CONTROL OF MICROBIAL AND ENZYMATIC CHANGES IN INTERMEDIATE MOISTURE SUN-DRIED FIGS by MILD HEATING AND HYDROGEN PEROXIDE DISINFECTION

Dilek DEMİRBÜKER

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Control of Microbial and Enzymatic Changes in Intermediate Moisture Sun-Dried Figs by Mild Heating and Hydrogen Peroxide Disinfection

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Dilek DEMİRBÜKER

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We approve the thesis of **Dilek DEMİRBÜKER**

Date of Signature

21.08.2003

Assoc. Prof. Ahmet YEMENİCİOĞLU

Supervisor

Department of Food Engineering

21.08.2003

Asst. Prof. Figen TOKATLI

Co-Supervisor

Department of Food Engineering

21.08.2003

Prof. Şebnem HARSA

Department of Food Engineering

21.08.2003

Prof. Taner BAYSAL

Department of Food Engineering

Ege University, Faculty of Engineering

21.08.2003

Asst. Prof. Sami DOĞANLAR

Department of Biology

21.08.2003

Prof. Şebnem HARSA

Head of Department

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ABSTRACT

During cold storage, the enzyme pectin methylesterase (PME) caused softening and loss of desired gummy texture in rehydrated intermediate moisture (IM) sun-dried figs. Heat inactivation studies indicated that the purified PME can be inactivated rapidly at 80 ^{o} and 90 ^{o}C. However, at or below 70 ^{o}C the enzyme showed activation by heating and inactivated very slowly. The in-situ activation of PME occurred much more extensively when sun-dried figs were rehydrated between 70^{o} and 90 ^{o}C to produce IM figs with approximately 30 % moisture and this prevented the effective inactivation of enzyme even by rehydrations conducted at 80 ^{o} and 90 ^{o}C. The partial reduction of PME enzyme activity (almost 30 %) by rehydration of figs at 80 ^{o}C for 16 min may be used to delay undesirable textural changes in cold stored IM figs for 3 months. However, for longer storage periods hot reyhdration alone is not sufficient to prevent softening. No considerable yeast and mold growth was detected in IM figs cold stored 3-3.5 months. However, in some samples rehydrated in water at 80 ^{o}C, the total mesophilic aerobic counts and total yeast and mold counts showed a considerable increase when storage time exceeded 3-3.5 months. The rehydration of IM figs in 2.5 % H_2O_2 for 16 min at 80 ^{o}C reduced the total mesophilic aerobic microbial count of figs almost 90 %. Due to bleaching caused by H_2O_2, the brown fig color turned to a desirable and stable yellow-light brown as well. However, during cold storage the O_2 gas released due to the decomposition of H_2O_2 by in situ fig catalase, accumulated within figs and caused some physical defects. Also, the residual level of H_2O_2 in the homogenates of disinfected figs was too much (300 ppm) and it seemed unlikely to eliminate this amount of H_2O_2 by physical or chemical means during processing. Pureeing IM figs eliminated residual H_2O_2 very rapidly. The application of rehydration first in 2.5 % H_2O_2 solution at 80 ^{o}C for 4 or 8 min and then in hot water at the same temperature for 12 or 8 min, respectively, also reduced the amount of residual H_2O_2 in IM figs considerably. Besides, these two-stage rehydration procedures eliminated the physical defects occurred in IM figs due to O_2 gas release and gave firmer IM figs. To reduce the initial microbial load of IM figs, 4 and 8 min disinfections conducted in H_2O_2 solutions were less effective than 16 min disinfection in H_2O_2 solution. However, both 4 and 8 min disinfections effectively suppressed microbial load for at least 3.5 months and they may be used in the production of SO_2 free light colored fig products.

ÖZ

Rehidre edilerek orta nemli hale getirilmiş incirlerde soğukta depolama sırasında ortaya çıkan en belirgin sorunlardan birisinin pektin metilesteraz (PME) enziminin neden olduğu yumuşama olduğu belirlenmiştir. Söz konusu yumuşama incirlerde arzu edilen sakızımsı tekstürün ortadan kalkmasına neden olmakta ve önemli bir kalite kaybına yol açmaktadır. Kuru incirlerden ekstrakte edilmiş ve kısmi olarak saflaştırılmış PME enzimi 80 ^{o} ve 90 ^{o}C` lerde süratle inaktive edilebilmekte, ancak buna karşın 70 $^{o}$C ve bu derecenin altındaki sıcaklıklarda yavaş bir şekilde inaktive olmakta ve önemli düzeyde aktivasyon göstermektedir. Nem düzeyi % 30 olacak şekilde incirlerin 70-90 $^{o}$C`ler arasında sıcak su içerisinde rehidre edilmesi sırasında incir dokularında bulunan PME enziminde görülen ısıyla aktivasyon, saflaştırılmış olan enzime göre çok daha fazla gerçekleşmekte ve bu durum enzimin incirlerde büyük oranda aktif kalmasına neden olmaktadır. İncirlerin 80 ^{o}C de 16 dakika rehidre edilmesi PME enzimini kısmi olarak inaktive edebilmekte (yaklaşık % 30 düzeyinde) ve bu durum soğukta 3 ay kadar depolanmış incirlerde yumuşamayı geciktirebilmektedir. Ancak, depolama süresinin 3-3.5 ayı aşması durumunda yalnızca sıcak rehidrasyon uygulayarak yumuşamanın önlenmesi mümkün görülmemektedir. Yürütülen mikrobiyolojik sayımlar 3-3.5 ay soğukta depolanmış, ısıtılmış incirlerde herhangi bir küf veya maya gelişmesi meydana gelmediğini göstermiştir. Ancak bu sürenin aşılmasıyla 80 ^{o}C'de rehidre edilmiş incirlerin toplam mezofilik aerobik mikroorganizma sayısında ve toplam maya ve küf sayısında önemli artışlar olabilmektedir. 80 ^{o}C `deki rehidrasyon işleminin % 2.5 $H_2O_2$ çözeltisi içerisinde yürütülmesi toplam mezofilik aerobik mikroorganizma sayısında % 90`lık bir azalma meydana getirmiş ve kullanılan H_2O_2 incirlerin renginde oldukça arzulanan stabil bir sarı-açık kahve rengin oluşmasını sağlamıştır. Ancak, bu uygulamayla dezenfekte edilmiş incirlerde bulunan katalaz enziminin kalıntı H_2O_2`i parçalamasıyla oluşan ve bitkisel dokuda biriken O_2 gazı, soğukta depolama sırasında incirlerde birtakım fiziksel hasarlara yol açmıştır. Ayrıca bu uygulama ile dezenfekte edilmiş incirlerden elde edilen homojenatlarda herhangi bir fiziksel veya kimyasal yöntemle zor giderilebilecek düzeyde yüksek (300 ppm) H_2O_2 kalıntısı bulunmuştur. Ancak incirlerin püreye işlenmesiyle ortamda bulunan H_2O_2 kalıntısı katalaz enzimi etkisiyle kısa sürede yok olabilmektedir. Ayrıca, rehidrasyon işleminin sırasıyla önce 4 veya 8 dakika 80 ^{o}C deki %2.5`luk H_2O_2 içerisinde ve daha sonra 12 veya 8 dakika aynı derecedeki sıcak su içerisinde gerçekleştirilmesiyle, kalıntı H_2O_2 miktarının büyük

oranda azaltılması ve depolama sırasında oluşan fiziksel hasarların tamamen giderilmesi mümkündür. Ayrıca bu iki aşamalı dezenfeksiyon işlemleriyle elde edilmiş incirlerde daha az yumuşama belirlenmiştir. % 2.5 `luk H_2O_2 çözeltileri içerisinde 4 veya 8 dakika dezenfeksiyon uygulanması, aynı çözelti içerisinde 16 dakika dezenfeksiyon uygulanmasına göre incirlerin başlangıç mikrobiyal yükü üzerinde daha az bir etki göstermektedir. Ancak her iki uygulama da soğukta depolanan orta nemli incirlerde mikrobiyal yükün en az 3,5 ay boyunca başarıyla baskılanmasını sağlamakta ve SO_2 içermeyen açık renkli incir ürünlerinin üretilebilmesini mümkün kılmaktadır.

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Chapter 1

INTRODUCTION

Turkey with its 300.000 metric tones of annual production is the largest producer of figs in world (Cabrita et al., 2001). Most of the figs are produced in the Aegean region of Turkey, around the city of İzmir whose ancient name is Smyrna, and the dominating cultivar grown is Sarılop cultivar. Almost all of the figs grown have been destined for sun-drying that is conducted after the fruits left on trees dry partially and fall down. Traditionally, the sun-drying is carried out in field by spreading the figs on mats for 8-10 days (Cemeroğlu, 1986). Currently, the drying is also conducted in some simple tunnel driers that accelerate the drying process and increase the microbial quality of figs.

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

To obtain a microbial stability at room temperature, intermediate moisture foods (IMF) are stabilized by different chemical preservatives such as sorbates, sulfates and benzoates (Cemeroğlu, 1986; de Daza et al., 1997). However, due to the increased health concerns the use of such chemical preservatives has been limited, banned or discouraged. For example, because of their asthmatic reactions FDA banned the use of sulfites in fresh fruit and vegetables (Labuza et al., 1992). Also, there is a great pressure from consumers to reduce or abandon the use of sulfites in dehydrated or sun-dried fruits (Özkan and Cemeroğlu, 2002). The American dried-fruit industry has also

developed some hazard analysis and critical control point programs to find an alternative to sorbates used in exportation products (Simmons et al., 1997).

Recently, some successful studies were conducted to reduce the microbial load of IM fruits such as raisins and plums with vapor-phase H_2O_2 disinfection (Simmons et al., 1997; Sapers and Simmons, 1998). Also, many other successful applications of liquid phase H_2O_2 disinfection were demonstrated for fresh fruit and vegetables (Sappers and Simmons, 1998). H_2O_2 is a GRAS (Generally Recognized as Safe) chemical and FDA approved the direct use of this chemical in the disinfection of different food products at the concentrations ranging from a high of "sufficient for purpose" to a low of 0.04 % (Code of Federal Regulations, 2000a). However, FDA requires that the residual H_2O_2 in disinfected food be removed by appropriate physical and/or chemical means following disinfection.

Recently, the potential application of hurdles such as cold storage, mild heating and H_2O_2 disinfection for the preservation of IM sun-dried figs at 30 % moisture content were investigated. During cold storage, one of the biggest problems observed was softening and loss of desired gummy texture of IM figs in several months. The enzyme pectin methylesterase (PME) plays a central role in the softening of fruits and vegetables by reducing the degree of pectin methylation and making it a substrate for polygalacturonases (PG) that depolymerize the pectin (Pressey and Woods, 1992; Thakur et al., 1996). Thus, after the determination of considerable amounts of PME activity in softened figs it was decided that in addition to the control of microbial load, the control of PME action is also essential to obtain good quality IM sun-dried figs. In this thesis, the activity and thermal properties of PME in sun-dried figs have been investigated and the potential application of hot rehydration alone or in combination with H_2O_2 to control PME mediated textural changes and microbial load during cold storage of IM sun-dried figs was tested.

Chapter 2

HURDLE CONCEPT AND FRUIT PRESERVATION TECHNOLOGIES

2.1. Hurdle Concept

Since many years foods have been preserved by traditional methods such as adding chemical preservatives, canning, freezing, drying, chilling, fermentation, etc. Today, these preservation methods are still employed extensively to obtain numerous products. Thus, food technologists have still been developing and characterizing the effects of traditional preservation methods on microbial safety, sensory attributes and nutritional quality of foods to assure public health and consumer satisfaction.

Most traditional methods of food preservation provide sufficient safety by effectively killing or preventing the growth of pathogenic and spoilage microorganisms. However, when they applied alone almost all of them cause some changes in the sensory attributes of food such as texture, flavor and color. Also, the use of chemical preservatives at high concentrations causes some health concerns and reduces the consumer acceptance of foods. Thus, in recent years many efforts have been spent to develop some alternative preservation technologies that provide sufficient microbial safety, maintain the sensory attributes and minimize health concerns of consumers. Hurdle technology has appeared as a result of these intensive studies. In this technology carefully selected and combined preservative factors are applied to obtain the indicated benefits. There are more than 60 potential hurdles that may be used in this technology (Leistner, 2000). However, the most important hurdles used in food preservation are heating, water activity (a_w), acidity (pH), redox potential, refrigeration and competitive microorganisms (e.g., lactic acid bacteria). The other hurdles include; oxygen tension (low or high), modified atmosphere (carbon dioxide, nitrogen, oxygen), pressure (high or low), radiation (UV, microwaves, irradiation), ohmic heating, pulsed electric fields, pulsed light, ultrasonication and new packaging (e.g., selective permeable films, advanced edible coating) methods.

Some hurdles are very effective and they may influence both the microbiological safety and flavor of foods positively when used properly. However, the same hurdles, when their intensity is increased too much, may cause a negative effect on the foods. Thus, considering the safety and quality, it is very critical to keep hurdles at the optimum

range. The kind of hurdle differs according to the type of food. One or set of hurdles may be used to obtain high quality and food safety by keeping the normal population of the microorganisms under control. At this point, the initial microbial quality of the food is important. In fact, this is one of the main factors determining the intensity of the hurdles.

2.2. Application of Hurdle Concept in Different Fruit Preservation Technologies

2.2.1. Intermediate moisture food (IMF) technology

IMF technology is considered as one of the major fruit preservation technologies. IMFs have no precise definition based on their water activity (a_w) and water content. However, generally they include the products that have a_w between 0.75-0.92 (Welti-Chanes et al., 1997). These products are obtained by adjusting their a_w to the given range by different methods such as dehydration, osmotic dehydration and dry infusion. Although different sources report varying water contents for intermediate moisture (IM) fruits, the water content between 20 and 50% may be accepted as the intermediate moisture level which makes fruit soft, moist, and ready to eat (Cemeroğlu, 1986).

Because of their suitable a_w the IM fruits may easily be spoiled by the action of fungi. Thus, they should be stabilized by use of different preservative factors. In 1980s, the committee for IMF of France's National Center for Coordination of Research on Food and Nutrition proposed the following comprehensive definition for intermediate moisture foods; *"Food products of soft texture, subjected to one or more technological treatments, consumable without further preparation and with a shelf stability of several months, assured without thermal sterilization, nor freezing or refrigeration, but an adequate adjustment of their formulation: composition, pH, additives, etc. and mainly a_w, which must be between 0,6 and 0,84 (measured at 25 ^oC)"*.

2.2.1.1. Methods of a_w reduction

The major hurdle used to prevent microbiological spoilage of IMF is a_w control. The methods to reduce a_w are classified into four groups:

2.2.1.1.1. Partial drying

For partial drying the most frequently used method for fruits is sun-drying which is simple and cheap. In Turkey, this method is still used extensively for figs apricots and raisins. Commercial dehydrators can also be used to reduce the water content of fruits and other food and to control their a_w. This method is applied generally to pears, raisins, peaches and apples.

2.2.1.1.2. Osmotic drying (moist infusion)

In this method food pieces are soaked in solutions of different humectants such as sugars, alcohols, polyols, organic acid salts, proteins, etc. Difference between osmotic pressure of food and solution provides a driving force. Thus, water in food particles diffuses into solution and humectant diffuses into food particles. This method is applied in the production of candied fruits by using sugar as a humectant in soaking solution.

2.2.1.1.3. Dry infusion

In this method food pieces are first dehydrated and then they are soaked in humectant solution at the desired a_w. This is the most energy intensive method of IMF production but it gives high quality products.

2.2.1.1.4. Blending (formulation)

In this method, which is currently very popular, foods and various ingredients including humectants are mixed and different processes such as extrusion, cooking and baking are applied to mixture to reach the target a_w. This is a fast and energy-efficient method that is more flexible than others in using different ingredients and it is employed both for traditional IMF (confectionaries and preserves) and novel IMF (snacks and pet foods).

