Received: 10 September 2022 | Revised: 19 February 2023 | Accepted: 23 March 2023

DOI: 10.1111/1541-4337.13154

Comprehensive REVIEWS in Food Science and Food Safety WILEY

COMPREHENSIVE REVIEW

Mechanistic insights into the changes of enzyme activity in food processing under microwave irradiation

Hongwei Cao[1,2] | **Xiaoxue Wang**[1] | **Jing Liu**[3] | **Zhu Sun**[4] | **Zhiquan Yu**[4] | **Maurizio Battino**[5,6,7] | **Hesham El-Seedi**[8,9] | **Xiao Guan**[1,2]

[1]School of Health Science and Engineering, University of Shanghai for Science and Technology, Shanghai, P. R. China

[2]National Grain Industry (Urban Grain and Oil Security) Technology Innovation Center, Shanghai, P. R. China

[3]College of Information Engineering, Shanghai Maritime University, Shanghai, P. R. China

[4]Inner Mongolia Yangufang Whole Grain Industry Development Co., Ltd., Shanghai, P. R. China

[5]Department of Clinical Sciences, Polytechnic University of Marche, Ancona, Italy

[6]International Joint Research Laboratory of Intelligent Agriculture and Agri-products Processing, Jiangsu University, Zhenjiang, P. R. China

[7]Research Group on Foods, Nutritional Biochemistry and Health, Universidad Europea del Atlántico, Santander, Spain

[8]Division of Pharmacognosy, Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden

[9]International Research Center for Food Nutrition and Safety, Jiangsu University, Zhenjiang, P. R. China

Correspondence
Xiao Guan, School of Health Science and Engineering, University of Shanghai for Science and Technology, Shanghai, P. R. China.
Email: gnxo@163.com

Funding information
National Natural Science Foundation of China, Grant/Award Number: 32102140; Young Elite Scientists Sponsorship Program by CAST, Grant/Award Number: 2021QNRC001; Shanghai Sailing Program, Grant/Award Number: 20YF1433400; Domestic Science and Technology Cooperation Projects of Shanghai, Grant/Award Number: 21015801100

Abstract

Microwave (MW) and enzyme catalysis are two emerging processing tools in the field of food industry. Recently, MW has been widely utilized as a novel type of green and safe heating energy. However, the effect of MW irradiation on enzyme activity is not described clearly. The intrinsic mechanisms behind enzyme activation and inactivation remain obscure. To apply better MW to the field of enzyme catalysis, it is essential to gain insights into the mechanism of MW action on enzyme activity. This review summarizes the changes in various enzyme activity during food processing, especially under MW irradiation. The intrinsic mechanism of thermal and nonthermal effects of MW irradiation was analyzed from the perspective of enzyme reaction kinetics and spatial structure. MW irradiation temperature is a vital parameter affecting the catalytic activity of enzymes. Activation of the enzyme activity is achieved even at high MW power

NOMENCLATURE/ABBREVIATIONS: A, frequency factor (–); APX, ascorbate peroxidase; $CALB_{ex}$10000 LA, *Candida antarctica* lipase B expressed in yeast cells; CAT, catalase; Cat L, cathepsin L; CTH, conventional heating; D_R (D_1) and D_L (D_2), the time required for inactivation of 90% of the activity of the thermosensitive and thermoresistant fractions, respectively (s); D_w, the time for the first decimal reduction (s); $D_{w,\,ref}$, the time for the first decimal reduction at T_{ref} (s); E_a, energy of activation (J/mol); Fermase CALB10,000, *Candida antarctica* lipase B immobilized on microporous and hydrophobic polyacrylate beads using covalent binding; k, rate constant (min^{-1}); k_R (k_1) and k_L (k_2), the inactivation rate constants for heat-resistant and heat-labile enzyme fractions, respectively (s^{-1}); LA, lipase activity; LA QLM, thermophilic lipase QLM from *Alcaligenes* sp.; MWH, microwave heating; Novozym 435, *Candida antarctica* lipase B immobilized using an acrylic resin support; POD, peroxidase; PPO, polyphenol oxidase; SOD, superoxide dismutase; TG, transglutaminase; T_{ref}, equivalent isothermal processing time at a given reference temperature (°C); z_w, the temperature increase that reduces D_w by 90% (°C); β, the shape parameter of the Weibull distribution (–); ΔG, Gibbs free energy (kJ/mol); ΔH, enthalpy change (kJ/mol); ΔS, entropy change (kJ/mol/K); δ_T, the time for the first decimal reduction at a given temperature T (s).

Hongwei Cao and Xiaoxue Wang contributed equally to this work.

when the enzyme is operating at its optimum temperature. However, when the temperature exceeds the optimum temperature, the enzyme activity is inhibited. In addition to MW dielectric heating effect, nonthermal MW effects also alter the microenvironment of reactive system. Taken together, enzyme activity is influenced by both thermal and nonthermal MW effects.

KEYWORDS
enzyme activity, mechanism, microwave irradiation, reaction kinetics, structure

1 | INTRODUCTION

How to maintain the color, aroma, taste, and structure of the products in food processing is a very critical issue, so severe chemical reactions should be avoided (Jing, 2020). Enzyme, as an efficient, gentle, and green biocatalyst, is replacing traditional chemical agents with its unique advantages and is increasingly widely used in the food industry (Bashari et al., 2016). It is undeniable that the role of enzyme in food processing is two-sided. Some enzymes are favorable, such as esterase, which affects the ripening and flavor of fruits and vegetables and proteases and transglutaminase (TG) for food structural modification (Yang et al., 2015). However, some enzymes are detrimental to food processing. For example, endogenous polyphenol oxidase (PPO) and peroxidase (POD) initiate the browning reaction and produce undesirable flavors (Hachicha Hbaieb et al., 2017). Endogenous lipase activity (LA) and lipoxygenase (LOX) cause oxidative rancidity of oils and fats (Ramírez et al., 2016). Therefore, it is necessary not only to make use of the beneficial aspects of the enzyme but also to avoid the undesirable changes caused by the enzyme, which is conducive to achieve the purpose of production.

Traditionally, conventional (CT) heat treatments, including water baths, hot air, and steam, are the most commonly used methods of food processing (Tang, 2015). However, as the energy transfer is based on "heat transfer from outside to inside," it leads to uneven heating of the material, longer processing times, and greater energy consumption (Bornhorst et al., 2017). Presently, microwave (MW) as an innovative thermal processing technique is widely used in food processing. MW is a type of electromagnetic wave that oscillates when it penetrates through the food materials, thus generating heat energy (El Khaled et al., 2018). Compared with the CT conduction heating, MW instantaneously acts on each polar molecule in the material and simultaneously heats the materials in a "volumetric heating" process that significantly shortens the heating time. Electromagnetic wave penetrates into all parts of the object and generates heat, which is a benefit to improve the uniformity of heating. Due to MW only heating those materials that could absorb MW energy, promising the high thermal efficiency was acquired. Moreover, MW heating (MWH) directly uses electric energy, and there is no contaminant generated; therefore, it is a green, environmental, and clean heating technology (Costa et al., 2021).

As a kind of protein with catalytic properties, the enzyme has certain polarity. According to the principle of MWH, MW directly affects the enzyme function (Wang, Liu, et al., 2018). It was found that MW irradiation enhanced LA and stability, thereby promoting enzyme-catalyzed hydrolysis, esterification, and ester exchange reactions (Chen et al., 2016; Yadav & Lathi, 2005). However, in the last few years, the increasing number of studies demonstrated that MW irradiation was effective in inactivating most endogenous protease, PPO, and POD activity enzymes in foods (Wang et al., 2021; Yuan et al., 2021; Zhong et al., 2017). It is well known that the induction of the active site of the enzyme and the substrate is a prerequisite for enzyme catalysis (Ma et al., 2021). Owing to the diverse physical properties of specific substrates and solvents, the effect of physical field on enzyme also was disparate (Iqbal et al., 2019). Furthermore, because of the different thermal stability of the substances, the enzyme activity varies under different processing factors, such as MW processing parameters, enzyme type, and other conditions (Yuan et al., 2021). Therefore, the effect of MW irradiation on enzyme activity is not described clearly. The intrinsic mechanism behind enzyme activation and inactivation is still obscure. The current research about the mechanism of the effect of MW on enzyme activity mainly focuses on the thermal and nonthermal effects of MW (Guo et al., 2021). Thermal effect is caused by the ability of dielectric materials to absorb MW energy and convert it into heat. Dielectric properties and penetration depth are key factors affecting MWH and its thermal distribution (Clodoveo et al., 2016). Dielectric properties reflect the response of the system to MWs, thereby determining the penetration depth of MWs. Dielectric properties are influenced by the MW frequency, system composition, temperature, and other factors (Tao et al., 2020). As different types of enzymes have various dielectric

properties, MW field also has different thermal effects on them. Some researchers consider that only the thermal effect contributes to the deactivation of the enzyme because MW does not have enough energy to break covalent bonds or some secondary bonds (Cavalcante et al., 2021; Siguemoto et al., 2018). However, other researchers believe that changes in enzyme activity are affected by the nonthermal effect of MW. Nonthermal MW effect is assumed to be the result of the direct and stable interaction between MW field and specific polar molecules in the reaction medium, which is independent of the macroscopic temperature effect (Xue et al., 2021). Whether the change of enzyme activity is attributed to MW thermal effect or nonthermal effect, or the combination of the two, is also the focus of our discussion. The enzymatic properties and structure of the enzyme molecules are changed by MW treatment. The direct action of MW leads to nonequilibrium state and the rapid rise of temperature, which affects the reaction kinetics of the whole system (Herrero et al., 2008). The application of MW treatments changes the enzyme structure to a much greater extent, which was directly related to enzyme activity (Radoiu et al., 2021). Therefore, an in-depth understanding of the effects of MW irradiation on the reaction kinetics and spatial structure of enzymes plays an important role in analyzing the mechanism of MW electromagnetic fields.

