

Research Article

Effects of Low-dose Microwave Radiation on Catalase and Lactate Dehydrogenase

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Abstract: The most frequent microbial contamination found in cereal were mainly caused by moulds which cell metabolic activities were closely related to catalase and lactate dehydrogenase. Here, we studied the effects of Low-Dose Microwave Radiation (LDMR; 2450 Hz, 2.4 W/g) on physicochemical characteristics of catalase and lactate dehydrogenase and compared them to the effects of conventional heating treatment (water bath), to provide a theoretical basis for using LDMR for moulds control in cereal. When catalase or lactate dehydrogenase was subjected to LDMR, the enzyme's sulfhydryl content, hydrophobic value and decreasing rate of enzyme activity were universally higher than when the enzyme was subjected to conductive heating. The activity of catalase and lactate dehydrogenase decreased with the rise in temperature. After LDMR exposure or heat conduction treatment, the secondary structure of catalase and lactate dehydrogenase were changed. Moreover, the heat inactivation temperature and conformation transition temperature of catalase were higher than those of lactate dehydrogenase.

Keywords: Catalase, lactate dehydrogenase, microwave, mould

INTRODUCTION

Mould contamination in cereal grains, which can occur before or after harvest as well as during transportation or storage, decreases their nutritional value and constitutes health hazards (Paolesse *et al.*, 2006; Conkova *et al.*, 2006). Microwave radiation as an alternative method for controlling mildew and insects has been gaining increasing recognitions because it offers several advantages over the conventional method such as security, high efficiency and ensuring food quality (Zhao *et al.*, 2007a; Fang *et al.*, 2011; Lu *et al.*, 2010; Zhao *et al.*, 2007b; Vadivambal *et al.*, 2007). Low-Dose (about 2.0 kW/kg) Microwave Radiation (LDMR) is claimed to have better effect on maintaining food quality and can also kill pests and molds in comparison with high-dose microwave (Fang *et al.*, 2011; Lu *et al.*, 2010). Microwave sterilization has been proposed and applied for a variety of foods, moreover, it is well known that it is effective against a wide range of microorganisms (Giuliani *et al.*, 2010). Although studies to differentiate the thermal and nonthermal effects of microwaves on microbiological systems have been investigated and discussed (Mervine and Temple, 1997; Gedye, 1997; Canumir *et al.*, 2002; Sasaki *et al.*, 1998; Velizarov *et al.*, 1999; Zielinski *et al.*, 2007; Yaghmaee and Durance, 2005), the mechanism of lethality of microwaves for microorganisms has not been resolved.

Catalase and lactate dehydrogenase play a very important role in some physiological activities such as biological oxidation, detoxification and participating in active oxygen metabolism (Zamocky and Koller, 1999; Chelikani *et al.*, 2004; Legrand *et al.*, 1992). Therefore, both enzymes are closely related to the growth and metabolism of moulds. Research has revealed that the microwave can inhibit or destroy the activity of the enzyme related to biological oxidation, breathing metabolism and energy generation in the cell leading to microbial death (Fu *et al.*, 2003).

In this study, catalase and lactate dehydrogenase were exposed to LDMR (2.4 kW/kg), respectively. The effects of LDMR on the molecular structure and activity of both enzymes were studied with conventional heating (water bath) as a control, in order to provide a theoretical basis for prevention and control of grain mildew.

MATERIALS AND METHODS

Materials: Catalase and lactate dehydrogenase were obtained from Sigma Chemical Co (St Louis, Mo., U.S.A.). All reagents used were of analytical grade.

Test methods:

Preparation and pre-process of the enzyme solution: Solution of catalase and lactate dehydrogenase were respectively prepared as following: 1 mg of enzyme

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stock was diluted in 1 mL of distilled water to obtain enzyme solution. Both enzyme solution were placed into EP tubes which disinfected by high temperature (121°C, 15 min) and stored at a low temperature (4°C).

For the LDMR treatment, samples were irradiated using a QW-15HM microwave oven (Guangzhou Kewei Microwave Oven Energy, China) with a 240 W capacity and 2.45 GHz frequency. Samples were microwaved for different times to obtain a range of final solution temperatures (30, 40, 50, 60 and 70°C). The temperature of the solutions was measured immediately after irradiation with a JDDA80 point thermometer (Wuhan, Jingda Instrument FactoryCo., China) at different times. To determine the effect of conductive heat treatment, the enzyme solutions subjected to a constant temperature of 30, 40, 50, 60 and 70°C in water bath.

