Potential Application of Hot Rehydration Alone or in Combination with Hydrogen Peroxide to Control Pectin Methylesterase Activity and Microbial Load in Cold-stored Intermediate-moisture Sun-dried Figs

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ABSTRACT: Sun-dried figs contain a considerable amount of pectin methylesterase (PME) activity (22 μ*M* COOH/min/g). The enzyme causes softening and loss of desired gummy texture in cold-stored intermediate-moisture (IM) sun-dried figs brought to a 28% to 29% moisture range. Partial reduction of PME activity (28%) delayed undesirable textural changes in IM figs rehydrated at 80 °C for 16 min. The heat treatment did not cause a considerable reduction in microbial load. However, the addition of 2.5% H_2O_2 to the rehydration medium at 80 °C reduced the initial total mesophilic aerobic count of figs by at least 90% and turned the figs from a brown color to a desirable and stable yellow–light brown. The in situ fig catalase remains after rehydration at 80 °C. Thus, by reducing the contact period of figs with H_2O_2 or by pureeing figs, it is possible to eliminate residual H_2O_2 and to obtain safe and SO_2-free light-colored fig products.

Keywords: fig, pectin methylesterase, microbial load, rehydration, hydrogen peroxide

Introduction

Turkey, with its 300000 metric tons of annual production, is the largest producer of figs in the world (Cabrita and others 2001). Most figs are produced in the Aegean region of Turkey around the city of Izmir, ancient Smyrna. The dominant cultivar grown in Turkey is Sarilop. Almost all figs grown are destined for sun-drying, which takes place after the fruits partially dry and fall from the trees. Traditionally, sun-drying is carried out in the field by spreading the figs on mats for 8 to 10 d (Cemeroglu 1986). In recent years, the drying also has been conducted in some simple tunnel driers that accelerate the drying process and increase the microbial quality of the figs.

Sun-dried figs generally contain 15% to 20% moisture (Desai and Kotecha 1995) and, with their characteristic gummy texture, they may be consumed as is or may be used in products such as breakfast cereals, cereal bars, and confectionaries. In recent years, industry and consumer demand for intermediate-moisture (IM) fruits has increased the rehydration of sun-dried fruits to 25% to 40% moisture (Cemeroglu 1986; Desai and Kotecha 1995; Simmons and others 1997). IM fruits are more suitable for direct consumption, and they may also be used in the production of dairy and bakery products. Moreover, IM fruit pieces or purees may be used in salads, fruit drink formulations, preserves, jams, or jellies (de Daza and others 1997).

Because of their higher moisture contents, IM fruits are generally stabilized by different chemical preservatives such as sorbates, sulfites, or benzoates (Cemeroglu 1986; de Daza and others 1997). These chemicals effectively inhibit the growth of most pathogenic and spoilage microorganisms in IM fruits and provide a reasonable shelf-life at room temperature (de Daza and others 1997). Thermal processing is also used to obtain shelf-stable IM fruits. For example, Cemeroglu (1986) reported the pasteurization of IM dates. Also, it was reported that dried prunes and figs are heat-treated with steam or boiling water to increase their water content to 40% (Desai and Kotecha 1995). However, the increasing health concerns of consumers toward foods stabilized by chemical preservatives, as well as the unsatisfactory sensory and nutritional properties of thermally processed foods, have forced the dried-fruit industry to seek alternative preservation methods (de Daza and others 1997; Welti-Chanes and others 1997).

Recently, some studies were conducted in which researchers successfully reduced the microbial load of dried fruits such as raisins and plums with vapor-phase H_2O_2 disinfection (Simmons and others 1997; Sapers and Simmons 1998). In most countries, H_2O_2 has been approved for use in different food products as an antimicrobial agent. In fact, some H_2O_2-containing disinfectants approved by ministries of health in Europe and Israel are still being used in drinking water and food industries as an alternative to chlorine (Fallik and others 1994). The United States Food and Drug Administration (FDA) has approved the use of H_2O_2 for the treatment of milk for use in cheese, and for the preparation of modified whey and thermophile-free starch. Recently, the FDA also approved the use of H_2O_2 in a mixture of disinfectants for red meat carcasses (Mermelstein 2001). Moreover, the United States Dept. of Agriculture (USDA) allows the use of H_2O_2 for the pasteurization of egg

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white (Muriana 1997). H_2O_2 is a GRAS (Generally Recognized as Safe) chemical, but the FDA requires that residual H_2O_2 be removed by appropriate physical and chemical means during processing (CFR 2000).

Although H_2O_2 may effectively be used to reduce the microbial load of fruits and vegetables, its application should be supported by additional procedures to prevent the reproduction of survivor microorganisms during storage (Simmons and others 1997; Sapers and Simmons 1998). Thus, recently we investigated the combined application of hot rehydration, liquid-phase H_2O_2 disinfection, and cold storage to produce rehydrated IM sun-dried figs. During cold storage, in addition to nonenzymatic browning and microbial development, another major problem we observed was softening and loss of desired gummy texture of IM figs in several months. By deesterifying the pectin in plant cell walls and making it a substrate for depolymerizing polygalacturonases (PG), the enzyme pectin methylesterase (PME) plays a central role in the softening of fruits and vegetables (Pressey and Woods 1992; Thakur and others 1996). Thus, for the production of good-quality IM sun-dried figs, the control of PME action is also essential. In this study, we investigated the activity and thermal properties of PME in sun-dried figs and tested the potential application of hot rehydration alone or in combination with H_2O_2 to control PME activity and microbial load during cold storage of IM sun-dried figs.