Enzymatic reactions are widespread in the food industry; accordingly, it is essential to study the effect of MW treatment on enzyme systems for MW applications in food processing. However, few studies have been conducted to clearly describe the effect of MW irradiation on enzyme activity. The intrinsic mechanism behind enzyme activation and inactivation remains obscure. Therefore, the aim of this review is to explain the effects of MW irradiation on various enzyme activities in food processing and the mechanism of electromagnetic fields. The intrinsic mechanism of enzyme activation or inactivation was discussed from the perspective of enzyme reaction kinetics and spatial structure. This review provides a reference for understanding the biological effects of MW field on enzyme catalysis, which is of guiding significance for understanding the development and optimization of MW processing products.

2 | EFFECT OF MICROWAVE IRRADIATION ON ENZYME ACTIVITY

2.1 | Activation of enzyme activity under microwave irradiation

The special catalytic function of enzyme has been widely used in food processing. The addition of glucoamylase, amylases, and dextranases was used to clarify and increase the juice yield of fruit juices (Bashari et al., 2016). Streptomycin protease, papain, and alkaline protease were added to hydrolyze whey protein to reduce the antigenicity of milk protein (Izquierdo et al., 2008). Adding protease and TG changed the tissue structure and made meat tender (Cao et al., 2019). LA catalyzed hydrolysis, esterification, transesterification, transesterification, and acidolysis (Chen et al., 2016). Therefore, the demand for these enzymes with high activity is the main trend in food industry. Previous studies have shown that MW irradiation technology enhanced the catalytic activity of enzyme, effectively improving the reaction and conversion rate or yield. Table 1 summarizes some relevant studies about the effect of MW irradiation on enzyme activation in food processing.

Recent studies about the effect of MW on food-related enzyme activity mainly focused on the activation of enzyme activity by MW treatment parameters (power, time, and temperature). Appropriate MW treatment power and time effectively improved antioxidant enzyme activity and enhanced the antioxidant capacity of materials (Wang et al., 2021). For example, Bian et al. discussed the effects of different MW irradiation powers and time on the antioxidant enzyme activity in Tartary buckwheat buds. The results showed that the optimum MW irradiation condition for superoxide dismutase (SOD), catalase (CAT), POD, and ascorbate peroxidase (APX) was at the power of 300 W for 75 s, and their activities were higher than those of the control (without MW treatment) (Bian et al., 2020). It had been reported that appropriate MW irradiation enhanced the ability to eliminate free radicals of seedlings for wheat (Chen et al., 2009) and buckwheat (Li et al., 2018) and significantly improved the activity of SOD, POD, CAT, APX, GSH-Px, and other antioxidant enzymes in wheat seeds (Qiu et al., 2013). It was found that appropriate MW treatment conditions activated the enzyme activity and strengthened the gel properties of the material. Qin et al. (2016) discovered that MW treatment, with an increase of the power (0–700 W), remarkably increased the gel strength of soybean protein and wheat protein catalyzed by TG. MW pretreatment (75°C, 15 min) promoted the cross-linking reaction between whey protein isolate and TG, which enhanced the gel characteristic (Zhang et al., 2022). Analogously, compared with conventional heating (CTH) pretreatment, MWH pretreatment (90°C) promoted the formation of polymers in laccase-cross-linked α-La (Jiang et al., 2021). Upon the addition of MW-treated TG into surimi, the gel strength increased, showing that the activity of TG was indeed enhanced by MW treatment (Cao et al., 2019). TG activity was affected by MWH power and time. Low power and short time (5 W/g, 20 min) increased TG activity, which was related to the optimum temperature

TABLE 1 Key parameters and findings about the effect of microwave (MW) irradiation on enzyme activation in food processing.

Enzyme type	MW parameters	Irradiation time	Irradiation temperature (°C)	Effect	Reference
Antioxidant enzymes	300 W	75 s	25 ± 2	Enzyme activity improved	Bian et al. (2020)
TG	5 W/g	20 min	40	The activity of TG enhanced	Cao et al. (2018)
Microbial TG	30 W	1 h	30	The reaction time was reduced	Chen & Hsieh (2016)
Pronase, etc.	213 W	5 min	40–50	The efficiency of enzymatic hydrolysis increased	Izquierdo et al. (2008)
Pronase	30 W	10–20 min	40		Izquierdo et al. (2005)
α-Chymotrypsin	15 W				
LA	1.35 kW	30 s	–	The hydrolysis rate increased by 7–12 times	Bradoo et al. (2002)
Novozyme 435	–	–	70	The conversion increased from 60% to 96%	Yadav and Pawar (2012)
	50 W	8 h	60	The conversion of 92% was achieved	Bhavsar and Yadav (2018a)
	700 W	2 h	60	Higher yield (95%) in a shorter time (120 min) was achieved	Bansode and Rathod (2018)
	50 W	40 min	50	The conversion of 69.2% was achieved	Bhavsar and Yadav (2018c)
Fermase CALB1000	–	25 min	60	An equilibrium conversion of 97.1% was obtained	Khan and Rathod (2020)
Fermase CALB	700 W	10 min	45	The maximum conversion of 98.2% was obtained	Jaiswal and Rathod (2021)
$CALB_{ex}10000$	500 W	70 min	60	The highest conversion of 85% was achieved	Nhivekar and Rathod (2021)
Immobilized LA CSL	400 W	4 h	35	The CSL exhibited a satisfying enzyme activity	Wang, Zhang, Zheng et al. (2018)
Immobilized LA QLM	640 W	3 h	70	The enzyme activity was enhanced about 9.6-fold	Wang, Zhang, Zhang et al. (2018)

Abbreviations: LA, lipase activity; TG, transglutaminase.

of TG. The optimum temperature of endogenous TG with the highest enzyme activity was about 40°C. In the MW heating process, the temperature gradually reached TG's optimum reaction temperature, resulting in an increase in enzyme activity. However, the activity of TG was almost not affected by conduction heating. MW irradiation also raised the activity of LA and increased its hydrolysis rate. The rate of hydrolytic reaction for various lipases (LAs) was increased by 7–12-fold when performed in the presence of MW irradiation as compared to CTH methods (Bradoo et al., 2002). Some LAs exhibited better catalytic activity at temperatures higher than the conventional temperature (37°C). MWH was usually performed at temperatures exceeding 37°C and thus definitely led to a higher reaction rate (Chen et al., 2016). Temperature was a vital parameter affecting the catalytic activity of enzymes under MW irradiation. With the increase of MW power and time, the reaction temperature gradually increased, which reached the optimum temperature of enzyme reaction, thereby increasing the chemical reaction rate.

Novozym 435 was a thermostable agent with maximum activity in the range of 50–70°C under MW irradiation. Yadav et al. reported that Novozym 435 catalyzed transesterification of ethyl 3-phenylpropionate with *n*-butanol under controlled MW irradiation. Compared with CTH, the reaction rate changed by 1.4 times. With the rise in temperature (40–70°C), the conversion rate increased from 60% to 96% (Yadav & Pawar, 2012). MW-assisted Novozym 435 synthesis of various esters was also faster and more efficient than CTH at the same temperature (Bansode & Rathod, 2018; Bhavsar & Yadav, 2018a, 2018c). Temperature is the key factor of enzyme's esterification reaction. When the temperature was raised from 30 to 60°C, the conversion rate of *n*-butyl propionate synthesized by MW-assisted enzymatic method altered from 40% to 92%. The maximum conversion of ethyl valerate was 69.2% at 50°C. When the temperature reached 60°C, the maximum conversion rate of isoamyl butyrate was 95% within 120 min under MW irradiation. Khan et al. used Fermase CALB1000 LA to catalyze the MW synthesis of *n*-butyl palmitate and found that the maximum activity was observed at 60°C for 25 min (Khan & Rathod, 2020). When ethyl laurate was synthesized at 35–45°C, the conversion boosted from 65.98% to 98.01% in 10 min (Jaiswal & Rathod, 2021). The $CALB_{ex}$10000 LA catalyzed the synthesis of polyethylene glycol stearate, and the maximum conversion was 85% at 60°C for 70 min (Nhivekar & Rathod, 2021). It was also found that the enzyme activity was heightened with the increase in the MW power. In the LA-catalyzed regioselective acylation of resveratrol, the enzyme activity was the highest when the power was 400 W (Wang, Zhang, Zheng, et al., 2018). LA QLM had the highest activity at 640 W. The immobilized QLM exhibited high enzyme activity in the temperature range of 65–80°C, and its optimal temperature was 70°C (Wang, Zhang, Zhang, et al., 2018). High power caused faster movements of the molecules that in turn increased the reaction temperature (Young et al., 2008). Therefore, we have reason to believe that the activation of the enzyme can be conspicuously achieved when the MW radiation temperature is controlled within the optimal temperature range for the enzyme.