Determination of the enzyme's sulfhydryl content:

The sulfhydryl content was determined as described elsewhere (Thannhauser *et al.*, 1984). The molar concentration of -SH was quantified with the following equation:

$$C_0 = \frac{A}{\xi} \times D$$

where,

C_0 = The molar concentration of -SH (mol/L)

A = The OD value at 412 nm

D = The dilution factor

ξ = The molecular absorption factor (13600 mol/L·cm)

Determination of the enzyme's hydrophobic value:

The hydrophobic value was determined by ANS (1-nilinoaphthalene-8-sulfonic acid) fluorescent probe method as described elsewhere (Alizadeh and Li, 2000). The Relative Fluorescence Intensity (RFI) of samples was measured on a Shimadzu RF-5301PC (Shimadzu Corp., Kyoto, Japan) spectrofluorometer at room temperature, with excitation and emission wavelength set at 289 nm and 337 nm.

Determination of enzyme activity:

The catalase activity was assayed by using a catalase analysis kit (Beyotime Biotechnology, China) according to the instructions of manufacture. The lactate dehydrogenase activity was determined with a ultraviolet spectrophotometric method as described elsewhere (Chen *et al.*, 1989). The enzymatic activity was calculated from the steady-state portion of the absorbance curve.

Determination of the enzyme's secondary structure:

The secondary structure of enzyme was determined by Jasco J-810 Circular Dichroism (CD) spectropolarimeter (JASCO Corporation, Japan) at

room temperature. All the samples through different heat treatment were determined after spending a night at 25°C. Spectra shown are the average of 4 scans, with a scan rate of 500 nm/min and a quartz cell length of 1 mm.

After obtaining the CD spectrum, each spectrum analysed for the contents of alpha-helix (X_H), beta-sheet (X_β) and random coil (X_R) using a method described elsewhere (Tetin *et al.*, 2003).

Data analysis: Data were analyzed with Microsoft Excel 2003 and SAS (SAS Institute, 1989). Differences were considered significant at $p < 0.05$.

RESULTS

Sulfhydryl content of catalase and lactate dehydrogenase:

The effect of LDMR and conventional heating on the sulfhydryl content of catalase is illustrated in Fig. 1 (I). In general, the sulfhydryl content of catalase increased with the rise in temperature. The effects of LDMR on the sulfhydryl content of catalase were negligible below 50°C, but sharply increased at 60 and 70°C.

Figure 1 (II) shows the effect of LDMR and conventional heating on the sulfhydryl content of lactate dehydrogenase. The sulfhydryl content of lactate dehydrogenase first rose and then declined with increasing temperature. After LDMR exposure, the sulfhydryl content of lactate dehydrogenase arrives at its peak at 40°C and declined sharply at 60 and 70°C. Probably it is because lactate dehydrogenase occurred thermal denaturation at both temperature (Jacobson and Braun, 1977).

When the two kinds of approaches achieved the same physical temperature, the sulfhydryl contents of both enzymes treated with LDMR were higher than those treated with conventional heating. This could be due to two types of microwaves effects (thermal and non-thermal) which triggered alterations of protein structure (Banik *et al.*, 2003).

Hydrophobic value of catalase and lactate dehydrogenase:

The effects of LDMR and microwave heating on hydrophobic values of (I) catalase and (II) lactate dehydrogenase are shown in Fig. 2.

We can see from Fig. 2 (I) that the hydrophobic value of catalase first increased and then decreased as the temperature increased. As we know, hydrophobic residues are hidden due to the protein folding originally (Hashemnia *et al.*, 2006). When the temperature of the enzyme solution increased, the structure of catalase was destructed, which may result in an unpredictable fashion, in the exposure of the internal residues of the protein to the matrix (Cardamone and Puri, 1992). However, higher temperature may cause protein

