RETARDATION OF BROWNING AND SOFTENING OF THERMALLY PROCESSED PEARS PACKED IN RETORTABLE POUCHES

by

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Abstract of the Thesis

Retardation of Browning and Softening of Thermally Processed Pears

Packed in Retortable Pouches

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Thesis Director:
Dr. Mukund V. Karwe

Wet pack fruit rations have been included in the U.S. Army Meals Ready-to-Eat

(MRE) program menus since 1992, when they replaced freeze-dried fruits. These

rations, designed for soldiers in the field, have high acceptability, are very convenient to

carry, and provide important nutrients. The required shelf life for MRE wet pack fruit

rations is 36 months at 26.7ºC or 6 months at 37.7ºC. However many of the wet pack

fruits, especially pears in syrup, turn brown and mushy during storage before the

required shelf life, and get rejected by the soldiers.

Preliminary experiments had suggested that the oxygen left in the headspace of

the pouches after vacuum packaging had a significant role in the browning, and that

softening was due to canned pears being used instead of fresh pears as starting material. Therefore, our objective was to develop strategies to slow down the browning of MRE pears by reducing the available oxygen, and to find a year-long available fresh pear that could be used as starting material to avoid double processing.

Accelerated storage studies were carried out at 48.8ºC for 45 days, and samples were withdrawn periodically to measure headspace volume and composition, color, ascorbic acid concentration and hardness of the pears. Sensory evaluation by a trained panel was done at the U.S. Army Natick Soldier Research, Development and Engineering Center.

The control formulation showed a significant consumption of oxygen in the headspace, degradation of ascorbic acid in the product, and formation of carbon dioxide during storage. Based on these findings we think that browning is mainly due to ascorbic acid degradation. Minimizing the residual headspace by pulling vacuum for a longer time during packing and doing agitated retorting reduced ascorbic acid degradation by approximately 87% after thermal processing and decreased browning by approximately 34% after 30 days of storage. Oxygen scavenger films were also used to reduce the oxygen in the headspace, but the results were inconsistent. Using fresh D'Anjou pears instead of canned Bartlett pears was enough to increase the hardness of the MRE rations to acceptable levels according to the sensory analysis.

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## 1.1	Pears

Pears (*Pyrus communis)*, along with apples, are among the oldest of the world's fruit crops (Jackson, 2003). The earliest authentic record of pears being cultivated in the United States is from the year 1630 at Salem, Massachusetts (Childers, 1983). The main pear producing countries are China, Italy, and USA (Jackson, 2003). Farming of pears is concentrated in the temperate regions of the world because the tree requires a certain amount of hours below 7ºC during the day to bear fruit (Salunkhe and Kadam, 1995). In the United States the leading region in pear production is Washington State, which produces more than 35% of the pears (Jackson, 2003); California and Oregon State are other major producers. Annual pear production in the United States has fluctuated between 700,000 and 1,000,000 MT during the last ten years, as shown in **Table 1.1**. Out of the total pears consumed in the United States, approximately 60% are consumed fresh and 40% are consumed processed (as canned fruit, juice, puree, etc), as shown in **Table 1.2**.

Table 1.1: Pear Production in the US.

Year	Production (MT)
2000	877,380
2001	912,460
2002	787,840
2003	846,630
2004	823,760
2005	748,720
2006	757,780
2007	791,930
2008	790,020
2009	848,490

Source: Crop Production. National Agricultural Statistic Service.

Table 1.2: Pear utilization in the US.

Year	Consumption as fresh (MT)	Consumed as Processed (MT)	Total (MT)
2000	520,490	365,050	885,550
2001	516,030	382,370	898,400
2002	476,190	330,630	806,820
2003	508,430	334,600	843,030
2004	466,960	326,090	793,050
2005	458,000	288,080	746,080
2006	454,650	300,000	754,660
2007	501,180	290,460	791,640

Source: Economic, Statistics and Market Information System – USDA.

1.1.1 Pear Cultivars

The leading cultivar in the United States, both for fresh consumption and canning, is Bartlett, which has accounted for over 80% of the commercial pear production (Childers, 1983). The fruits are medium-large, yellow blushed with brownish-red spots (Jackson, 2003). They are harvested from mid August to early September and have a shelf life of 3 months when stored between -1.1ºC and -0.56ºC (30ºF and 31ºF) (Childers, 1983).

Another cultivar of importance is D'Anjou, which is the main winter pear in the Pacific Northwest of the United States (Jackson, 2003). Its harvesting season starts in late August and goes through late September, and the pears have a shelf life of 7 months when stored between -1.1ºC and -0.56ºC (30ºF and 31ºF) (Childers, 1983). Other pear cultivars of importance in the world are Hardy, Bosc, Comice, Conference, Packham and Coscia (Jackson, 2003). **Figure 1.1** shows the main pear cultivars in the United States.

a)   b)

c)   d)
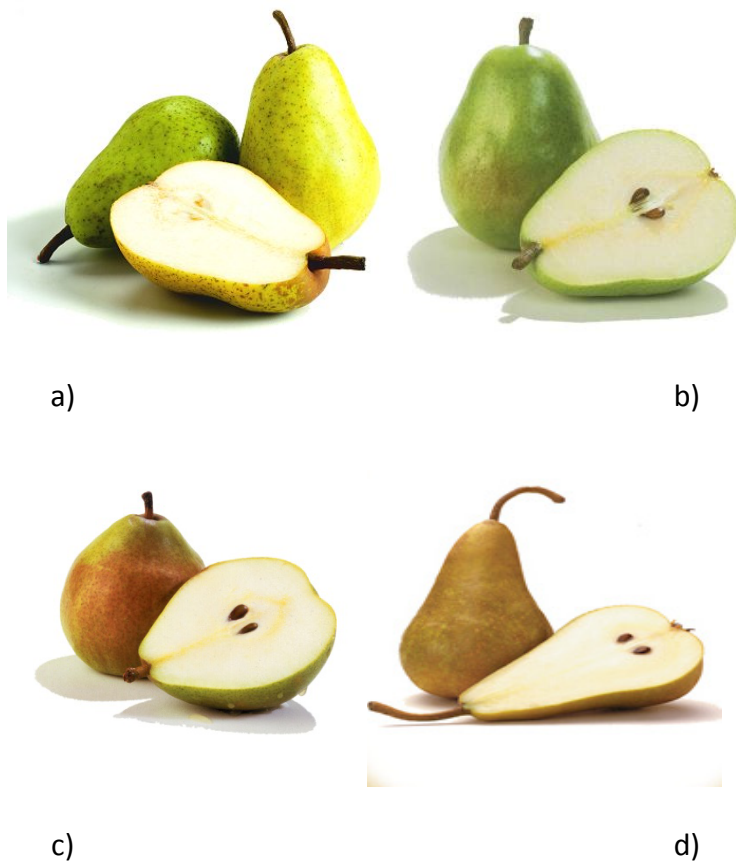
Figure 1.1: Major pear cultivars in the United States

a) Bartlett Pear , b) D'Anjou Pear, c) Comice Pear, d) Bosc Pear.

(Bartlett pear image from: http://www.worldwidegourmet.com,

other images from: http://trade.usapears.com)

1.1.2 Composition of pears

Table 1.3 shows the main constituents and nutrients found in most varieties of pears. Pears are considered a good source of fiber and potassium (Salunkhe and Kadam, 1995; TanrІöven and Eksi, 2005; Schieber et al., 2001). The sugars in pears are primarily fructose and glucose; sucrose is also present but in a very small amount. The main polyphenol in pears is chlorogenic acid.

Table 1.3: Composition of pears (no cultivar specified)

Constituent	Content
Water	83.71%
Protein	0.38%
Fat	0.12%
Carbohydrates (by difference)	15.46%
Fiber	3.1%
Sugars	9.8%
Fructose	6.23%
Glucose	2.76%
Sucrose	0.78%
Potassium	1,190 ppm
Phosphorus	110 ppm
Magnesium	70 ppm
Calcium	90 ppm
Copper	0.82 ppm
Ascorbic acid	42 ppm
Vitamin K	45 ppb

Source: USDA National Nutrient Database for Standard Reference, 2009.

1.2 Meals Ready-to-Eat (MRE)

MRE are the first type of rations used for feeding deployed troops, before field kitchens are setup. Their use started in the early 1980s and since then they have been continuously modified to improve the quality and give variety to the menu. After Operation Desert Storm, it was suggested that taking into account the U.S. Army soldiers' preferences would increase their consumption and assure the nourishment of the troops, so feedback from the soldiers has been used to replace components from the rations and improve the quality. **Figure 1.2** shows different types and presentations of MRE rations, which include cold and warm entrees, snacks, and desserts.

