

Effects of Dill Pickling Process, H₂O₂ and Storage Duration on Lipoxygenase, Peroxidase and Catalase Activities in Cucumber and Brine

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Abstract : Lipoxygenase, peroxidase and catalase activities were determined in tissues and brines of refrigerated dill pickling cucumbers in response to pickling process, storage and H₂O₂. Lipoxygenase was almost inactivated within 1 day exposure to dill pickling brine, and then gradually declined during storage. In contrast, peroxidase activity in cucumber tissue decreased steadily for 4 days after exposure to dill pickling brine. Catalase was present in fresh cucumber tissues, but only slight activity was observed after submerging cucumbers in pickling brine. Lipoxygenase, peroxidase and catalase activities were rapidly inactivated in cucumbers exposed to brine containing H₂O₂. (Received April 23, 1996; accepted June 7, 1996)

Introduction

Disruption of many plant tissues gives rise to rapid hydrolytic and oxidative degradation of endogenous lipids to volatile compounds, responsible for either desirable or undesirable flavor.¹⁻⁵ The typical flavor of fresh cucumber is generated enzymatically by lipoxygenase (linoleate: oxygen oxidoreductase, EC 1.13.11.12), hydroperoxide cleavage and isomerase enzymes when the cucumber fruit tissue is cut or mechanically ruptured in the presence of oxygen. However the flavor volatiles which develop during storage are often undesirable. The fatty acid in cucumber is first attacked by lipoxygenase and product, hydroperoxide, undergoes cleavage and isomerization to form volatiles.

Peroxidase (donor: hydrogen peroxide oxidoreductase, EC 1.11.1.7) decomposes hydrogen peroxide in the presence of a hydrogen donor, which exists naturally in nearly all plants, animals and microorganisms and is particularly related to the production of off-flavor and off-colors in raw and unblanched vegetables.^{6,7} High levels of residual peroxidase activity in pasteurized fresh-pack cucumber pickles were associated with off-flavors. Nebesky et al. found high peroxidase activity in fresh pack Kosher pickles and moderate peroxidase activity in processed dill pickles.⁸

Development of adverse flavor, odor and color during storage of fresh pack Kosher pickles was associated with high peroxidase activity.⁸ Buescher and McGuire repor-

ted that peroxidase activity was present in several commercial retail and food service pickle products.⁹ Also, peroxidase activity gradually declined during fermentation, storage and after processing.¹⁰

Catalase (hydrogen peroxide: hydrogen peroxide oxidoreductase, EC 1.11.1.6) is also found in most animals, plants and aerobic microorganisms. It is primarily located in peroxisomes. It has certain similarities in the action between catalase and peroxidase but has large differences in protein characteristics.

Catalase and peroxidase as hemoproteins are the powerful lipid oxidation catalysts in many foods.⁸ Catalase has a heme group (iron porphyrin group) and is involved in both enzymatic and nonenzymatic oxidation reactions. The nonenzymatic reaction occurs at the iron porphyrin group in hemo protein and catalyzes the oxidation of unsaturated fatty acid.

The objectives of this study were to determine lipoxygenase, peroxidase and catalase activities in cucumber tissues and brine as affected by dill pickling process, storage duration and H₂O₂.

Materials and Methods

Pickling process and brining

Freshly harvested pickling cucumbers (*Cucumis sativus*) were washed and sorted for uniformity in diameter (3.2±0.5 cm) and defects. Cucumbers were sliced longitudinally into halves and packed (563±12 g) into 1 liter

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glass jars. A commercially prepared mixture of dry spices (5 g) was added to each jar. Subsequently, jars were filled with pickling brine, and then sealed tightly. Brine contained on a per liter basis 0.15 g of sodium benzoate, 70 g of NaCl, 9 g of glacial acetic acid, 0.64 g of dill emulsion, 0.17 g of garlic, 0.02 g of onion concentrate and 0.11 g of turmeric acid. All the ingredients were obtained from commercial sources.

Effect of pickling process and storage duration

Activities of lipoxygenase, peroxidase and catalase in tissues or brine samples were determined after dill pickling process within 20 days. These activity values were used as the controls to compare with the values obtained after H₂O₂ treatment for the same storage duration. Brine samples from pickling jars were filtered and used for assaying enzymes activities. Samples of 2 jars for each storage time after pickling process were prepared and refrigerated until assayed.