2.2.1.2. Stability of IMF

In the IMF technology, reduction of a_w reduces the amount of free water participating in chemical and biochemical reactions. Although, this does not slow down some deteriorative reactions it may prevent the growth of most microorganisms in food and increases the stability of IMF (Figure 2.1). For a microbiologist a_w is water availability for microbial growth. The a_w is measured as equilibrium relative humidity (ERH), the percent relative humidity of an atmosphere in contact with a product at the equilibrium

water content (Toledo, 1994). a_w is also the ratio of the partial pressure of water in the headspace of a product (P) to the vapor pressure of the pure water (P^o).

$$a_w = ERH = P/P^o$$

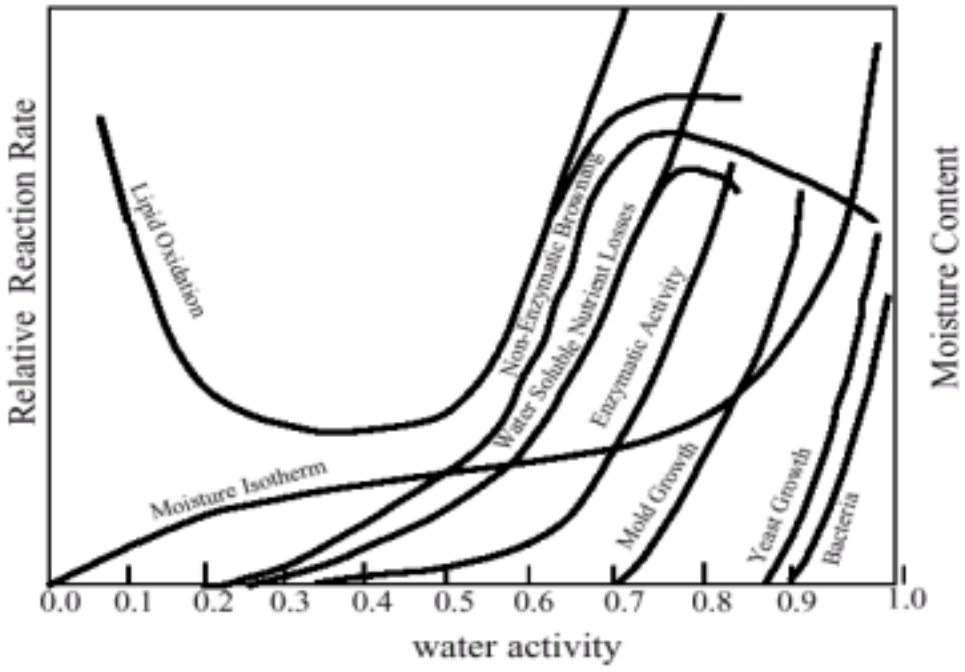

Figure 2.1.The effect of a_w on chemical and biochemical reactions in foods (www.fsci.umn.edu/Ted-Labuza / papers / IMF.pdf).

2.2.1.2.1. Microbial stability of IMF

Although the reduction of a_w in IMF prevents the growth of most pathogenic microorganisms, there are still some microorganisms that can cause spoilage and health problems when conditions are favorable. One of the major concerns of IMF is *Staphylococcus aureus*. This microorganism is able to grow at a_w above 0.84-0.85 if the pH is favorable (www.fsci.umn.edu/Ted-Labuza / papers / IMF.pdf). Thus, formulation of IMF at the highest possible moisture content, for improved texture and palatability, requires additional measures for the inhibition of *S. aureu*s. The other bacteria that can be problem in IMF are *Streptococcus faecalis* and *Lactobacillus* spp. However, these two bacteria can grow in IMF only when a_w is above 0.87-0.88. Thus, they are less important.

Another concern is common *Aspergillus* and *Penicillium* species that can grow at a_w above 0.77-0.85. The minimum a_w for mycotoxin production by these molds is usually higher. Osmophilic yeast, *Saccharomyces rouxii,* and molds such *as Aspergillus echinulatus* and *Monascus bisporus* cause spoilage between 0.6 and 0.65 a_w, whereas Xerophilic molds such as *Aspergillus chavalieri, Aspergillus candidus* and *Wallamia sebi* cause spoilage between 0.65 and 0.75 a_w (www.fsci.umn.edu/Ted-Labuza / papers / IMF.pdf). In dried fruits such as figs and dates different species of *Zygosaccharomyces* and *Hanseniaspora* are important agents causing spoilage, whereas *Saccharomyces rouxii, Aspegillus glaucus* and *Xeromyces bisporus* cause spoilage mostly in plums (Cemeroğlu, 1986). Thus, besides a_w , some additional hurdles should also be used for the microbial stabilization of IMF.

The second most important hurdle for the stabilization of IMF is the use of chemical preservatives. The most frequently applied chemical preservatives are sulfites, sorbic, citric, benzoic, propionic, phosphoric and ascorbic acids (Welti-Chanes et al., 1997). Also, propylene glycol, a humectant with specific antimicrobial activity is used in the stabilization of IMF. The effective mold inhibitors are sorbates and propionates, whereas propylene glycol is effective on *S. aureus*. Above pH 5.4 and in 0.86-0.90 a_w range most chemical additives show very little antimicrobial effect. However, at these conditions propylene glycol may inhibit *S. aureus* and molds such as *Aspergillus glaucus* and *Aspergillus niger*. At higher a_w values at pH 5.4 propylene glycol should be combined with mold inhibitors such as sorbates and propionates. At high a_w values generally organic acids are more effective than phosphoric acid. But at low a_w values this inorganic acid is more effective then the organic acids (www.fsci.umn.edu/Ted-Labuza / papers / IMF.pdf).

Other hurdles used in IMF technology are pH and heat treatment. Although, thermal processing is not specified in the hurdles applied to IMF, pasteurization is sometimes used to obtain IMF products. For example, Cemeroğlu (1986) reported the pasteurization of intermediate moisture dates.

2.2.1.2.2. Chemical and biochemical stability of IMF

In the a_w range of IMF the rates of some deteriorative chemical reactions increase dramatically. In fact, this is the main disadvantage of IMF. The main chemical reactions

in IMFs are non-enzymatic browning and lipid oxidation. However, due to the very low amount of lipids in fruits non-enzymatic browning is the major deteriorative reaction in IM fruits. Also, some enzymatic changes may cause the loss of IM fruit quality if they are not controlled.

Non-enzymatic browning: In dried fruits and IM fruits the reducing sugars produce some undesirable brown pigments. These pigments are formed especially during long-term storage. However, thermal processing of foods may accelerate the brown pigment formation by reducing sugars. The reaction that leads the formation of brown pigments occurs by the interaction of carbonyl groups of reducing sugars, mainly D-glucose, with amino groups of amino acids or free amino groups of amino acid residues in proteins and it is called the Maillard reaction or non-enzymatic browning (Davidek et al, 1990) The flavor, aroma and color of brown pigments may be desirable for some foods such as chocolate and caramels. However, in IM fruits the formation of brown pigments is undesirable.

The formation of brown pigments by Maillard reaction occurs at different steps. In the first step of reaction the reducing sugar reacts with amine reversibly to produce the glycosylamine. This undergoes a reaction called Amadori rearrangement to give some products that turn intermediates and then dehydrate to some cyclic furan derivative. In the case of glucose the amadori rearrangement gives a derivative of 1-amino-1-deoxy-D-furanose and when dehydrated this produces the furan derivative 5-hydroxymethyl-2-furaldehyde (HMF) that polymerizes quickly to dark-colored pigments (BeMiller and Whistler, 1996; Davidek et al., 1990).

In IMF these brown pigments may also cause the formation of off-flavors that are not acceptable by consumers. Moreover, the reaction of reducing sugars with amino acids destroys the amino acids. This is particularly important for the lysine that is an essential amino acid important for the nutritive value of proteins. However, considering the low lysine content of plant proteins this may not be a considerable problem in fruit products.

The reactivity of different sugars to form brown pigments is as follows: ribose > xylose > arabinose > galactose > glucose > fructose > galactose > mannose > glucose >

fructose > lactose > saccharose (Davidek et al., 1990). Thus, when non-enzymatic browning is a problem pentoses such as ribose, xylose and arabinose should not be used in the formulation of IM fruits.

For the rate of Maillard reaction the a_w of food is very critical. Between 0.6-0.7 a_w the reaction occurs with maximum rate. However, at lower and higher a_w values the reaction slows down. Thus, control of a_w may be an effective method to limit non-enzymatic browning. In fact, the use of sulfites is the most effective method to prevent non-enzymatic browning. However, due to their adverse health effects, the use of these chemicals has been discouraged.

Enzymatic browning: The enzymatic browning catalyzed by enzyme polyphenol oxidase (PPO) is one of the biggest problems observed during processing of fruits. Processes such as cutting, pitting and peeling cause disruption of plant cells and contact of phenolic compounds in vacuols and PPO in cytoplasm in the presence of air starts enzymatic oxidation. The oxidized phenolic compounds are not stable and turn spontaneously to dark brown melanins. Compared to non-enzymatic browning, the reaction occurs very fast and it causes the loss of food sensory properties such as color and flavor. The enzymatic browning also causes the reduction of the nutritive value of foods by causing the exhaustion of antioxidants such as ascorbic acid. Thus, during processing the PPO is generally inactivated by heat treatment. In fact, PPO enzymes do not belong to an "extremely heat-stable enzyme" group and short exposures of product to temperatures between 70 ^{o} and 90 ^{o}C are sufficient to inactivate them. However, in some *Prunus* fruits such as cherries, plums and apricots PPO may have a considerable thermostability (Vamos-Vigyazo, 1981). In particular, the thermostability of apricot PPO has been known for a very long time (Ponting et al., 1954). Thus, during processing of apricots the heat treatment may be combined by browning inhibitors.

Besides their considerable effect on non-enzymatic browning reactions, sulfites are also used effectively to inhibit enzymatic browning. However, as indicated above due to the health concerns, there have been great efforts to minimize or eliminate the use of sulfites in food technology. This has encouraged the use of sulfite alternatives, such as ascorbic acid and its derivatives, β-cyclodextrin, L-cysteine, and 4-hexylresorcinol (Sapers and Miller, 1992; Santerre et al., 1991; Gunes and Lee, 1997). These chemicals

are less effective compared with sulfites. However, when they used in combination with complementary treatments such as packaging under nitrogen atmosphere and/or use in combination with heat treatments, acidic solutions or polyphosphates they became more effective (Sapers and Miller, 1992; Sapers and Miller, 1995; Gunes and Lee, 1997).

Enzymatic softening: By acting as a cement material between the plant cells, pectin plays an important role for the firmness of plant tissues. During processing and storage, pectic enzymes such as pectin methylesterase (PME) and polygalacturonase (PG) may cause the softening of IM fruits by the degradation of pectin. The enzyme pectin methylesterase (PME) plays a central role in the softening process (Pressey and Woods, 1992; Thakur et al, 1996). This enzyme demethylates pectic polysaccharides and makes them suitable for the action of PG that degrades low methoxy pectin chains by hydrolysis (Cemeroğlu et al., 2001). Today, there is no commercial inhibitor for pectic enzymes. Thus, the heat treatment of fruits is sometimes desired for the inactivation of these enzymes.

2.2.1.3. Advantages of using IMF technology

The traditional methods such as canning, refrigeration and freezing are energy intensive methods. Thus, compared to these methods IMF production requires less energy. This is the main advantage of IMF technology and it is important especially in countries with tropical climates and third world countries where refrigeration is scarce. The other advantages of IMF are; (1) they are ready to eat foods and need no preparation, (2) because of their high plasticity they can easily be shaped as needed and packed uniformly, (3) the appropriate hurdles applied during their production make these food safe, (4) IMF contains concentrated nutrients. Thus, compared to fresh fruits they provide more nutrients and energy. With all these properties IMF are also very suitable for military purposes and for use at times of natural disasters.

2.2.2. High moisture fruit products (HMFP) technology

The HMFP technology has recently been developed for the preservation of fruits. The moisture level of HMFP is considerably higher than those of IMF, but it is lower than those of the fresh products. Although, the a_w of HMFP varies in the range of 0.93-0.98, they are shelf-stable at room temperature (de Dazza et al, 1997). Thus, compared to

IMF technology the hurdles should be combined more carefully to obtain the desired microbiological stability in these products.

The main hurdles used in the production of HMFP are a_w , pH, preservatives and mild heating and this technology aims processing fresh fruits to stable fruit products with maximum retention of their sensory properties. Thus, intensity of each hurdle should be selected very carefully. In the application of HMFP technology, the a_w of the product is reduced by blending or by immersion in solutions of sucrose, glucose, maltodextrins, etc., whereas the pH is adjusted to low levels (between 3.0-4.1) without flavor impairment. The preservative effect of this technology depends mainly on the principle that a slight reduction in a_w decreases the range of pH that allows the microbial growth. Thus, the intensity of these two hurdles should be adjusted according to the a_w-pH resistance of pathogenic and spoilage microorganisms (Table 2.1). Antimicrobial agents such as potassium sorbate or sodium benzoate between 0-1500 ppm concentrations and a slight thermal treatment with saturated steam at 100 ^oC and hot filling are also employed to obtain the desired shelf-life.

Table 2.1. Minimal a_w and pH for growth of bacteria in fruit products (de-Dazza et al., 1997).

Microorganism	**a_w**	**pH**
Clostridium butyricum	0.945- <0.965 (glucose)	-
	0.935- <0.950 (glycerol)	-
Clostridium pasteurianum	0.985	3.5-4.5
Bacillus coagulans	0.94 (glucose)	3.8-4.8
Bacillus licheniformis	>0.89- < 0.91 (NaCl or glucose)	4.2-4.4
Bacillus stearothermophilus	> 0.97 (NaCl or glucose)	>5.0-< 6.0
Lactobacillus species	> 0.94 (glycerol)	3.8-4.4
Lactobacillus plantarum	0.94	-
Leuconostoc mesenteroides	0,94 (NaCl)	-
Streptococcus faecalis	0.94	4.4-4.7
Salmonella species	0.95	3.7-4.5
Salmonella oranienberg	0.95 (NaCl); 0.935 (glycerol)	-

2.2.2.1. Stability of HMFP

Because of the low pH of HMFP, the pathogenic bacteria may not be considered a major problem. However, to minimize the contamination of osmotolerant / osmophilic and nonosmotolerant / osmophilic yeasts they should be processed, packaged and stored carefully. *Z. rouxii* and *Z. bailli* are among the most potential agents causing spoilage in HMFP. These yeasts may develop resistance to preservatives such as sorbates and cause spoilage in HMFP and in other foods and beverages containing preservatives. Especially, *Z. bailli* with its strong fermentation activity, ability to grow in hostile environments and higher resistance to preservatives may cause major spoilage in fruit products (de-Dazza et al, 1997).

Since HMFP are mostly heated by saturated steam, the quality degrading enzymes in these products are inactivated. However, sulfites at the concentration of 0-150 ppm are added to minimize non-enzymatic browning and to support the other preservatives that used as hurdle (de-Daza et al., 1997).

2.2.3. Minimally processed foods (MPF) technology

This technology is developed to meet the consumer demand to fresh or fresh-like food products. At the beginning, the MPF technology is mainly applied for the fresh meat products. However, in recent years the main developments in MPF technology have been on fruit and vegetables (Welti-Chanes et al., 1997).

The equivalent terms used for minimal processing are "partial preservation treatment" and "invisible processing", whereas those terms used for minimally processed foods are; "partially processed foods" and "high moisture shelf-stable foods". Welti-Chanes et al. (1997), reported many different definitions for minimal processing. For example, one of the early definitions is that; *"minimal processing includes all the operations (washing, selection, peeling, slicing, etc.) that must be carried out before blanching in a conventional processing line that keep the food living tissue*". Minimal processing is also defined as *"procedures that cause fewer possible changes in the food quality (keeping their freshness appearance), but at the same time provide the food enough useful life to transport it from the production site to the consumer*". de-Dazza et al. (1997) reported that the condition of keeping product cell tissues alive may not be required, if products have fresh-like appearance. However, in most cases the life

permanence in the biological tissues is one of the main elements that distinguish minimally processed fruits and vegetables.

The most important hurdle used in all minimal processing applications is refrigeration. In fact, this is one of the main points that make MPF different from IMF and HMFP technologies (Figure 2.2). Other hurdles used frequently are disinfection to reduce microbial load, addition of chemical additives (by direct incorporation, osmotic processes or vacuum infusion), pH control and modified atmosphere packaging (Welti-Chanes et al., 1997; Brody, 1998; Barry-Ryan and O`Beirne, 1999). Heat treatment is not included to most minimal processing applications. However, a very mild heating may be used to control undesirable enzymatic changes (Kim et al., 1993; Sapers and Miller, 1995; Saltveit, 2000; Yemencioğlu, 2002). Also, some new technologies may be used alone or in combination to form a hurdle effect for the preservation of MPF. Such new technologies include the use of natural antimicrobials (mostly phenolic compounds), competitive flora (lactic acid bacteria), non-thermal processes (pulsed electric fields, high or ultra high pressure, irradiation, oscillating magnetic fields, etc.) and new thermal processing methods (light pulses) (Welti-Chanes et al., 1997; Breidt and Fleming, 1997). Moreover, in future biopreservatives such as lactoperoxidase, lysozym, lactoferrin and lactoferricin may be used in the minimal processing of fruits and vegetables.