Figure 1.2: Different MRE army rations (credit: http://www.mreinfo.com)

1.2.1 Packaging

According to the military performance specification MIL-F-44067D, MRE rations should be packed in individual flexible pouches of 148 mL (5 oz) or 237 mL (8 oz) of capacity. The pouches should be formed using a multilayer material that should have an

oxygen transmission rate of no more than 0.06 mL/m^2/day/atm and water vapor

transmission rate of no more than 0.01 g/m^2/day (temperature unspecified). For fruit

rations, the headspace after packaging should not be higher than 10 mL of air. The

pouch should be able to withstand the thermal processing required by the product

without delaminating or losing the integrity of the seals. Fruit rations are pasteurized at

maximum external temperatures of around 100ºC.

   In general, retortable pouches are made of polyester film, aluminum foil and

polypropylene, which allow the pouches to be processed to temperatures up to 135ºC

(Downing, 1996). A typical configuration is an outside layer of polyethylene terephtalate

(PET), middle layers of aluminum foil, biaxially oriented nylon, and an internal layer of

polypropylene (Holdsworth, 2007). According to the military specifications MIL-F-

44067D, a multilayer material made with a layer 0.076 – 0.1 mm thick of polyolefin on

the external side, a layer 0.009 – 0.017 mm thick of biaxially oriented polyamide type 6,

a layer 0.015 mm thick of aluminum foil and an internal layer 0.012 mm thick of

oriented polypropylene would comply with the requirements for MRE rations.

Polypropylene is used for its tolerance to thermal processing and heat sealability

properties. Aluminum foil, being a metal, gives the high barrier properties to the film.

Polyamide gives high puncture resistance to the film and is also heat resistant (Lee et al.,

2008). Polyolefin is used for its low surface energy, which allows the pouch to be water

impermeable.

1.2.2 Thermal processing

Although in general terms thermal processing of flexible pouches is no different than thermal processing of metallic containers, some additional considerations have to be given. The pressure in the headspace of the pouches will increase due to heating, which has to be compensated with the pressure in the heating medium so the integrity of the seal is not compromised. The highest pressure differential is at the start of the cooling cycle, so pressurized air sometimes has to be used to maintain a high pressure outside the pouch (Downing, 1996). Due to the high surface area and short characteristic dimension, heat transfer to the coldest point is faster in flexible containers than in cans. Similar to metallic containers, agitation can accelerate heat penetration by enhancing convection heat transfer; however, the pouches need to be properly confined to avoid damage due to the seal loosing strength at high temperature. Downing (1996) points out that at 121ºC the seal can lose about 75% of its strength compared to ambient temperature.

1.3 Browning Reactions

Brown pigments are the end product of polymerization reactions that start with different substrates. Based on the composition of fruits and processing conditions, the possible reactions would be enzymatic and phenolic browning, ascorbic acid browning, and Maillard browning. Caramelization could not take place even though the reactants (sucrose and monosaccharides) are present because it requires high temperatures, around 200ºC (deMan, 1999), which are never reached during thermal processing.

1.3.1 Enzymatic and phenolic browning

Enzymatic browning is the rapid darkening of tissues (mainly fruits, vegetables and mushrooms) when exposed to the air after being damaged, due to the conversion of phenolic compounds to brown melanins (Eskin, 1990). This reaction in fresh fruits is catalyzed by the enzyme polyphenol oxidase (PPO), also known as phenoloxidase, phenolase, monophenol oxidase, diphenol oxidase and tyrosinase (Marshal et al., 2000). Several isozymes have been identified in fruit samples, each one exhibiting specificity to different phenolic substrates (Eskin, 1990). These enzymes have a type 3 binuclear copper active site (Damodaran et al., 2007), which is crucial for the activity of the enzyme. Several methods have been identified to inhibit PPO activity, heat denaturation being the most suitable for processed fruit. Temperatures between 70ºC and 90ºC generally give complete inactivation of PPO enzyme (Marshal et al., 2000).

PPO catalyzes two reactions: 1) hydroxylation of monophenols to o-diphenols, and 2) oxidations of o-diphenols to o-benzoquinones (**Figure 1.3**). Quinone formation is both enzyme and oxygen dependant; the subsequent reactions occur spontaneously and no longer depend on these two factors (Eskin, 1990). Quinones can also be formed without the aid of enzymes (Schüsler-Van Hees et al., 1985), but at much lower rates. Cilliers and Singleton (1989) studied the autoxidation of caffeic acid and found the reaction to be of first order with a rate highly dependent on pH and temperature (16.5 times faster at 35ºC compared to 5ºC at pH 5, and 44.5 times faster at pH 8 compared to pH 4 at 20ºC). Cornwell and Wrolstad (1981) studied the browning of pear juice

concentrate (water activity of 0.73) and found that autoxidation of phenols had no

effect on the formation of pigments.
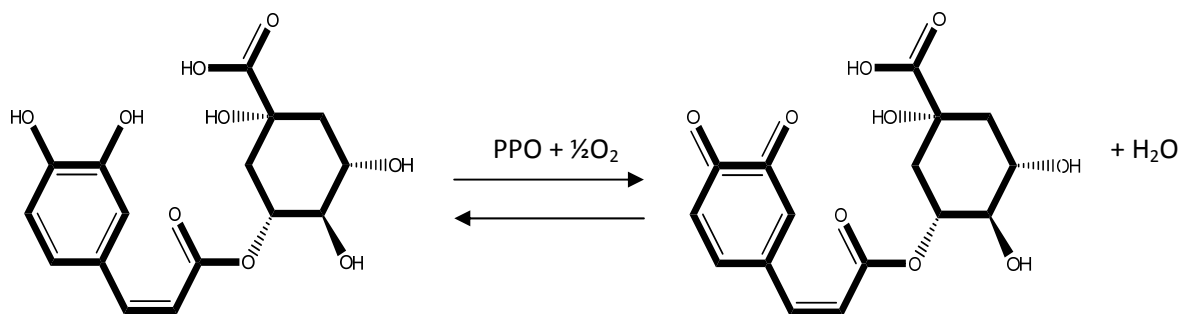
Figure 1.3: Oxidation reaction of chlorogenic acid, an o-diphenol (left), to

chlorogenic acid quinone (right).

Several methods are available for inactivating PPO enzymes. In processed

products, such as MRE rations, heat would inactivate the enzyme, as the required

temperatures are met during processing. According to Halim and Montgomery (1978),

inactivation of PPO in D'Anjou pears follows a first order kinetics, and 50% of the activity

is lost after 1.1 minutes of processing at 85ºC. Inhibition through additives is also a

common practice, ascorbic acid being the most commonly used. Ascorbic acid reverts

the initial reaction by reducing the o-benzoquinones back to monophenols, as shown in

Figure 1.4 (Eskin, 1990). A second inhibition mechanism by ascorbic acid and ascorbate

is the reduction of the copper ion in the PPO enzyme, from Cu^{2+} and Cu^{+} (Hsu *et al.*,

1988).
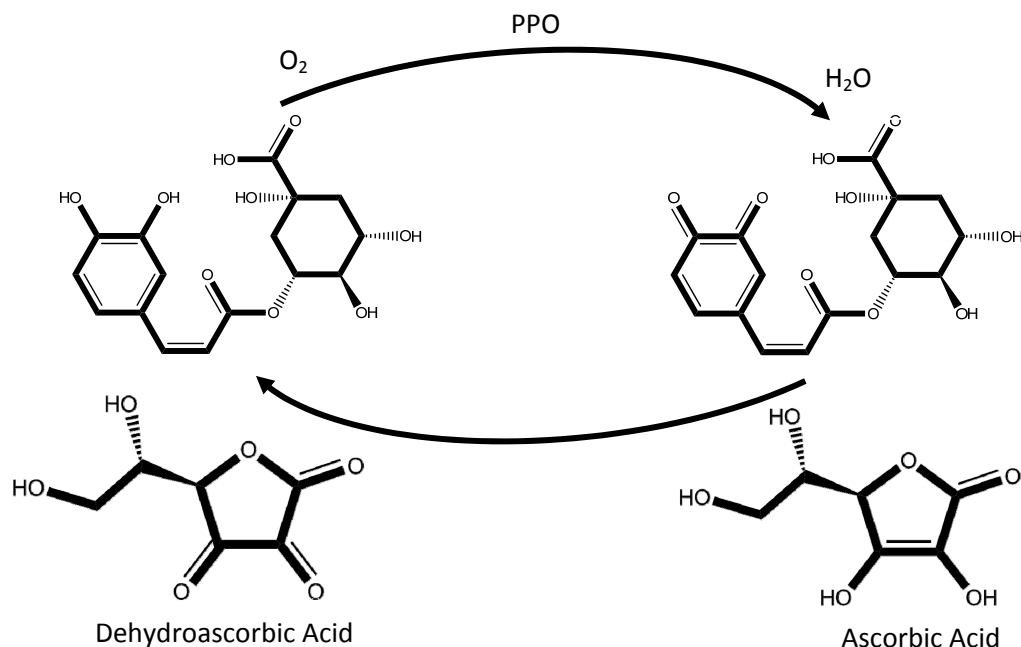
Figure 1.4: Inhibition mechanism of enzymatic browning by ascorbic acid.