For to compare enzymes activities between fresh cucumber and cucumber pickles after pickling process, activities of lipoxygenase, peroxidase and catalase were determined from fresh cucumber tissue extracts as the control which was expressed as 0 day after processing in Table or Fig.

Effect of H₂O₂ and storage duration

In the first study, H₂O₂ (5 mM, 0.57 g of 30% H₂O₂) was added to a sample of brine to determine the effects of H₂O₂ on lipoxygenase, peroxidase and catalase activities. Control treatments had no H₂O₂, but stored for same times. Samples of 2 jars for each storage time were prepared and stored. Assays for lipoxygenase, peroxidase and catalase activities in tissue or brine were conducted after H₂O₂ treatment within 20 days. Since the response of peroxidase to H₂O₂ was observed to be very rapid in the first experiment, the second study was established to observe the changes in peroxidase activity at shorter time intervals. Thus, peroxidase activity in tissue extracts was determined at 0~4 hours after treatment with 2.5 or 5 mM H₂O₂. In this case, sample of 3 jars for each storage time and H₂O₂ concentration were prepared.

Enzymes extraction, preparation of substrate and assay solution

General extractions, preparation of substrate, assay solutions and assay conditions were described in the previous report for lipoxygenase, peroxidase and catalase.¹¹

¹²⁾ Activity of lipoxygenase was expressed as $\mu\text{g O}_2$ consumed/min/g fresh wt. or 0.1 ml of brine. Peroxidase activity was expressed as the increase in absorbance (460 nm)/min/0.1 ml of extract or 0.1 ml of brine. Catalase

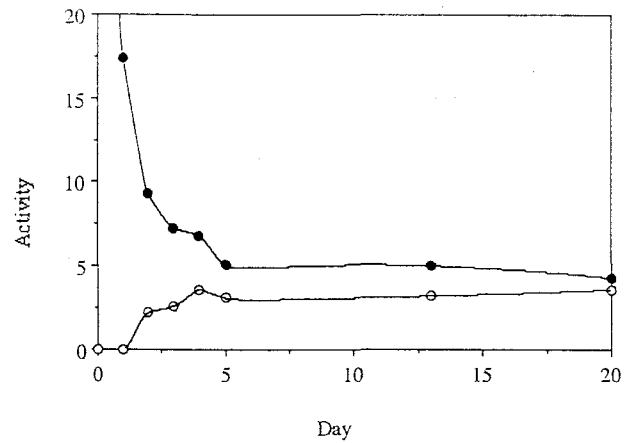


Fig. 1. Effect of pickling process on lipoxygenase activity in refrigerated dill pickle tissue and brine. Lipoxygenase activity is expressed as $\mu\text{g O}_2$ consumed/min/g fresh wt. or 0.1 ml brine (100% activity = 82.3 $\mu\text{g O}_2$ consumed). ●-●; tissue, ○-○; brine.

activity was expressed as the decrease in absorbance (240 nm)/min/0.1 ml of extract or 0.1 ml of brine.

Results

Effect of pickling process, H₂O₂ and storage duration on lipoxygenase

Lipoxygenase activity declined rapidly in cucumbers exposed to refrigerated dill pickle, a nonpasteurized fresh pack product, brine for 1 day, and then gradually declined with increasing storage time (Fig. 1). After exposure to brine for 1 day at 7°C, lipoxygenase lost its activity 79% of initial activity of freshly harvested cucumber. Only 21% of initial activity was remained after 1 day exposure to pickling brine. Activity of lipoxygenase in tissues decreased steadily between 1~5 days, but there was no further decrease in activity after cucumbers were in brine for 5 to 20 days. Lipoxygenase activity decreased from 100% in fresh tissue to about 5% after 5 days in pickling brine.

When filtered brine samples from pickle jars were assayed for lipoxygenase activity, no activities of lipoxygenase were found in brine samples after 2 days pickling process (Fig. 1). Lipoxygenase activities in brine samples were increased steadily for 2~5 days, but there was no further change in lipoxygenase activity in brine samples 5 days after submerging the cucumbers.