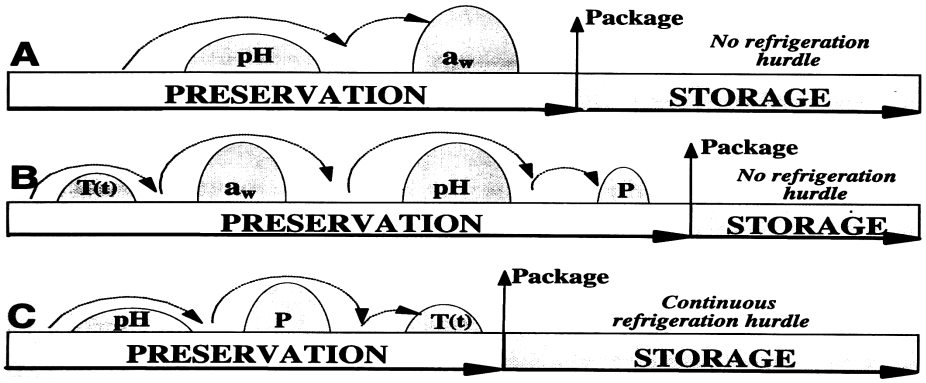

Figure 2.2. Hurdles applied in different fruit preservation systems (Welti-Chanes et al., 1997): A: Intermediate moisture fruits B: High moisture fruits. C: Minimally processed refrigerated fruits and vegetables.

2.2.3.1. Modified atmosphere packaging (MAP)

Nowadays the combination of MAP with minimal processing is very popular. MAP is a packaging technology that shelf-live of foods is increased by modification of package gas atmosphere. For fruits and vegetables the gas composition desired to increase the shelf-life consists of low O_2 and high CO_2. Generally, increase of air CO_2 concentration and reduction of air O_2 concentration around 5 % reduce the respiration rates of most fruits and vegetables. The reduction of respiration rate slows down the metabolic processes in plant tissues and increases their shelf-life. To obtain the desired shelf-life in MAP, the refrigeration of products after packaging is essential. By refrigeration the control of fruit or vegetable respiration rate is achieved more easily. Also, low temperature reduces microbial growth and minimizes spoilage.

In MAP the package atmosphere is modified by passive or active modification methods.

2.2.3.1.1. Passive modification

In this method the fruits and vegetables are packed with a suitable packaging film. During their respiration, fruits and vegetables consume O_2 and produce CO_2. Thus, modification of the package atmosphere occurs by the respiration of the packed fruits or vegetables. However, to achieve the desired equilibrium O_2 and CO_2 concentrations in package within a short time period, packaging film used should be semi-permeable. It is reported that the packaging film should let the permeation of sufficient amounts of O_2 from air to package and CO_2 from package to air (Cemeroğlu et al., 2001). Otherwise, the exhaustion of O_2 and/or accumulation of CO_2 in package cause(s) the initiation of anaerobic respiration in fruits and vegetables. This is undesirable, because it causes the formation of off-flavors in fruits and vegetables due to the accumulation of excessive amounts of alcohols and acids in their tissues (Yahia and Rivera, 1992; Yemenicioğlu and Cemeroğlu, 1996). The most frequently used packaging materials for passive modification are polyethylene (PE) and low-density polyethylene (LDPE) films (Labuza and Breene, 1989).

During their respiration, besides CO_2, fruit and vegetables produce also H_2O and some respiration metabolites such as ethylene. The production of too much H_2O may increase the risk of microbial growth at product surface, whereas the presence of ethylene increases the respiration rate. Thus, some small sachets containing ethylene absorbers

(active char-coal) or scavengers (potassium permanganate) and H_2O absorbers such as NaCl and silicagel are also placed in package to increase the shelf life of products (Yemenicioğlu and Cemeroğlu, 1996; Yahia and Rivera, 1992).

2.2.3.1.2. Active modification

In active modification, the desired gas atmosphere (low O_2 and high CO_2) in package is formed by two different methods. In one of these methods the air in packages is first evacuated by vacuum, and then the desired gas mixture is flushed into packages (Figure 2.3), whereas in the other the air in packages is swept by continuous flushing of desired gas mixture into packages. In active modification, packaging films with suitable permeability should also be used to maintain the flushed gas mixture within acceptable limits and to enable the formation of equilibrium conditions.

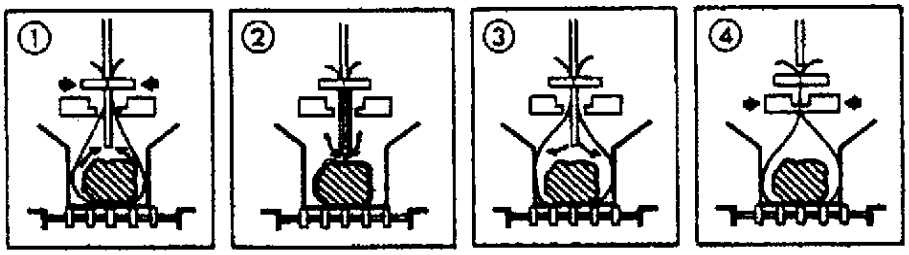

Figure 2.3. Active modification by vacuum + gas flushing (Cemeroğlu et al., 2001) 1. Placing material into packaging equipment 2.Vacuum application 3.Gas flushing 4. Sealing.

The application of active modification to fresh fruit and vegetables has been studied intensively (Cemeroglu et al., 2001). However, there are very limited studies related to the use of active modification as a hurdle for the preservation of HMFP and IM fruits. The only study that has been found is that of El Halouat et al (1998). These researchers reported that modified atmospheres containing 40 % CO_2-60 % N_2 or 80 % CO_2-20 % N_2 in combination with the addition of 417 and 343 ppm potassium sorbate or 383 and 321 ppm sodium benzoate inhibited the growth of *Z. rouxii* and extended the shelf life of high moisture (a_w: 0.84-0.87) prunes and raisins at 30 ^oC for at least 6 months. These results are very promising for the application of MAP in the preservation of IM fruits and HMFP. However, further studies should be conducted related to the effects of high CO_2 concentrations on fruit flavor.

Chapter 3

THE USE OF H_2O_2 DISINFECTION AS A HURDLE

3.1. Physical and Chemical Properties of H_2O_2

H_2O_2 is a clear and colorless chemical with a pungent odor. It is nonflammable and very stable at high temperatures (Özkan and Kırca, 2001). It is also totally miscible with water and commercially sold as 30, 35 or 50 % solutions. Some physical and chemical properties of H_2O_2 were given in Table 3.1.

Table 3.1. Some physical and chemical properties of H_2O_2 (www.H2O2.com).

Properties	Concentration (%)	
	35	50
Active oxygen content	16,5	23,5
pH	2-3	1-2
Acidity (mg. L^{-1} ,H_2SO_4)	<50	<50
Freezing point (^oC)	-33	-52
Boiling point	108	114
Vapor pressure (mmHg, 30 ^0C)	23	18
Viscosity (cp)		
0 (^oC)	1,81	1,87
20 (^oC)	1,11	1,17

H_2O_2 is one of the most powerful oxidizers known. Its oxidation potential is stronger than those of chlorine, chlorine dioxide, and potassium permanganate (Table 3.2).

Table 3.2. Different oxidants and their oxidation potentials (Özkan and Kırca, 2001).

Oxidant	Oxidation potential (V)
Chlorine	3.0
Hydroxyl radical	2.8
Ozone	2.1
Hydrogen peroxide	1.8
Potassium permanganate	1.7
Chlorine dioxide	1.5
Chlorine	1.4

In aqueous solution H_2O_2 decomposes to a more powerful oxidizer, hydroxyl radical ($^{\bullet}OH$), and some other reactive compounds such as perhydroxyl anion and perhydroxyl radical (Özkan et al., 2002).

$H_2O_2 \rightarrow H^+ + HOO^-$ (perhydroxyl anion)……...Dissociation

HOOH $\rightarrow$ $^{\bullet}OOH$ (perhdroxyl radical) + $^{\bullet}H$..…....Homolytic cleavage of O-H bond

HOOH $\rightarrow$ 2 $^{\bullet}OH$ (hydroxyl radical)..........……..Homolytic cleavage of O-O bond

The presence of metal atoms such as iron, copper and manganese in medium encourages the decomposition of H_2O_2 to its more reactive hydroxyl radical and increases its antimicrobial effect considerably (Brul and Coote; 1999; Neyens and Baeyens; 2003). The decomposition of H_2O_2 in the presence of iron occurs by Fenton reaction as given below;

$H_2O_2 + Fe^{+2} \rightarrow Fe^{+3} + OH^- + {}^{\bullet}OH$ (hydroxyl radical)…….Fenton reaction

3.2. Mode of Action

Some microorganisms protect themselves against the harmful effects of H_2O_2 by their antioxidant enzymes such as catalase and peroxidase. However, in biological systems there are no enzymes to degrade the more reactive hydroxyl radical formed by the decomposition of H_2O_2 (Vattanaviboon and Mongkolsuk, 1998). Thus, antimicrobial effect of H_2O_2 is mainly due to its highly reactive hydroxyl radical ($^{\bullet}OH$) that diffuses microbial cells and damages their DNA. The oxidation of sulfhydryl groups and double bonds in proteins, lipids and surface membranes of microbial cells is also effective on the death of microbial vegetative cells.

Besides vegetative cells H_2O_2 shows antimicrobial action also on bacterial and fungal spores. The mechanism of the sporicidal action of H_2O_2 has not been fully understood. In fact, today there is still a great discussion on this phenomenon. H_2O_2 is a small molecule about twice the size of water and might be expected to pass readily through biological membranes to sensitive targets within the cytoplasm of bacterial cells.

However, there have been suggestions that bacterial spores may have low permeability to H_2O_2 and that this low permeability contributes to resistance. Recently, Riesenman and Nicholson (2000) reported an increased sensitivity of decoated *B. subtilis* spores to H_2O_2. Thus, it was thought that the spore coats could potentially act as a barrier to H_2O_2 entry. In contrast, Rutherford et al. (2000) reported that chemical decoating of *B. megaterium* spores had minor effect on their sensitivity to H_2O_2. According to Khadre and Yousef (2001), DNA damage is the main reason of the inactivation of spores by H_2O_2. However, there are some contrary reports to this hypothesis that DNA in spores is not affected from H_2O_2 due to the protective effects of some small acid-soluble spore proteins (Riesenman and Nicholson, 2000). Also, it was showed that the mechanism of sporicidal action of H_2O_2 may be due to its inhibitory action on some enzymes responsible from the germination and outgrowth in spore core (Rutherford et al., 2000). Thus, further studies should be conducted to clarify the mode of H_2O_2 action on microbial spores.

3.3. Factors Effecting Antimicrobial Power

The antimicrobial power of H_2O_2 is highly affected from its concentration and temperature and pH of the medium. The effect of H_2O_2 concentration on microbial death has been investigated in details. At very high concentrations, especially at elevated temperatures, H_2O_2 causes major dissolution of spores with loss of the structures of their coat, cortex and core. However, at much lower concentrations, H_2O_2 kills spores without inducing microscopically evident cytological changes (Rutherford et al., 2000). This indicates that the lytic action of H_2O_2 has a secondary importance on its antimicrobial effect. In literature, there are different reports about the effective concentrations of H_2O_2. For example Davidson et al. (1993) reported that the concentrations of H_2O_2 between 0.001-0.1 % are sufficient to inhibit the growth of most bacteria and fungi at room temperature. The same authors reported the concentration of hydrogen peroxide to obtain a bactericidal or fungicidal effect at room temperature to be at least 0.1 %. On the other hand, according to Vijayakumar and Wolf-Hall (2002) in strains of *Escherichia coli* that cause diseases in humans, the minimum bacteriostatic and bactericidal concentrations of H_2O_2 at 35 ^oC are 0.3-0.4% and 0.4% for commercial H_2O_2 solutions, respectively.

For killing spores, long contact times and 3 % or greater concentrations of H_2O_2 are required (Table 3.3, Figure 3.1).However, the contact times may be shortened considerably by increasing the temperature (Table 3.4). It was found that for each 10 ^oC increase in temperature, destruction of spores increased by one third to one half using 1% H_2O_2 (Davidson et al., 1993).

Table 3.3. The effect of different H_2O_2 concentrations and contact times on *B. subtilis* spores (Davidson et al., 1993).

H_2O_2 concentration (%)	**Exposure time (min)**		
	2	**30**	**60**
3	85[a]	22	2
10	35	0.0027	0
15	22	0.0022	0

[a] percentage of survivors

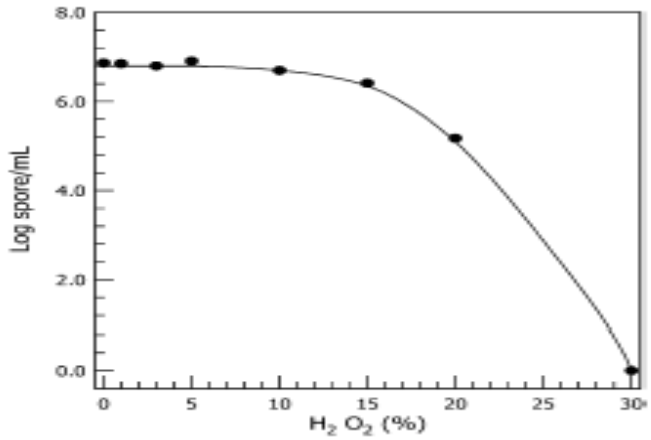

Figure 3.1. Inactivation of *B. subtilis* spores treated with varying concentrations of H_2O_2 at 20 ^oC for 1 min (Khadre and Yousef, 2001).

Besides temperature and concentration, pH of medium is also effective on the antimicrobial power of H_2O_2. In acidic pH, H_2O_2 is more effective on microorganisms. As pH increases, higher concentrations of H_2O_2 are required to obtain the same lethality. For example, 5 ppm H_2O_2 may inhibit the growth of *P. aeruginosa* at pH 5, but to obtain the same inhibitory effect 10 and 50 ppm H_2O_2 is required at pH 6.7 and pH 8.0, respectively (Davidson et al., 1993).

Table 3.4. The effect of temperature on number of decimal reductions obtained for *B. subtilis* spores at different H_2O_2 concentrations (Cemeroğlu and Karadeniz, 2001).

Number of decimal reduction	**Time to achieve the given decimal reduction (seconds)**					
	15 % H_2O_2			**20 %** H_2O_2		
	80 °C[1]	**90 °C[2]**	**95 °C[2]**	**80 °C[1]**	**90 °C[2]**	**95 °C[2]**
3	17	10	9	11	7	5
4	23	14	11	15	9	7
5	39	18	14	19	12	9
6	35	21	16	23	14	11

[1]Values determined experimentally; [2]Values determined by extrapolation

3.4. Disinfection of Food and Food Contact Surfaces with H_2O_2

Hydrogen peroxide (H_2O_2) has been used in foods and food-packaging materials for various purposes in many European countries for over 30 years (Andres, 1981; Wang and Toledo, 1986). It has major advantages for sterilization of packaging materials for aseptic products in that it is both bactericidal and sporicidal, but does not leave toxic residues that could adversely affect human health (Rutherford et al., 2000). In the US, FDA approved H_2O_2 for the sterilization of polyethylene food-contact surfaces only after February 1981 (Nielson et al., 1993). From this date, H_2O_2 has been the choice of chemical sterilant for treatment of plastic packaging materials used in aseptic processing systems (Tillotson, 1984; Wang and Toledo, 1986; Kunz and Binnig, 1987; Mitchell, 1988).

In aceptic systems H_2O_2 concentrations between 15 and 30 % and temperatures between 60^o and 90 ^{o}C are generally applied for the disinfection of food contact packaging material surfaces. (Özkan and Kırca, 2001; Cemeroğlu et al., 2001). FDA regulation currently limits the residual H_2O_2 to 0.5 ppm, leached into distilled water, in the finished food packages (Code of Federal Regulations, 2000b). Thus, excessive H_2O_2 is removed form the food contact surfaces by pressure roller in combination with scrappers and subsequent drying with sterile hot air at 180° - 205°C (von Bockelman and von Bockelman, 1986).