Ascorbic acid oxidizes to dehydroascorbic acid, reducing quinones back to phenols

(adapted from Eskin, 1990).

Peroxidase is another enzyme that could cause browning. Its mechanism is similar to PPO, but instead of reducing dissolved oxygen it uses hydrogen peroxide or other organic peroxides as oxidizing agent. Flavonoids and other phenols can serve as substrates (Eskin, 1990). Peroxidases are considered to be more heat stable than PPO (**Figure 1.5**) and can partially recover their activity after thermal processing (Gibriel et al., 1978; Moulding et al., 1989; McLellan and Robinson, 1981). Although fruits normally have low levels of hydrogen peroxide (Richard-Forget and Gauillard, 1997), which would limit the activity of peroxidase, it can be produced from other reactions, like ascorbic acid oxidation.
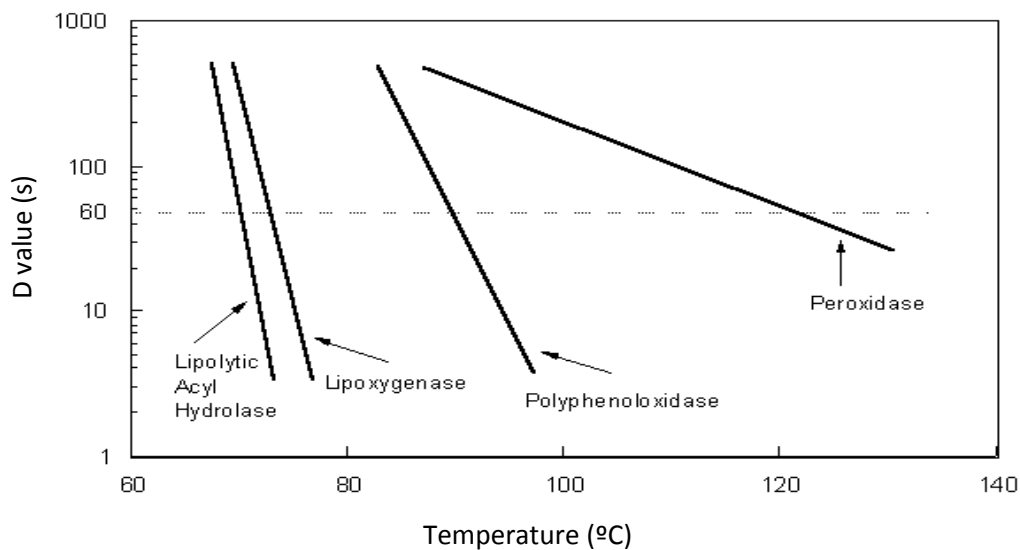
Figure 1.5: Thermal inactivation of different enzymes from potato

(Svenson, 1977)

1.3.2 Maillard browning

Also referred as non enzymatic browning, it is the reaction involving compounds with amino groups and carbonyl groups (Eskin, 1990). In the case of pears, the carbonyl groups are provided by reducing sugars, mainly fructose and glucose. Pilipenko et al. (1999) studied Bere pears and found that the most abundant free amino acids were asparagine (0.056%), phenylalanine (0.024%), isoleucine (0.017%), and lysine (0.015%). Lysine is considered the most reactive amino acid (deMan, 1999).

The first step of the Maillard browning reaction is the reversible formation of N-substituted glycosylamine by condensation of the carbonyl from a reducing sugar with the amine group of an amino acid; the condensed molecule, known as Schiff's base, undergoes a cyclation in the sugar fraction to form the N-substituted glycosylamine. This step requires that the carbonyl group be available for reaction, which doesn't happen under furanose or pyranose form, and is favored by a pH above the isoelectric point of the amino acid (Eskin, 1990). The second step of the reaction is called Amadori rearrangement if the N-substituted glycosylamine was formed from an aldose or Heyns rearrangement if it was formed from a ketose. In Amadori rearrangement, the N-substituted glycosylamine goes through an internal rearrangement by changing the sugar fraction to a ketose. In Heyns rearrangement, the sugar fraction goes from ketose to aldose with the amino group in carbon 2. The Amadori compound is very stable and it was found to reach a maximum value of concentration in processed apricots before brown color developed. After the Amadori compound is formed the reaction can take different pathways depending on the reactants and the environment conditions (Eskin, 1990; deMan, 1999).

The factors that affect Maillard reaction kinetics and pathways are:

a) Temperature: Maillard reaction is extremely sensitive to temperature changes, its Q_{10} value is around 3 to 4 (Taub, 1998).

b) pH: A basic pH usually favors the reaction as it puts amino acids in basic form and increases the amount of reducing sugars in open chain (Eskin, 1990). An exception to

this is high sucrose systems (or other disaccharides or polysaccharides), in which a lower pH promotes hydrolysis of non reducing sugars to glucose and fructose (both reducing sugars), therefore favoring the reaction (Eskin, 1990). Raisi and Aroujalian (2007) reported a slight increase in browning in corn syrup 42DE when stored at pH 4 compared to pH 5.

c) Moisture content and water activity: Studies have shown that the reaction is fastest at water activity levels around 0.6 to 0.7 (Eskin, 1990). This is probably because several steps involve dehydration reactions, which is favored by dryer environments, but conditions drier than the optimum would affect molecule mobility. After the Amadori or Heyns rearrangement, the formed compound may either loose three water molecules to form a Schiff base of furfural or hydroxymethylfurfural (HMF) or lose two water molecules and form reductones; additionally it may just split and form fission products (acetol, diacetyl, pyruvatealdehyde, etc.) (deMan, 1999).

d) Metals: The formation of metal complexes between the amino acids can influence the reaction (Eskin, 1990); some ions accelerate the reaction while others inhibit it. Bohart and Carson (1955) found that manganese (added as manganese chloride) under very little concentration could greatly decrease the formation of brown pigments in a glucose-glycine system in air or oxygen, but found no effect under nitrogen. Iron (as ferric chloride), on the other hand, was found to increase the color development by four or five times when added at levels of 2 ppm under oxygen conditions. Markuze (1963) also studied the effects of metals on Maillard reaction and concluded that iron and cupper in metallic and ionic form increased the reaction

rates, manganese and tin in ionic form acted as inhibitors, and tin in metallic form had no effect.

e) Other conditions: Environment gases and light have also been reported as affecting Maillard browning, but not much research has been published. Bohart and Carson (1955) reported that nitrogen flushed sealed samples of glucose-glycine developed two to three times as much color as samples sealed with air. They also found that light accelerated the reaction under absence of oxygen, but it bleached partially browned samples at ambient temperature when oxygen was available; this last observation was also made by Markuze (1963). Raisi and Aroujalian (2007) obtained contradictory results, they found that increasing oxygen concentration (from 0 to 21%) slightly increased the reaction rate in corn syrup 42DE regardless of pH and temperature, but they also found a higher reaction rate at 100% N_2 atmosphere compared to vacuum packing.

1.3.3   Ascorbic acid browning

It is a very important browning mechanism for processed fruits, along with Maillard browning (Adams and Brown, 2007). Substantial research on browning of thermally processed fruits and fruit products has been published in the last 50 years, and much of it points at ascorbic acid degradation as the origin of browning (Wong and Stanton, 1989; Kacem et al., 1987a and 1987b; Shinoda et al., 2004). However, some also points at Maillard reaction (Cornwell and Wrolstad, 1981; Ibarz et al., 1999; Rattanathanalerk et al., 2005); it is important to point out that these experiments were

done either on low water activity samples (Cornwell and Wrolstad, 1981) or with

samples being processed at high temperatures (55ºC – 95ºC) and long times (80 min –

500 min) (Ibarz et al., 1999; Rattanathanalerk et al., 2005).

Two pathways are distinguished for ascorbic acid degradation: aerobic

degradation and anaerobic degradation; the later approximately 10 times slower than

the first (Eskin, 1999). The rate of ascorbic acid browning is inversely proportional to pH

in the range of 2 to 3.5 (Braverman, 1963), however Taqui Khan and Martell (1967a)

found that the rate of uncatalyzed ascorbic acid oxidation increased with pH in the

range of 2 to 6, and concluded that only the monoionic form is susceptible to oxidation.