With addition of 5 mM H₂O₂ in pickling brine, lipoxygenase activity in tissue within the first day rapidly declined to 3.6% of initial activity of fresh cucumber tissues and it had no further effect on lipoxygenase activity during storage (Fig. 2). Residual lipoxygenase activity of about 3% was remained within 20 days of period time after dill pickling process. Compared to the 21% of activity in pickled cucumber for 1 day exposure to brine,

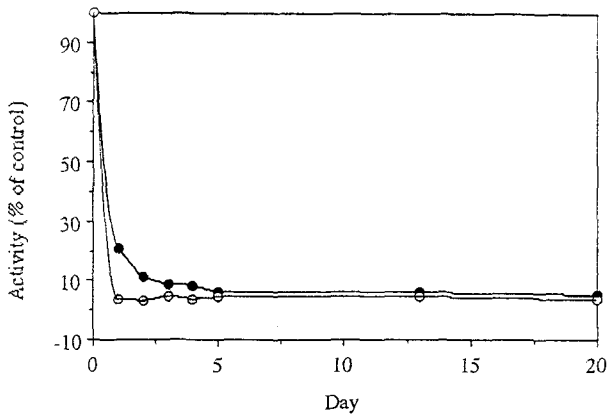


Fig. 2. Effect of pickling process and H_2O_2 on lipoxygenase activity in refrigerated dill pickle tissues. Lipoxygenase activity is expressed as % of control (100% = 82.3 $\mu g O_2$ consumed/min/g fresh wt). ●—●; no H_2O_2 , ○—○; H_2O_2 .

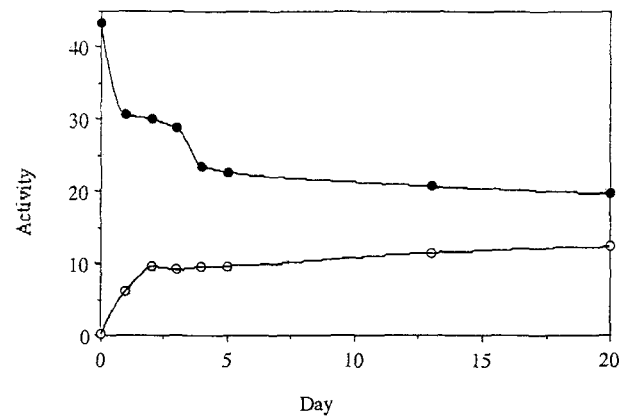


Fig. 3. Effect of pickling process on peroxidase activity in refrigerated dill pickle tissue and brine. Peroxidase activity is expressed as $\Delta A_{460}/\text{min}/0.1\text{ml}$ extract or 0.1 ml brine. ●—●; tissue, ○—○; brine.

Table 1. Effects of pickling process and H_2O_2 on lipoxygenase and peroxidase activities in refrigerated dill pickle brine.

Days after processing	Lipoxygenase ^a		Peroxidase ^b	
	Control	$H_2O_2^c$	Control	H_2O_2
0	ND ^d	ND	0.1	0.1
1	ND	ND	6.2	ND
2	2.2	ND	9.6	ND
3	2.6	ND	9.2	ND
4	3.1	ND	9.6	ND
5	3.1	ND	9.7	ND
13	3.2	ND	11.5	ND
20	3.6	ND	12.4	ND

^aLipoxygenase activity is expressed as g O_2 consumed/min/g fresh wt. or /0.1 ml brine. ^bPeroxidase activity is expressed as $\Delta A_{460}/\text{min}/0.1\text{ml}$ extract or /0.1 ml brine. ^cThe initial concentration of H_2O_2 in brine was 5 mM which was anticipated to equilibrate to 2 mM. ^dND: Not detected.

H_2O_2 in brines altered the loss of lipoxygenase activity in pickles. In contrast, H_2O_2 treatment in brines caused filtered brine samples to have no lipoxygenase activity at any of the observation times (Table 1). The decline in lipoxygenase was accelerated by the presence of H_2O_2 in dill pickling brine, although minimum levels were similar in both tissues and brine samples.