In addition to its successful applications for the disinfection of food packaging materials, in most countries H_2O_2 is also approved for use in different food products as an antimicrobial agent. FDA approved the use of H_2O_2 for treatment of milk for use in cheese, preparation of modified whey and preparation of thermophile-free starch (Sapers and Simmons, 1998). Recently, FDA also approved the use of H_2O_2 in a mixture of disinfectants for red meat carcasses (Mermelstein, 2001). Moreover, the United States Department of Agriculture (USDA) approved the use of H_2O_2 for the pasteurization of egg white (Muriana, 1997). For these and other food applications of H_2O_2 the Food and Drug Administration (FDA) in the United States requires that residual H_2O_2 be removed by appropriate physical or chemical means during processing. Out of US, H_2O_2 is used more extensively for the disinfection of food. In fact, some H_2O_2 containing disinfectants approved by the ministry of health in Europe and Israel have still been used extensively in drinking water and food industries (Fallik et al., 1994).

Today, there are extensive studies to develop different protocols for the H_2O_2 disinfection of foods. Recently, as an alternative to chlorine, H_2O_2 has been recommended for the surface disinfection of fruits and vegetables to inhibit the post-harvest decay during storage (Fallik et al., 1994; Sapers and Simmons, 1998). Thus, many experimental studies had been carried out related to the disinfection of table grapes (Forney et al., 1991), sweet red pepper and eggplant (Fallik et al., 1994), dried prunes (Simmons et al., 1997), mushrooms, melon, cucumber, zucchini, green bell pepper and raisins (Sapers and Simmons, 1998).

During disinfection, H_2O_2 may be applied as vapor or liquid phase. In vapor phase application, H_2O_2 solution is volatilized into a stream of dried air until this mixture reaches the desired composition. For this application which a chamber and H_2O_2 vapor generator are required, the main difficulty is to obtain constant air-H_2O_2 vapor composition. The boiling point of H_2O_2 is 150,2 ^oC at atmospheric pressure. Thus, H_2O_2 vapor shows a great tendency to condense in treatment chamber that kept at near-ambient temperatures. Wang and Toledo (1986) by first heating air to a temperature same as H_2O_2 solution and then bubbling air into liquid H_2O_2 reduced this problem. In fact, the American Steriliser Company (AMSCO) has developed a patented vapor phase H_2O_2 generator for commercial sterilisation of medical devices and clean rooms

(Simmons et al., 1997). This machine is now used in different experiments to optimize the vapor phase H_2O_2 disinfection of different foods. However, these systems are still expensive, slow working and very complicated. During disinfection the operating parameters such as air flow rate, H_2O_2 concentration, vapor injection rate, air dehumidification time etc. should be controlled very carefully. Thus, commercialization of such systems for food disinfection still needs some time.

On the other hand, the use of liquid phase H_2O_2 during disinfection is fast, easier to control H_2O_2 concentration and apply commercially. In aqueous application, H_2O_2 solution can be sprayed onto food surface or food can be dipped in H_2O_2 solution. Since it is more effective and has some advantages, dipping is the most commonly applied method. The main parameters of dipping are treatment time and concentration of H_2O_2. Thus, this method is a very practical low-cost method that requires no complex machinery. Sapers et al (2001b) successfully built the first continuous, commercial-scale washing facility that will be used for the disinfection of fresh mushrooms. This was a great achievement to commercialize the use of liquid phase H_2O_2 disinfection in fruits and vegetables. Thus, it is expected that this chemical will be alternative to chlorine in a near future.

Besides vapor and liquid phase disinfection, H_2O_2 producing bacteria can also be added to foods for preservation. Lactic acid bacteria have ability to produce H_2O_2 even during cold storage and in some cultures, H_2O_2 may accumulate to inhibitory levels. It was reported that *Lactobacillus delbrueckii* subspecies produced sufficient amount of H_2O_2 to kill cells of *Escherichia coli* O157:H7 on refrigerated raw chicken meat (Villegas and Gilliland, 1998).

3.5. Removal of Residual H_2O_2 from Disinfected Food

As indicated above, FDA requires the removal of residual H_2O_2 in food following its application. The residual H_2O_2 in foods may be removed effectively by using H_2O_2 decomposing enzyme catalase. This enzyme decomposes H_2O_2 to water and oxygen ($H_2O_2 \Rightarrow H_2O + ½\ O_2$) and it exists in many foods. Therefore, in most cases the residual H_2O_2 in food disappears without an additional treatment of the food. This enzyme exists also in saliva of humans and this provides an extra protection against

H_2O_2 residues. In fact, this is why H_2O_2 is safely used in tooth pastes to obtain a better antimicrobial effect and washing effect.

The antioxidant chemicals such as ascorbic acid and its derivatives and sulfites may also be used to eliminate residual H_2O_2. In addition to the in situ catalase, Sapers and Simmons (1998) used 4,5 %, pH 5,5 Na-erythorbate solution to better eliminate the residual H_2O_2 in fresh fruit and vegetables. In some cases, the residual H_2O_2 in food may be eliminated by washing. For example, after dipping to 5 % H_2O_2 solution, residual H_2O_2 in cucumbers and melons may be removed completely by washing with water for 5 and 20 min, respectively (Sapers and Simmons, 1998).

3.6. Potential Effects of H_2O_2 on Food Quality

The use of H_2O_2 in foods may cause the oxidation of some sensitive food components. For example, the deleterious effect of H_2O_2 on anthocyanins is well-known. The degradation of anthocyanins by H_2O_2 has been demonstrated in strawberry, pomegrenate and sour cheery juices (Sondheimer and Kertesz, 1952; Özkan et al., 2000; Özkan et al., 2002). Thus, application of H_2O_2 disinfection may not be suitable for some fresh cut fruits rich in these color pigments. However, when whole fruits are disinfected, the waxy peel of some fruits may prevent the penetration of H_2O_2 to fruit flesh and this eliminates the possible discoloration. For example, Forney et al. (1991), applied vapor phase H_2O_2 disinfection, observed no discoloration in Red globe grapes. Sapers and Simmons (1998) also did not report the bleaching of sweet cherry anthocyanins while strawberry and raspberry anthocyanins showed bleaching. Simmons et al. (1997) indicated that prunes exposed to vapor phase H_2O_2 became lighter because of bleaching and blistering occurred at long exposures.

Besides anthocyanins H_2O_2 shows bleaching also on carotenoids. For example, Özkan and Cemeroğlu (2002), showed the bleaching of sun-dried apricots treated with 0.5-1.5% H_2O_2. A slight bleaching was also observed in cantaloupes that were treated with H_2O_2 (Sapers and Simmons, 1998). However, compared to anthocyanins carotenoids are considerably more resistant to the oxidative effects of H_2O_2. Thus, the partial bleaching of carotenoids may not result the rejection of products by the consumers.

Another possible undesirable effect of H_2O_2 may be the oxidation of vitamin C. Johnson and Toledo (1975) reported that the half-life of ascorbic acid in orange juice concentrate was only 21 days at 24°C when the aseptic chamber was pre-sterilized with H_2O_2 and 42 days when it was pre-sterilized with steam. Thus, the disinfected foods may be supplemented with additional vitamin C to recover the portion degraded by H_2O_2.

In literature the studies related to the effect of H_2O_2 on food nutrients are very limited. Sapers et al. (1999) reported that the washing of mushrooms with 5% H_2O_2 caused no considerable change in the composition and nutrient content of mushrooms. In contrast, Özkan and Kırca (2001) reported the reduced quality of proteins in peanuts especially due to the oxidation of cysteine, methionine, lysine and trionine.

3.7. Advantages of Using H_2O_2 as a Disinfectant

The use of H_2O_2 as a disinfectant brings many advantages. First of all H_2O_2 is a GRAS chemical which shows strong bactericidal and sporicidal effects without leaving toxic residues. The instability of H_2O_2 in a medium enables the elimination of its residues following the disinfection. In fact, that is one of the main reasons that make H_2O_2 an alternative to clorine which currently used extensively for the disinfection of food and drinking water. Clorine when reacts with food constituents may form toxic by-products named trihalomethans (THMs). Therefore, environmental and health communities have expressed concerns about the residual by-products of chlorine and questioned its future applications (Xu, 1999).

In commercial applications, H_2O_2 is usually added into washing water of fruits and vegetables. By washing in the presence of H_2O_2, adhering dirt and soil on food can be removed very effectively. This is because of the excessive O_2 gas release during decomposition of H_2O_2. The presence of catalase enzyme activity in disinfected food increases the gas release and the mechanical action helps to remove adhering dirt and soil from food surface more effectively (Sapers and Simmons, 1998). This is an important advantage for the disinfection of sun-dried fruits that left in field for a very long time and contaminated with dirt and soil.

Another advantage of using H_2O_2 is that it eliminates some undesirable chemical residues from food. Altuğ et al. (1990) showed the degradation of aflatoxin B1 in figs in

the presence of H_2O_2. Different studies about reduction of aflatoxin level in groundnuts and peanuts by H_2O_2 were also reported by Özkan and Kırca (2001). McFeeters (1998) successfully applied H_2O_2 to remove sulfites from fresh cucumbers, whereas Özkan and Cemeroğlu (2002) used H_2O_2 to reduce sulfur level in apricots. The degradation of pesticide residues by H_2O_2 is also reported by Doong and Chang (1998) and Fallmann et al. (1999).

In nature many microorganisms produce H_2O_2 as a natural metabolite, and some microorganisms have enzyme systems that decompose H_2O_2 to O_2 and H_2O. Also, H_2O_2 does not cause a selection on microorganisms and it is environmentally safe. In fact, H_2O_2 is used for the bioremediation of contaminated sites (Neyens and Baeyens, 2003).

Chapter 4

MATERIALS AND METHODS

4.1. Materials

Sun-dried figs (cultivar Sarılop from Aydın, Turkey) were supplied by TARİŞ (İzmir, Turkey), the cooperative for marketing agricultural products grown in Aegean region. Two different parties of sun-dried figs were supplied at two different seasons (2001 and 2002). Fresh figs (cultivar Sarılop from Aydın, Turkey) were obtained from a local market in İzmir and kept frozen at – 25 ^{o}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 PVPP (polyvinylpolypyrrolidone), and Horseraddish peroxidase (Type II) were purchased from Sigma Chem. Co. (St. Louis, MO). H_2O_2 test strips were obtained from Macherey-Nagel Co. (Dueren, Germany) or Merck (Darmstadt). 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). Chloramphenicol antibiotic was kindly donated by Borkim Chem Co. (İzmir, Turkey) or purchased from Oxoid (Hampshire, England). All other chemicals were reagent grade.

4.2. Methods

4.2.1. PME extraction

For the extraction of PME enzyme, 30-50 g sun-dried or fresh figs were homogenized in 180-200 mL cold 0.02 M sodium phosphate buffer (pH 7.0) containing 1 M NaCl for 1.5 min by using a Waring blender. 2 % PVPP was also added to medium to absorb the phenolic compounds during homogenization. The slurry obtained was then filtrated through a four layers of cheese-cloth and used in this study after centrifugation. This enzyme extract containing ionically bound + 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.

4.2.2. Partial purification

In partial purification studies, obtained crude PME extract was centrifuged at 4000 g for 30 min (+ 4 ^{o}C). For the partial purification, solid $(NH_4)_2SO_4$ was added slowly to enzyme extract at + 4 ^{o}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 ^{o}C) was dissolved in minimum amount of deionized water. The enzyme extract was then dialyzed for 24 h at + 4 ^{o}C by two changes of 2000 mL of deionized water and used in heat inactivation studies.

4.2.3. PME activity

For the determination of PME enzyme activity spectrophotometric or titrimetric methods were used. 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 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 working at 30 ^{o}C, and enzyme activity was determined from the slope of the initial linear portion of abs versus 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 and 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 Yemenicioğlu (2002). The fig homogenate was obtained by homogenizing 50 g of figs with 150 mL of 8.8 % NaCl. For the determination of covalently bound PME activity the residues obtained from the filtration and centrifugation of crude PME extract were combined and suspended in some deionized water. The reaction mixture contained 1.5 mL of enzyme extract (or 0.8-4 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 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 ^{o}C and enzyme activities were expressed as percent initial activity or μmol of liberated carboxyl groups per minute per mL enzyme extract. All activity measurements were done at least three times and averages were calculated.

4.2.4. Heat inactivation of PME

The temperature profiles were determined by heating 1.5 mL of crude PME enzyme extract (centrifuged at 3000 g and + 4 ^{o}C for 15 min) in thermal inactivation time (TIT) tubes (i.d., 9 mm; wall thickness, 1mm) for 5 min over 50-70 ^{o}C. The tubes were then cooled in an ice water bath and the residual enzyme activities were assayed by the titrimetric method.

The heat inactivation of partially purified PME was studied over the temperature range of 60-90 ^{o}C. To minimize the lag phase, 0.3 mL of enzyme extracts were pipetted into preheated TIT tubes. After heating for a given period, the tubes were cooled in an ice water bath and immediately assayed for PME activity by the spectrophotometric method. All heat inactivation studies were conducted as three replicates and averages were calculated.

4.2.5. Determination of protein content

Protein was determined by the Lowry method by using bovine serum albumin as standard (Harris, 1987). For the assay 0.2 mL samples, diluted to 25-200 μg. mL^{-1} protein, were mixed with 2.1 mL of alkaline copper reagent prepared by mixing 1 % $CuSO_4.5H_2O$ and 1% NaK tartrate. $4H_2O$ in a 1:1 ratio and diluting to 100 mL with 2 % Na_2CO_3 (in 0.1 M NaOH). After 10 minutes incubation, 0.2 mL commercial folin-ciocalteau reagent diluted 1:1 with bidistilled water was added to medium and the mixture was incubated at dark for 1h. At the end of incubation period the absorbances of samples were read at 750 nm against blanks prepared by adding 0.2 water to reaction mixture in place of samples. The concentrations of protein standards were between 25-200 μg. mL^{-1} (10-40 μg. 0.2 mL^{-1}). For the calibration curve see Appendix A1.

4.2.6. Microbiological tests

For these tests 50 or 60 g fig pieces (1/4 pieces obtained from 10 figs) were put into flasks containing 200-250 mL 0.1 % (w/w) peptone water. For counting osmotrophs 0.1 % (w/w) peptone water was supplemented with 20% sucrose. The flasks were shaken by hand for 2 min and 0.1 ml samples were spread onto the surface of agar plates. 1/10 dilutions were performed when needed by using 0.1 % peptone water (supplemented with 20 % sucrose when dilution was conducted for osmotrophs). The total number of mesophilic aerobic microorganisms and total number of yeasts and molds were determined by using PCA and PDA (acidified to pH 3.5 with 10 % tartaric acid or supplemented with 100 $mg.L^{-1}$ chloramphenicol) agars, respectively. The PCA plates were incubated at 35 ^{o}C for 48 h, whereas PDA plates were incubated at 25-28 ^{o}C for 5 days. The averages of three or five plate counts were used in all microbiological tests.

4.2.7. Selection of suitable rehydration conditions

To determine suitable rehdration temperatures and times required to obtain IM figs with approximately 30 % moisture content, the rehydration curves of samples at 30 ^{o}, 70 ^{o}, 80 ^{o} and 90 ^{o}C were determined using a circulating water bath (Polysience, Model 71). In these experiments, 200-250 g figs were put into sucks made from cheese-cloth 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 weighting (± 0.01 g) the sucks at different time intervals. The initial moisture content of figs was determined by the standard vacuum oven method for dried fruits (AOAC, 1995). During rehydration studies the temperature profiles of three figs were also determined by placing a 0.9 mm diameter thermocouple in their geometric center and by using a portable temperature recorder (Cole Parmer, DualLogR). 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 if necessary. The most suitable rehydration temperature was selected according to the amount of residual PME activity remained in IM figs after rehydration.

4.2.8. Rehydration of figs in hot water

After evaluating the remaining PME activities in IM figs rehydrated at different temperatures, the most suitable rehydration temperature was selected as 80 ^{o}C. In both season 2001 and 2002 the time to bring sun-dried figs to 30 % moisture content at 80 ^{o}C was almost 16 min. The figs were rehydrated as 1.2 kg parties and all rehydrations were at least duplicated. The results of PME activity and microbiological tests were given separately for each trial and IM figs rehydrated at 30 ^{o}C were used as control. For figs obtained in season 2001 and 2002 the rehydration times of controls were 65 and 51 min, respectively.