In later experiments, Buettner (1988) demonstrated that the monoionic form does not

autoxidize without the presence of metals. A scheme of the basic initial steps of the

aerobic degradation reaction is shown in **Figure 1.6**. Besides xylosone and carbon

dioxide, diketo gulonic acid can also degrade into oxalic acid and other carboxylic acids,

or into furfural, and hydroxyfurfural (Eskin, 1990; Shinoda et al., 2004). Ranganna and

Setty (1974) showed that carbon dioxide was the main by-product of ascorbic acid

degradation in aqueous medium.
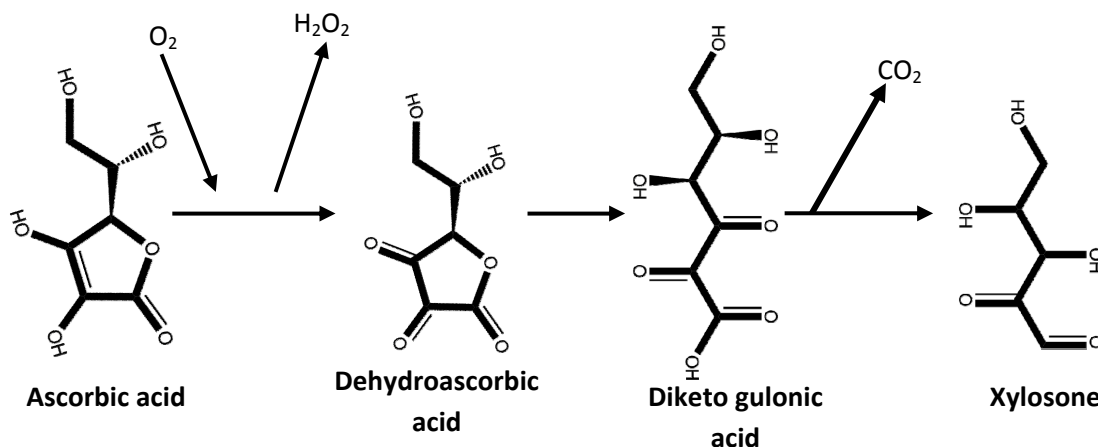
Figure 1.6: Aerobic degradation of ascorbic acid (adapted from Damodaran, 2008)

The initial oxidation to dehydroascorbic acid is easily reversible by reducing agents, so most of the dehydroascorbic acid is still considered to have vitamin C activity; however the opening of the lactone ring to form 2,3 diketo gulonic acid is irreversible, and once it happens vitamin C activity is lost (Damodaran, 2008). This initial oxidation step is catalyzed by the presence of metal ions and, to a lesser degree, by metal chelates. Taqui Khan and Martell (1967a and 1967b) studied the effect of cupric and ferric ions and chelates, and found that both Cu^{2+} and Fe^{3+} significantly increase the reaction rate (5 times faster with ferric ion and 10 times faster with cupric ion). They also concluded that ferric ion has more effect on the fully protonated form and that cupric ion has more effect on the monoionic form. Regarding the ferric and cupric chelates, these had negligible effects at pH 2.25 – 3.45 but had a significant effect at higher pH, indicating that chelated metals can oxidize the monionic form of ascorbic acid. Shinoda et al. (2004) also found that metals promoted browning in model orange juice systems.

An important by-product from the oxidation of ascorbic acid is hydrogen peroxide, as it could be used by peroxidase enzyme to oxidize phenols, which would then be reduced by ascorbic acid, therefore producing more dehydroascorbic acid. As mentioned before, peroxidase has been shown to survive thermal processing and even recover activity during storage.

Amino acids have also been shown to have an important effect on the degradation of ascorbic acid. Kacem et al. (1987a and 1987b) concluded that increasing levels of aspartic acid and arginine promoted the degradation of ascorbic acid and browning of orange juice (pH 3.8). Ranganna and Setty (1968) worked with dried cabbage and showed that the browning came from oxidized ascorbic acid (either dehydroascorbic acid or diketo gulonic acid) reacting with amino acids following Strecker degradation reaction. This reaction was found only at high pH (above 3.5, optimum at 5.3 to 6.8) and low moisture conditions (both of which favor Maillard reaction). Citric acid has been shown to promote the formation of brown pigments from ascorbic acid (Clegg, 1966), but the mechanism was not elucidated. Shinoda et al. (2004) also found that citric acid promoted ascorbic acid browning in model orange juice systems.

The anaerobic degradation pathway for ascorbic acid has only been partially described, and is only of importance once the oxygen has been fully consumed (Eskin, 1990; Damodaran, 2008). It has been shown to be pH dependant, with maximum rate at pH 3 to 4, and also to be promoted by fructose (Huelin, 1953). Huelin et al. (1971) found out that carbon dioxide and furfural were by-products of anaerobic degradation of ascorbic acid, and that the yield of carbon dioxide to ascorbic acid was almost 1:1 on a molar basis regardless of the pH (on a range from 2 to 6).

1.4   Texture Loss

Softening of fruit tissues is due to loss of cellular integrity, which takes place mainly during thermal processing above 55ºC (Rosenthal, 1999). This loss of cellular integrity comes from cellular breakdown, caused by disruption of the cell membrane and structural changes in the cell wall, as well as cellular separation due to breakdown and solubilization of the pectin in the middle lamellae, which is the structure that maintains the cells together. High methoxyl pectin is less water soluble than low methoxyl pectin; so ripened fruits, in which pectin is mostly demethylated due to ripening, typically soften more after thermal processing than less ripened fruits, unless the demethylated sites are used to cross-link the pectin chains with calcium and increase the rigidity of the lamellae. **Figure 1.7** shows the schematic of pectin chains cross-linked with calcium molecules.
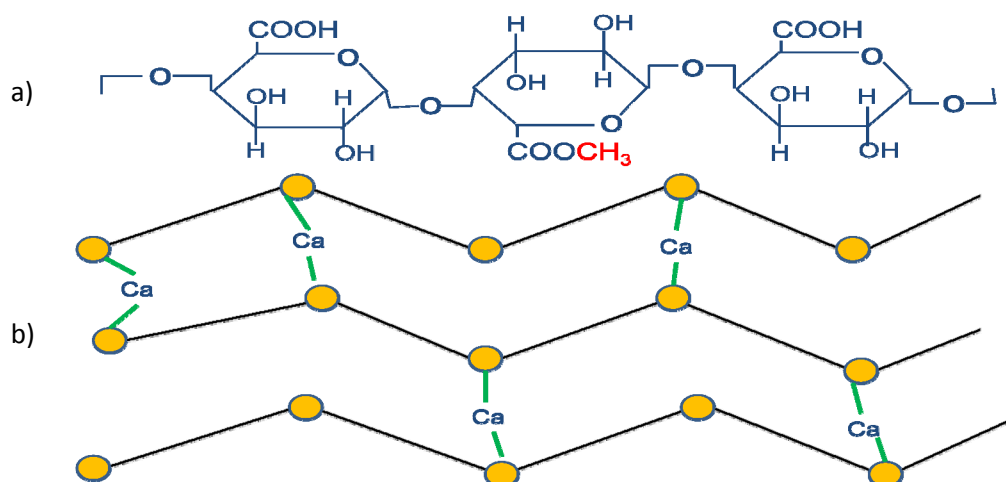
Figure 1.7: a) Pectin structure.

b) Pectin chains cross-linked by calcium ions.

1.5 Rationale

This study aims at increasing the shelf life of MRE rations of pears in syrup. Pear

rations are highly appreciated by the soldiers in the field and are an important source of

nutrients but get rejected if they are too degraded, which happens even within the

required shelf life of 3 years at 26.7ºC. **Figure 1.8** shows the MRE pears at the beginning

and at the end of their shelf life.

a)   b)

Figure 1.8: a) MRE pears immediately after processing,

b) MRE pears at the end of their shelf life of 3 years at 26.7ºC.

Canned fruits are generally considered to be acceptable for consumption within

3 years of manufacturing. Therefore, one or more degradation process taking place in

pears packed in MRE pouches have to be inhibited in pears packed in cans.

Understanding the color and texture degradation mechanisms taking place in MRE pear

rations and which one should be inhibited to maintain the product acceptable

throughout its shelf life would allow the findings to be useful for other MRE wet pack

fruit products, which may also face the same problems.

1.6   Problem Statement

MRE rations of pears in syrup do not comply with the shelf life of 3 years at 26.7ºC required by the U.S. Army. This shelf life is necessary due to the unpredictable nature of the Army operations, which require stockpiles of military rations, and the harsh conditions of the war theatres in the Middle East, which significantly shorten the shelf life of the rations. It is necessary to find a way to delay the color and texture degradation processes in MRE pear rations so they comply with the required shelf life and remain acceptable for the soldiers.