Effect of pickling process, H_2O_2 and storage duration on peroxidase

Activity of peroxidase in cucumber tissues gradually decreased during 4 days storage after dill pickling process, and then it remained fairly constant (Fig. 3). Peroxidase activity declined rapidly to 70% of initial activity of fresh cucumbers after 1 day in pickling brine. Actually, there was 30% loss of peroxidase activity. The loss in peroxidase activity was not high in comparison to that

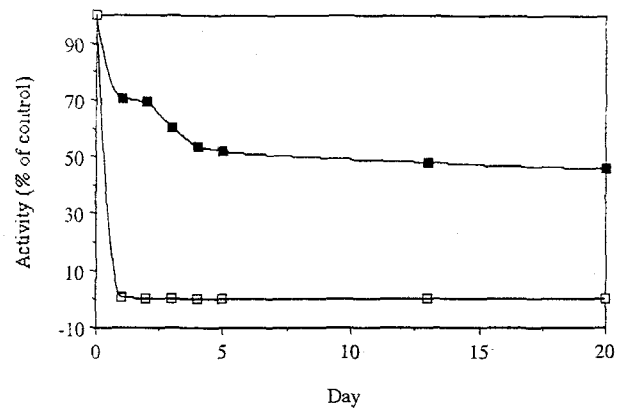


Fig. 4. Effect of pickling process and H_2O_2 on peroxidase activity in refrigerated dill pickle tissues. Peroxidase activity is expressed as % of control (100% = 43.3 $\Delta A_{460}/\text{min}/0.1\text{ml}$ extract). ■—■; no H_2O_2 , □—□; H_2O_2 .

of lipoxygenase activity after fresh cucumbers were processed into dill pickle product. In case of lipoxygenase, there was 70% loss of its activity 1 day after exposure to brine. Peroxidase lost its activity from 30% to 47% after cucumbers were in brine for 1 to 4 days. In contrast to lipoxygenase, peroxidase activity in pickles was fairly high even after exposure to brine for 20 days, and had about 50% of residual activity remained.

Peroxidase activity increased steadily in filtered brine samples from 1 to 20 days storage (Table 1). Activity of peroxidase was detected in brine samples one day after dipping fresh cucumbers in brine. Compared to lipoxygenase, peroxidase was released rapidly from tissues into brines.

H_2O_2 treatments of brines caused tissues and brines to have no peroxidase activities. With 1 day exposure to H_2O_2 in brine, peroxidase activities in tissue extracts were only 0.9% of initial activity of fresh cucumbers (Fig.

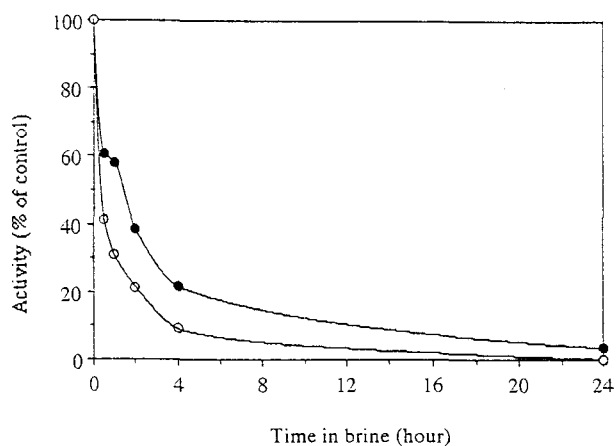


Fig. 5. Rapid effects of 2.5 mM or 5.0 mM H₂O₂ in brine on peroxidase activity. Activity is expressed as % of control (100% = 44.2 $\Delta A_{460}/\text{min}/0.1 \text{ ml}$ extract). ●—●; 2.5 mM H₂O₂, ○—○; H₂O₂.

4). After 1 day, no peroxidase activity was detected in tissue extracts. Also, no peroxidase activity was detected in brine sample of H₂O₂ treatment from 1 to 20 days of storage (Table 1). In the second experiment with short-time exposure to H₂O₂, peroxidase activities in tissues with 2.5 and 5 mM H₂O₂ treatment for 30 min were 60.8% and 41.1% of the initial activity of fresh cucumbers, respectively (Fig. 5). 21.7% and 9% of the initial peroxidase activity remained in tissues 4 hours after submerging sliced cucumbers into brines containing 2.5 and 5 mM H₂O₂, respectively. These results indicated that the response of peroxidase to H₂O₂ was very rapid and that it was affected by storage time and H₂O₂ concentration.

