose were found to increase however the viscosity was observed to decrease in both the treated samples. The shelf life of 15, 90 and 270 days under refrigerated conditions was achieved in untreated, only irradiated and in combined steamed and irradiated treated samples respectively by Youssef et al. [100]. The combination of irradiation with steam treatment enhanced the microbiological quality of the pulp.

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