1.7   Objectives

- To develop a strategy to slow down the browning reactions in MRE pear rations as compared to product manufactured according to the current industry procedures.

- To decrease the softening of the MRE pears in syrup rations as compared to product manufactured according to the current industry procedures.

2.   Materials and Methods

2.1   Materials

2.1.1   Diced pears

Two types of diced pears were used for this study:

a) <u>Fresh D'Anjou pears:</u>

Provided by Truitt Bros Inc., a fruit processor from Salem, Oregon, and used for large-scale experiments which were carried out at their facility. The whole pears were stored at refrigeration and controlled atmosphere and diced on the day of processing. Their parameters measured on the day of processing were:

- Hardness: 3.4 +/- 0.2 kg (7.5 +/- 0.5 Lb)

- Brix: 13 – 13.2º

- Ascorbic acid content: 75 +/- 25 ppm

- Moisture: 84.16% (w.b.).

b) <u>Canned diced Bartlett pears:</u>

Manufactured by Del Monte (Modesto, CA) and used for small-scale experiments carried out at Rutgers Food Manufacturing Technology (FMT) in Piscataway, NJ. The diced pears were canned in a size 10 tin can with light corn syrup. Their parameters were:

- Hardness: 69 +/- 13 g

- Brix: 10 – 14º

- Ascorbic acid content: 50 ppm

- Moisture: 78% (w.b.).

2.1.2 Pouches

Two types of pouches were used to pack MRE pears:

- Regular MRE pouches

- Oxygen scavenger pouches.

Both pouches had a bottom preformable layer and a top flat layer, and were assembled using the horizontal form fill sealers. The oxygen scavenger pouches only had the top layer made from oxygen scavenging film, since no preformable oxygen scavenging film was available for this project; the bottom layer was made from regular MRE film. The regular MRE film used was Flexalcon®, manufactured by Alcan Packaging Singen GmbH (Germany); the oxygen scavenger film was Ageless OMAC ®, manufactured by Mitsubishi Gas Chemical Company Inc. (Tokyo, Japan). **Figure 2.1** and **Figure 2.2** show the structure of the regular MRE films; no detailed description of the structure of the oxygen scavenger film was available.
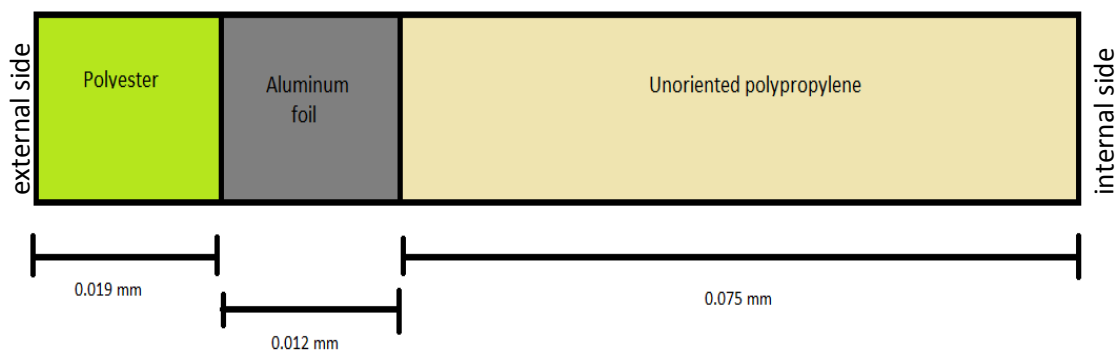
Figure 2.1: Structure of the regular MRE film (Flexacon®)

for the top layer of the pouches
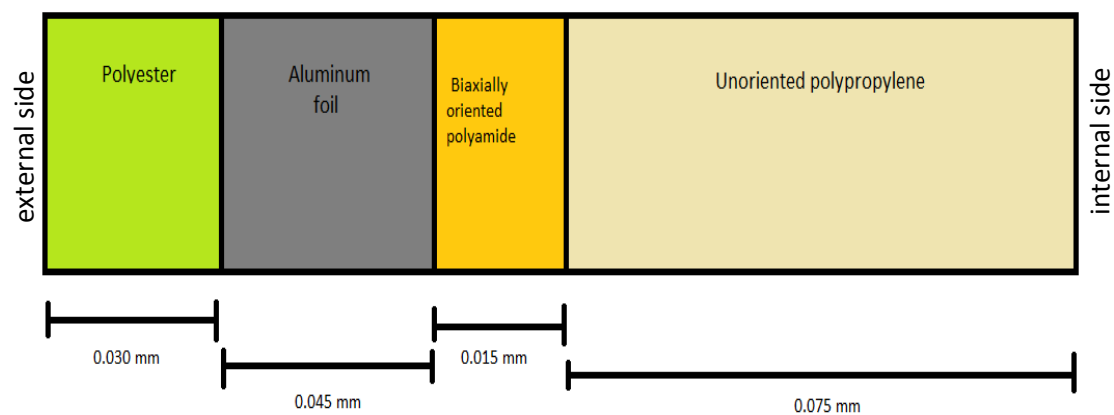
Figure 2.2: Structure of the regular MRE film (Flexacon®)

for the bottom layer of the pouches

The specifications of the oxygen scavenger film provided by the manufacturer (Mitsubishi Gas Chemical Company Inc.) are:

- Oxygen absorbing layer: Polypropylene with embedded iron particles

- Sealant layer: Polypropylene

- Oxygen absorption performance during retorting (121.1ºC): 266 mL/m^2/hr at 100% relative humidity

- Minimum water activity required for the product: 0.85.

The regular MRE film complied with the military specification MIL-F-44067D that limits the oxygen transmission rate to 0.06 mL/m^2/24 hrs/atm. Based on the pouch size and ambient conditions, this rate would be 0.16 mL of oxygen per year.

2.1.3   Reagents and supplies

a) EM Quant® ascorbic acid test spatulas (EMD Chemicals, NJ), for ascorbic acid measurements.

b) 2,6 dichlorophenol indophenol (Acros Organics, NJ), for ascorbic acid measurement

c) Metaphosphoric acid (Acros Organics, NJ), for ascorbic acid measurement

d) Acetic acid glatial (Fisher Scientific, NJ), for ascorbic acid measurement

e) L-ascorbic acid (Fisher Scientific, NJ), for ascorbic acid measurement

f) 2-N-Morpholinolethanesulfonic acid (Acros Organics, NJ), for peroxidase assay

g) Hydrogen peroxide at 30% (Fisher Scientific, NJ), for peroxidase assay

h) o-Phenylene-diamine (Acros Organics, NJ), for peroxidase assay

i) Sodium phosphate monobasic (EMD Chemicals, NJ), for peroxidase assay

j) Sodium fluoride (Fisher Scientific, NJ), for peroxidase assay

k) Chelex 100 (Bio-Rad Laboratories, CA), for peroxidase assay

l) Ascorbate oxidase spatulas (Roche Applied Science, IN), for peroxidase assay.

2.1.4   Equipment and Instruments

a) CFS Tiromat (Frisco, TX) Tiromat 300 horizontal form fill sealer

b) Stock America (Grafton, WI) Rotomat batch retort

c) Wagner Instruments (Greenwich, CT) fruit penetrometer with a 0.5'' (12.7 mm) diameter probe

d) Reichert Anaytical Instruments (Depew, NY) r^2mini handheld digital refractometer

e) Nova Analytics Corp. (Woburn, MA) Scholar 425 pH meter

f) Mocon (Minneapolis, MN) PalCheck 325 portable gas analyzer

g) Konica Minolta (Ramsey, NJ) CR-400 colorimeter

h) Kinematica AG (Lucerne, Switzerland) Polytron PT 1600 E high shear homogenizer

i) International Equipment Co. (Needham, MA) clinical centrifuge model CL

j) Biotek (Winooski, VT) Synergy-HT UV-Visible/Fluorescence microplate reader

k) Texture Technologies (Scarsdale, NY) TA-XT2i texture analyzer, with a 2 mm diameter probe and a 25 kg cell.

2.2   Procedures

2.2.1   Sample preparation

Samples were prepared using fresh D'Anjou pears and canned Bartlett pears so when 36 g of syrup was mixed with 107 g of diced pears in the pouches the final product would equilibrate and comply with the MIL-F-44067D Military Specification for Pears in MRE rations:

- Minimum total weight: 127.57 g (4.5 oz)

- Minimum drained weight: 99.22 g (3.5 oz)

- Brix: 18 – 22º

- pH: 3.85 – 4.15

- Ascorbic acid: 200 – 800 ppm.