4.2.9. Disinfection of figs with H_2O_2

The disinfections were conducted by adding H_2O_2 to rehydration water immediately before dipping sun-dried figs. All disinfections were conducted at 80 ^{o}C and at 2.5 % H_2O_2 concentration. However, the contact period of figs with H_2O_2 was changed. Some of the samples were brought to 30 % moisture content directly by rehydrating them in 2.5 % H_2O_2 solution at 80 ^{o}C for 16 min, whereas others were brought to the same moisture content by rehydrating them first in 2.5 % H_2O_2 solution at 80 ^{o}C for 4 or 8 min and then in water at 80 ^{o}C for 12 or 8 min, respectively. All rehydrations were at least duplicated and the results of PME activity and microbiological tests were given separately for each trial.

4.2.10. Storage studies

Following rehydration the figs were rinsed, spread over trays and incubated 12 min at 100 ^{o}C to remove free water from their surface. After that the samples were separated to 300 g groups, each group was put into zipped polyethylene bags and cold stored at +4 ^{o}C for 1, 3, 3.5 or 5 months. Although the free surface water of figs was removed by the oven treatment, after rehydration some figs may entrap water and this may cause leakage during storage. To prevent this, test tubes containing 4 g of silicagel were also placed into bags before closing them. These tubes were remained in packages when IM figs were stored for 1 or 3 months. However, when storage period exceeded 3 months the tubes were removed from the packages under aceptic conditions after 15 days storage. The summary of the processes applied to IM figs were given in Figure 4.1.

SUN-DRIED FIGS

|

REHYDRATION to 30 % MOISTURE CONTENT*

|

SPREADING OVER TRAYS AND INCUBATION FOR 12 MIN AT 100 ^{o}C

|

PACKAGING AND COLD STORAGE

Figure 4.1. The summary of the processes applied to sun-dried figs (*Rehydration procedures;(1) Rehydration in water at 30 ^{o}C for 65 or 51 min (Controls); (2) Rehydration in water at 80 ^{o}C for 16 min; (3) Rehydration in 2.5 % H_2O_2 solution at 80 ^{o}C for 16 min; (4) Rehydration in 2.5 % H_2O_2 solution at 80 ^{o}C for 4 min + Rehydration in water at 80 ^{o}C for 12 min; (5) Rehydration in 2.5 % H_2O_2 solution at 80 ^{o}C for 8 min + Rehydration in water at 80 ^{o}C for 8 min).

4.2.11. Examination of texture and color

In addition to the samples rehydrated, packed and stored for monitoring PME activity and microbial load, some samples rehydrated at different conditions were also packed and stored separately for color and texture analysis. On this purpose almost 40-50 figs were rehydrated at the same time. These figs were then divided into two groups, packed and stored for 3.5 or 5 months. The textural examinations were conducted by some simple tests performed with hand or with a fruit harness tester (Nippon-1kg/Model FHR-5 equipped with a cone type tip, base diameter: 12 mm, height: 10 mm). In tests conducted by hand, the figs were firstly examined by the classical thumb test to detect whether they softened. Secondly, to detect any sticky and gel like structure formation the figs were halved and their fleshes were squeezed by using thumb and forefinger. Thirdly, with thumb the internal surface of halved figs was went-over to see whether it was rubbed-off from the peels easily. When storage period exceeds 3 months the firmnesses of figs were determined with a fruit hardness tester. Before penetration tests the fruits were shaped by hand like discs to form a homogenous surface. The neck and eye of shaped fruits always remained at the edges and the penetrations were always conducted close the middle part of flattened fruit surfaces. For each fruit, the number of penetrations conducted was 6 and the points of measurement were equally spaced to

form 2 columns and 3 rows. The effect of storage and H_2O_2 on fig color was monitored by taking photographs of samples with a digital camera (Nikon, PIX995 or Sony, DCR-PC115E).

4.2.12. Determination of residual H_2O_2

The residual H_2O_2 in disinfected figs was determined by using semi-quantitative Quantofix test stripes which can detect residual H_2O_2 in the range of 1-100 mg H_2O_2. L^{-1}. During tests 25g figs, chilled by holding 10 min at –25 ^oC, were homogenized with 200 mL 0.05M cold Na-phosphate buffer (pH 7.0) or bidistilled water for 1.5 min at low speed by using a Waring blender. In this study, considerable amount of catalase enzyme activity was determined in sun-dried figs. Thus, to prevent the degradation of residual H_2O_2 by catalase, the slurry obtained was very rapidly filtrated through a single layer of cheese-cloth and a test stripe was immediately dipped to the filtrate. The residual H_2O_2 in filtrate was determined by comparing the intensity of blue color developed on test stripes and color-concentration scale given and it was expressed as ppm.

The disinfected figs were tested for residual H_2O_2 immediately after the oven treatment applied following the rehydration process and during cold storage for different time periods, until no residual H_2O_2 was detected by the test stripes. The disappearance of residual H_2O_2 in IM figs during cold storage was also confirmed qualitatively by the more sensitive enzymatic H_2O_2 determination method. In this method the residual H_2O_2 is determined by the color change formed by peroxidase enzyme. In the presence of residual H_2O_2, this enzyme forms a very distinguishable brown color from guaiacol. The reaction mixtures used in this study were formed by mixing 4mL of filtrate prepared as described above, 0.25 mL peroxidase (almost 42 purpurogallin unit) prepared in 0.05M , pH 7.0 phosphate buffer and 0.2mL 0.5 % guaiacol (prepared in 50 % ethanol). The blanks for comparison were prepared by using 0.2mL phosphate buffer in place of guaiacol.

4.2.13. Catalase activity

The presence of catalase activity in sun-dried IM figs was controlled qualitatively. On this purpose the figs rehydrated at 30 ^oC or 80 ^oC were halved and dipped to a 2.5 %

H_2O_2 solution at room temperature. The gas evolution and foaming observed were accepted as the indication of catalase activity.

Chapter 5

RESULTS AND DISCUSSION

5.1. Rehydration Studies

In this thesis, one of our purposes' is to use hot rehydration as a hurdle for the preservation of IM figs. A carefully selected rehydration temperature may be used to control or at least minimize enzymatic and microbial changes in IM figs during cold storage. In the preliminary studies, a very rapid yeast and mold growth was determined in cold stored IM figs when their moisture content exceeded 30 %. For example, at 35 % moisture content the total mesophilic aerobic count and total yeast and mold count of samples cold stored for 2.5 months were $> 1.3 \times 10^4$ and 3×10^3 CFU.g^{-1}, respectively. Thus, during cold storage it was decided to keep the moisture content of figs almost at 30 %.

To find the times to reach the desired moisture content at different temperatures, the rehydration kinetics of sun-dried figs were studied. Figure 5.1 and Figure 5.2 show the rehydration curves of sun-dired figs obtained in 2001 and 2002 seasons, respectively.

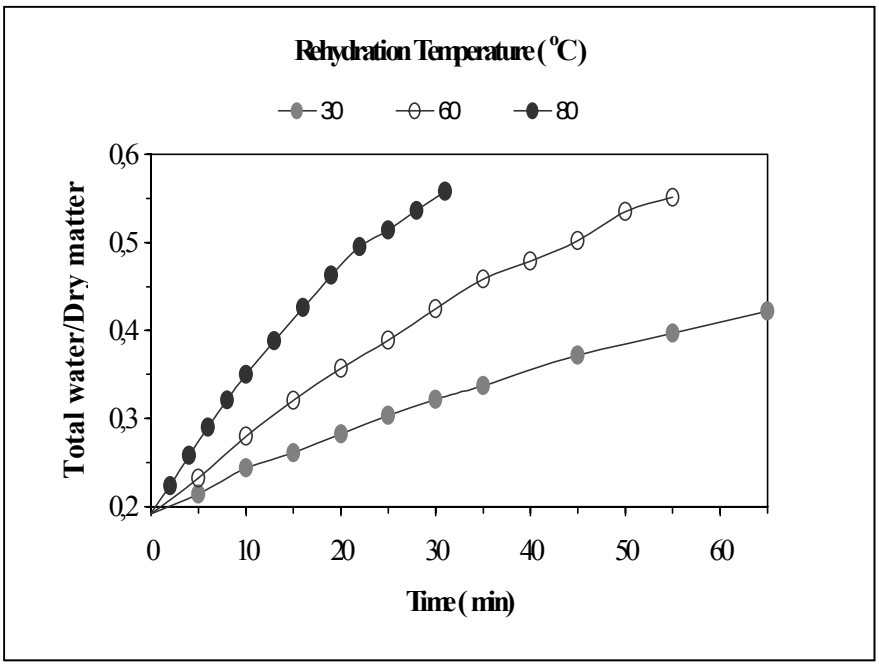

Figure 5.1. Rehydration curves of sun-dried figs at different temperatures (Season 2001, the percent initial moisture content of figs was 15.4 ± 0,2).

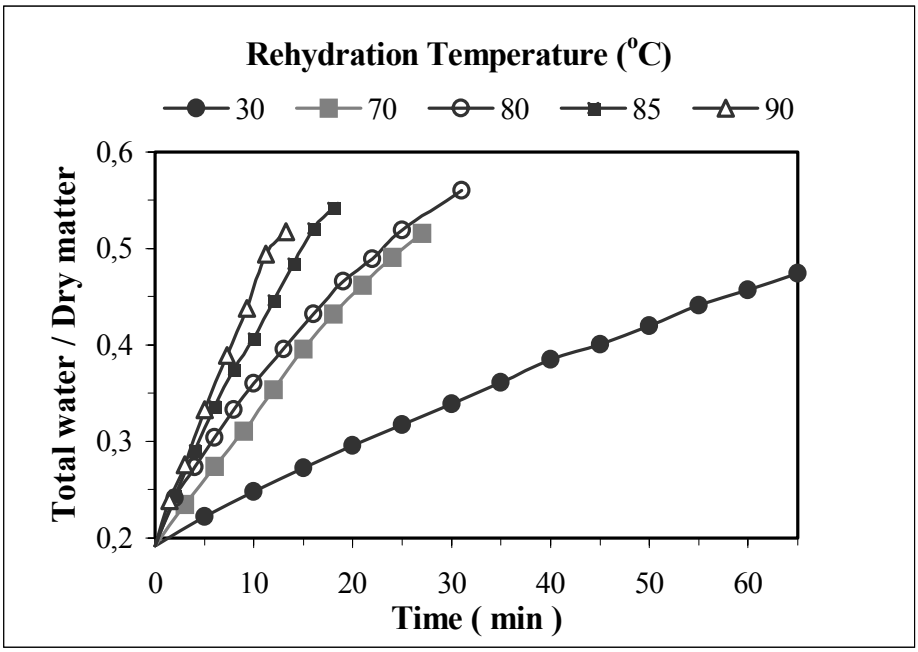

Figure 5.2. Rehydration curves of sun-dried figs at different temperatures (Season 2002, the percent initial moisture content of figs was 16.1 ±0.07).

Table 5.1. Rehydration times at different temperatures to bring the moisture content of figs to 30 %.

Temperature (°C)	Reyhdration time (min)	
	Season 2001	Season 2002
30	65	51
60	30	-
70	-	17.5
80	16	16
85	-	11
90	-	8.8

Note: The times were read from the rehydration curves

As given in Table 5.1, for sun-dried figs produced at different seasons, the times to bring the moisture level of figs to 30 % vary slightly at 30 °C. However, at 80 °C the samples have the same rehydration times. In fact, for season 2002 there are also no

considerable differences in rehydration times between 70 ^{o} and 80 ^{o}C and rehydration times between 85^{o} and 90 ^{o}C.

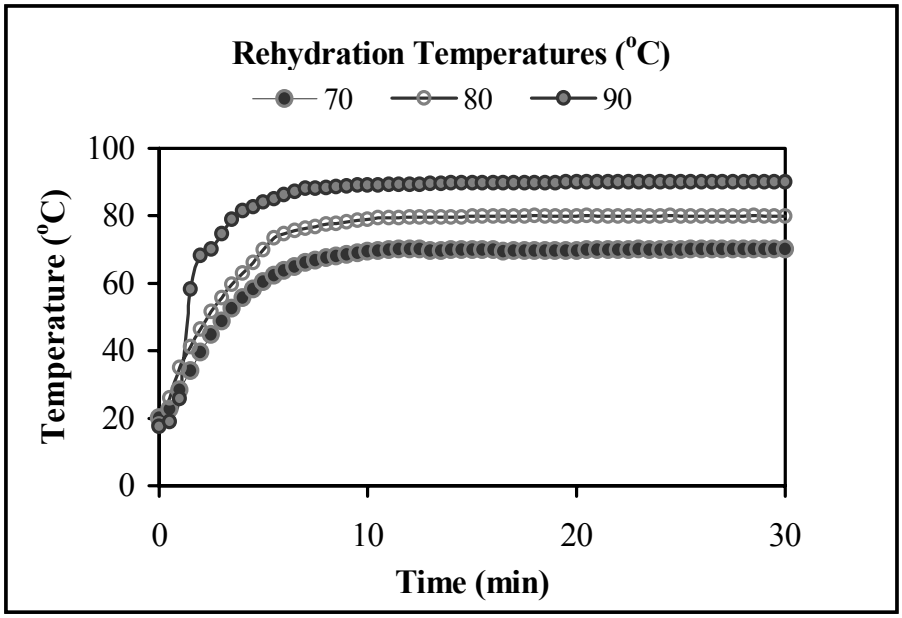

Figure 5.3. Heat penetration curves of sun-dried figs during rehydration at different temperatures.

The temperature changes at the geometric center of average figs during rehydration were also given in Figure 5.3. For most enzymes and vegetative cells 55 ^{o}C is a critical temperature that starts the inactivation. As seen in this figure, between 70 ^{o} and 90 ^{o}C it takes almost 10 min to bring the temperature of the geometric center of figs to rehydration temperature. During rehydration at 70 ^{o}, 80 ^{o} and 90 ^{o}C, the times above 55 ^{o}C, were approximately 13.5, 13 and 7 min, respectively.

5.2. Possible Mechanisms of Textural Change During Cold Storage

In season 2001, during cold storage of IM sun-dried figs, extensive softening was determined in samples rehydrated at 30 ^{o}C. The softening, initiated at the end of first month of the storage, increased dramatically at the end of third month of the storage. When softened figs were halved and examined carefully by hand, the substantial reduction in the consistency of their fleshes was felt easily. The fleshes of softened fruits were also very sticky and gel like and this caused loss of their desired gummy texture.

The properties, solubility and amount of pectic compounds are the primary factors determining the texture of fresh and processed fruits and vegetables (Cemeroğlu et al. 2001). Thus, because of its central role in the modification of pectin, PME affects the textural properties of these products considerably (Castaldo et al., 1989, Thakur et al., 1996, Alonso et al., 1997). The PME enzyme is capable to catalyze pectin demethylation even at low storage temperatures, while depolymerization enzymes such as PG slows down under the same conditions (Marangoni et al., 1995; Artes et al., 1996). Thus, it seems that during cold storage PME reduced the degree of pectin methylation in figs. This possibly enabled the interaction of pectin and divalent ions such as Ca^{++} and Mg^{++} and caused the gel formation. The enzyme PG should have also degraded pectin molecules partially and this reduced the consistency of the fruit fleshes and induced the formation of a very sticky structure.

5.3. The Origin of PME in Sun-dried Figs

The ability of different bacteria and fungi to produce pectic enzymes was reported by different workers (Liu and Luh, 1978, Hao and Bracket, 1994). However, the presence of PME enzyme activity in sun-dried figs immediately after rehydration indicated that the enzyme was not formed by some microorganisms during cold storage. 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 ^{o}C and cold stored for 3 months were compared (Fig 5.4).

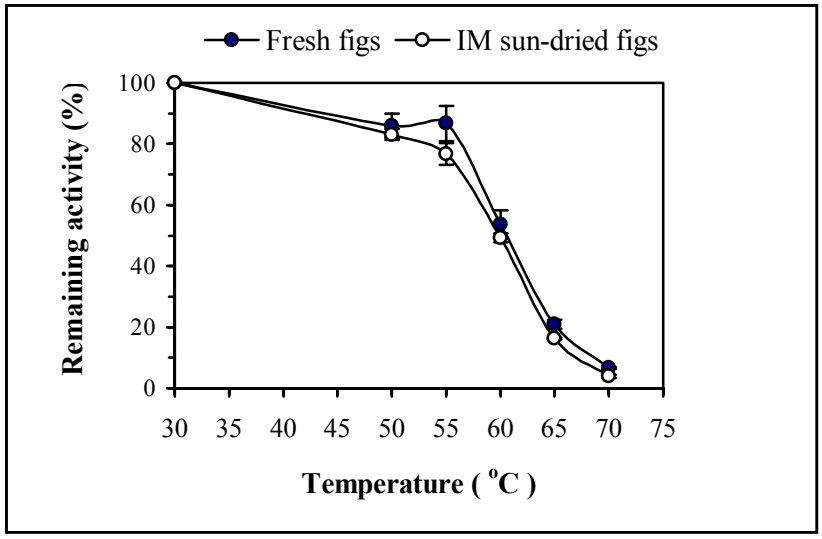

Figure 5.4. Temperature profiles of crude PME from fresh figs and 3 months cold stored softened intermediate moisture sun-dried figs (Season 2001).