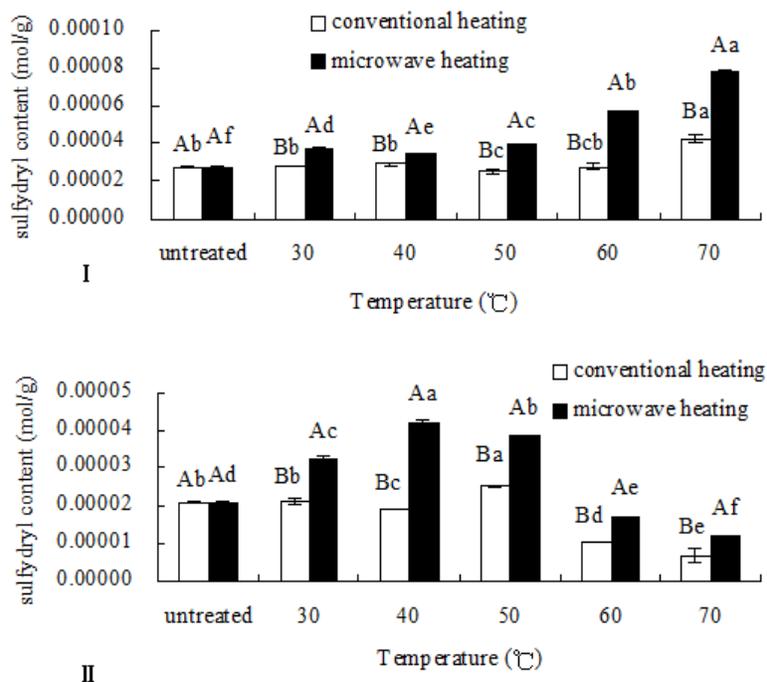


Fig. 1: Effects of microwave heating and conventional heating on sulfhydryl contents of (I) Catalase and (II) Lactate Dehydrogenase (n = 3, $\bar{X} \pm SD$); Bars denote standard deviation of the mean; Different capital letters (A and B) indicate significant differences in-SH contents at the two treatment methods under different temperature; Different lowercase letters (a-f) indicate significant differences in-SH contents at the same treatment methods under different temperature

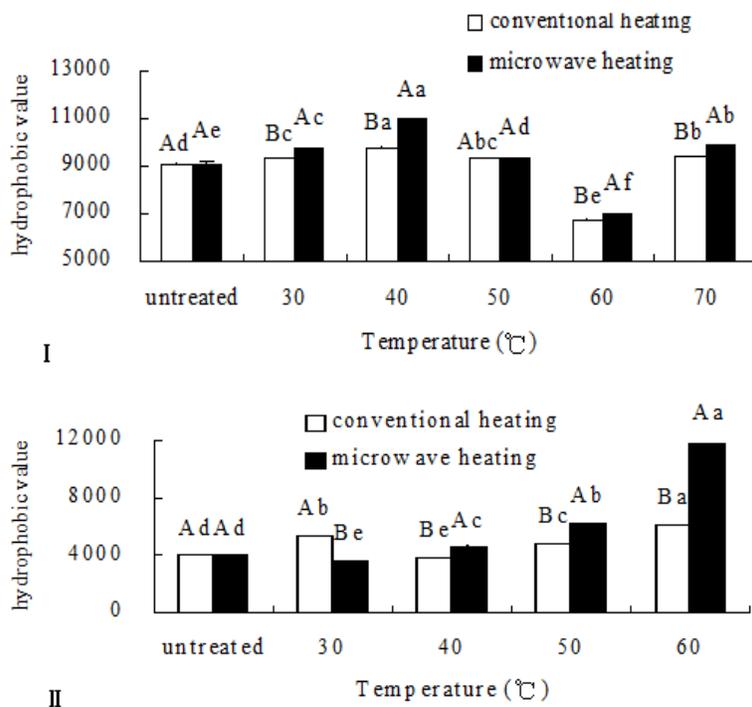


Fig. 2: Effects of microwave heating and conventional heating on hydrophobic values of (I) Catalase and (II) Lactate Dehydrogenase (n = 3, $\bar{X} \pm SD$); Bars denote standard deviation of the mean. Different capital letters (A and B) indicate significant differences in hydrophobicity values at the two treatment methods under different temperature; Different lowercase letters (a-f) indicate significant differences in hydrophobicity values at the same treatment methods under different temperature ($p < 0.05$; LSD)