Materials and Methods

Materials

Sun-dried figs (cultivar Sarilop from Aydin, Turkey) were supplied by TARIS (Izmir, Turkey), the cooperative for marketing agricultural products grown in the Aegean region. Fresh figs (cultivar Sarilop from Aydin, Turkey) were obtained from a local market in Izmir and kept frozen at –25 °C until used in the experiments. The dialysis tubing (prepared as described in the product information), citrus pectin (galacturonic acid content, 79%; methoxy content, 8%), insoluble polyvinylpolypyrrolidone, horseradish peroxidase (type II), and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). H_2O_2 (30%, extra pure), ammonium sulfate (for biochemistry), plate count agar (PCA), potato dextrose agar (PDA), and tartaric acid were purchased from Merck (Darmstadt, Germany). The chloramphenicol antibiotic was kindly donated by Borkim Chemical Co (Izmir, Turkey). H_2O_2 test strips were obtained from Macherey-Nagel Co. (Dueren, Germany).

In this study, the samples used in extraction of PME, determination of PME activity and residual H_2O_2 were prepared by taking pieces from about 10 figs.

PME extraction and partial purification

For the extraction of PME enzyme, 30 to 50 g samples of sun-dried or fresh figs were homogenized in 180 to 200 mL of 0.02 *M* sodium phosphate buffer (at pH 7.0, 4 °C) containing 1 *M* NaCl for 1.5 min using a Waring blender (no load speed 18000 rpm). Two-percent polyvinylpolypyrrolidone was also added to the medium to absorb the phenolic compounds during homogenization. The slurry obtained was then filtered through 4 layers of cheesecloth and used in this study after centrifugation. This enzyme extract containing ionically bound and soluble enzymes was designated crude PME extract. The residues obtained from the filtration and centrifugation of this extract were combined and used for the determination of covalently bound PME enzyme activity.

When it was used in partial purification studies, the crude PME extract had been centrifuged at 4000 × *g* for 30 min (4 °C). For the partial purification, solid $(NH_4)_2SO_4$ was added slowly to enzyme extract at 4 °C up to 90% saturation. The mixture was stirred slowly for 1 h and the precipitate collected by 45 min centrifugation at 4000 × *g* (4 °C) was dissolved in a minimum amount of deionized water. The enzyme extract was then dialyzed for 24 h at 4 °C by 2 changes of 2000 mL of deionized water and used in heat inactivation studies.

PME activity

For the determination of PME enzyme activity we used spectrophotometric or titrimetric methods. In the spectrophotometric tests, the method of Hagerman and Austin (1986) was used with slight modifications to determine enzyme activity in crude or partially purified PME extracts. The reaction mixture was formed by mixing 2.3 mL 0.5% pectin solution prepared in 0.1 *M* NaCl, 0.5 mL of 0.01% bromothymol blue prepared in 0.003 *M* sodium phosphate buffer (pH 7.5), and 0.2 mL of crude or partially purified enzyme extract. The decrease in absorbance at 620 nm was monitored by using a Shimadzu (Model 2450) spectrophotometer, equipped with a constant-temperature cell holder at 30 °C, and enzyme activity was determined from the slope of the initial linear portion of the absorbance against time curve. All activities measured were corrected by determining spontaneous decreases in absorbance by using the reaction mixture containing boiled enzyme extract. In heat inactivation and partial purification studies, the enzyme activities were expressed as percent initial activity or units, respectively. One unit was defined as that amount of enzyme that caused 0.001 change in absorbance in 1 min.

The activities of crude PME, covalently bound PME, and fig homogenates were determined by the modification of the titrimetric method given in Yemenicioglu (2002). The fig homogenate was obtained by homogenizing a 50 g fig sample with 150 mL of 8.8% NaCl. To determine covalently bound PME activity, the residues obtained from the filtration and centrifugation of crude PME extract were combined and suspended in deionized water. The reaction mixture contained 1.5 mL of enzyme extract (or 0.8 to 4 g of homogenate or suspension) and 20 mL of 0.5% pectin solution prepared in 0.1 *M* NaCl. The pH of reaction mixture was brought to 7.5 with 0.1 *N* NaOH and kept constant for 10 min by titrating slowly with 0.01 *N* or 0.05 *N* NaOH. The titrations were conducted in a double-walled magnetically stirred cell connected to a circulating water bath working at 30 °C, and enzyme activities were expressed as percent initial activity or μM COOH groups liberated per minute per gram of figs. All activity measurements were done at least 3 times and averages were calculated.

Protein content

Protein was determined by the Lowry method by using bovine serum albumin as standard (Harris 1987).

Heat inactivation of PME

The temperature profiles were determined by heating 1.5 mL of crude PME enzyme extract (centrifuged at 3000 × *g* and at 4 °C for 15 min) in thermal inactivation time (TIT) tubes (9-mm inner dia; 1-mm wall thickness) for 5 min at 50 to 70 °C. The tubes were then cooled in an ice water bath for 10 to 15 min, and the residual enzyme activities, assayed by the titrimetric method, were given as percent initial activity.

The heat inactivation of partially purified PME was studied over the temperature range of 60 to 90 °C. To minimize the lag phase, 0.3-mL aliquots of enzyme extract were pipetted into preheated TIT tubes. After heating for a given period, the tubes, cooled for 10 to 15 min in an ice water bath, were immediately assayed for PME activity by the spectrophotometric method. All heat-inactivation studies

were conducted as 3 replicates, and averages of activities were calculated and expressed as percent initial activity.

Rehydration and storage studies

To find the rehydration times required to bring IM figs to approximately 30% moisture content at different temperatures, we determined the rehydration curves of samples at 30, 70, 80, and 90 °C in a circulating water bath (Polysience, Model 71). The times required to bring figs to 30% moisture content at the given temperatures were 65, 17.5, 16, and 8.8 min, respectively. In these kinetic experiments, 200 to 250 g of figs were put into sacks made from cheesecloth and rehydrated at the given temperatures. In all rehydration studies, the fig/water ratio was set to 0.1 (w/w), and the increase in the weight of samples was monitored by draining and weighing (± 0.01 g) the sacks at different time intervals. The initial moisture content of figs was determined by the standard vacuum oven method of AOAC (nr 934.06) for dried fruits (AOAC 1996). During rehydration studies, the temperature profiles of 3 figs were also determined by placing a 0.9-mm-dia thermocouple in their geometric center and by using a portable temperature recorder (Cole Parmer, DualLogR, Vernon Hills, U.S.A.). The moisture analysis, rehydration experiments, and temperature measurements were repeated for each party of figs separately, and the rehydration times to obtain 30% moisture figs at different temperatures were modified when needed.