For samples made with canned Bartlett pears, these were first drained, then the soluble solids (ºbrix), moisture, and ascorbic acid of the canned pears were measured. The amount of sucrose and ascorbic acid to be added to the drained syrup so the MRE specifications would be met were calculated using the following equations:

$$Sug = \frac{°B_i \times M \times 107}{1 - °B_i}$$

$$°B_T^S = \frac{36 \times °B_T^R + \left(B_T^R - 1\right) \times Sug + 107 \times M \times B_T^R}{36}$$

$$AA_T^S = \frac{0.143 \times AA_T^R - 0.107 \times AA_i}{0.036}$$

where *Sug* is the sugar content of the canned pears, *M* is the moisture, $°B_i$ is the soluble

solids of the canned pears, $°B_T^S$ is the target soluble solids for the adjusted syrup, $°B_T^R$ is the

target soluble solids for the MRE rations (20º brix), AA_T^S is the target ascorbic acid (ppm) for the

adjusted syrup, AA_T^R is the target ascorbic acid (ppm) for the MRE rations, and AA_i is the

ascorbic acid (ppm) of the canned pears.

For pH adjustment, 107 g of pears were ground with 36 g of syrup, and citric acid

or sodium citrate were added to decrease or increase the pH as needed until it met the

MRE specifications. The same amount of citric acid or sodium citrate was added to 36 g

of syrup, and the pH of the syrup was recorded. Finally, the pH regulator was used to

adjust the pH of the whole lot of syrup to the recorded pH, so the pH of the formed

ration would be within the specifications. The target pH was 4.0, which according to

Kluter et. al. (1996) is the optimum value, as a lower pH would have an adverse effect

on color and texture. A higher pH would pose a risk of the product being low acid and

therefore being unsafe after thermal processing.

For fresh D'Anjou pears, the syrup was prepared from scratch using water,

sucrose, ascorbic acid and sodium citrate as pH regulator. Soluble solids (ºbrix),

moisture, and ascorbic acid of the pears were measured to calculate the parameters of

the syrup to be prepared. Additionally, D'Anjou pears required flavor enhancing, which

was done with Bartlett pear flavor (Flavor Solutions, Natural Pear Flavor ID#: 112430B)

dissolved into the syrup at a level of 0.4% based on the whole MRE ration.

Cups were manually filled with 107 g of diced pears and 36 g of syrup, and

poured over preformed trays in the horizontal form fill sealers (Tiromat 300 at FMT) for

vacuum packaging. Vacuum pressure was set at 88 kPa and exposure times of 3 s and 6 s

were used; it was determined that 3 s would result in a headspace of 4 to 7 mL in the

pouch, which is the current industry standard, and that 6 s would result in almost no

residual headspace (full vacuum packaging).

The pouches were processed in full water immersion retorts. The process was

designed to achieve an F value during hold time of no less than 2.5 min. Reference

temperature was 93.3ºC and z value was 8.8ºC, water at 100ºC was used as heating

medium. Stationary thermal process, which is the industry standard, and agitated

thermal process (rotation speed of 10 RPM) were used. Heat penetration curves were

obtained by fixing a thermocouple at a center of a pear dice, which was located at the

center of an overfilled pouch (10% above standard weigh of pear dices) placed at the

cold spot of the retort (previously determined). **Figure 2.3** and **Figure 2.4** show the MRE

pears ready to be packed and the retort used for MRE pouches.

Figure 2.3: Preformed trays with mixture of diced pears and syrup

Figure 2.4: Water-immersion retort. The lower-left corner shows the cage

and trays used to hold the MRE pouches

2.2.2   Storage studies

Retorted pouches were stored at 48.8ºC (120ºF) for 45 days and analyzed at 15

days intervals for headspace volume and composition (oxygen and carbon dioxide),

color, ascorbic acid concentration, and hardness. Additionally, sensory analysis was

performed at U.S. Army Natick Soldier Center. The initial analysis (day 0) were actually

done after 3 days at ambient temperature, which is required for sucrose, ascorbic acid,

and pH to equilibrate between the pears and the syrup. Analysis (not shown) carried

out on samples produced at Truitt Bros. after 3 days of production and after 10 days of

production (transit time between Oregon and New Jersey) show no significant

degradation of MRE pears when kept at ambient temperature.

2.3   Methods

2.3.1   Ascorbic Acid

Ascorbic acid of the MRE pear rations was measured according to AOAC Method

43.060 (1980). Syrup from MRE pears was filtered using Whatman filter paper Nº 1

(Whatman International, England). 1 to 3 mL of syrup were diluted with 5 mL of

extracting solution (3% metaphosphoric acid and 8% acetic acid in distilled water) and

titrated with 2,6 dichlorophenol indophenol solution, which was previously standardized

with 1 mL of a 1,000 ppm L-ascorbic acid solution (Fisher Scientifics, NJ, U.S.A.) diluted

in 5 mL of extracting solution. Ascorbic acid of the canned or fresh pears that were used

as starting material was measured using ascorbic acid test spatulas; for canned pears

the spatula was immersed in the syrup and the concentration was determined with the color scale provided by the manufacturer, for the fresh pears the spatula was immersed in puree made with ground pears.

2.3.2 Headspace analysis

Headspace volume was measured by opening 2 to 3 pouches in a water sink and collecting the headspace gas in a graduated inverted cylinder using a funnel. Analysis was carried out with a portable headspace analyzer by inserting the probe through a hole in the cylinder which had been covered with a septum; the instrument expressed the results as percentage of oxygen and carbon dioxide in the headspace. The setup for this analysis is shown in **Figure 2.5**. Volume and composition were used to calculate the amount of micromoles of each gas present in the headspace by:

$$\mu mols_{gas} = \frac{\%_{gas} \times headspace(mL)}{100} \times \frac{P}{R \times T}$$

Where P is the atmospheric pressure (101,325 Pa), R is the gas constant (8.314 J/mol·K), and T is the temperature (298 K).

Measurements were only performed on pouches packed with headspace, as no quantifiable headspace could be collected from the pouches packed with full vacuum at any point during the shelf life.
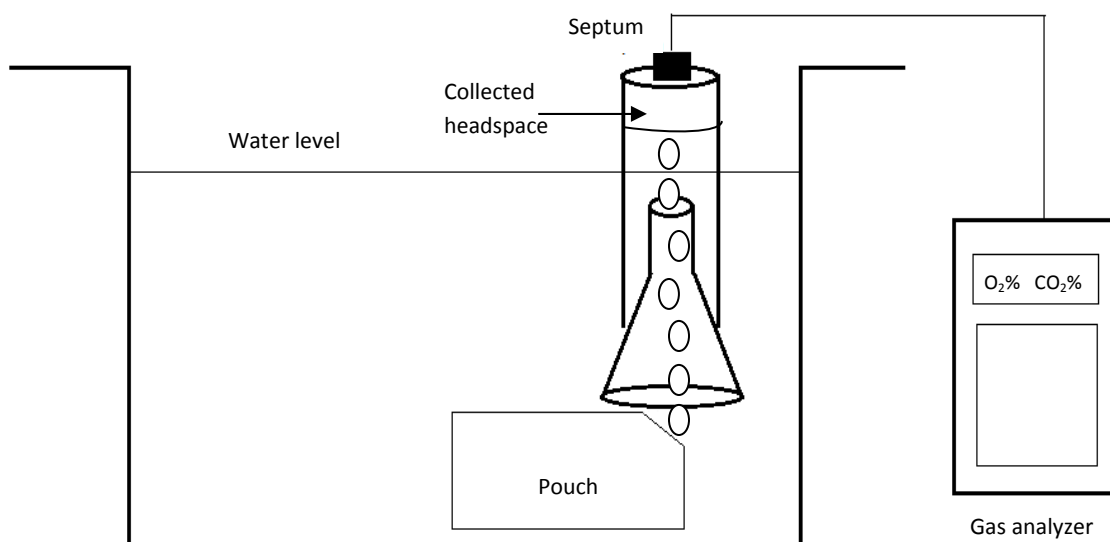
Figure 2.5: Setup for headspace measurement and analysis

2.3.3   Color Analysis

Color was measured using a Konica Minolta CR-410 handheld colorimeter

(Konica Minolta, Tokyo, Japan). D65 standard illuminant and 2º observer angle were

used, calibration was done with a white standard Y = 94.7, x = 0.3156 and y = 0.3319.

Between twenty-four and twenty-seven pear dices obtained from four to nine pouches

per data set were used.