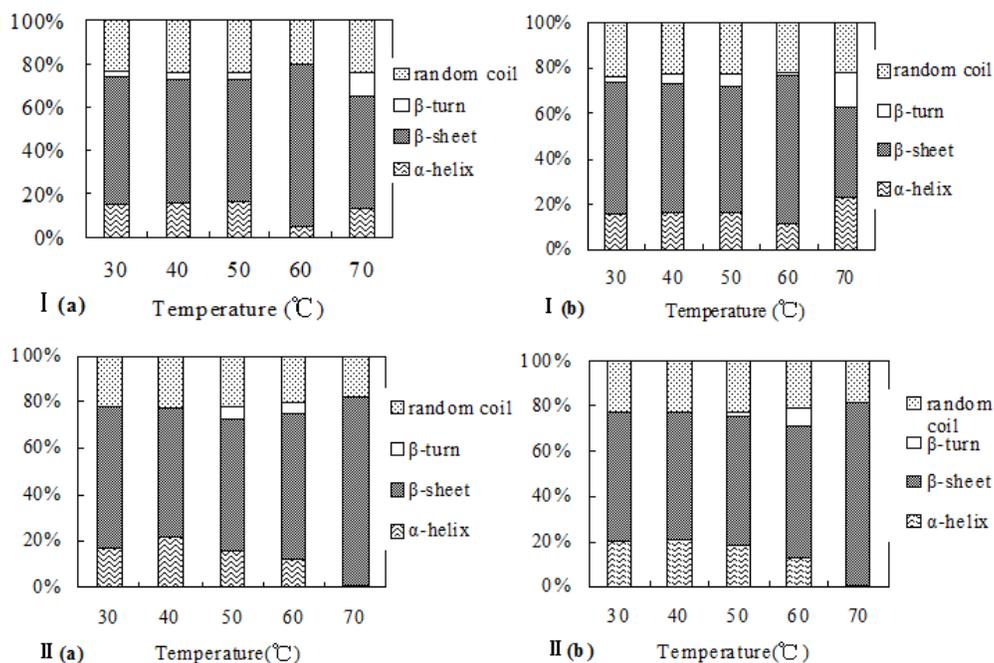


Fig. 3: Effects of microwave heating and conventional heating on secondary structures of (I) Catalase and (II) Lactate Dehydrogenase; (a): Microwave heating; (b): Conventional heating

aggregation (Risso *et al.*, 2008), which leading to the downtrend to the hydrophobic value of catalase. Thereafter, the hydrophobic groups being due to the steric hindrance caused by aggregation peptide chains, are not accessible to interact with each other (Choi *et al.*, 2011), as a result, the hydrophobic value of catalase increased in a manner.

From Fig. 2 (II), the hydrophobic value of lactate dehydrogenase increased sharply above 50°C. This suggested that the longer the enzyme solution exposed to microwave radiation, the more hydrophobic residues being buried in the core of the enzyme exposed.

When the two kinds of approaches achieved the same temperature, the hydrophobic values of specimens treated with LDMR were universally higher than those treated with conventional heating, which illustrated LDMR was more effective in changing the hydrophobicity of catalase and lactate dehydrogenase than conventional heating.

Secondary structure of catalase and lactate dehydrogenase: Figure 3 presents the effects of LDMR and conventional heating on secondary structures of catalase and lactate dehydrogenase.

From Fig. 3 (I), we can see a progressive alteration of catalase's conformation as the temperature increased. When the temperature was below 50°C, progressive decreased in the β -sheet content were accompanied by progressive increased in the β -turn content. When the temperature was above 50°C, β -sheet content first increased (60°C) and then decreased (70°C), whereas

content of β -turn and α -helix first decreased (60°C) and then increased (70°C). In addition, change of random coil content was unobvious. There were no significant differences in random coil contents between LDMR (I a) and conventional heating (I b). When the temperature was above 50°C, β -sheet contents were higher and contents of β -turn and α -helix were lower when catalase samples were exposed to LDMR than to conventional heating.

From Fig. 3 (II), we can see a progressive alteration of lactate dehydrogenase's conformation as the temperature increased. Content of α -helix declined and β -turn content first increased and then decreased, whereas β -sheet content first decreased and then increased. In addition, change of random coil content was unobvious. There were no significant differences in random coil contents between LDMR (II a) and conventional heating (II b). In general, β -sheet contents were higher and α -helix contents were lower when lactate dehydrogenase samples were exposed to LDMR than to conventional heating.