To obtain the IM figs used in the storage experiments, almost 1.2-kg figs were rehydrated at 30 or 80 °C. The figs, drained and cooled at room temperature, were then spread over trays and incubated for 12 min at 100 °C in a forced hot air oven to remove free water from their surfaces. At the 6th min of drying, the figs were turned over to obtain homogeneous drying. This treatment resulted in 1.38% ± 0.28% moisture loss from the figs and brought their moisture content to a range of 28% to 29%. The samples were then separated into approximately 300-g groups, and each group was put into zipped polyethylene bags and cold stored between 4 and 7 °C for 1 to 3 mo. Although we removed the free surface water of the figs by the oven treatment, after rehydration some figs entrapped water and this caused water leakage and wetting of figs during storage. To prevent this, test tubes sealed with cotton tabs and containing 4 g of silicagel were also placed into the bags before closing them. For 3-mo cold storage, the possible effect of these absorbers to IM fig moisture content was insignificant (< 0.12%). All rehydrations were repeated twice and the results of microbiological tests were given separately for each trial.

At the end of 3 mo of storage, the figs were carefully examined for texture by 3 simple tests conducted by hand. In test 1, the figs were examined by the classical thumb test to detect whether they softened. In test 2, to detect any sticky and gel-like structure formation, the figs were halved and their flesh was squeezed by using thumb and forefinger. In test 3, the internal surface of halved figs was smoothly rubbed with the thumb to see whether it rubbed off from the peels easily. All tests were performed by the same trained person.

Disinfection of figs with H_2O_2

The disinfection of figs was conducted by rehydrating them at 80 °C in 2.5% H_2O_2 (w/v) solution for 16 min. The rehydrations were repeated twice by using 1.2-kg figs at each trial and by maintaining the 0.1 (w/w) fig/H_2O_2 solution ratio. After rehydration, the figs were drained and cooled at room temperature, treated in the oven, packed as described previously, and cold stored for 1 or 3 mo. The effect of H_2O_2 on fig color was monitored by taking the photographs of packed figs with a digital camera (Nikon PIX995, Japan).

Determination of residual H_2O_2

The residual H_2O_2 was determined by using semiquantitative Quantofix test strips, which can detect H_2O_2 at 1 to 100 mg·L^{-1}. During tests with whole figs, 25-g sample was homogenized with 200 mL 0.05 *M* Na-phosphate buffer (at pH 7.0 and 4 °C) for 1.5 min in a Waring blender (no load speed 18000 rpm). When fig purees were tested, 25-g sample was homogenized with 150 mL distilled water (at 4 °C) for 0.25 min at the same blender speed setting. To prevent the degradation of residual H_2O_2 by in situ catalase, the slurries obtained were rapidly filtered through cheesecloth, and a test strip was immediately dipped into the filtrates. The concentration of the residual H_2O_2 in filtrates, determined by comparing the intensity of blue color developed on test strips and the color-concentration scale provided with the test strips, was given as mg·kg^{-1}.

The homogenates of disinfected figs were tested for residual H_2O_2 immediately after the oven treatment and during cold storage at different time intervals until no residual H_2O_2 was detected by the test strips. The disappearance of residual H_2O_2 in IM figs was also confirmed qualitatively by the more sensitive enzymatic H_2O_2 determination method. The reaction mixtures used in the enzymatic method were formed by mixing 4 mL of filtrate prepared as described previously with phosphate buffer, 0.25 mL peroxidase (almost 42 purpurogallin Unit prepared in 0.05 *M*, pH 7.0 phosphate buffer), and 0.2 mL 0.5% guaiacol (prepared in 50% ethanol). The blanks for comparison were prepared by using 0.2 mL phosphate buffer in place of guaiacol.

Catalase activity

The presence of catalase enzyme activity in sun-dried IM figs was determined qualitatively. For this test, after oven treatment, the figs that had been rehydrated at 30 °C or 80 °C were halved and dipped into a 2.5% H_2O_2 solution at room temperature. The observation of gas evolution and foaming was accepted as the indication of catalase activity.

Microbiological tests

For microbiological tests, approximately 60 g of fig sample (quartered pieces obtained from 10 figs) was put into flasks containing 250 mL 0.1% (w/w) peptone water. The flasks were shaken by hand for 2 min, and 0.1-mL samples were spread onto the surface of agar plates. One-tenth dilutions were performed when needed by using 0.1% peptone water. The total number of mesophilic aerobic microorganisms and yeasts and molds were determined by using PCA and PDA (acidified to pH 3.5 with 10% tartaric acid or supplemented with 100 mg/L chloramphenicol) agars, respectively. The PCA plates were incubated at 35 °C for 48 h, whereas PDA plates were incubated at 25 to 28 °C for 5 d. The averages of 3 plate counts were used in all microbiological tests.

Statistical analysis

The effects of 2 factors, rehydration conditions and storage time, on total mesophilic aerobic load of IM figs were determined by analyses of variance (ANOVA). At an alpha level of 0.05, a significant effect was concluded if results showed $P \leq 0.05$. Because of their relatively low numbers, total yeast and mold counts were not statistically analyzed.

Results and Discussion

Possible mechanisms of textural change during cold storage

The molecular properties, solubility and amount of pectic compounds, are the primary factors determining the texture of fresh

and processed fruits and vegetables. (Cemeroglu and others 2001). Thus, because of its central role in the modification of pectin, PME affects the textural properties of fruit and vegetable products considerably (Castaldo and others 1989; Thakur and others 1996; Alonso and others 1997). The PME enzyme is capable of catalyzing pectin demethylation even at low storage temperatures, whereas depolymerization enzymes such as PG slow down under the same conditions (Marangoni and others 1995; Artes and others 1996). Thus, it seems that during cold storage, PME reduced the degree of pectin methylation in IM figs. This possibly enabled the interaction of pectin and divalent ions such as Ca^{++} and Mg^{++} and caused the gel formation. During storage, the enzyme PG should also have slowly degraded pectin molecules, and this may have reduced the consistency of the fruit flesh and induced the formation of a very sticky structure.