2.2 | Inactivation of enzyme activity under microwave irradiation

During the storage and processing of food, the presence of some enzymatic activity causes oxidation, discoloration, and spoilage of many food products. For example, PPO initiates the browning reaction during fruit and vegetable processing. POD induces undesirable changes in the color, flavor, and texture of food. LA and LOX render the oxidative rancidity of oil-processing raw materials and products. Therefore, the inactivation by thermal processing of spoilage enzymes that trigger negative changes in foods during processing and storage has become an important method to improve product quality. Compared to CTH treatment, MWH offers many advantages in effectively inactivating enzymes and maintaining the freshness and nutritional and organoleptic qualities of foods (Xanthakis et al., 2018). Table 2 summarizes some relevant studies about the effect of MW irradiation on enzyme inactivation in food processing.

It was found that MW irradiation effectively reduced the activity of various oxidases, thereby preventing the browning reaction. Yuan et al. (2021) studied the changes in PPO activity during wine impregnation under different MW treatment conditions. With the extension of power, temperature, and heating time, the inactivation rate of PPO activity markedly goes up. Under the conditions of 500 W, 50°C, and 8 min, the PPO activity was reduced by 39.58%. When the temperature was above 40°C, the activity of PPO began to diminish. The MW temperature exceeded 50°C, and the activity of PPO dropped dramatically, which proved that higher temperature has a substantial influence on PPO activity. When the power density was 11 W/g and treatment time was 80 s, the PPO activity of defatted soy sauce had a reduction of 80% during storage (Zhou et al., 2016). When the power amounted to 937 W, the PPO in Mamey fruit was completely inactivated at 70°C after 165 s MW treatment (Palma-Orozco et al., 2012). Lopes et al. (2015) identified that the loss of horseradish peroxidase (HRP) activity was found to grow with the rising temperature and power of the MW treatment. The minimum residual activity of HRP was only 16.9% at 60°C/60 W/30 min. Latorre et al. (2012) also found that

TABLE 2 Key parameters and findings about the effect of microwave (MW) irradiation on enzymes inactivation in food processing.

Enzyme sources	Enzyme type	MW parameters	Irradiation time	Irradiation temperature (°C)	Effect	Reference
Nigella sativa L. seeds	LA	1100 W	3.5 min	157.26	The enzyme activity was the lowest	Mazaheri et al. (2019)
Wheat germ		300 W	10 min	70	The enzyme completely inactivated	Meriles, Penci et al. (2022) and Meriles, Steffolani et al. (2022)
Grape	PPO	500 W	8 min	50	The activity decreased by 39.58%	Yuan et al. (2021)
Defatted avocado puree		11.0 W/g	80 s	70	The activity decreased by 80%	Zhou et al. (2016)
Mamey fruit		937 W	165 s	70	The enzyme completely inactivated	Palma-Orozco et al. (2012)
Tomato puree	PPO	3150 W	150 s	–	The residual activity was 11.72%	Arjmandi et al. (2017)
	PG			–	The residual activity was 29.22%	
	Pectin methylesterase (PME)			–	The residual activity was 14.652%	
Kiwifruit puree	POD	1000 W	340 s	–	The activity decreased by 90.7%	Benlloch-Tinoco et al. (2013)
	PPO				The activity decreased by 97.5%	
	PME				The activity decreased by 77.2%	
Horseradish roots	HRP	60 W	30 min	60	The lowest residual activity was only 16.9%	Lopes et al. (2015)
Surimi	Cat L	9 W/g	80 s	60	The enzyme activity decreased to 28.66%	Cao et al. (2020)

Abbreviations: Cat L, cathepsin L; LA, lipase activity; POD, peroxidase; PPO, polyphenol oxidase.

the PPO inactivation level of red beet and green coconut water was more elevated after long-term MW irradiation. In addition, MW irradiation power and treatment time had interaction on enzyme inactivation. In the samples with longer processing time, compared with the kiwifruit mud with shorter processing time, the level of PPO inactivation was accelerated with increasing power. In higher power and MW treatment, time was relatively long, and the enzyme activity dropped sharply. Excessive MW power dramatically inflated the temperature of the enzyme solution, which disrupted the molecular structure of the enzyme and denatured and inactivated it (Benlloch-Tinoco et al., 2013). CTH treatment was considered to require the use of high temperatures and long periods of time to stabilize the food, which led to a noticeable deterioration of product quality. Compared with CTH, the highest MWH power combined with shorter processing time resulted in higher inactivation rates of POD, PME, and PG. It also maintained physical, nutritional, and organoleptic qualities that were closer to those of fresh food (Arjmandi et al., 2017; Benlloch-Tinoco et al., 2013). MW irradiation enhanced storage stability by inhibiting endogenous LA to maintain raw material properties. After MW treatment for only 3.5 min, the temperature of the sample reached 157.26°C, and the LA in *Nigella sativa* L. seeds was the lowest. However, the temperature of traditional conduction heating was 144.70°C in 8 min (Mazaheri et al., 2019). Therefore, the abatement of enzyme activity under MW irradiation was directly related to the rapid increase of temperature. At 50°C, more than 75% residual LA was maintained in wheat germ. However, after 10 min of treatment at 70°C, the enzyme was completely inactivated, and the physical properties of wheat germ remained almost unchanged (Meriles, Penci, et al., 2022; Meriles, Steffolani, et al., 2022). MWH inhibited the activity of cathepsin L (Cat L) and facilitated the formation of surimi gel. When the temperature exceeded 60°C, the enzyme activity weakened faster with higher power. The downward trend was apparent, especially at 9 W/g; the relative enzyme activity of Cat L was down to 28.66% (Cao et al., 2020). When temperatures were low, MW or heating times had little effect on Cat L inactivation. The optimum temperature of the endogenous protease of surimi was usually 60°C, the activity of the endogenous protease was higher at this temperature (Wang et al., 2019).

The above analyses demonstrate that the temperature of the MW irradiation directly determines the catalytic activity of the enzyme. Activation of the enzyme activity is achieved even at high MW power when the MW irradiation temperature is maintained within the optimum temperature range for the enzyme. However, when the temperature of MW irradiation exceeds the optimum temperature, the enzyme activity decreases. Therefore, the purpose of activating or passivating enzyme activity would be achieved by optimizing MW irradiation process parameters in actual industrial production. But what is the intrinsic mechanism behind enzyme activation and inactivation? The following sections try to analyze the mechanism of enzyme activity changes under MW irradiation from the perspective of enzyme reaction kinetics and spatial structure changes.

3 | EFFECT OF MICROWAVE IRRADIATION ON KINETICS OF ENZYME REACTIONS

Enzyme reaction kinetics is important to reveal the mechanism of enzyme catalysis and to determine the most efficient reaction system and reaction conditions (Juárez-Enríquez et al., 2022). The study of the effect of MW irradiation on the kinetics of enzyme reactions will contribute to the understanding of the mechanism of changes in enzyme activity in reaction systems. The mechanism of MW effects on enzyme thermodynamics and kinetics is illustrated in Figure 1.

3.1 | Thermodynamics of microwave-assisted enzyme catalysis

The study of MW effect on LA-catalyzed systems was an area of great interest. It was found that the combination of MW and biocatalytic technology revealed better performance in terms of reaction and conversion rate than CT biocatalytic system (Bansode & Rathod, 2018; Bhavsar & Yadav, 2018a; Khan & Rathod, 2020). MW energy gave rise to effective internalized heating by allowing the coupling of MW with the reaction medium (Khan & Rathod, 2018). MW transmitted energy through different media in a short time of 10^{-9} s, and the molecular relaxation time was about 10^{-5} s. The time required for the energy to advance was quicker than the molecular relaxation time. This resulted in high-temperature conditions, which added to the molecular collisions and thus to the reaction kinetics (Leonelli & Mason, 2010; Polshettiwar & Varma, 2008). However, most analyses of the mechanism of MW-assisted lipase-catalyzed chemical reactions had been carried out from a thermodynamic point of view. The thermodynamics parameters of various lipase-catalyzed chemical reactions under MWH and CTH treatments are discussed in Table 3.

In the study of the synthesis of *n*-butyl palmitate catalyzed by LA under MW treatment, the kinetic parameters were estimated by pseudo-first-order kinetics (Badgujar & Bhanage, 2015). The reactants needed to overcome the transition state to form products and the energy involved

TABLE 3 Summary of representative studies of the thermodynamics parameters of various lipase-catalyzed chemical reactions under microwave heating (MWH) and conventional heating (CTH) treatments.