Studies have put forward that the microwaves either caused ions to accelerate and collide with other molecules or caused dipoles to rotate and line up rapidly with electric field resulting in a change in secondary and tertiary structure of proteins of microorganisms (Banik *et al.*, 2003).

Activity of catalase and lactate dehydrogenase: The effects of LDMR and conventional heating on activities of (I) catalase and (II) lactate dehydrogenase are shown in Fig. 4.

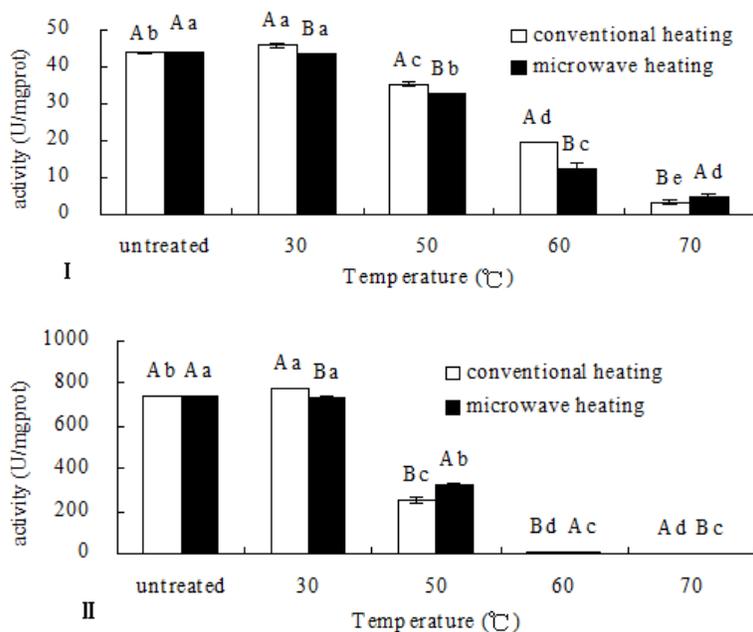


Fig. 4: Effects of microwave heating and conventional heating on activities of (I) Catalase and (II) Lactate Dehydrogenase (n = 3, $\bar{X} \pm SD$); Bars denote standard deviation of the mean; Different capital letters (A and B) indicate significant differences in activity at the two treatment methods under different temperature; Different lowercase letters (a-f) indicate significant differences in activity at the same treatment methods under different temperature

We can see from Fig. 4 (I) that both LDMR and conventional heating treatments led to the activity of catalase decreased as the increasing temperature. Moreover, the catalase activity declined noticeably after 50°C. The activity of catalase was decreased by about 90% when the treatment temperature achieved 70°C, indicating that most catalase was inactivated. When the two kinds of approaches achieved the same temperature, the catalase activities treated with LDMR were universally lower than those treated with conventional heating, which illustrated LDMR was more effective in enzyme inactivation.

From Fig. 4 (II), the activity of lactate dehydrogenase decreased as the increasing temperature after both LDMR and conventional heating treatments. In particular, the lactate dehydrogenase activity sharply declined after 30°C. The activity of lactate dehydrogenase was decreased by about 99% when the treatment temperature achieved 60°C, indicating that most catalase was inactivated.

We can see from Fig. 3 and 4 that the conformation transition temperature and heat inactivation temperature of catalase were higher than those of lactate dehydrogenase. The results showed that the structure of enzyme was closely related to its activity. After LDMR treatment, a progressive alteration of enzyme conformation was accompanied by a progressive inactivation of enzyme.

CONCLUSION

Microwave and conventional heating were both effective at increasing the sulfhydryl content and

hydrophobic value of catalase and lactate dehydrogenase, disrupting secondary structure of the enzyme and decreasing enzyme activity. The activity of catalase and lactate dehydrogenase declined with the increasing temperature. The conformation transition temperature and heat inactivation temperature of catalase were higher than those of lactate dehydrogenase. The sulfhydryl content, hydrophobic value and decreasing rate of enzyme activity were universally higher when catalase samples or lactate dehydrogenase samples were exposed to LDMR than to conventional heating.

In conclusion, the results indicated that LDMR was more effective at inactivating enzymes which were closely related to cell metabolic activities of moulds. LDMR could progressively alter enzyme conformation, to inactivate enzyme which may resulting in the death of moulds. We conclude that LDMR could eventually present a clean and effective means of controlling cereal mildew, but further study will be needed.

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