The origin of PME in sun-dried figs

The ability of different bacteria and fungi to produce pectic enzymes was reported by different researchers (Liu and Luh 1978; Hao and Brackett 1994). However, the presence of PME enzyme activity in sun-dried figs indicated that the enzyme was not formed by some microorganisms during cold storage of rehydrated IM figs. The sun-dried figs contained both ionically bound and soluble and covalently bound PME enzyme fractions, and activity of these enzymes were 16.6 and 5.7 μM COOH/min/g, respectively. To obtain a better proof related to the origin of this enzyme, the temperature profiles of crude PME obtained from healthy fresh figs and softened IM sun-dried figs rehydrated at 30 °C and cold stored for 3 mo were also compared (Figure 1). As seen in Figure 1, the temperature profiles of crude PME in fresh and softened IM sun-dried figs are quite similar. Thus, this result suggests that the enzyme is fig PME that remained after sun-drying.

Partial purification of PME

As seen in Table 1, 0% to 90% $(NH_4)_2SO_4$ precipitation and dialysis of PME extracted from fresh and sun-dried figs gave 132% and 169% yields, respectively. The high yields obtained after dialysis suggest the removal of a PME enzyme inhibitor by partial purification. Because of their lower moisture content, the total PME activity purified from 30-g sample of sun-dried figs was almost 1.6-fold higher than that purified from the same amount of fresh figs. However, the high activity determined in sun-dried figs indicated that the enzyme is very stable under low a_w conditions. The total protein contents in the crude extracts of fresh and sun-dried figs were almost the same. However, after ammonium sulfate precipitation and dialysis, more protein remained in the partially purified extract obtained from fresh figs. This suggests the partial hydrolysis of the proteins in sun-dried figs by in situ proteases and explains the higher purity of this enzyme extract after partial purification.

Heat inactivation of PME

To determine a suitable rehydration temperature, the heat inactivation kinetic of partially purified PME from sun-dried figs was investigated between 60 and 90 °C. The inactivation of PME from sun-dried figs followed a 1st-order reaction kinetic, and the biphasic inactivation curves of the enzyme indicated that it contained heat-labile and heat-stable enzyme fractions (Table 2). The enzyme also showed activation by heating and this occurred particularly at 60 and 70 °C (Figure 2). However, at 80 and 90 °C, the activation was not observed and PME showed rapid inactivation. Thus, it seems that the rehydration temperature should be above 70 °C to achieve faster enzyme inactivation and minimize activation.

For the calculation of the enzymes' heat inactivation parameters, the residual enzyme activities determined at different temperatures were plotted on semi-log curves. However, the points of activation were not considered during calculation of D values. For comparison, the heat inactivation kinetic of partially purified PME from fresh figs was also studied (Figure 3). Although the D values of PME from fresh figs were almost 2-fold higher than those of PME from sun-dried figs, both enzymes showed almost the same activation and inactivation patterns. In particular, the activation and inactivation patterns observed at 70 °C were quite similar. Also, the z values of the enzymes heat labile and heat stable portions between 70 and 90 °C were almost the same. Thus, these results confirm that the PME in sun-dried fruits is fig PME that remained after sun-drying.

To determine the heat inactivation behavior of enzyme in fig tissues, we also compared the residual activity of PME in ionically bound and soluble enzyme extracts and covalently bound en-

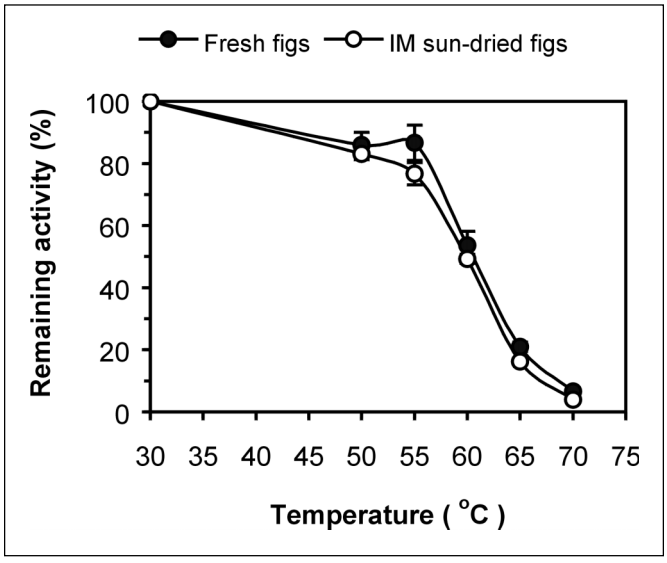

Figure 1—Temperature profiles of crude pectin methylesterase from 3 mo of cold-stored, softened intermediate-moisture sun-dried figs and fresh figs

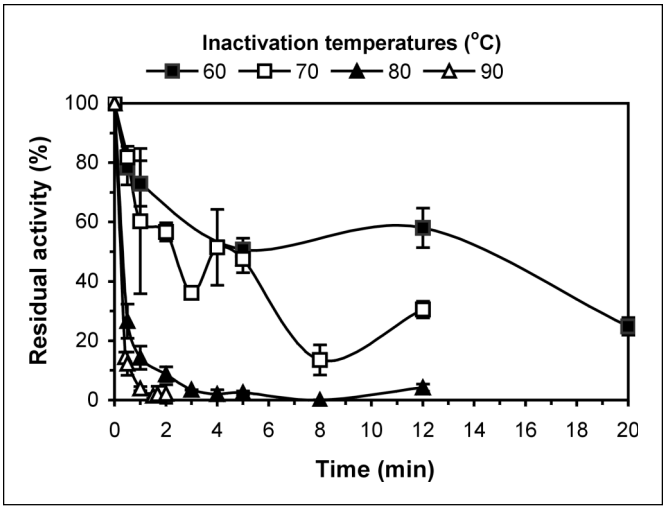

Figure 2—Heat inactivation curves of partially purified pectin methylesterase from sun-dried figs