Enzyme type	Products/target	Heating method	Temperature (K)	Kinetics parameters (ΔH) kJ/mol; (ΔS) kJ/mol/K; (ΔG) kJ/mol		Reference
Fermase CALB 10000	*n*-Butyl palmitate	MWH	313	$\Delta H = -16.18$; $\Delta S = -0.25$; $\Delta G = 71.64$	$E_a = -13.58$ kJ/mol	Badgujar and Bhanage (2015)
			323	$\Delta H = -16.26$; $\Delta S = -0.25$; $\Delta G = 72.86$		
			333	$\Delta H = -16.35$; $\Delta S = -0.25$; $\Delta G = 73.55$		
			343	$\Delta H = -16.43$; $\Delta S = -0.26$; $\Delta G = 74.72$		
		CTH	313	$\Delta H = 30.10$; $\Delta S = -0.28$; $\Delta G = 108.96$	$E_a = 32.7$ kJ/mol	
			323	$\Delta H = 30.01$; $\Delta S = -0.27$; $\Delta G = 112.15$		
			333	$\Delta H = 29.93$; $\Delta S = -0.27$; $\Delta G = 115.77$		
			343	$\Delta H = 29.85$; $\Delta S = -0.26$; $\Delta G = 119.66$		
Fermase CALB	Ethyl laurate	MWH	313	$\Delta H = -20.41$; $\Delta S = -0.2356$; $\Delta G = 47.18$	$E_a = -31.98$ kJ/mol	Jaiswal and Rathod (2021)
			318	$\Delta H = -20.37$; $\Delta S = -0.2357$; $\Delta G = 48.46$		
			323	$\Delta H = -20.33$; $\Delta S = -0.2359$; $\Delta G = 49.74$		
			328	$\Delta H = -20.29$; $\Delta S = -0.246$; $\Delta G = 50.02$		
		CTH	–		$E_a = 20.95$ kJ/mol	
CAL-Bex10000	Polyethylene glycol stearate	MWH	333	$\Delta H = -40.7128$; $\Delta S = -0.076$; $\Delta G = -15.40$	$E_a = -37.94$ kJ/mol	Nhivekar and Rathod (2021)
			338	$\Delta H = -40.75$; $\Delta S = -0.076$; $\Delta G = -15.20$		
			343	$\Delta H = -40.796$; $\Delta S = -0.0693$; $\Delta G = -17.02$		
		CTH	333	$\Delta H = 17.1601$; $\Delta S = -0.29$; $\Delta G = 96.62$	$E_a = 19.92$ kJ/mol	
			338	$\Delta H = 17.11$; $\Delta S = -0.29$; $\Delta G = 115.17$		
			343	$\Delta H = 17.07$; $\Delta S = -0.29$; $\Delta G = 116.55$		
Novozym 435	Isoamyl butyrate	MWH	313	$\Delta H = 5.151$; $\Delta S = -0.274$; $\Delta G = 90.796$	$E_a = 7$ kJ/mol	Bansode and Rathod (2018)

(Continues)

TABLE 3 (Continued)

Enzyme type	Products/target	Heating method	Temperature (K)	Kinetics parameters (ΔH) kJ/mol; (ΔS) kJ/mol/K; (ΔG) kJ/mol		Reference
			323	$\Delta H = 5.067$; $\Delta S = -0.273$; $\Delta G = 93.295$		
			333	$\Delta H = 4.984$; $\Delta S = -0.273$; $\Delta G = 95.778$		
		CTH	313	$\Delta H = 13.715$; $\Delta S = -0.28$; $\Delta G = 62.203$	$E_a = 16$ kJ/mol	
			323	$\Delta H = 13.631$; $\Delta S = -0.281$; $\Delta G = 67.373$		
			333	$\Delta H = 13.548$; $\Delta S = -0.285$; $\Delta G = 71.613$		
	Isoamyl myristate	MWH	–	$E_a = 4.95$ kJ/mol		Yadav and Thorat (2012)
		CTH		$E_a = 4.72$ kJ/mol		
	Ethyl-3-phenylpropanoate with *n*-butanol	MWH	–	$E_a = 4.6$ kJ/mol		Yadav and Pawar (2012)
		CTH		$E_a = 5.3$ kJ/mol		
	Ethyl valerate	MWH	–	$E_a = 8.36$ kJ/mol		Bhavsar and Yadav (2018a)
		CTH		$E_a = 6.59$ kJ/mol		
	Citronellyl Acetate	MWH	–	$E_a = 8.57$ kJ/mol; $A = 0.319$		Yadav and Borkar (2009)
		CTH		$E_a = 8.28$ kJ/mol; $A = 1.7 \times 10^{-3}$		
	n-Butyl dipheyl methyl mercapto acetate	MWH	–	$E_a = 8.9$ kJ/mol		Yadav and Lathi (2007)
		CTH		$E_a = 7.9$ kJ/mol		

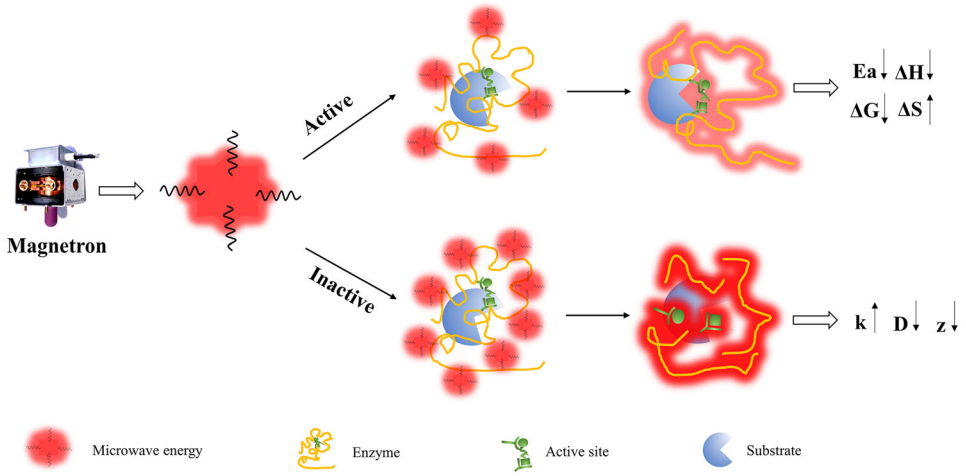

FIGURE 1 Proposed mechanisms of microwave irradiation on enzymes thermodynamics and kinetics.

in the process was termed as the energy of activation denoted as E_a. The value of E_a under MW treatment was −13.58 kJ/mol. The negative value of E_a suggested that MW diminished the energy barriers and improved the mass transfer of the reaction remarkably (Gawas & Rathod, 2018). In the MW system, the E_a was much lower than that in the CT system, which proved that the reaction was faster, and the product was formed faster. Similar results were obtained during the synthesis of ethyl laurate and polyethylene glycol stearate catalyzed by MW-assisted LA in solvent-free system (Jaiswal & Rathod, 2021; Nhivekar & Rathod, 2021). The highest E_a of biocatalytic synthesis of isoamyl butyrate in solvent-free system was 7 kJ/mol, which was much lower than 16 kJ/mol in the CT system (Bansode & Rathod, 2018). Novozym 435 MW-assisted transesterification of ethyl 3-phenylpropionate with *n*-butanol yielded similar results. From CTH to MWH treatment, the E_a decreased from 5.3 to 4.6 kcal/mol, suggesting that the reaction rate was accelerated (Yadav & Pawar, 2012). The net enthalpy change (ΔH) of the reaction was negative for the MW systems. The negative value of ΔH was attributed to the creation of high energetic species due to the increased collision of molecules. The ΔH in the MW system was low, indicating that the reaction was spontaneous, and the enzyme–substrate complex was formed rapidly. The low value of ΔH also suggested easy elongation and compression of chemical bonds to attain the state of transition with less energy uptake (Choudhury et al., 2013; Kadkhodaee & Povey, 2008). In the LA-catalyzed synthesis of isoamyl butyrate, MW energy decreased the ΔH of the system at different reaction temperatures, that is, 313, 323, and 333 K. The high positive ΔH values in the CT system specified random and inefficient collisions of substrate molecules and enzymes, requiring high energy to carry out the reaction. The negative value of entropy (ΔS) suggested the spontaneity of the reaction system. MW irradiation increased the ΔS of the system by accelerating the collision between molecules (Bansode & Rathod, 2018). Gibb's free energy of activation (ΔG) was defined as the free energy available to take the reaction toward the equilibrium. It demonstrated the feasibility of the reaction and reached the lowest value when the reaction reached equilibrium. The lower value of ΔG in the MW system represented the spontaneity of the reaction by the easy formation of product by enzyme–substrate complex breakdown (Gawas & Rathod, 2018). The frequency factor (A) for the enzyme-catalyzed synthesis of citronellyl acetate under MWH was improved by a factor of 190 compared to CTH treatment. MWH enhanced intermolecular collisions and system ΔS, resulting in an increase in the A. The E_a (s) of the two systems were similar, explaining that the heating method did not change the reaction mechanism (Yadav & Borkar, 2009). Similarly, in enzymatic synthesis of isoamyl myristate and *n*-butyl diphenyl methyl mercapto acetate, the E_a (s) of MWH and CTH were almost equivalent to those of most LA-catalyzed reactions (Yadav & Lathi, 2007; Yadav & Thorat, 2012). However, the rate constant (k) increased under MWH due to the higher collision frequency of the reacting molecules. From the "dipole polarization mechanism" point of view, the rate enhancement was due to the combined effect of the polarity of the reaction mixture and the MW absorption characteristics of the ionic properties. Compare to CTH treatment, MWH influenced the enzymatic reaction by its interaction first with lipase as a biocatalyst and then with the polar substrate molecules (Bansode & Rathod, 2018). Unlike the conduction mode of heating through CTH, MW energy was an outcome of interactions within dielectric substance. MW influenced the polar molecules that possessed high dipole moment and aligned to the external electric field, thus producing

their rotations (Shinde & Yadav, 2015). Appropriate MW energy interfered with the presence of weakly polar hydrogen bonds in the lipase protein cluster, which induced a conformational flip in the active site, resulting in the activation of lipase. Overall, MW accelerated the local heating of molecules thereby increasing the kinetic energy and adding the friction between molecules. At the optimum MW irradiation temperature, the enzyme molecular structure became stretched, thus enhancing the interaction between the enzyme and the substrate. These were not just the result of the thermal effect of MWs.