Effect of pickling process, H₂O₂ and storage duration on catalase

Catalase activity was present in fresh cucumber tissues, but it was observed to be very low after submerging cucumbers in dill pickling brines for 1–3 days. No activity was detected after 3 days exposure to brines (data not shown). With 5 mM H₂O₂ in brine, no catalase activities were detected in cucumber pickles at any of the sampling times. Filtered brine samples of both pickling process and H₂O₂ treatment had no catalase activity at all.

Discussion

Lipoxygenase and peroxidase activities declined in cucumber tissues exposed to pickling brine during the first day of storage while activities increased in brine samples. According to these results, lipoxygenase and peroxidase seemed to be released from tissues into brine.

H₂O₂ (5 mM) in brine accelerated the decline in lipoxygenase activity. Even though lipoxygenase activity in

refrigerated dill pickles declined during storage, more reduction of lipoxygenase activity was observed due to H₂O₂ treatment. H₂O₂ in dill pickling brine also strongly inactivated peroxidase and catalase. With 2.5 mM H₂O₂, peroxidase activity was reduced, but it was not as much as with 5 mM H₂O₂. The mechanism of inactivation may be explained that H₂O₂ oxidized metal and/or sulfhydryl (–SH) groups of these oxidases. Lipoxygenase requires nonheme iron (Fe⁺⁺⁺) for activation.^{2,13,14}

Peroxidase and catalase contain heme as a prosthetic group.^{6,7,15} These active sites are probably oxidized by H₂O₂ or free radicals generated by H₂O₂ and thus the reactions are not performed. It is unknown if activity can be protected or restored by reducing substances.

Mitsuda *et al.* reported that H₂O₂ caused a significant loss of lipoxygenase activity from defatted soybean meal. Lipoxygenase activity declined by 50% with 6×10^{-6} M H₂O₂.¹⁶ Sumner and Gjessing reported that peroxidase can be inactivated by an excess of H₂O₂.⁸ However, activity was restored if the level of H₂O₂ is reduced by catalase. In dill pickling brine, catalase is rapidly inactivated, thus it was unable to reduce levels of H₂O₂.

In summary, lipoxygenase, peroxidase and catalase were rapidly inactivated in cucumbers exposed by dill pickling brines and H₂O₂ treatment. Lipoxygenase and peroxidase activities in tissues rapidly declined and their activities after being released from tissues into brines gradually increased to a certain values and remained constant over 20 days storage. In the presence of H₂O₂ in pickling brine, the reduction of lipoxygenase and peroxidase activities were accelerated. In case of catalase, pickling process and H₂O₂ treatment in brine caused complete inactivation of its activity in cucumbers.

Thus, cucumber lipoxygenase and peroxidase appear to be capable of surviving pickling process and brine storage. The residual activities of these two enzymes may be responsible for off-flavor generation in pickle products during storage. It appears that knowledge of residual lipoxygenase and peroxidase activities of a non-pasteurized fresh pack product may be useful for evaluating product quality stability.

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Pickle 제조과정, H₂O₂, 저장기간에 따른 refrigerated dill pickle과 brine종의 lipoxygenase, peroxidase, catalase 활성의 변화

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초록: 제조과정, 과산화물 및 저장기간에 따른 냉장 Dill 피클과 brine에 함유된 lipoxygenase, peroxidase, catalase 효소의 활성 변화를 조사하였다. 오이를 Dill 피클용 brine에 1일 저장시의 lipoxygenase는 거의 비활성화 되었으나, 1일 이후에는 lipoxygenase의 활성은 서서히 감소하였다. 반면, 오이 tissue에 존재하는 peroxidase의 활성은 피클 제조후 4일 저장기간 동안 서서히 감소 현상을 나타내었다. 신선한 오이 tissue에서 추출된 catalase는 높은 활성을 가지나, 오이를 피클용 brine에 담갔을 경우에는 소량의 catalase 활성만 보였다. Brine에 과산화물을 첨가시, tissue에서 추출된 lipoxygenase, peroxidase, catalase 활성은 저장 기간과 과산화물 농도에 따라 지속적인 감소를 보였다.

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