Table 1—Partial purification of pectin methylesterase (PME) from fresh and sun-dried figs

Purification step	Volume (mL)	Total activity (units)	Total protein (mg)	Specific activity (units/mg)	Yield (%)	Purity (fold)
Fresh figs						
Crude extract	111	10900	230	47	100	1.0
0% to 90% $(NH_4)_2SO_4$ precipitation and 24-h dialysis	65	14400	42	343	132	7.3
Sun-dried figs						
Crude extract	76	12900	262	49	100	1.0
0% to 90% $(NH_4)_2SO_4$ precipitation and 24-h dialysis	48	21800	25	872	169	17.8

zyme extracts obtained from sun-dried figs rehydrated at different temperatures (Figure 4). The covalently bound enzyme is heat labile and lost almost 50% of its activity when rehydration was conducted at 80 or 90 °C. The ionically bound and soluble PME, on the other hand, showed almost 25% and 30% inactivation after 80 and 90 °C rehydrations, respectively. Thus, for PME inactivation, rehydration at 90 °C has almost no benefits. The times of rehydration to achieve 30% moisture content at 80 and 90 °C were approximately 16 and 8.8 min, respectively (Figure 5). According to the results of heat-penetration studies (Figure 6) and the heat inactivation data obtained, these temperatures and times should be enough to achieve at least 1 logarithmic reduction of ionically bound and soluble PME activity. However, the increase of ionically bound and soluble PME enzyme activity after rehydration at 70 °C clearly indicated the activation of in situ PME in whole figs by heating. The activation of this enzyme fraction was also observed during the heat inactivation studies conducted with partially purified ionically bound and soluble PME enzyme extracts. However, during these studies, activation at 70 °C did not cause an increase of enzyme activity over initial activity. Thus, it appears that the limited enzyme inactivation by hot rehydration was due to the considerably greater activation of in-situ PME in whole figs. It is likely that when fig tissue is homogenized for enzyme extraction the enzyme comes into contact with some inhibitors that limit its activation during heating. Also, it is well known that the heat inactivation of enzymes is affected by their solubility (Wasserman 1984; Weng and others 1991). Thus, the PME in sun-dried figs may be immobilized by the concentrated cellular matrices such as

Table 2—Heat inactivation parameters of partially purified pectin methylesterase (PME) from fresh and sun-dried figs

Enzyme	Temperature or temp. range (°C)	*D* value (min) Heat labile	*D* value (min) Heat stable
PME from sun-dried figs	60	7.3	42.0
	70	4.6	10.5
	80	1.2	2.7
	90	0.5	1.1
	60 to 90	z = 24.5 °C (0.971)[a]	z = 18.6 °C (0.992)
	70 to 90	z = 20.8 °C (0.984)	z = 20.2 °C (0.989)
PME from fresh figs	60	Activated	Activated
	70	2.7	26.0
	80	2.0	5.5
	90	0.3	2.9
	70 to 90	z = 20.8 °C (0.853)	z = 21.1 °C (0.948)

[a]Correlation coefficients.

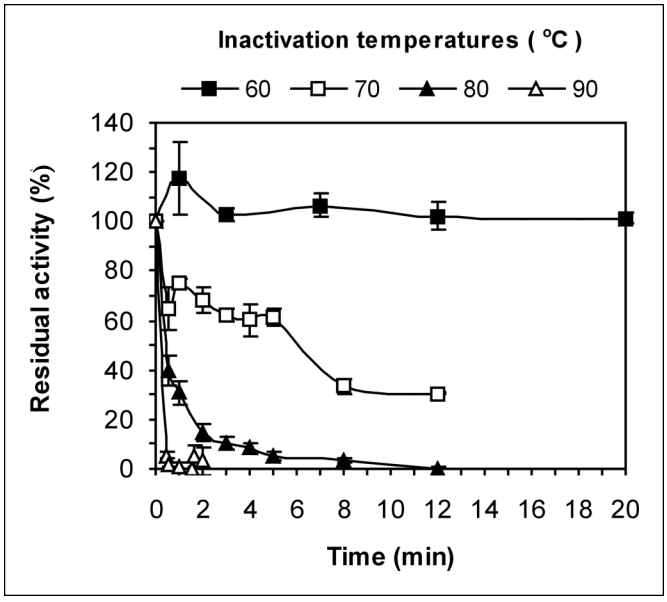

Figure 3—Heat inactivation curves of partially purified pectin methylesterase from fresh figs

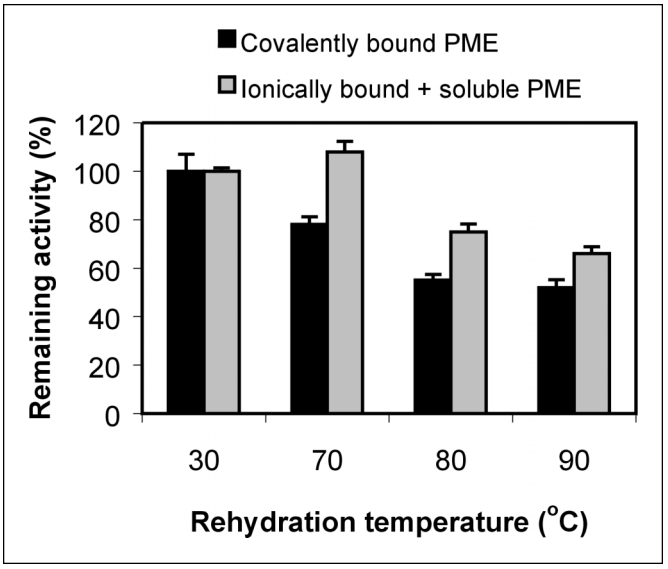

Figure 4—Residual activities of ionically bound and soluble pectin methylesterase (PME) and covalently bound PME in intermediate-moisture sun-dried figs rehydrated at different temperatures

pectic compounds and this may increase the activation and thermal stability of the enzyme.