3.2 | Kinetics of enzyme thermal inactivation

The front was the thermodynamic analysis of enzyme activation, and the inactivation kinetics of enzymes was an essential approach to reveal the mechanism of MW enzyme inactivation. In the theoretical study of thermal inactivation enzymes, researchers mostly used the first-order reaction kinetics to study the inactivation of enzymes (Xanthakis et al., 2018). The kinetic parameters of enzyme inactivation under MWH and CTH treatments are discussed in Table 4. However, as MWH was non-thermostatic, the primary passivation kinetics were evaluated mainly by setting a reference temperature (T_{ref}) for non-thermostatic heating. Moreover, other models of enzyme inactivation kinetics had been extensively investigated in recent years, such as the Weibull model.

POD was considered to be one of the most thermostable enzymes. Owing to the existence of isozymes with variable thermal stability, thermal inactivation of POD generally occurred in the biphasic stages (Siguemoto et al., 2018). Previous investigations reported a biphasic first-order model for the thermal inactivation curve of POD in carrots, which consisted of heat-labile and heat-resistant fractions (Soysal & Söylemez, 2005). It was determined that MWH was more effective in inactivating the enzyme than CTH treatment. In MWH, time required for approximately 90% reduction in POD activity was shorter than CTH treatment at same temperature. Brugos et al. considered two components of PME (heat sensitive and heat resistant) in orange juice by using the first-order multicomponent kinetic model. The parameters generated for the PME kinetics in the CTH process at $T_{ref} = 90°C$ were: $z_1 = 13.1°C$, $z_2 = 21.7°C$, $D_1 = 0.258$ s, and $D_2 = 97.1$ s with $\alpha = .93$. For the MWH treatment conducted in a focused reactor (MW) at $T_{ref} = 90°C$ were: $z_1 = 13.7°C$, $z_2 = 20.3°C$, $D_1 = 0.231$ s, and $D_2 = 130.8$ s with $\alpha = .95$. Under the same conditions, the MW-assisted heating process was more effective than CTH process, suggesting that there may be some additional nonthermal effects in the MWH system (Brugos et al., 2018). Thermal inactivation of POD and PPO in açai-berry pulp was well represented by the two-component first-order model. The inactivation behavior depended on the heating technology, demonstrating the presence of a specific effect of MW. However, the differences were less significant at higher temperatures (85°C) (Costa et al., 2021). Cavalcante et al. described the inactivation of PPO from two different sources (mushroom tyrosinase in buffer and coconut juice) by CTH and MWH under similar conditions by the Weibull model. At temperatures above 70°C, the predicted inactivation of PPO by MWH was higher than that by CTH, which proved that there was nonthermal effect at higher power levels (Cavalcante et al., 2021). Some published investigations reported that the enzyme was more rapidly deactivated under MWH and proposed that the nonthermal effect of MWH played a major role (Han et al., 2018; Lopes et al., 2015). Conversely, Siguemoto et al. investigated the inactivation of PME, PPO, and POD in cloudy apple juice under CTH and MWH, and the kinetic model could be adjusted using the whole time–temperature curve of the sample instead of assuming isothermal conditions. The two-component first-order inactivation model exhibited a credible fit. The comparison between CTH and MWH did not reveal significant differences in enzyme inactivation rate. Therefore, no evidence of nonthermal or enhanced MW effects was observed in the inactivation of PME, PPO, and POD in cloudy apple juice (Siguemoto et al., 2018). Xu et al. concluded that CTH and MWH treatments were equally effective in inactivating LA and LOX in wheat germ. The thermal effect of MW irradiation was the main cause of enzyme inactivation. The E_a values required to inactivate the same enzymes were similar for both methods and the results for z values were consistent. Therefore, MWH and CTH have the same enzyme inactivation effect, but in different ways (Xu et al., 2016). Kubo et al. (2021) also argued that the MWH and CTH treatments were identical in terms of LOX and trypsin inhibitor inactivation in soymilk, and thus, no additional effects of MW were found. Chen et al. (2016) claimed that the difference observed between MWH and CTH actually was explained by the difference of reaction temperature, rather than the result of nonthermal effect. The experiment was carried out in the MW equipment made in the laboratory, and the test temperature may be difficult to control precisely. As highlighted by Kubo et al. (2021), the accurate control of electromagnetic field distribution and reliable monitoring of food internal temperature during MW treatment are nontrivial but essential for a fair comparison. Therefore, MW nonthermal effects may render the enzyme inactivation efficiency higher than that of CTH. However, these plausible effects were inconsistent in the literature and were explained by inadequate temperature measurements. It was certain that the enzymatic

TABLE 4 Summary of representative studies of the kinetic parameters of enzyme inactivation under microwave heating (MWH) and conventional heating (CTH) treatments.

Enzyme source	Enzyme type	Heating method	Kinetics model	Kinetics parameters	Reference
Carrot	POD	MWH	Biphasic first-order	70 W: k_R (min^{-1}) = 0.153 ± 4.08 × 10^{-3}; k_L (min^{-1}) = 0.078 ± 1.59 × 10^{-3}; D_R (min) = 15.33 ± 0.42; D_L (min) = 29.38 ± 0.583	Soysal and Söylemez (2005)
				210 W: k_R (min^{-1}) = 1.429 ± 4.24 × 10^{-4}; k_L (min^{-1}) = 0.526 ± 1.69 × 10^{-2}; D_R (min) = 1.612 ± 4.95 × 10^{-4}; D_L (min) = 4.381 ± 0.141	
			Monophasic first-order	350 W: k_R (min^{-1}) = 1.861 ± 5.59 × 10^{-3}; D_R (min) = 1.237 ± 3.69 × 10^{-3}	
				700 W: k_R (min^{-1}) = 2.593 ± 1.93 × 10^{-2}; D_R (min) = 0.888 ± 6.65 × 10^{-3}	
		CTH	Biphasic first-order	E_a (J/mol) = 14.8 × 10^4	
			Monophasic first-order	E_a (J/mol) = 8.96 × 10^4	
Cloudy apple juice	PME	MWH	Two-fraction first-order kinetic model (T_{ref} = 80°C)	$D_{1,\,ref}$ (s) = 22.3; $D_{2,\,ref}$ (s) = 776 z_1 (°C) = 20.1; z_2 (°C) = 33.0	Siguemoto et al. (2018)
		CTH		$D_{1,\,ref}$ (s) = 30.1; $D_{2,\,ref}$ (s) = 584 z_1 (°C) = 28.8; z_2 (°C) = 42.6	
	PPO	MWH		$D_{1,\,ref}$ (s) = 48.9; $D_{2,\,ref}$ (s) = 57.8 z_1 (°C) = 7.42; z_2 (°C) = 94.6	
		CTH		$D_{1,\,ref}$ (s) = 27.7; $D_{2,\,ref}$ (s) = 42.3 z_1 (°C) = 3.52; z_2 (°C) = 50.7	
	POD	MWH		$D_{1,\,ref}$ (s) = 3.69; $D_{2,\,ref}$ (s) = 93.9 z_1 (°C) = 9.78; z_2 (°C) = 9.35	
		CTH		$D_{1,\,ref}$ (s) = 4.64; $D_{2,\,ref}$ (s) = 215 z_1 (°C) = 10.6; z_2 (°C) = 12.7	
Açai-berry	POD	MWH	First-order with two components (T_{ref} = 80°C)	D_s (s) = 9695; z_s (°C) = 3.081 D_l (s) = 119.2; z_l (°C) = 8.398	Costa et al. (2021)
		CTH		D_s (s) = 1.239 × 10^6; z_s (°C) = 2.440 D_l (s) = 909.9; z_l (°C) = 4.271	
	PPO	MWH		D_s (s) = 562.6; z_s (°C) = 6.229 D_l (s) = 3.321; z_l (°C) = 9.842	
		CTH		D_s (s) = 609.5; z_s (°C) = 7.084 D_l (s) = 0.04906; z_l (°C) = 3.102	
Orange juice	PME	MWH	First order with two components (T_{ref} = 90°C)	z_1 (°C) = 13.7; z_2 (°C) = 20.3 D_1 (s) = 0.231; D_2 = 130.8	Brugos et al. (2018)