As seen in this figure, temperature profiles of crude PME in fresh and softened IM sun-dried figs are quite similar. Thus, this result suggests that the enzyme was fig PME that survived from sun-drying.

5.4. Partial Purification of PME

The crude PME from sun-dried figs and fresh figs was also partially purified to compare their purification parameters. As seen in Table 5.2, 0-90 % $(NH_4)_2SO_4$ precipitation and dialysis of PME extracted from fresh and sun-dried figs gave 132 % and 168 % recoveries, respectively. During partial purification of enzymes, the increase of recovery over 100 % has been observed by many workers (Benjamin and Montgomery, 1973; Hara et al, 1989; Söderhall and Söderhall, 1989). Segel (1976) explained this by the removal of an enzyme inhibitor by partial purification. However, this may also be due to the activation of the enzyme during partial purification. The total PME activity purified from sun-dried figs was almost 1.6 fold higher than that purified from fresh figs. Thus, it seems that the enzyme concentrated by drying is very stable under low a_w conditions and it may show activation.

Table 5.2. Partial purification of pectin methylesterase from fresh and sun-dried figs (Season 2001).

Purification step	Volume (mL)	Total activity (Units)	Total protein (mg)	Specific activity (Units . mg $^{-1}$)	Recovery (%)	Purity (Fold)
Fresh figs						
Crude extract						
	111	10934	230	48	100	1.0
0-90 % $(NH_4)_2SO_4$ precipitation and 24 h dialysis						
	65	14398	42	343	132	7.2
Sun-dried figs						
Crude extract						
	76	12943	262	49	100	1.0
0-90 % $(NH_4)_2SO_4$ precipitation and 24 h dialysis						
	48	21782	25	871	168	17.8

The total protein contents in the crude extracts of fresh and sun-dried figs were almost 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 action of proteases and explains the higher purity of this enzyme extract after partial purification.

5.5. Heat Inactivation of PME

One of the main objectives of this study was to test the potential application of hot rehydration to control PME catalyzed undesirable textural changes in IM figs. Thus, to determine a suitable rehydration temperature, the heat inactivation kinetic of partially purified PME from sun-dried figs between 60 ^{o} and 90 ^{o}C was investigated. The inactivation of PME from sun-dried figs followed a first order reaction kinetic, and the biphasic inactivation curves of enzyme indicated that it contained heat labile and heat stable enzyme fractions (Fig. 5.5). The enzyme also showed activation by heating and this occurred particularly at 60 ^{o} and 70 ^{o}C. However, at 80 ^{o} and 90 ^{o}C the activation was not observed and PME showed rapid inactivation. Thus, it seems that the rehydration temperature should be above 70 ^{o}C to achieve faster enzyme inactivation and minimize activation.

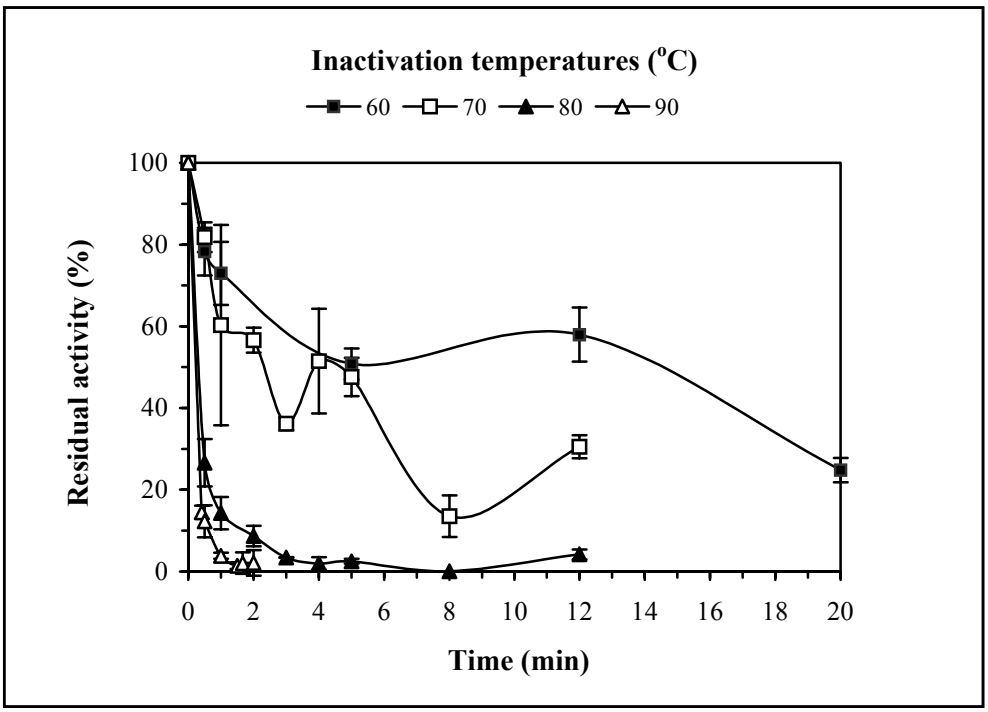

Figure 5.5. Heat inactivation curves of partially purified PME from sun-dried figs (Season 2001).

Table 5.3. Heat inactivation parameters of partially purified PME in sun-dried and fresh figs (Season 2001).

Enzyme	Temperature or temperature range (^{o}C)	*D* value (min)	
		Heat labile	**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
	60-90	z = 24.5 ^{o}C (0.971)	z = 18.6 ^{o}C (0.992)
	70-90	z = 20.8 ^{o}C (0.984)	z = 20.2 ^{o}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
	70-90	z = 20.8 ^{o}C (0.853)	z = 21.1 ^{o}C (0.948)

For the calculation of enzyme`s 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 (Table 5.3). For comparison, the heat inactivation kinetic of partially purified PME from fresh figs was also determined (Fig 5.6). Although the *D* values of PME from fresh figs were almost two folds higher than those of PME from sun-dried figs, both enzyme showed almost the same activation and inactivation patterns. In particular, the activation and inactivation patterns observed at 70 ^{o}C were quite similar. Also, the *z* values of enzyme`s heat labile and heat stable portions between 70-90 ^{o}C were almost same. Thus, these results confirm that the PME in sun-dried fruits is fig PME survived from sun-drying.

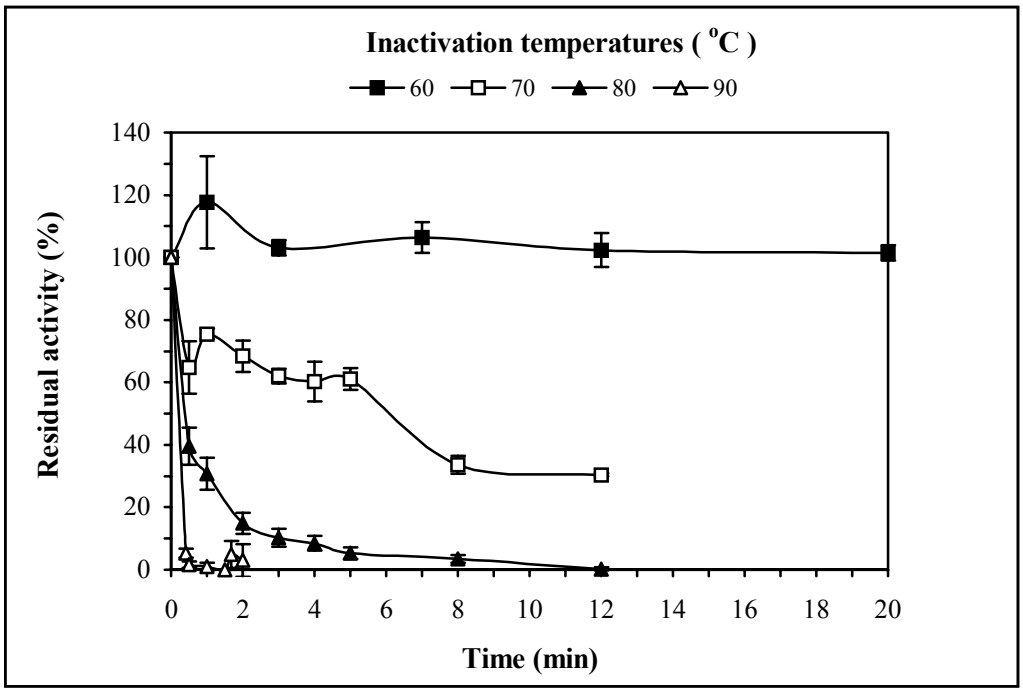

Figure 5.6. Heat inactivation curves of partially purified PME from fresh figs (Season 2001).

5.6. Effect of Hot Rehydration on IM Fig Texture, Color and Residual PME Activity

The heat inactivation studies conducted with partially purified extracts indicated that hot rehydration of sun-dried figs above 70 °C may be used to control PME catalyzed undesirable textural changes in IM figs during cold storage. Thus, in season 2001 figs were rehydrated at 30 ° and 80 °C to 30 % moisture content and their textural properties and remaining PME activities were compared after 3 months cold storage. During examinations, the apparent browning occurred in IM figs rehydrated either at 30 ° or 80 °C was observed clearly (See photos A2 and A3 in Appendix). The softening of figs rehydrated at 30 °C was felt clearly by the thumb test. In contrast, figs rehydrated at 80 °C were found firmer. When all figs were halved and examined by hand, the fleshes of most figs rehydrated at 30 °C were found very sticky. In contrast, the fleshes of most figs rehydrated at 80 °C were considerably more consistent, less sticky and they were not easily rubbed-off from the peels with thumb. These results suggest that the problem of softening of IM figs may be controlled for 3 months by hot rehydration at 80 °C.

It is interesting to note that at the end of 3 months storage, compared to controls rehydrated at 30 °C, only 28 % less PME activity was found in the homogenates of IM

figs rehydrated at 80 ^{o}C (Table 5.4). In heat inactivation studies, fig PME was found heat labile. Thus, this result clearly showed the considerably different inactivation kinetic of PME in whole figs and in partially purified extracts. In contrast, the control of undesirable textural changes in IM figs for 3 months 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.

Although we investigated the thermal properties of crude and partially purified ionically bound + soluble enzyme extracts in season 2001 with details, we have no information related to the presence or thermal stability of covalently bound PME enzyme in sun-dried figs. Thus, in season 2002, to find the reason of limited PME inactivation by hot rehydration at 80 ^{o}C, the residual activities of PME in ionically bound + soluble enzyme extracts and covalently bound enzyme extracts were also compared immediately after rehydration of sun-dried figs at different temperatures (Fig 5.7).

Table 5.4. Residual PME activities in the homogenates of IM sun-dried figs rehydrated at different conditions and cold stored for 3 months (Season 2001).

Type of rehydration	**Activity (μmol COOH min^{-1} g^{-1})**
Control / 65 min in water at 30 ^{o}C	
1	25,73 ±0,2
2	22,42 ±0,6
	Average 24.1 (100)
16 min in water at 80 ^{o}C	
1	17,24 ±1,5
2	17,58 ±0,3
	Average 17,4 (72)*
16 min in 2.5% H_2O_2 solution at 80 ^{o}C	
1	16,47 ±0,6
2	15,92 ±1,2
	Average 16,2 (67)

* Percentage of remaining PME activity in IM figs as compared to activity of control

As expected sun-dried figs contained also covalently bound PME activity (5.7 μM COOH . min^{-1}. g^{-1}). This enzyme forms almost 25 % of the total PME activity in sun-dried figs. However, compared to inonically bound + soluble PME that showed 16.6 μM COOH . min^{-1}. g^{-1}activity, it is heat labile and lost almost 50 % of its activity when rehydration was conducted at 80 ^{o} or 90 ^{o}C. Thus, the limited PME inactivation observed in IM figs rehydrated in water at 80 ^{o}C was not due to the presence of an extremely thermostable covalently bound enzyme fraction. The ionically bound + soluble PME, on the other hand, showed almost 25 and 30 percent inactivation after 80 ^{o} and 90 ^{o}C rehydrations, respectively. Thus, for PME inactivation, rehydration at 90 ^{o}C has almost no benefits. The times of rehydration to achieve 30 % moisture content at 80 ^{o} and 90 ^{o}C were almost 16, 8.8 min, respectively (Table 5.1). According to the results

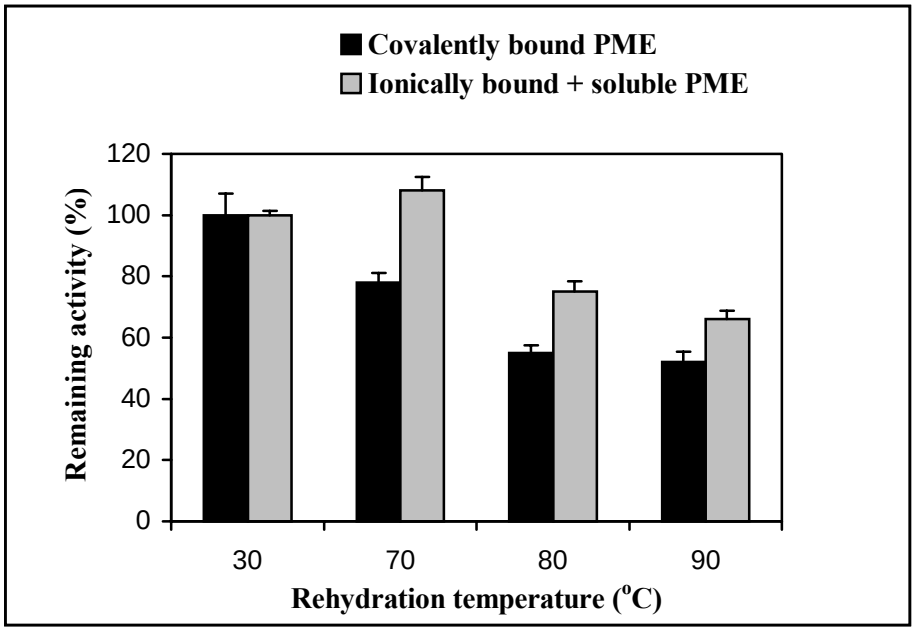

Figure 5.7. Residual activities of ionically bound + soluble PME and covalently bound PME in IM figs rehydrated at different temperatures (Season 2002).

of heat penetration studies (Fig 5.3) and heat inactivation data obtained (Table 5.3), these temperatures and times should be enough to achieve at least 1 decimal or more inactivation of ionically bound + soluble PME fraction. However, the increase of ionically bound + soluble PME enzyme activity after rehydration at 70 ^{o}C clearly indicated the in situ activation of this PME fraction 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 + soluble PME enzyme extracts. However, during these studies the activation at 70 ^{o}C did not cause the increase of enzyme activity over initial activity. Thus, it appears that the limited enzyme inactivation by hot rehydration was due to the considerably more activation of in-situ PME in whole figs. It is very difficult to explain how ionically bound + soluble PME showed more activation in whole figs. However, it is likely that when fig tissue was homogenized for enzyme extraction the enzyme came into contact with some inhibitors that limited its activation during heating. Also, it is well known that the heat inactivation of enzymes is affected from their solubility (Wasserman, 1984; Weng et al., 1991). Thus, the PME in sun-dried figs may be immobilized by the concentrated cellular matrices such as pectic compounds and this may increase the activation and thermal stability of enzyme.

As indicated above rehydration at 80 ^{o} or 90 ^{o}C has almost the same effect on PME activity. Thus, in 2002 season all hot rehydrations were applied at 80 ^{o}C. However, to see the effect of longer storage periods on remaining PME activity and IM fig texture, this time IM sun-dried figs were stored for more than 3 months. As seen in Table 5.5, after rehydration the average PME activity in the homogenates of IM figs rehydrated in water at 80 ^{o}C was almost 25 % less than that of the homogenates of IM figs rehydrated in water at 30 ^{o}C. This result complies well with previous findings of this study related to the inactivation of PME in whole IM figs rehydrated at 80 ^{o}C (Figure 5.7). As given before, in season 2001 the PME activity of IM figs rehydrated in water at 80 ^{o}C and cold stored for 3 months was 28 % lower than that of controls stored for the same time period. However, in 2002 season these differences between the activities of cold stored control and heat treated IM figs were smaller. This was because of the slight increase and reduction in the PME activities of heat treated and control samples during cold storage, respectively. As seen in Table 5.5 after 3.5 and 5 months cold storage the PME activity of IM figs rehydrated in water at 80 ^{o}C was almost 14 % and 21 % lower than those of the controls stored for the same time periods, respectively.