Effect of hot rehydration and cold storage on IM fig color, texture, and PME activity

To evaluate the potential application of hot rehydration to control PME-catalyzed undesirable textural changes in IM figs during cold storage, sun-dried figs were rehydrated at 30 or 80 °C, and their textural properties and remaining PME activities were compared after 3 mo of cold storage. During examinations, the browning that occurred in IM figs rehydrated either at 30 or 80 °C was observed clearly (Figure 7). The figs rehydrated at 30 °C became shiny, looked as if they were wet, and their softening was felt clearly by the thumb test. In contrast, figs rehydrated at 80 °C were not shiny and wet, and they were firmer. When figs were halved and their flesh was examined by hand, the major difference detected between figs rehydrated at 30 and 80 °C was the sticky and nonsticky nature of their flesh, respectively. Also, the flesh of figs rehydrated at 80 °C was considerably more consistent than that of figs rehydrated at 30 °C. Thus, in heated figs, the flesh was rubbed off from the peels with the thumb with more difficulty. These results suggest that the problem of softening of IM figs may be controlled for at least 3 mo by hot rehydration.

At the end of 3 mo of storage, compared with controls rehydrated at 30 °C, only 28% less PME activity (17.4 μM COOH/min/g) was found in the homogenates of IM figs rehydrated at 80 °C. The control of undesirable textural changes in IM figs by partial PME inactivation suggests that the loss of desired textural properties occurs when enzyme PME reduces the degree of pectin methylation below a critical level.

Effect of hot rehydration and cold storage on microbial load

To determine the effect of hot rehydration and storage time on total mesophilic aerobic load, the data given in Table 3 for IM figs rehydrated in water at 30 °C or 80 °C and cold-stored for 0, 1, and 3 mo were statistically analyzed by ANOVA. The results of 2 factor analysis showed that the rehydration of figs in water at 80 °C and storage time do not have significant effects on the total mesophilic aerobic load of IM figs (P values, >0.05).

In microbiological tests conducted during cold storage of IM figs rehydrated in water at 30 and 80 °C, no mold growth had been observed on petri dishes. In the literature there are reports related to the possible negative effects of tartaric acid, used for the acidification of PDA, on mold growth (Taniwaki and others 1999). Thus, as suggested by Farber (1997), the 3-mo-stored fig samples were also tested on PDA, supplemented with chloramphenicol antibiotic. However, mold growth did not occur also on these petri dishes. This shows the effective washing and separation of contaminated figs under ultraviolet (UV) light in the factory. The yeast counts of IM figs rehydrated in water at 30 °C and 80 °C were also very low. In fact, except for the low counts of the 1st trial of 30 °C rehydration, no yeast counts were determined for the IM figs during cold storage. These results showed the suitable moisture range selected in this study. However, when the moisture content of figs is sufficient, there is always a risk of fungal development. In the preliminary studies to select a good moisture level, sun-dried figs were reyhdrated to 35% moisture at 30 °C and cold-stored without removing their free surface water by the oven treatment (see "Materials and Methods" section). However, at the end of 2.5-mo cold storage, the total mesophilic aerobic count and total yeast count of these samples were > > 1.3×10^4 and 3×10^3 colony-forming units (CFU)/g, respectively.

Effect of H_2O_2 and cold storage on IM fig color, texture, and PME activity

To increase the benefits of hot rehydration, the effects of hot rehydration at 80 °C in the presence of 2.5% H_2O_2 were also investigated. The addition of H_2O_2 to rehydration medium caused the bleaching of figs and turned the brown fruits to golden yellow–light brown. The light-colored figs were more attractive than the brown figs rehydrated in water, and they maintained this desirable color even after 3 mo of storage. Traditionally, the light color of IM or high-moisture fruits is maintained by adding 0 to 150 ppm SO_2 during packaging of the products (de Daza and others 1997). Thus, by the application of H_2O_2 disinfection, the use of sulfites may be minimized or eliminated completely.

Also, at the end of 3 mo of storage, no apparent softening was detected in IM figs rehydrated in 2.5% H_2O_2 at 80 °C. The fruit flesh maintained its consistency and was not sticky. Also, at the end of 3 mo of cold storage, the PME activity (16.2 μM COOH/min/g) in the

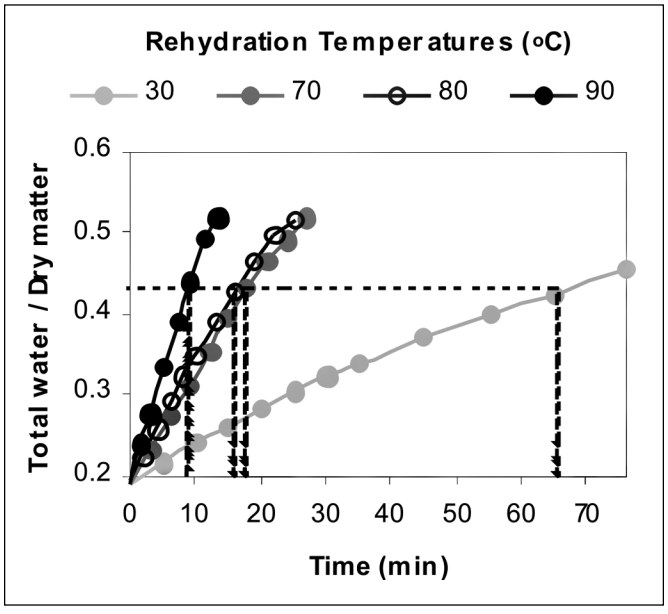

Figure 5—Rehydration curves of sun-dried figs at different temperatures (the percent initial moisture contents were 15.4% ± 0.2% for figs rehydreted at 30 and 80 °C and 16.1% ± 0.07% for figs rehydrated at 70 and 90 °C).