(Continues)

TABLE 4 (Continued)

Enzyme source	Enzyme type	Heating method	Kinetics model	Kinetics parameters	Reference
		CTH		z_1 (°C) = 13.1; z_2 (°C) = 21.7 D_1 (s) = 0.258; D_2 (s) = 97.1	
Wheat germ	LA	MWH	First-order	E_a (kJ/mol) = 21.275; Z (°C) = 5.0	Xu et al. (2016)
		CTH		E_a (kJ/mol) = 21.719; Z (°C) = 4.9	
	LOX	MWH		E_a (kJ/mol) = 29.988; Z (°C) = 3.5	
		CTH		E_a (kJ/mol) = 32.910; Z (°C) = 3.2	
Soymilk	LOX	MWH	First-order with two fractions	$D_{90°C,\,R}$ (s) = 38.79; Z_R (°C) = 12.19$D_{90°C,\,L}$ (s) = 1.20; Z_L (°C) = 11.35	Kubo et al. (2021)
		CTH		$D_{90°C,\,R}$ (s) = 24.97; Z_R (°C) = 20.21$D_{90°C,\,L}$ (s) = 0.10; Z_L (°C) = 4.31	
	TI	MWH	Weibull	$\delta_{90°C}$ (s) = 17098; z (°C) = 29.81; β = .48	
		CTH		$\delta_{90°C}$ (s) = 20514; z (°C) = 32.66; β = .47	
Buffer	PPO	MWH	Weibull	β = .266; Z_w (°C) = 3.56; $D_{w,70°C}$ (s) = 10.2	Cavalcante et al. (2021)
		CTH		β = .543; Z_w (°C) = 9.79; $D_{w,70°C}$ (s) = 26.7	
Coconut water		MWH		β = .456; Z_w (°C) = 7.89; $D_{w,70°C}$ (s) = 1028	
		CTH		β = .499; Z_w (°C) = 16.3; $D_{w,70°C}$ (s) = 509	

Abbreviations: LA, lipase activity; LOX, lipoxygenase; POD, peroxidase; PPO, polyphenol oxidase.

molecular system became destabilized when the MW input energy was too excessive. The inhibition of enzyme activity was mainly due to the rapid heating rate of MW in the sample, which led to the complete denaturation of enzyme molecules and inactivation of enzyme.

Ultimately, thermodynamics and kinetics are both manifestations of the process of MW energy conversion. The strength of the energy intensity provided by MW leads to distinctions in the structural transformation of the enzyme. In the optimum temperature range, MW causes the molecular structure of the enzyme to expand, which is thermodynamically expressed as an increase in enzyme activity. When MW input energy is excessively high and exceeds the energy range that the enzyme molecule is capable of accepting, the enzyme molecular system becomes destabilized. MW leads to the disruption of the enzyme molecular structure, which kinetically manifests in an inhibition of the enzyme activity. Enzymes belonging to proteins are mostly very sensitive and have highly complicated spatial structures in their folded states (Attri et al., 2012). There is a unique relationship between enzyme activity and spatial structures of enzymatic protein. The following section attempts to analyze further the intrinsic mechanism of enzyme activity changes by studying the effect of MW irradiation on enzyme spatial structure and functional groups.

4 | EFFECTS OF MICROWAVE IRRADIATION ON STRUCTURE OF ENZYME

It was well known that enzyme relied on its structural conformation to catalyze biological reactions. The small discrepancies in the higher order structures led to apparent variations in the biological properties of enzyme, such as changes in enzyme activity and stability (Yi et al., 2020). The changes of secondary and tertiary structures of proteins were the determinants of enzyme activity (Yan et al., 2014). The active site was an important structural unit where the catalytic reactions of enzyme occurred (Chai et al., 2020). This part had specific functional group(s) to react with specific substrate(s) (Liu et al., 2018). MW facilitated the unfolding of proteins by breaking hydrogen, disulfide, and other weaker bonds through thermal and electromagnetic effects, thus changing the secondary and tertiary structure of proteins (Fan et al., 2016; Han et al., 2018). Structural changes in MW-treated enzyme highlighted the cascade of events at the molecular level and provided information for further explanation. Therefore, the spatial structure and functional groups of enzymes induced by MW irradiation were important aspects to be presented.

4.1 | Effect of microwave irradiation on the secondary structure of enzyme

Preliminary research studies highlighted that MW irradiation altered the secondary structure of enzyme molecules (Gomaa et al., 2016). The secondary structure referred to the spatial arrangement of molecules in the polypeptide main chain, and its changes were qualitatively evaluated by the content of α-helix, β-sheet, β-turn, and random coil (Battisti et al., 2017). Numerous techniques provided information on secondary structure variations of enzymes, such as circular dichroism spectroscopy and Fourier transform infrared spectroscopy (Baltacıoğlu et al., 2017).

MW treatment revealed alterations in the secondary structure of TG and glucoamylase (Cao et al., 2018; Zhang et al., 2012). The α-helical content reduced, whereas the proportions of β-sheets and β-turns increased gradually, and some ordered structures of protein became random coils. These structural changes allow the enzyme to be more spacious, and the enzyme activity center was easier to bind to the substrate, thereby improving the activity of the enzyme. The secondary structures of enzyme protein were maintained by the hydrogen bonds between carbonyl and amide groups on the peptide (Yi et al., 2020). MW field activated LA by interfering with the weak polar hydrogen bonds in the protein cluster of LA, resulting in the conformational inversion of the active site. This promoted the binding of the LA to the substrate and the release of the active site product (Gupta & Rathod, 2018; Pellis et al., 2016). Lopes et al. found that the decrease in α-helical content at 30 and 40°C was accompanied by a strong activation of the enzyme. Modifications in HRP secondary structure occurred at specific sites of the enzyme, depending on the temperature at which it was exposed. Slight changes in the secondary structure of the enzyme at low temperatures (30–45°C) had an activating effect, whereas at higher temperatures (mainly at 60°C), the effect on the enzyme was deleterious regarding its activity (Lopes et al., 2015). The decrease of enzyme activity by MWH was also closely related to the change of secondary structure (Zhao & Yang, 2009). The relationship between α-helical structures and active sites of enzyme was stated by Zhang et al. (2015), who reported that some amino acids consisting of the active sites were all involved in the constitution of the α-helical structures. Decreasing in α-helical structure played an effective role in the inactivation of enzyme activity (Zhang et al., 2015). MWH treatment led to the change of the secondary structure of Cat L, mostly manifesting the decrease of α-helix structure. The variation trend of the residual enzyme activity of Cat L after MWH and CTH was the same as that of α-helix. Moreover, after treatment, the random coil of Cat L molecules gradually raised, and the activity gradually weakened. The molecular chain configuration of

Cat L revealed that the molecular structure of Cat L was curled up, and active center was buried, which resulted in the inhibition of Cat L. MWH caused the polar groups on the Cat L molecules to vibrate violently, modifying the conformation of the Cat L molecules. Alternating electric field produced by MWH destroyed the protein α-helix structure due to the superposition of all the dipole moments of peptide bonds in α-helix, which resulted in the strong directional dipole moments and the coupling between external alternating electric field and directional dipole moments (Cao et al., 2020). Under MW irradiation, the α-helix content of α-amylase reduced sharply, and the secondary structure was disordered. MW irradiation interfered with hydrogen bonding, hydrophobic bonding, and van der Waals forces. The subsequent loosening, cleavage, and compounding of hydrogen bonds transformed the orientation of the original hydrogen bonds that initially retained a stable secondary structure. As a result, the hydrogen bonds were redistributed to change the conformation and activity of enzyme protein, so as to reduce the structure stability of α-amylase and improve its flexibility (Zhang et al., 2011). Therefore, MW irradiation altered the secondary structure of enzyme molecules to enhance or inhibit their activity.

4.2 | Effect of microwave irradiation on the tertiary structure of enzyme

The tertiary structure of protein is a complex three-dimensional structure that is stabilized due to different bonding interactions among side chains of amino acids. The hydrophobic side chains are buried inside the protein molecule to prevent from aqueous medium, whereas acidic or basic hydrophilic side chains of amino acids are exposed on protein surface (Liu et al., 2018). The tertiary structure changes are reflected by the intensity of endogenous and exogenous fluorescence. The endogenous fluorescence is directly linked to aromatic amino acids, such as tryptophan, tyrosine, or phenylalanine, whereas the exogenous fluorescence is usually characterized by the change of surface hydrophobicity of proteins (Karoui & Blecker, 2011).