After 3.5 months cold storage when IM figs rehydrated in water at 80 ^{o}C and 30 ^{o}C were examined by the classical thumb test, an apparent softening was detected in both groups. Although, it is very difficult to determine which group was firmer, it seemed

that the controls were slightly firmer. When the firmnesses of IM figs were determined by fruit hardness tester this difference was observed more clearly (Table 5.6).

Table 5.5. Residual PME activities in the homogenates of IM sun-dried figs rehydrated at different conditions and cold stored for different time periods (Season 2002).

Type of rehydration	**Activity (μM COOH. min^{-1}. g^{-1})**		
	Storage time (months)		
	0	**3.5**	**5**
Control / 51 min in water at 30 ^{o}C			
1	24,16 ±1,1	26,09 ±0,2	24,83 ±0,9
2	26,35 ±0,6	22,75 ±1,1	23,78 ±0,5
3	25,90 ±0,3	22,23 ±0,2	26,52 ±0,7
	Average 25,5 (100)	**Average 23,69 (93)**	**Average 25,04 (98)**
16 min in water at 80 ^{o}C			
1	20,22 ±0,4	20,40 ±0,6	19,08 ±0,3
2	18,53 ±0,3	21,30 ±0,5	21,78 ± 0,4
3	18,62 ±0,4	19,27 ±0,4	18,27 ±1,2
	Average 19,1 (75)*	**Average 20,32 (80)**	**Average 19,71 (77)**
4 min in 2.5% H_2O_2 solution at 80 ^{o}C + 12 min in water at 80 ^{o}C			
1	20,87 ±0,3	19,93 ±0,4	22,08 ±0,4
2	17,27 ±0,1	18,65 ±0,7	19,05 ±0,4
3	19,17 ±0,5	18,32 ±0,5	19,47 ±0,9
	Average 19,1 (75)	**Average 18,97 (74)**	**Average 20,20 (79)**
8 min in 2.5% H_2O_2 solution at 80 ^{o}C + 8 min in water at 80 ^{o}C			
1	16,85 ±0,3	19,75 ±0,4	19,68 ±0,4
2	16,91 ±0,3	21,69 ±0,2	20,54 ±1,0
3	19,51 ±0,3	19,47 ±0,2	19,49 ±0,5
	Average 17,8 (70)	**Average 20,30 (80)**	**Average 19,90 (78)**

* percentage of remaining PME activity as compared to initial activity of control

When storage period was increased to 5 months and the same textural examinations were repeated, further softening was determined in samples. In almost 77 % of IM figs

rehydrated at 30 ^{o}C the fruit fleshes were rubbed-off form the peels very easily. On the other hand, in IM figs rehydrated in water at 80 ^{o}C, the percentage of fruits that fleshes rubbed-off from the peels remained at 60 % (Table 5.7).

As indicated above, in season 2002 the beneficial effect of hot rehydration in water at 80 ^{o}C was not observed when IM figs were cold stored for 3.5 months. It seems that this occurred because of the activation of PME enzyme during cold storage. In season 2001 such an activation was not observed in PME activity at the end of 3 months cold storage and hot rehydration in water at 80 ^{o}C was found beneficial. Thus, it may be concluded that the partial inactivation of PME by hot rehydration in water at 80 ^{o}C may be beneficial only when IM figs are cold stored for short storage periods.

Table 5.6. The firmnesses of IM sun-dried figs cold stored for different time periods (Season 2002).

Sample	**Firmness (kg)**	
	Storage time (months)	
	3.5	**5**
Control / 51 min in water at 30 ^{o}C		
	0,349 ± 0,101 (294)*	0,294 ±0,065 (318)
16 min in water at 80 ^{o}C		
	0,308 ±0,058 (318)	0,251 ±0,041 (288)
4 min in 2.5% H_2O_2 solution at 80 ^{o}C + 12 min in water at 80 ^{o}C		
	0,339 ±0,051 (288)	0,324 ±0,054 (306)
8 min in 2.5% H_2O_2 solution at 80 ^{o}C + 8 min in water at 80 ^{o}C		
	0,446 ±0,111 (288)	0,319 ±0,047 (306)

*The number of penetrations

During cold storage an apparent browning also occurred in 2002 season IM figs rehydrated in water at 30 ^{o}C or 80 ^{o}C. Particularly the IM figs rehydrated at 80 ^{o}C were darker after 3.5 and 5 months cold storage (see photographs A4 and A5 in Appendix). Thus, it is clear that the use of antibrowning agents is essential for heat-treated IM figs.

5.7. Effect of H_2O_2 on IM Fig Texture, Color and Residual PME Activity

In season 2001, to search the possibility of increasing the shelf-life and safety of IM figs during cold storage, the effects of hot rehydration at 80 ^oC in the presence of 2.5 % H_2O_2 were also tested. The addition of H_2O_2 to rehydration medium caused the bleaching of figs and turned the brown color of fruits to golden yellow-light brown (see photograph A6 in Appendix). The light colored figs were more attractive than the brown figs rehydrated in water and they maintained this desirable color even after 3 months cold storage.

Table 5.7. Some characteristics of IM figs rehydrated at different conditions and cold stored for different time periods (Season 2002).

A[a] (%)		**Comments**	
		Storage time (months)	
3.5	**5**	**3.5**	**5**
Control / 51 min in water at 30 ^oC			
51 (49)[b]	77 (53)	Brown-dark brown, some fruits had sticky and gel like fleshes, most fruits lost their volumes, became disc like and softened	Dark brown, almost all fruits had very sticky and gel like fleshes and they lost their volumes, became disc like and very softened
16 min in water at 80 ^oC			
48 (48)	60 (49)	Dark brown, some fruits had sticky and gel like fleshes, most fruits lost their volumes, became disc like and softened	Dark brown, most fruits had sticky and gel like fleshes, and they lost their volumes, became disc like and softened
4 min in 2.5% H_2O_2 solution at 80 ^oC + 12 min in water at 80 ^oC			
40 (48)	53 (51)	Light brown, some fruits had sticky and gel like fleshes, most fruits maintained their volume and shape, only several fruits became disc like and softened	Light brown-brown, some fruits had sticky and gel like fleshes, most fruits maintained their volume and shape, some fruits became disc like and softened
8 min in 2.5% H_2O_2 solution at 80 ^oC + 8 min in water at 80 ^oC			
31 (51)	55 (51)	Light brown-yellow, some fruits had sticky and gel like fleshes, most fruits maintained their volume and shape, only several fruits became disc like and softened	Light brown, some fruits had sticky and gel like fleshes, most fruits maintained their volume and shape, some fruits became disc like and softened

[a]A: Percentage of fruits that fleshes can easily be rubbed-off from the peels when they went-over with thumb; [b] Number of fruits tested

Traditionally, the light color of IM or high moisture fruits is maintained by adding 0-150 ppm SO_2 during their packaging (de Daza et al., 1997). Thus, by the application of H_2O_2 disinfection the use of sulfites may be minimized or eliminated completely.

At the end of 3 months storage, no apparent softening was detected in IM figs rehydrated in 2.5 % H_2O_2 at 80 ^oC. The fruit fleshes maintained their consistency and their fleshes were not sticky. Also, the PME activities in the homogenates of disinfected figs were 33 and 5 percent lower than those in the homogenates of figs rehydrated in water at 30 ^{o} and 80 ^oC, respectively (Table 5.4). 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. Also during storage, in some other figs the gas formed exhausted from the fruit eye and this caused the accumulation of white foam at this location. 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.

In season 2002, to reduce the contact period of figs with H_2O_2 and to eliminate undesirable physical defects some alternative treatments were tested. In these treatments, the moisture content of figs was brought to 30 % by rehydrating them first in 2.5 % H_2O_2 solutions at 80 ^oC for 4 or 8 min and then in water at 80 ^oC for 12 or 8 min, respectively. When figs were disinfected and cold stored for 3.5 or 5 months by using these two stage rehydration procedures, no blowing and foam formation were observed in IM sun-dried figs during cold storage. Also, at the end of 3.5 months the IM figs rehydrated for 4 or 8 min in H_2O_2 solution had a light brown and light brown-yellow color, respectively (see photographs A7 and A8 in Appendix). After 5 months storage IM figs disinfected 4 min in H_2O_2 showed slight darkening. However, 8 min disinfected IM figs were still light colored (see photographs A9 and A10 in Appendix).

After rehydration, the PME activities of IM figs rehydrated at two stages were almost same with those of IM figs rehydrated in water at 80 ^oC (Table 5.5). During cold storage a slight PME activation was observed also in disinfected IM figs. However, the disinfected IM figs maintained their textural properties better than the controls and samples rehydrated in hot water at 80 ^oC. As seen in Table 5.6, after 3.5 months cold

storage the IM figs disinfected 4 min in H_2O_2 had almost the same firmness with control IM figs. The IM figs disinfected for 8 min in H_2O_2, on the other hand, were firmer than both of these IM fig groups. After 5 months cold storage the disinfected IM figs showed only slight further softening, whereas controls and IM figs rehydrated in water at 80 ^oC softened considerably. When fleshes of 4 and 8 min disinfected IM figs were went over with thumb after 3.5 months cold storage, in almost 60 and 70 % of the fruits the fleshes were not rubbed off from the peels easily (Table 5.7). Also, after five months cold storage almost half of the fruits maintained the integrity of their fleshes. This indicates the additional benefits of H_2O_2 on fig texture. As indicated in Table 5.7 the figs disinfected with H_2O_2 maintained their volumes and shapes during cold storage. It appears that the O_2 gas, formed due to the decomposition of H_2O_2 by in situ catalase, is responsible from the increased volume of disinfected figs. During cold storage this probably helped fruits to maintain their original shapes and reduced the deformation of fruits by their own weight and by the weights of other fruits. It is well known that the deformation of cells increases the contact of PME and pectic compounds and accelerates softening. Thus, it is clear that the application of hot rehydration in combination with H_2O_2 is very beneficial to control PME mediated softening in IM figs.

5.8. Effect of Hot Rehydration on Microbial Load

During cold storage, the total mesophilic aerobic count and total yeast and mold count were monitored for IM figs rehydrated in water at 30 ^{o} and 80 ^oC. Table 5.8 shows the microbial counts of 2001 season IM figs immediately after rehydration and after 1 and 3 months cold storage. As seen in this table the initial total mesophilic aerobic counts of figs rehydrated at 30 ^oC varied considerably for the first and second trials. At the end of the first month of cold storage, the total number of mesophilic aerobic counts of IM figs did not change considerably, except the substantial reduction (almost two decimals) in the microbial count of IM figs obtained by the first rehydration trial at 30 ^oC. During storage period such a considerable drop was observed only in these samples. Thus, the higher count obtained after the first rehydration trial at 30 ^oC may be due to the presence of several spoiled figs in these samples. Considering this observation, it is unlikely to report that hot rehydration at 80 ^oC had a substantial effect on total mesophilic aerobic load of IM figs. Between 1 and 3 months of cold storage, the total mesophilic aerobic counts of control IM figs did not change considerably. In contrast, in the same period, the total mesophilic aerobic counts of IM figs rehydrated at 80 ^oC

increased almost 2-2.6 folds. Thus, for longer storage periods, further increases may be expected in the microbial load of IM figs rehydrated in water at 80 ^{o}C.

On the other hand, during cold storage of IM figs rehydrated in water at 30 ^{o}C or 80 ^{o}C, no mold growth on petri dishes was observed. In literature there are reports related to the possible negative effects of tartaric acid, used for the acidification of PDA, on mold growth (Taniwaki et al., 1999). Thus, as suggested by Farber (1997) the 3 months cold stored IM figs were also tested on PDA supplemented with chloramphenicol antibiotic. However, no mold growth was observed also in these counts. This shows the effective washing and separation of contaminated figs under UV light in the factory. On the other hand, except the figs obtained in the first trial of 30 ^{o}C rehydration, the total number of yeasts had drop continuously during cold storage.

In season 2002, storage tests of IM figs rehydrated in water at 30 ^{o}C and 80 ^{o}C were repeated. However, this time IM figs were stored for 3.5 or 5 months. As seen in Table 5.9, in season 2002, the total mesophilic aerobic count of control IM figs rehydrated in water at 30 ^{o}C varied between $1x10^{3}$ and $1,9x10^{3}$ CFU.g^{-1}. Thus, compared to season 2001 the total mesophilic aerobic counts of IM figs were slightly lower and varied in a very narrow range. Considering the average of controls ($1,6x10^{3}$ CFU.g^{-1}), in two of the separate rehydration trials conducted in water at 80 ^{o}C the total mesophilic aerobic count of IM figs reduced between 70 and 80 % after rehydration. However, in one of the trials conducted in water at 80 ^{o}C, the number of total mesophilic aerobic count is slightly higher than those of controls. Most probably this occurred because of the high microbial count of several figs in hot rehydrated samples. Thus, it is clear that the application of hot rehydration at 80 ^{o}C can not always reduce the microbial load of IM figs below 10^{3} CFU.g^{-1}. After 3.5 months cold storage, in 2 of the 3 trials of controls the total mesophilic aerobic counts of IM figs increased slightly over 2.5 x 10^{3} CFU. g^{-1}, whereas the total mesophilic aerobic count of the other trial remained in the range of initial microbial loads. It is interesting to note that in one of the groups (trial 2) of figs rehydrated in water at 80 ^{o}C, a very high total mesophilic aerobic count was determined after 3.5 months cold storage. As given in Table 5.8, in season 2001, during 3 months cold storage of IM figs rehydrated in water at 80 ^{o}C, an increasing trend was determined in the total mesophilic aerobic count of IM figs. These increases may be due to the effect of mild heating. This is because it is reported that mild heat treatments may

activate some microbial spores (Stumbo, 1965). It is likely that a mild heating and a following cold storage activated some microbial spores and this caused an increase in the total microbial load of IM figs rehydrated in water at 80 ^{o}C. On the other hand, the detection of high microbial counts only in some parties of stored IM figs rehydrated in water at 80 ^{o}C indicates the heterogeneous distribution of the microorganism capable to grow or to restore themselves in cold stored IM figs.

Table 5.8. The effect of hot rehydration at 80 ^{o}C alone or in combination with H_2O_2 on microbial load of intermediate moisture figs brought to 30 % moisture (Season 2001).

Type of rehydration	**Total mesophilic aerobic count (CFU . g $^{-1}$)**			**Total yeast and mold count (CFU . g $^{-1}$)**			
	Storage time (months)						
	0	**1**	**3**	**0**	**1**	**3**	**3[a]**
Control / 65 min in water at 30 ^{o}C							
1	2,0 .10^4 (± 6626)[b]	3,0 .10^2 (± 61)	4,2 .10^2 (± 405)	< 13	27 (± 46)	38 (± 66)	64 (± 44)
2	2,0 .10^3 (± 296)	1,6 .10^3 (± 676)	1,7 .10^3 (± 985)	14 (± 24)	< 15	< 15	<15
16 min in water at 80 ^{o}C							
1	1,0 .10^3 (± 177)	2,2 .10^3 (± 771)	4,3 .10^3 (± 703)	44 (± 44)	< 12	< 16	< 16
2	2,0 .10^3 (± 417)	4,5 .10^2 (± 234)	1,2 .10^3 (± 614)	< 14	< 13	< 13	<13
16 min in 2.5% H_2O_2 solution at 80 ^{o}C							
1	2,1 .10^2 (± 127)	1,2 .10^2 (± 68)	7,0 .10^2 (± 203)	< 14	< 13	109 (± 71)	16 (± 27)
2	1,5 .10^2 (± 67)	3,3 .10^2 (± 93)	4,4 .10^2 (± 85)	< 15	< 13	< 14	41[c] (± 71)

[a] Instead of tartaric acid, chloramphenicol antibiotic was added to the PDA agars; [b] standard deviations of microbial counts obtained from 3 plate counts; [c] indicates the only mold count (all other values were yeast counts)

Table 5.9. The effect of hot rehydration at 80 °C alone or in combination with H_2O_2 on total mesophilic aerobic count of intermediate moisture figs brought to 30 % moisture (Season 2002).