Results were obtained in the CIE L* a* b* system and converted to brow index

according to Buera (1986):

$$B.I. = 100 \frac{x - 0.31}{0.172}$$

Where x is the chromacity coordinate of a color, which can be calculated from:

$$x = \frac{a^* + 1.75L^*}{5.646L^* + a^* - 3.012b^*}$$

(Francis and Clydesdale, 1975)

2.3.4 Peroxidase activity measurement

The method developed by Dunne and Brack (1988) was used to measure peroxidase activity. 10 g of pears were homogenized with 35 mL of chilled extraction buffer (0.05 M phosphate and 0.1M sodium fluoride, at pH 7). Homogenization was carried out in two intervals of one minute each with the container surrounded by ice, after which the homogenate was transferred to 50 mL polypropylene tubes and centrifuged until a clear liquid was obtained. 10 mL of the liquid were transferred to a glass tube and treated with an ascorbate oxidase spatula for approximately 30 minutes, until ascorbic acid couldn't be detected. Additionally, the liquid was also treated with Chelex 100 resin using the batch method, to remove metals.

Peroxidase activity was measured by absorbance at 420 nm and 49ºC (to match the condition of the accelerated studies) every 30 seconds and determining the rate of change in absorbance over the linear portion of the curve. The substrate for the reaction was o-phenylene-diamine (10 mM solution), hydrogen peroxide (1 M) was used as oxidating agent, and 2-N-Morpholinolethanesulfonic acid (0.1 M and pH 5) was used as assay buffer. Sample controls and reagent control were also analyzed to account for

sample absorbance and autoxidation, respectively. **Table 2.1** shows the volume of each

component and the order as it were added to the cuvettes for the analysis.

Table 2.1: Peroxidase activity cuvette contents (250 μL total)

	Sample activity	Sample control	Reagent control
Substrate (μL)	12.5	-	12.5
Assay buffer (μL)	172.5	185	187.5
Pear supernatant (μL)	15	15	-
Hydrogen peroxide (μL)	50	50	50

Peroxidase activity was calculated by subtracting the sample control rate and the

reagent control rate from the sample activity.

2.3.5   Ripeness measurement

Ripeness was defined as the maximum force in pounds required for a 0.5'' (12.7

mm) diameter steel probe to pierce approximately 1 cm rapidly through the pear flesh.

A section of the fresh D'Anjou pear was peeled and the penetrometer probe was

manually pressed against the peeled surface at a 90º angle. According to Truitt Bros.,

the target value of ripeness for fresh pears to be mechanically diced and canned is 9 lb.

(4.1 kg). This analysis was not performed on canned pears because the dices were too

small for the penetrometer; instead hardness values were measured with the texture

analyzer.

2.3.6   Hardness measurements

The parameter hardness, defined by Rosenthal (1999) as the force required for compressing a food between the molars, was used to measure texture degradation. Instrumentally, hardness is defined as the peak force in the first cycle of a texture profile analysis, in which a probe is used to compress the samples and the force required for it to travel at a constant speed is recorded. **Figure 2.6** shows examples of force vs. time curves, from which hardness was determined. A 2 mm diameter cylindrical probe was used to assure that the contact area remained the same for all the analysis.

The following test parameters were used:

- Pre test speed: 10 mm/s

- Test speed: 5 mm/s

- Post test speed: 10 mm/s

- Start point: 25 mm

- Distance traveled: 30 mm
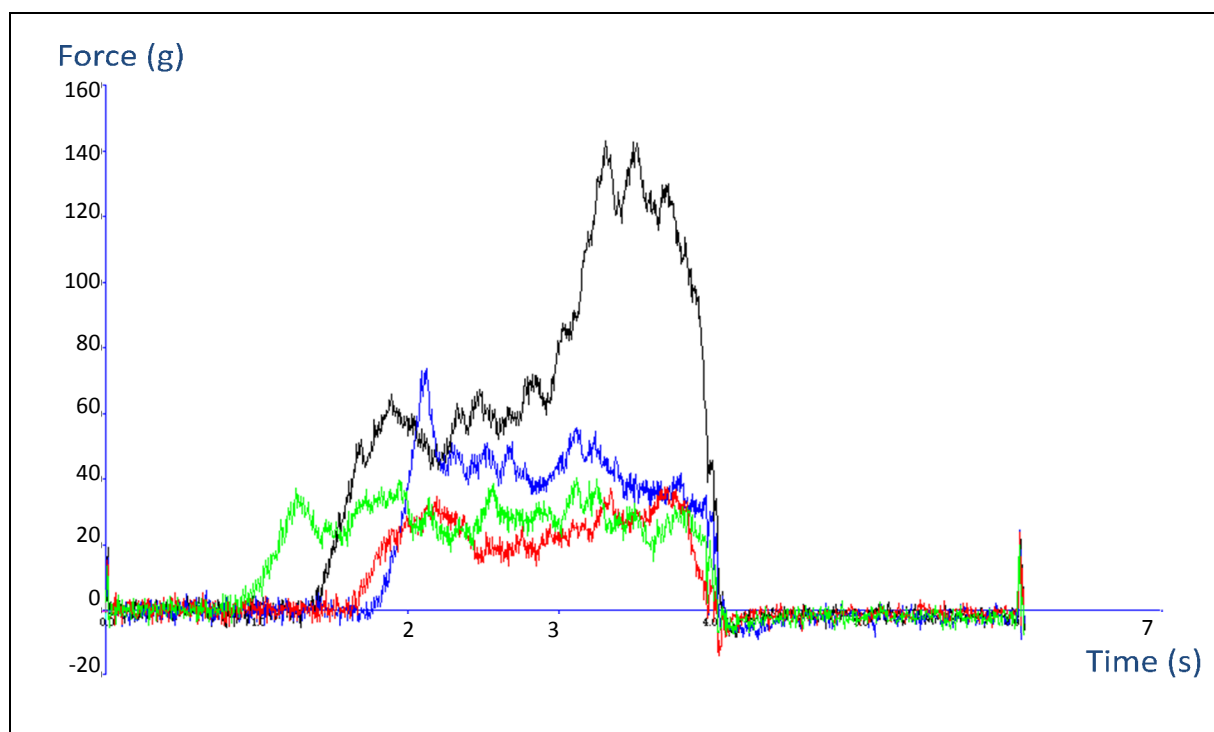
Figure 2.6: Typical force-time curves for MRE pears.

Each color represents a different sample

2.3.7 Sensory Analysis

A trained panel consisting of 10 to 13 panelists at the U.S. Army Natick Soldier Center graded the product on a 9 point hedonic scale for appearance, flavor, texture, odor, and overall preference. A score of 1 was assigned if the attribute was extremely disliked and 9 if it was extremely liked. The researchers in charge of the sensory evaluation indicated that appearance score was mostly related to the color of the pears. Due to the large amount of samples required for this study (50 pouches per variable), sensory analysis were only carried out for samples produced at Truitt Bros. Also, due to availability of resources, the samples produced with full vacuum and agitated retorting were not considered for sensory evaluation.

2.3.8   Soluble solids measurement

Soluble solids were measured on the pears that were used as starting material in order to calculate the sugar required so the MRE ration complied with the Army parameters. Soluble solids were measured with a handheld digital refractometer as ºbrix; for the canned pears the syrup was used as sample, for the fresh pears some dropplets of juice extracted from the pears were used.

2.3.9   Moisture measurement

Moisture of the pears was measured using a moisture balance, with a sample size of approximately 1 g. The samples were held at 110ºC and the moisture was read when less than 0.05% of change in weight per minute was detected by the balance.

2.3.10 Statistical Analysis

Analysis of variance (ANOVA) was carried out using Matlab for Windows 64-bits version 7.9.0.529 (R2009b); significant difference was found when the significance value was beneath $\alpha=0.05$. Tukey's test with confidence coefficient of 0.95 was used for paired comparison if factors at three or more levels show significant difference. Linear regression was also done with Matlab, confidence interval was calculated by hand using the mean square error provided by Matlab. Error bars in the figures represent standard error calculated with Excel 2007 SP2.