MW modified the microenvironment of the TG residues. After MWH, the TG molecules unfolded, and more tryptophan residues were exposed to the more polar environment of the molecular surface (Yin et al., 2011). Hydrophobic amino acid residues were located in the interior of protein molecules, and hydrophobic groups were exposed when protein molecules were subjected to MW (Qin et al., 2016). Moreover, laser light scattering revealed that the ionic bonds within the TG molecule were stretched and some hydrophobic groups were exposed under the action of MW. The positive and negative charges carried by the enzyme molecule repel each other and the enzyme molecule stretched the entire molecular chain in a flexible Gaussian chain state due to electrostatic interactions. The exposure of TG activity center was easier to bind to substrate, and the enzyme activity was improved (Cao et al., 2018). The inactivation of MW-treated HRP was related to the loss of tertiary structure, indicating changes around the tryptophan environment (Lopes et al., 2015). The tertiary structure of LA in wheat germ changed completely under MW irradiation at 60°C (Chen et al., 2017). At higher temperatures, the active site was gradually "removed" from the structure of the enzyme leading to the loss of the tertiary structure of the enzyme. After treatment, the protein became swallowed due to the exposure of amino acid to hydrophobic region. As a result, the buried fluorophore created an obstruction to the substrate binding site, which led to enzyme inactivation. Yuan et al. discovered that the fluorescence spectrum gradually redshifted, and the fluorescence intensity progressively degraded after MW treatment, consequently disrupting the tertiary structure of PPO protein. MW irradiation triggered the unfolding of PPO structure and exposure of aromatic amino acids in strong polar aqueous solution; on the other hand, MW treatment induced the formation of carbon-centered free radicals; the PPO structure integrity was damaged because of the presence of carbon-centered free radicals (Yuan et al., 2021). Therefore, MW irradiation mainly affected the tertiary structure of the enzyme by modifying the polarity of the microenvironment and the spatial position of the residues.

4.3 | Effect of microwave irradiation on functional groups of enzyme

In addition, MW was identified to alter enzyme activity by impacting specific functional groups in the enzyme, such as sulfhydryl, carbonyl, and amino groups (Higuera-Barraza et al., 2017; Hu et al., 2015).

The solvent (water) exposure of the hydrophobic core residue appeared to rise as the MW treatment time was extended. The disulfide bond of protein was broken under MW irradiation, resulting in an initial boost and then a reduction in the free sulfhydryl contents (Bi et al., 2015). When the MW treatment temperature was lifted from 30 to 60°C, the sulfhydryl content of the PPO during the maceration period of the grapes dropped, and the amino and carbonyl content rose. The increase of amino content was due to the fact that the increase of MW temperature accelerated the expansion of PPO and added the number of accessible sites of protease, resulting in the increase of free amino acid content. The sulfhydryl group

of PPOs generated the disulfide bond under the condition of MW treatment, leading to a decrement in the sulfhydryl content of PPO (Yuan et al., 2021). The exposed sulfhydryl content was usually accompanied by changes in the molecular structure of enzyme. MWH treatment reduced the total sulfhydryl content and raised the exposed sulfhydryl content of Cat L, demonstrating that the Cat L molecules unfolded or denatured (Cao et al., 2020). Another reason was that MW irradiation invoked a gradual growth of carbon-centered radicals with the rising temperature, which facilitated the formation of sulfone or sulfonic acid, consequently lessening its sulfhydryl content (Sun et al., 2019). As the temperature groped, the MW-induced carbon-centered radicals rose; thus, the oxidation characteristics and carbonyl content improved likewise. It was also noted that MW-induced elevated carbonyl content in rice proteins. Free radicals disrupted various interactions between protein molecules, including van der Waals forces, electrostatic and hydrophobic interactions, hydrogen bonds, disulfide bonds, and salt bridges, thereby altering the structure and functional properties of proteins (Fan et al., 2016; Han et al., 2018). It was reported that free radicals caused protein structure modification and oxidative degradation (Lyu et al., 2020). Yuan et al. confirmed that by using electron paramagnetic resonance (EPR) under different MW conditions, carbon-centered free radicals were induced. The EPR signal intensity of free radicals strengthened with the improvement of power, temperature, and time. The structural integrity of the PPO was somewhat compromised by the presence of the carbon-centric radicals. Water had a certain ability to quench free radicals. Water activity and MWH parameters affected protein free radicals (Yuan et al., 2021). Fan et al. studied the free radical change of rice protein under different MW powers and water activities. The results indicated that the intensity of protein radicals enhanced with the reduction of water activity (Fan et al., 2016). As water was a polar molecule, the upper water activity had a strong dielectric response in MW fields. In addition, the presence of higher MW power also raised the intensity of protein-free radicals. This was due to the conversion of electromagnetic energy into sufficient thermal and chemical energy at high power levels, which resulted in the formation of free radicals. The change of free radicals had a direct impact on protein structure. Therefore, it was definitely true that MW irradiation affected enzyme activity by acting specifically on the functional groups of the enzyme, and that a range of variations in sulfhydryl, amino, and carbonyl contents occur under different MW conditions.

In conclusion, the changes of protein secondary and tertiary structures and functional groups under MW irradiation directly influence the enzyme activity. MW irradiation alters the structure of enzyme molecules and their active sites, so that the active sites are exposed and potentially combine better with the substrate, showing an enhancement in enzyme activity. Some polar groups on the enzyme molecule are particularly reactive to MW fields. The rapid transformation of the alternating electric field inflicts mechanical damage to the molecular structure of the enzyme. The structural integrity of the enzyme molecules is destroyed, and the enzyme activity is reduced.

5 | EFFECTS AND MECHANISMS OF MICROWAVE IRRADIATION ON ENZYME

The clarification of the mechanism of MW affecting enzyme activity plays an important role in guiding the selection of specific conditions in food MW processing. It is generally believed that MW has thermal and nonthermal effects on enzyme activity. Proposed mechanisms for MW irradiation to activate or inactivate enzyme activity are illustrated in Figure 2.

5.1 | Thermal effect of microwave irradiation on enzyme

The most common MW frequency utilized in food processing was 2450 MHz. The MW photon energy at this power failed to break the hydrogen bond, and it was even more difficult to interfere with the covalent bonds in molecules (Tao et al., 2020). Consequently, it was impossible to absorb directly MW energy to promote chemical reactions (Datta & Rakesh, 2013). MW thermal effect referred to the phenomenon that MW energy was absorbed by dielectric materials and converted into thermal energy. It was shown as the total loss of MW energy in the material. Most mature chemical reactions involving MW were based on the mechanism of MW dielectric heating (El Khaled et al., 2018). The electric field was the main source of action of the thermal effect in MW electromagnetic complex fields, which were realized by two mechanisms: dipole polarization and ion conduction. Dipole polarization mechanism: The dipoles of matter were rearranged due to the electric field under MW irradiation, and the phase difference between the electric field direction and the induced dipoles caused MW energy to be consumed by molecular collisions and friction processes, which were converted into heat. Ionic conduction mechanism: MW caused ions in solution to undergo oscillations accompanied by changes in the electric field, resulting in collisions with surrounding atoms and molecules with each other. The resulting microscopic displacement induced heat (Alifakı & Şakıyan, 2017). Comparing the two mechanisms, the

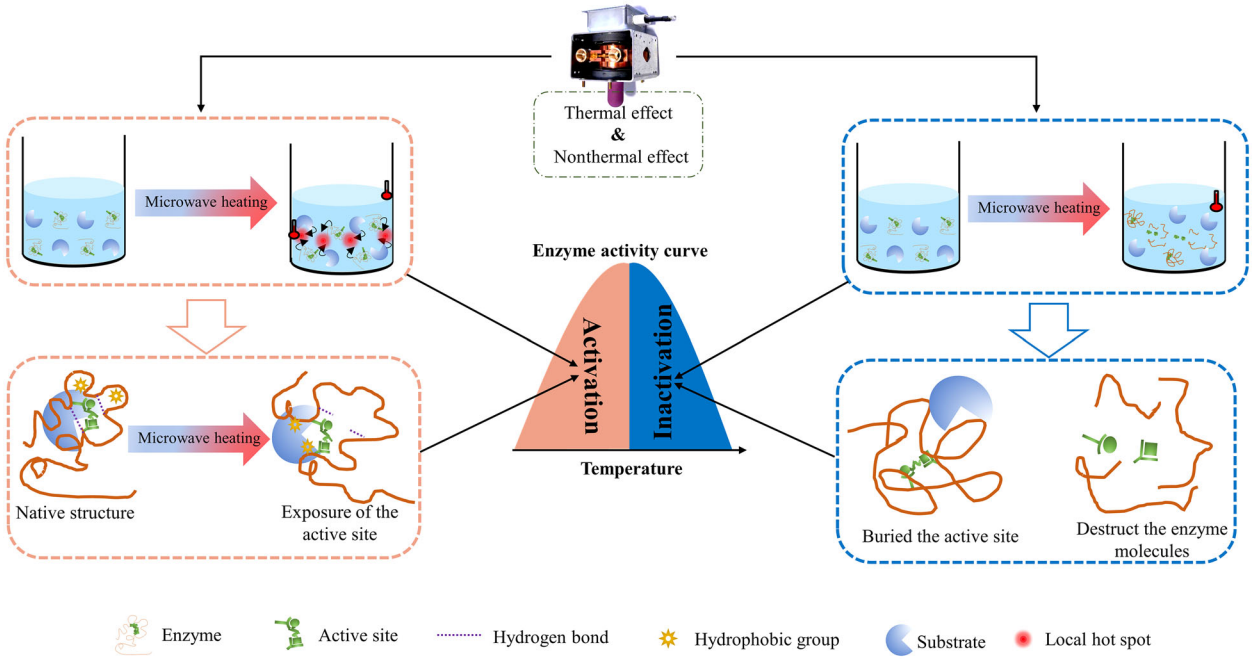

FIGURE 2 Proposed mechanisms for the activation or inactivation of enzymes under microwave irradiation.

ionic conduction process produced a more intense thermal effect.