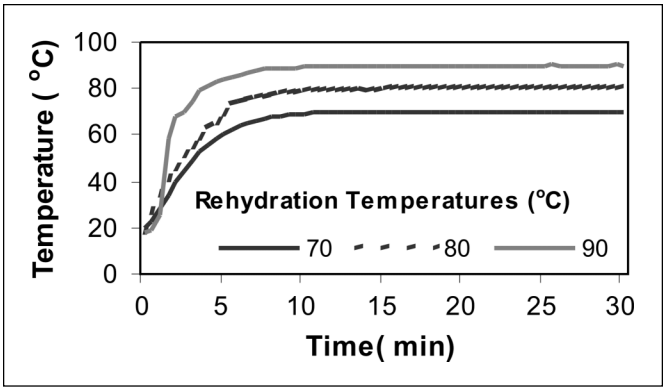

Figure 6—Heat penetration curves of sun-dried figs during rehydration at different temperatures

Table 3—The effect of hot rehydration at 80 °C alone or in combination with H_2O_2 on microbial load of cold stored intermediate-moisture (IM) sun-dried figs

	Total mesophilic aerobic count log_{10} (CFU/g)			Total yeast count (CFU/g)[a]			
	Storage time (mo)						
Type of rehydration	0	1	3	0	1	3	3[b]
65 min in water at 30 °C (1)	4.27 (± 0.15)[c]	2.46 (± 0.09)	2.48 (± 0.44)	<13 —	27 (± 46)	38 (± 66)	64 (± 44)
65 min in water at 30 °C (2)	3.3 (± 0.07)	3.16 (± 0.20)	3.18 (± 0.27)	14 (± 24)	<15 —	<15 —	<15 —
16 min in water at 80 °C (1)	2.99 (± 0.08)	3.33 (± 0.16)	3.63 (± 0.07)	44 (± 44)	<12 —	<16 —	<16 —
16 min in water at 80 °C (2)	3.29 (± 0.09)	2.61 (± 0.22)	3.02 (± 0.28)	<14	<13	<13	<13
16 min in 2.5% H_2O_2 solution at 80 °C (1)	2.26 (± 0.31)	2.03 (± 0.23)	2.83 (± 0.12)	<14 —	<13 —	109 (± 71)	16 (± 27)
16 min in 2.5% H_2O_2 solution at 80 °C (2)	2.14 (± 0.20)	2.51 (± 0.11)	2.64 (± 0.08)	<15 —	<13 —	<14 —	41[d] (± 71)

[a]CFU = colony-forming unit. Because of the very small numbers of yeast and mold counts, the logarithms of values were not taken.
[b]Instead of tartaric acid, chloramphenicol antibiotic was added to the potato dextrose agars.
[c]Standard deviations.
[d]Indicates mold count.

homogenates of disinfected figs was 33% and 5% lower than those in the homogenates of figs rehydrated in water at 30 and 80 °C, respectively. However, in some figs, the O_2 gas released by the action of residual in situ catalase caused some physical defects. For example, tiny gas bubbles formed and trapped within the viscous fruit flesh and in fruit center caused a substantial increase (blowing) in the volume of some figs during storage. In some other figs, the gas that formed exhausted from the fruit eye, and this caused the accumulation of a white foam at this location during cold storage. Thus, to eliminate these undesirable effects the concentration of H_2O_2 and/or the contact period of figs with H_2O_2 should be reduced.

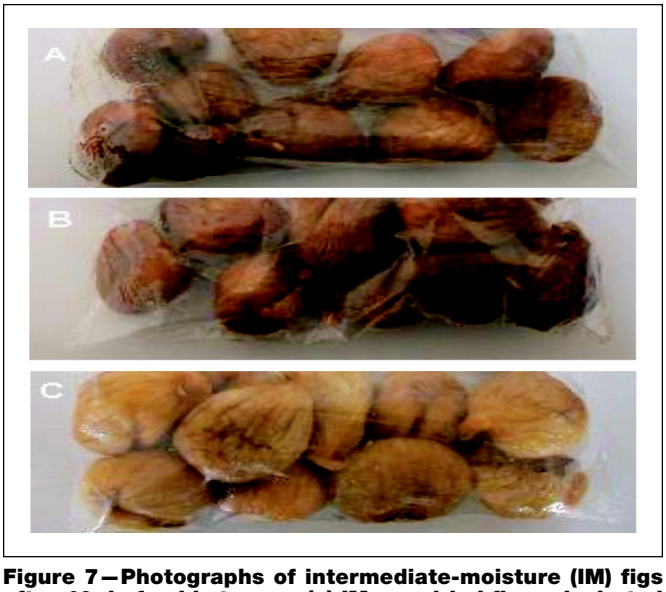

Figure 7—Photographs of intermediate-moisture (IM) figs after 40 d of cold storage. (a) IM sun-dried figs rehydrated for 65 min in water at 30 °C; (b) IM sun-dried figs rehydrated for 16 min in water at 80 °C; (c) IM sun-dried figs rehydrated for 16 min in 2.5% H_2O_2 at 80 °C

The effect of H_2O_2 and cold storage on microbial load

Statistical analysis to determine the effect of H_2O_2 showed that the total mesophilic aerobic counts of IM figs rehydrated for 16 min in 2.5% H_2O_2 solution at 80 °C and cold stored for 0, 1, and 3 mo were significantly lower than those of IM figs rehydrated in water at 80 °C and cold stored for the same time periods ($P < 0.01$). Thus, it can be concluded that the use of 2.5% H_2O_2 effectively reduces the microbial load of IM figs. In contrast, in the same statistical analysis, the effect of storage time on total mesophilic aerobic count of IM figs was found to be insignificant ($P > 0.05$).