Type of Rehydration	Total mesophilic aerobic count (CFU . g $^{-1}$)	
	Storage time (months)	
	0	3,5
Control / 51 min in water at 30 °C		
1	1,9 .10^3 (± 401)	1,4.10^3 (±581)
2	1,9 .10^3 (± 469)	2,8.10^3 (±229)
3	1,0 .10^3 (± 311)[b]	2,6.10^3 (±1635)
16 min in water at 80 °C		
1	0,5 .10^3 (± 80)	0,4.10^3 (±205)
2	0,3 .10^3 (± 101)	8,3.10^3 (± 916)
3	2,4 .10^3 (± 442)	0,4.10^3 (±255)
4 min in 2.5% H_2O_2 solution at 80 °C + 12 min in water at 80 °C		
1	1,0 .10^3 (± 289)	0,4.10^3 (±111)
2	1,0 .10^3 (± 120)	0,8.10^3 (±195)
3	1,3 .10^3 (± 289)	<8
8 min in 2.5% H_2O_2 solution at 80 °C + 8 min in water at 80 °C		
1	0,7 .10^2 (± 83)	0,7.10^3 (± 215)
2	4,4 .10^2 (± 212)	0,1.10^3 (± 73)
3	2,1 .10^3 (± 200)	0,2.10^3 (± 91)

[b] standard deviations of microbial counts obtained from 5 plate counts

The total yeast and mold counts of IM figs rehydrated in water at 30° and 80 °C were also given in Table 5.10. As seen in this table in most of the counts, the total number of yeasts and molds was higher when no sucrose was added to isolation medium. This suggests the presence of small number of osmotrophs in IM figs. The comparison of results clearly showed that the application of hot rehydration in water at 80 °C reduced the initial total number of yeasts and molds in IM figs. In controls and in two of the IM fig groups rehydrated in water at 80 °C the number of total yeasts and molds reduced or

almost unchanged by cold storage. However, in trial 3 of IM figs rehydrated in water at 80 ^{o}C the total yeast and mold count increased considerably by 5 months cold storage. In season 2001 after 3 months cold storage, slight increases in the number of yeast counts were determined in one of the control fig groups and in one of the fig groups disinfected by H_2O_2. Thus, for IM figs cold stored over 3-3.5 months there may be a risk of fungal growth.

Table 5.10. The effect of hot rehydration at 80 ^{o}C alone or in combination with H_2O_2 on total yeast and mold count of intermediate moisture figs brought to 30 % moisture (Season 2002).

Type of rehydration	**Total yeast and mold count (CFU . g $^{-1}$)**					
	Storage time (months)					
	0	**3,5**	**5**	**0[a]**	**3,5[a]**	**5[a]**
Control / 51 min in water at 30 ^{o}C						
1	333 (±115)[b]	40 (±40)	27 (± 23)	13 (± 23)	< 13	< 13
2	160 (± 69)	27 (±46)	27 (±23)	67 (±46)	27 (±23)	< 13
3	13 (± 23)	27 (±46)	< 13	< 13	27 (±46)	< 13
16 min in water at 80 ^{o}C						
1	< 13	13 (±23)	< 13	< 13	27 (±23)	< 13
2	< 13	<13	27 (±23)	< 13	13 (±23)	< 13
3	< 13	<13	4,9.10^{2} (± 266)	< 13	67 (±23)	1,1.10^{2} (± 61)
4 min in 2.5% H_2O_2 solution at 80 ^{o}C + 12 min in water at 80 ^{o}C						
1	< 13	13 (±23)	< 13	13 (± 23)	< 13	< 13
2	< 13	13 (±23)	< 13	< 13	< 13	13 (±23)
3	53 (± 61)	13 (±23)	13 (±23)	53 (± 61)	80 (± 40)	13 (±23)
8 min in 2.5% H_2O_2 solution at 80 ^{o}C + 8 min in water at 80 ^{o}C						
1	<13	27 (±46)	< 13	< 13	< 13	< 13
2	<13	< 13	< 13	13 (± 23)	< 13	13 (±23)
3	26 (± 23)	13 (±23)	13 (±23)	53 (± 61)	< 13	13 (±23)

[a] 20 % sucrose was added to medium during isolations and dilutions; [b] standard deviations of microbial counts obtained from 3 plate counts

5.9. Effect of H_2O_2 on Microbial Load

In season 2001, compared with IM figs rehydrated in water at 30 ^{o} or 80 ^{o}C, rehydration at 80 ^{o}C for 16 min in the presence of 2.5 % H_2O_2 reduced the initial total mesophilic aerobic count of IM figs almost 1 decimal (90 %). At the end of 3 months cold storage, the total mesophilic aerobic counts of disinfected IM figs increased almost 3-3.5 folds (Table 5.8). However, the counts were still below 10^{3} CFU. g^{-1}. After rehydration and at the end of 1 month storage, the total yeast and mold count of disinfected IM figs was very low, but at the end of 3 months storage, it increased slightly for the IM figs obtained in the first rehydration trial conducted in H_2O_2 solution. However, this increase was not observed clearly on the PDA plates supplemented with chloramphenicol antibiotic. For the microbiological studies carried out in 2001 season, the only mold count was obtained for one of the three plates of 3 months cold stored IM figs of second H_2O_2 rehydration trial. In literature, the effectiveness of vapor-phase H_2O_2 disinfection on fungi was reported for the dried plums (Sapers and Simmons, 1998), raisins (Simmons et al., 1997) and table grapes (Forney et al., 1991). However, because of the very low number of fungi in figs used in 2001 season, the effect of H_2O_2 disinfection and hot rehydration on these microorganisms could not have been determined clearly.

Although, rehydration at 80 ^{o}C for 16 min in 2.5% H_2O_2 solution reduced the microbial load of IM figs, as indicated before it causes blowing or foam formation in some figs during cold storage. Thus, in season 2002, we determined the effectiveness of two stage rehydration procedures conducted first in 2.5 % H_2O_2 solutions at 80 ^{o}C for 4 or 8 min and then in water at 80 ^{o}C for 12 or 8 min, respectively. As seen in Table 5.9 to reduce the initial total mesophilic aerobic load of figs, 4 or 8 min disinfection in %2.5 H_2O_2 are not more effective than hot rehydration in water at 80 ^{o}C. However, after 3.5 months cold storage, comparison of total mesophilic aerobic counts of disinfected IM figs with those of control figs and figs rehydrated in water at 80 ^{o}C, clearly showed the beneficial effect of H_2O_2 to suppress microbial growth. In both 4 and 8 min disinfected IM figs the total mesophilic aerobic counts were below 10^{3} after 3.5 months cold storage. This result showed that for 3-3.5 months cold storage there is almost no difference among the effects of 4, 8 or 16 min H_2O_2 disinfections on total mesophilic aerobic load of IM figs. It seems that disinfection with H_2O_2 gives an extra damage to microorganisms and prevents their growth under refrigerated conditions.

The effects of two stage rehydration procedures on total yeast and mold counts of IM figs were also determined. As seen in Table 5.10 the initial total yeast and mold counts of 4 and 8 min disinfected IM figs were low and they were almost unchanged during cold storage. These results proved the microbial safety of cold stored IM figs disinfected with H_2O_2.

5.10. Residual H_2O_2

After 16 min rehydration of sun-dried figs in 2.5 % H_2O_2 at 80 ^oC, considerable amount of residual H_2O_2 was determined in IM figs (Table 5.11). Almost 70 % and 99 % of the residual H_2O_2 in IM figs decomposed in 7 and 30 days, respectively. However, these long decomposition periods indicate the stability of residual H_2O_2 in IM figs. Also, because of the indicated physical defects occurred, it is not suitable to apply 16 min rehydration in 2.5 % H_2O_2 at 80 ^oC to whole figs.

Table 5.11. The amounts of residual H_2O_2 in filtered homogenates of IM figs rehydrated at different conditions.

Type of rehydration	**Residual H_2O_2 (ppm)**				
	Storage time (days)				
	0	**7**	**15**	**30**	**50**
4 min in 2.5% H_2O_2 solution at 80 ^oC + 12 min in water at 80 ^oC					
	10	1	1	< 1(nd)[a]	-
8 min in 2.5% H_2O_2 solution at 80 ^oC + 8 min in water at 80 ^oC					
	30-100	10-30	3	<1 (nd)	-
16 min in 2.5% H_2O_2 solution at 80 ^oC					
	300	100	30-100	3	< 1(nd)

[a] no residual H_2O_2 was detected by the qualitative enzymatic method (nd: not detected)

In qualitative tests to detect catalase activity in IM figs rehydrated in water at 80 ^oC, considerable amount of gas release was observed and this last continuously almost half an hour. The gas release in IM figs rehydrated in water at 30 ^oC occurred much more extensively and last almost 3 hours. This observation showed that the enzyme catalase was partially inactivated during hot rehydration. Most probably the inactivation

occurred at the outer tissues of figs that received more heat and this prevented or slowed down the decomposition of residual H_2O_2 at these locations. However, the in-situ catalase still exists in heat-treated figs and disintegration of fruit tissues may enable the contact of H_2O_2 with remaining enzyme. Thus, IM figs rehydrated in 2.5 % H_2O_2 may still be used in the production SO_2 free light colored fig purees. To see the stability of residual H_2O_2 in fig puree, following disinfection in 2,5 % H_2O_2 for 16 min, IM figs were pureed with a manual meat grinder. In pureed disinfected IM figs the residual H_2O_2 in filtered homogenates drop to 100 ppm. Also, 70 % of this residual H_2O_2 degraded when puree was stored at room temperature for 2 hours (Table 5.12). Further storage of fig puree at 4 ^oC for 3 and 22 hours, on the other hand, degraded almost 90 and 99 % of residual H_2O_2 in fig puree. Also, the total number of mesophilic aerobic counts and total yeast and mold counts of fig puree were very low.

Table 5.12. The amounts of residual H_2O_2 in filtered homogenates of fig purees obtained from IM sun-dried figs rehydrated for 16 min in 2.5 % H_2O_2 at 80 ^oC.

Time at different storage conditions after pureeing (hours)	**Residual H_2O_2 (ppm)**
Room temperature	
0	100
0.5	30-100
1	30-100
2	30
Cold storage	
3 (5)[a]	10
22 (24)	1
27 (29)	< 1
Total mesophilic aerobic count: 56 ± 60 CFU . g^{-1}	
Total yeast and mold count: $13^b \pm 23$ CFU . g^{-1}	

[a] Numbers in brackets indicate the hours elapsed after homogenization
[b] there is only mold growth

For the application of H_2O_2 disinfection in whole figs, the residual H_2O_2 was also tested in IM figs disinfected by two stage rehydration procedures. As seen in Table 5.11 two stage rehydration procedures 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 may also be achieved by the addition of very low dosages of H_2O_2 reducing chemicals such as sulfites (Özkan and Cemeroğlu, 2002) or ascorbic acid and its derivatives (Sapers and Simmons, 1998) to water used at the second stage of rehydrations. Also, the addition of $FeSO_4$ to second stage of rehydration may accelerate the decomposition of H_2O_2 in IM figs by causing Fenton reaction (see section 3.1). These results are very promising for the application of H_2O_2 to reduce the microbial load of IM figs or purees and to obtain light colored SO_2 free products.

Chapter 6

CONCLUSIONS

Sun dried figs contained considerable amount of PME activity and during cold storage this caused softening and loss of desired gummy texture of IM figs brought to 30 % moisture content. The enzyme was not heat stable but it showed activation by heating. Thus, it seems difficult to inactivate the enzyme by hot rehydrations conducted between 70 ^{o} and 90 ^{o}C. The partial inactivation of enzyme by hot rehydration at 80 ^{o}C and cold storage may only be used to delay PME catalyzed textural changes in IM figs when storage time is not longer than 3 months. For longer storage periods, partial inactivation of enzyme by hot rehydration alone has no benefits on maintaining desired textural properties.

The application of hot rehydration in water at 80 ^{o}C alone caused the activation of some microorganisms during cold storage. Thus, cold storage and hot rehydration in water may not be sufficient to obtain a shelf-life grater than 3 months. The rehydration of figs in 2.5 % H_2O_2 solution at 80 ^{o}C for 16 min reduced the microbial load of IM figs and suppressed microbial development during cold storage. Also, the light colored figs obtained by H_2O_2 disinfection were very attractive and needed no SO_2 treatment. However, this treatment left unacceptable levels of residual H_2O_2 in whole fig tissues and this causes formation of some physical defects in IM figs. The physical defects, appeared as blowing and foam formation at the eyes of some fruits, occurred due to the accumulation of O_2 gas released by in situ catalase. However, when figs are pureed the residual H_2O_2 was decomposed by the same mechanism. Thus, disinfection of sun-dried figs in 2.5 % H_2O_2 at 80 ^{o}C for 16 min can be suggested for the production of SO_2 free light colored fig purees.

By applying rehydrations first in 2.5 % H_2O_2 solutions at 80 ^{o}C for 4 or 8 min and then in water at 80 ^{o}C for 12 or 8 min, respectively, it is possible to reduce the residual amount of H_2O_2 in IM figs considerably. This eliminated the physical defects occurred in IM figs. Also, in these procedures further reduction of residual level of H_2O_2 may also be achieved by the addition of very low dosages of H_2O_2 reducing chemicals at the second stage of rehydrations. The two stage disinfections did not reduce the initial

microbial load of IM figs considerably. However, they effectively suppressed microbial development in IM figs during cold storage. The figs obtained by two stage rehydrations were also light colored and needed no SO_2 treatment.

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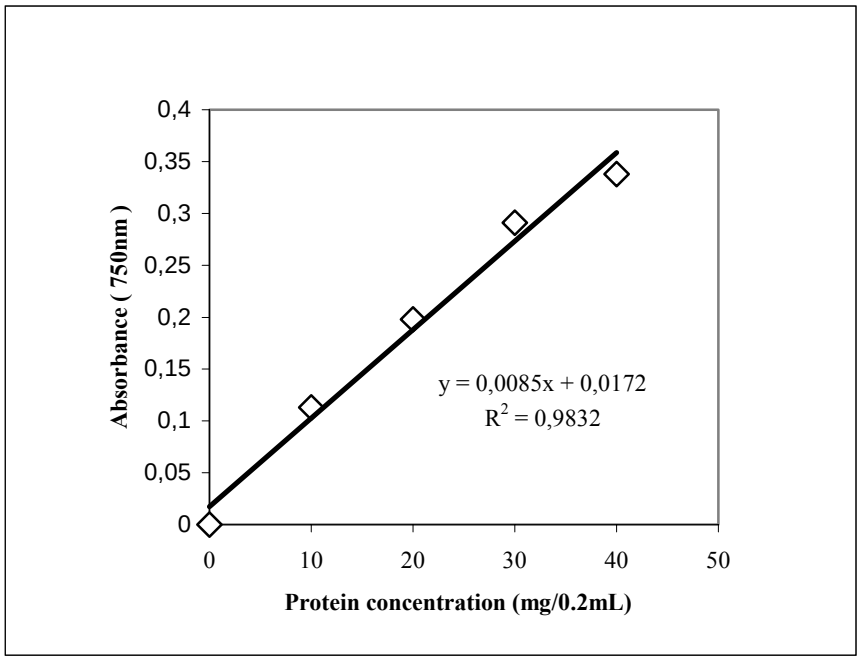
APPENDIX

A1. Standard curve for protein determination



A2. A photograph of IM figs rehydrated at 30 ^{o}C for 65 min and cold stored for 40 days

A3. A photograph of IM figs rehydrated at 80 ^{o}C for 16 min and cold stored for 40 days

A4. A photograph of IM figs rehydrated at 30 ^{o}C for 51 min and cold stored for 3.5 months

A5. A photograph of IM figs rehydrated at 80 ^{o}C for 16 min and cold stored for 3.5 months

A6. A photograph of IM figs rehydrated at 80 ^{o}C for 16 min in 2.5 % H_2O_2 solution and cold stored for 40 days

A7. A photograph of IM figs rehydrated first in 2.5 % H_2O_2 solution at 80 ^{o}C for 4 min and then in water at 80 ^{o}C for 12 min and cold stored for 3.5 months

A8. A photograph of IM figs rehydrated first in 2.5 % H_2O_2 solution at 80 ^oC for 8 min and then in water at 80 ^oC for 8 min and cold stored for 3.5 months

A9. A photograph of IM figs rehydrated first in 2.5 % H_2O_2 solution at 80 ^oC for 4 min and then in water at 80 ^oC for 12 min and cold stored for 5 months

A10. A photograph of IM figs rehydrated first in 2.5 % H_2O_2 solution at 80 ^oC for 8 min and then in water at 80 ^oC for 8 min and cold stored for 5 months.