MW irradiation directly acted on the molecules of the reaction substance, which was different from the conduction and convection of heat in CTH. MW irradiation directly acted on the polar functional groups of the enzyme when catalyzing the enzyme, so that the reaction system quickly reached the optimum reaction temperature of the enzyme. Activation of the enzyme activity was achieved when the enzyme was operating at its optimum temperature. As a result, the MW thermal effect was fast and comprehensive (Oliver Kappe, 2008). In addition, MW had the property of selectively heating substances, which was related to the dielectric constant (ε) of the substance. Dielectric constant was a physical quantity that characterizes the degree of polarization of a medium under the action of an external electric field. The dielectric constant was proportional to the absorption capacity of MW (El Khaled et al., 2018). It was observed that MW was able to target heating and had a more uniform heating pattern than CTH owing to the different dielectric properties of the enzyme molecules themselves. These were attributed to the specific heating mode of MW, which was still essentially a thermal effect (Meriles, Penci, et al., 2022; Meriles, Steffolani, et al., 2022). Manzocco et al. (2008) researched the effect of frequency on the kinetics of PPO and LOX and proved that the enzyme became inactive as a consequence of thermal effects. Thermal inactivation reduced the content of α-helix structure and improved the content of β-sheet of wheat germ LA after MWH and CTH treatments. MWH was slightly more effective in deactivating the enzyme. However, at ambient temperature (20°C), the activity and structure of LA remained invariant. Subsequently, it was effectively inactivated within 20 s after MW treatment at 60°C (Chen et al., 2017). Massa et al. examined the effects of MW (1.95 GHz, 25–30°C) on myoglobin. The structure of myoglobin remained relatively stable at ambient temperature (Bismuto et al., 2003). From the energy point of view, hydrogen bond was the main energy to maintain the secondary structure of protein, which was less than 0.44 eV but higher than the activation energy of MW at ambient temperature (10^{-3}–10^{-4} eV). Without heating support, MW hardly destroyed hydrogen bonds and secondary structures of enzymes (Chen et al., 2016). In summary, the dielectric heating effect of MW was instrumental in influencing enzyme activity. As shown in Section 2, the temperature of the MW irradiation directly determined the catalytic activity of the enzyme. When the temperature exceeded the optimal temperature range for the enzyme, the structure of the enzyme was damaged, and the enzyme activity was reduced.

5.2 | Nonthermal effect of microwave irradiation on enzyme

MW applications in the food sector were designed primarily on the basis of their dielectric heating effect, which were assessed by the temperature, and the time it was heated. However, during the MW treatment

process, researchers also discovered some phenomena that could not be explained by temperature and time, namely, the nonthermal or special effect. It was assumed that the main effect was the polarization of molecules by the MW electromagnetic field, which altered the original electron arrangement of the molecules. Additionally, the vibration and rotation of molecules due to the energy generated caused the breakage of chemical bonds or improved the activity of substances because of the selective heating properties of MW (Siguemoto et al., 2018).

Earlier studies confirmed that enzyme activity was enhanced by using controlled MW irradiation and suggested that these effects were nonthermal in origin (Herrero et al., 2008). The TG activity was higher with MW than with CTH at the same heating rate, which may be the result of the nonthermal effect of MW irradiation (Cao et al., 2018). There were several explanations for MW nonthermal effect in strengthening enzyme activity: Some polar amino acids on the enzyme molecules were exposed to more catalytic sites as the enzyme molecule structure unfolded under MW irradiation. The enzyme molecules were easier to combine with the substrate, thus accelerating the reaction rate. On the other hand, the secondary structure of enzyme was mainly maintained by hydrogen bonds, which were the main force to maintain the stability of the secondary structure of the protein in addition to hydrophobic interaction and van der Waals force. Electromagnetic fields caused the hydrogen bonds to be loosened, broken, and reorganized. As a result, the conformation and activity of the enzyme were modified. The conformational changes allowed the enzyme molecules to exhibit stronger adaptability and flexibility. The enzyme molecules were readily combined with the substrate, which ultimately manifested itself as an increase in enzyme activity (Kubo et al., 2020). These were summarized as the nonthermal effect of MWs causing a change in the conformation of the enzyme molecules that exposed the catalytic site of the enzyme. Some studies also demonstrated that the inactivation of PPO and POD was faster in the MWH mode than in the CTH mode because of the presence of the nonthermal effects (Latorre et al., 2012). The mechanism of nonthermal inactivation of enzymes was that some polar groups on enzyme molecules vibrate under MW field. The rapid change of alternating electric field provoked mechanical damage to the structure of enzyme molecules. Enzyme molecular structure curled and buried active center, resulting in an inhibition of enzyme activity. In addition, MWH entailed the polar groups on the enzyme molecule to vibrate violently, which directly destroyed the active center of the enzyme molecule.

Nevertheless, the existence of nonthermal effects has been questioned by researchers, and there are no definitive and uniform conclusions surrounding the experimental results of this investigation. The existence of a nonthermal MW effect has been demonstrated by two broadly based ideas. First, if a significant discrepancy in the results is still produced when there is the same thermal effect under MWH as under CTH conditions, this may prove that the existence of non-thermal effects is evident. The second is to enable the MW to exert a negligible thermal effect which would allow the differences it brings with the CTH method to be examined. When the reaction results under MW conditions are different from those under CTH at the same measured reaction temperature, this effect has been claimed. However, most studies used water as the medium, and the temperature control is not accurate, or the temperature rise rate varies with heating methods (Damm et al., 2012). From an experimental point of view, more rigorous experimental techniques and methods are demanded, especially for the fine measurement of the spatial distribution of temperature under MW irradiation in order to obtain reliable data. It is certain that MW energy penetrates the material and transfers heat from the center of the material to the surface during the heating process (Lerfall et al., 2018). This type of transfer results directly in a rapid rise in the temperature of the bulk material (Peng et al., 2017). Thus, if this particular effect exists, the thermal effect of MW is strongly interfered with by possible nonthermal effects, and the two are not easily separated from each other. The effect of MW on enzyme activity is affected by both thermal and nonthermal effects.

6 | CONCLUSIONS

MW irradiation as a novel environmentally friendly and safe heating energy source has been widely applied in the field of enzyme catalysis. MW irradiation temperature is a vital parameter influencing the catalytic activity of enzyme. Activation of the enzyme activity is achieved even at high MW power when the enzyme is operating at its optimum temperature. However, when the MW irradiation temperature exceeds the optimal temperature range for the enzyme, the enzyme activity is reduced. Therefore, the purpose of activating or passivating enzyme activity would be achieved by optimizing MW irradiation process parameters in actual industrial production.

The optimum MW energy enhances the collisions between polar molecules, which generate local hot spots. Subsequently, the enzyme activity is improved, and thus, the interaction between the enzyme and the substrate is better strengthened. The inhibition of enzyme activity is attributed primarily to the rapid rate of heat-up of MW in the sample, which results in complete denaturation of the enzyme molecules and enzyme inactivation. Structurally, MW alters the structure of enzyme molecules and

their active sites. The enzyme active site is exposed, which enhances its better binding to the substrate, thus displaying an improvement in enzyme activity. Conversely, MWH forces the polar groups on the enzyme molecule to vibrate violently, which directly destroys or buries the active center of the enzyme molecule, thereby inhibiting enzyme activity. These are not only the result of thermal effect but also may be the contribution of MW nonthermal effects.

AUTHOR CONTRIBUTIONS

Hongwei Cao: Investigation; Writing – original draft. **Xiaoxue Wang**: Writing – review & editing. **Jing Liu**: Visualization. **Zhu Sun**: Visualization. **Zhiquan Yu**: Visualization. **Maurizio Battino**: Conceptualization. **Hesham El-Seedi**: Formal analysis. **Xiao Guan**: Supervision; Resources.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (32102140), Young Elite Scientists Sponsorship Program by CAST (2021QNRC001), Shanghai Sailing Program (20YF1433400), and the Domestic Science and Technology Cooperation Projects of Shanghai (21015801100).

CONFLICT OF INTEREST STATEMENT

The authors declared that there is no conflict of interest.

ORCID

Hesham El-Seedi https://orcid.org/0000-0002-2519-6690
Xiao Guan https://orcid.org/0000-0001-9163-2430

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How to cite this article: Cao, H., Wang, X., Liu, J., Sun, Z., Yu, Z., Battino, M., El-Seedi, H., & Guan, X. (2023). Mechanistic insights into the changes of enzyme activity in food processing under microwave irradiation. *Comprehensive Reviews in Food Science and Food Safety*, 1–23. https://doi.org/10.1111/1541-4337.13154