After rehydration and at the end of 1 mo of storage, the total yeast count of disinfected IM figs was very low, but at the end of 3 mo of storage it increased slightly for the IM figs obtained in the 1st H_2O_2 rehydration trial. However, this increase was not observed clearly on the PDA plates supplemented with chloramphenicol antibiotic. In this study, the only mold count was obtained for 1 of the 3 plates of 3-mo cold-stored IM figs for the second H_2O_2 rehydration trial. In the literature, the effectiveness of vapor-phase H_2O_2 disinfection on fungi was reported for dried plums (Sapers and Simmons 1998), raisins (Simmons and others 1997), and table grapes (Forney and others 1991). However, because of the small number of fungi in figs, the effect of H_2O_2 disinfection, hot rehydration, and cold storage on these microorganisms could not have been determined in this study. Further microbiological studies should be conducted to determine the effect of H_2O_2 on fungi in figs.

Residual H_2O_2

After 16-min rehydration of sun-dried figs in 2.5% H_2O_2 at 80 °C, a considerable amount of residual H_2O_2 was determined in IM figs (Table 4). Almost 70% and >99% of the residual H_2O_2 in these IM figs decomposed in 7 d and 30 d, respectively. However, these long decomposition periods indicate the stability of residual H_2O_2 in IM figs. Also, because the previously mentioned physical defects occurred, it is not suitable to apply 16 min of rehydration in 2.5% H_2O_2 at 80 °C to whole figs. In qualitative tests to detect catalase activity in IM figs rehydrated in water at 80 °C, we observed a considerable amount of gas release that lasted continuously for almost

Table 4—The amounts of residual H_2O_2 in filtered homogenates of whole intermediate-moisture figs and fig purees during cold storage

Type of rehydration	Storage time (d):	Residual H_2O_2 ($mg \cdot kg^{-1}$) 0	7	15	30	50
4 min in 2.5% H_2O_2 solution at 80 °C + 12 min in water at 80 °C		10	1	1	<1[a]	—
8 min in 2.5% H_2O_2 solution at 80°C + 8 min in water at 80 °C		30 to 98	10 to 30	3	<1[a]	—
16 min in 2.5% H_2O_2 solution at 80 °C		560	186	30 to 98	3	<1[a]

Storage time (h)[b]:	0	0.5	1	2	5 (3)[c]	24 (22)	29 (27)
16 min in 2.5% H_2O_2 solution at 80 °C + pureeing	186	30 to 98	30 to 98	30	10	1	<1

[a]No residual H_2O_2 was determined by the qualitative enzymatic method.
[b]The 1st 2 h of storage was at room temperature.
[c]Numbers in parentheses indicate cold storage time.

half an hour. The gas release in IM figs rehydrated in water at 30 °C occurred much more extensively and lasted almost 3 h. This observation showed that the enzyme catalase was partially inactivated during hot rehydration. The inactivation probably occurred in the outer tissues of figs that received more heat, and this prevented or slowed the decomposition of residual H_2O_2 at these locations. However, the in situ catalase still exists in heat-treated figs, and the disintegration of fruit tissues may enable the contact of H_2O_2 with the remaining enzyme. Thus, IM figs rehydrated for 16 min in 2.5% H_2O_2 may still be used in the production of SO_2-free light-colored fig purees. To assess the stability of residual H_2O_2 in fig puree, IM figs were pureed with a manual meat grinder after disinfection. In pureed disinfected IM figs, the residual H_2O_2 in filtered homogenates dropped to 186 $mg \cdot kg^{-1}$. Also, almost 85% of this residual H_2O_2 degraded when the puree was stored at room temperature for 2 h. Further storage of fig puree between 4 and 7 °C for 3 h and 22 h, on the other hand, degraded almost 95% and >99% of residual H_2O_2, respectively. Also, the total mesophilic aerobic count (56 ± 60 CFU/g) and mold count (13 ± 23 CFU/g, no yeast growth) of fig puree were very low. These results are very promising for the application of H_2O_2 disinfection in fig puree production.

For the application of H_2O_2 disinfection in whole figs, we also tested some alternative treatments. For example, we brought the moisture content of figs to 30% by rehydrating them 1st in 2.5% H_2O_2 solutions at 80 °C for 4 or 8 min and then in water at 80 °C for 12 or 8 min, respectively. As described in the "Materials and Methods" section, after rehydration, the figs were drained and cooled at room temperature, treated in the oven, packed, and cold-stored for different time periods. These 2-stage rehydration procedures (4 + 12, 8 + 8) reduced the level of residual H_2O_2 in IM figs considerably. In these methods, further reduction of the residual level of H_2O_2 also may be achieved by the addition of very low dosages of H_2O_2-reducing chemicals such as sulfites (Özkan and Cemeroglu 2002) or ascorbic acid and its derivatives (Sapers and Simmons 1998) to the water used at the second stage of rehydrations. However, for exported products, the times needed for shipping and distribution seemed sufficient to eliminate residual H_2O_2 only by the action of in situ catalase. When they were disinfected and cold stored almost 3.5 mo using these 2-stage methods, no blowing and foam formation was observed in IM sun-dried figs during storage. Also, at the end of 3.5 mo, the IM figs rehydrated for 4 or 8 min in H_2O_2 solution had light brown and light brown-yellow colors, respectively. Further studies are now continuing to determine the effect of these alternative rehydration procedures on microbial quality of IM figs during cold storage.

Conclusions

Partial inactivation of PME by hot rehydration at 80 °C and cold storage can be used to delay PME catalyzed textural changes in IM figs. Hot rehydration alone is not sufficient to reduce the total mesophilic aerobic load of IM figs. However, in the presence of H_2O_2, the hot rehydration reduces the microbial load more effectively. Also, the light-colored figs obtained by H_2O_2 disinfection are very attractive and need no SO_2 treatment. The in situ catalase remains after hot rehydration at 80 °C. Thus, when IM figs are pureed, the residual H_2O_2 is decomposed rapidly by this enzyme. However, the concentration and/or treatment times of H_2O_2 should be arranged carefully to eliminate the residual H_2O_2 left in whole IM figs and to prevent physical defects that occurred due to O_2 gas formed by the in situ catalase.

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