3.   Results and Discussion

3.1   Correlation Between Instrumental Analysis and Sensory Analysis

Before discussing about the effects of the different variables of our study on the browning of the product it is important to establish if the way in which we measure browning, that is, the browning index calculated from L* a* b* values obtained from the colorimeter, actually reflect what a potential consumer would see as browning in the pears. **Figure 3.1** shows a correlation between the average brown index and the average appearance score. Each data point (brown index , appearance score) corresponds to the same set of rations stored at the same temperatures for similar period of time. A correlation coefficient of 0.871 is considered very high for sensory analysis (Gacula, 1997), so we can conclude that brown index is a good parameter to quantify browning in MRE pears.
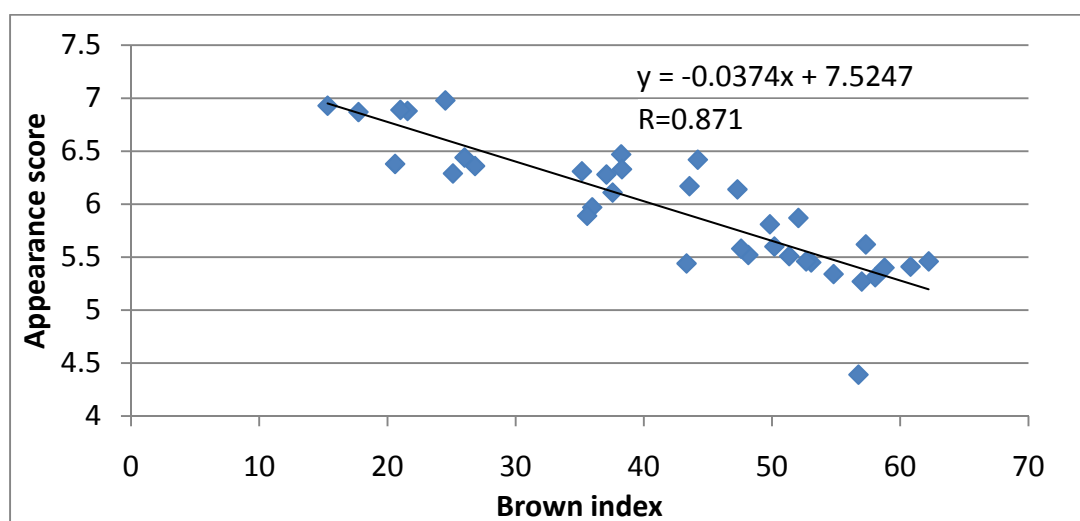
Figure 3.1: Correlation between appearance sensory score

and brown index for MRE pears

Based on the information provided by the U.S. Army Natick Soldier Center, a score of 6 after 30 days of storage at 48.8ºC (end of shelf life) was considered acceptable. The 95% confidence interval for brown index that would achieve such a value would be between 38.3 and 43.7, with a mean of 41.

A similar analysis was done for texture, between the average hardness of the pears and the average texture score in the sensory analysis. The results, seen in **Figure 3.2**, indicate that there is no correlation between these parameters. It is important to consider that the sensory analysis was for preference. Unlike appearance, where a browner pear would most definitely have a lower score or at best the same score as a lighter pear, consumers do not necessarily prefer a harder pear over a softer one. The fact that several samples had similar hardness and yet got very difference scores in the sensory analysis is an indication of that.
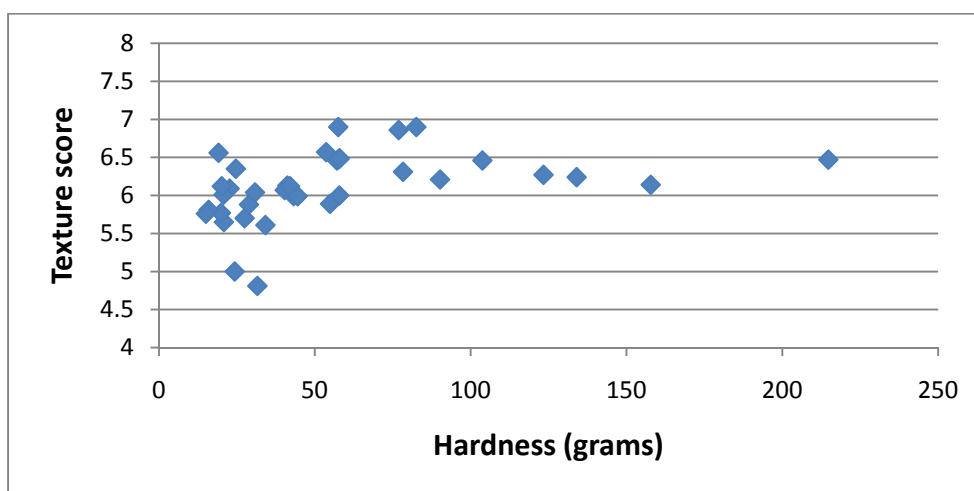
Figure 3.2: Correlation between texture sensory score and hardness for MRE pears.

3.2   Fresh D'Anjou Pears and its Effect on Color and Texture of MRE pears

Fresh D'Anjou pears were introduced as a variable in the study to avoid using already processed pears as a starting material and end up with a double processed pear in the MRE rations. The Army requires that the contractors be able to produce MRE pear rations anytime during the year, because the demand cannot always be anticipated, and since Bartlett pears have a short harvesting season and shelf life, year-long supply of fresh Bartlett pears is not possible. However, D'Anjou pears have a longer harvesting season and a much longer shelf life under refrigeration, so fresh D'Anjou pears can be available any time during the year.

Figure 3.3 shows the browning of MRE pears prepared with canned Bartlett pears and fresh D'Anjou pears; packaging was done in regular film and processed under regular conditions with 800 ppm of ascorbic acid. The difference in the initial measurements can be attributed to the thermal processing that Bartlett pears underwent during canning, so these measurements were not considered for statistical analysis, which shows no significant difference (**Table 3.1**).
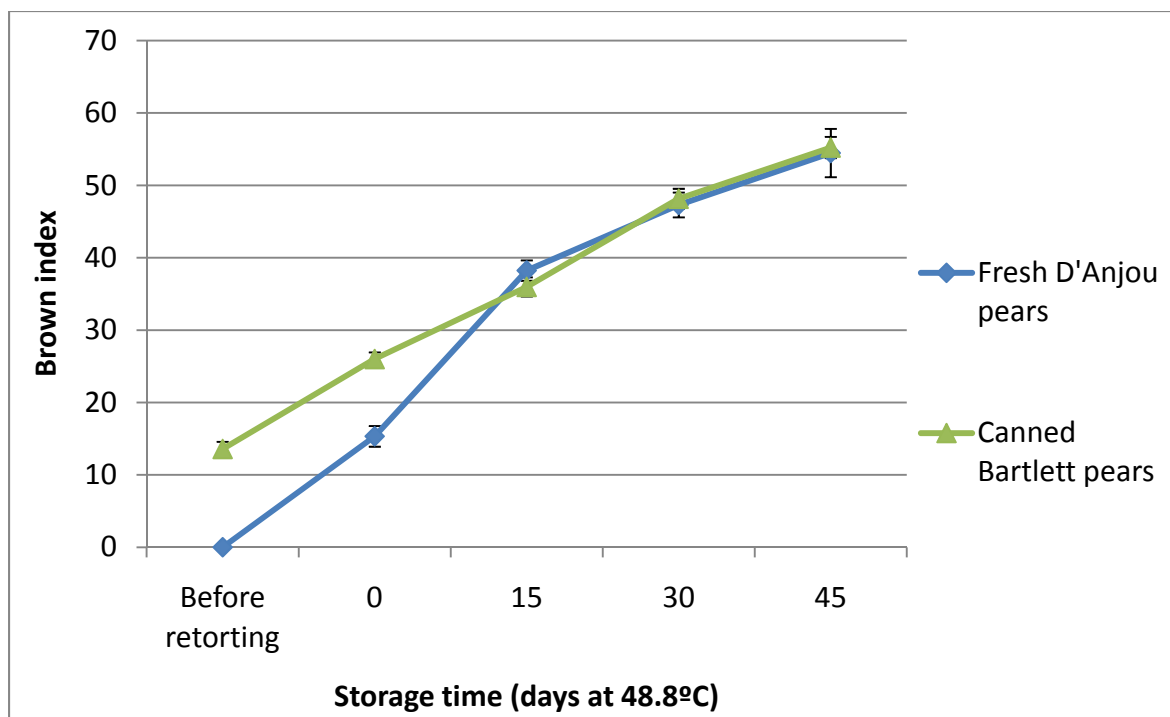
Figure 3.3: Browning of MRE pear rations produced using fresh D'Anjou pears

and canned Bartlett pears

Table 3.1: ANOVA for color of MRE pears prepared with canned Bartlett pears or fresh

D'Anjou pears

Source	Sums of squares	D.F.	Mean Square	F value	Significance
Storage time	3332.68	2.00	1666.34	21.29	0.00
Pear type	9.37	1.00	9.37	0.12	0.73
Storage time *Pear type	105.02	2.00	52.51	0.67	0.51
Error	6573.72	84.00	78.26		
Total	10019.66	89.00			

Texture, however, was greatly influenced by the type of pears used to prepare the MRE rations. **Figure 3.4** shows the results of hardness measurements, and **Table 3.2** shows that there was significant difference for the effect of pear type in the texture. This result was expected as it is known that thermal processing weakens the structure of plant cells, so double processing the pears rations would significantly affect the textural quality of the product. **Figure 3.2** shows that the highest sensory scores were achieved with pears of hardness between 50 and 100 g, which correspond to fresh D'Anjou pears.
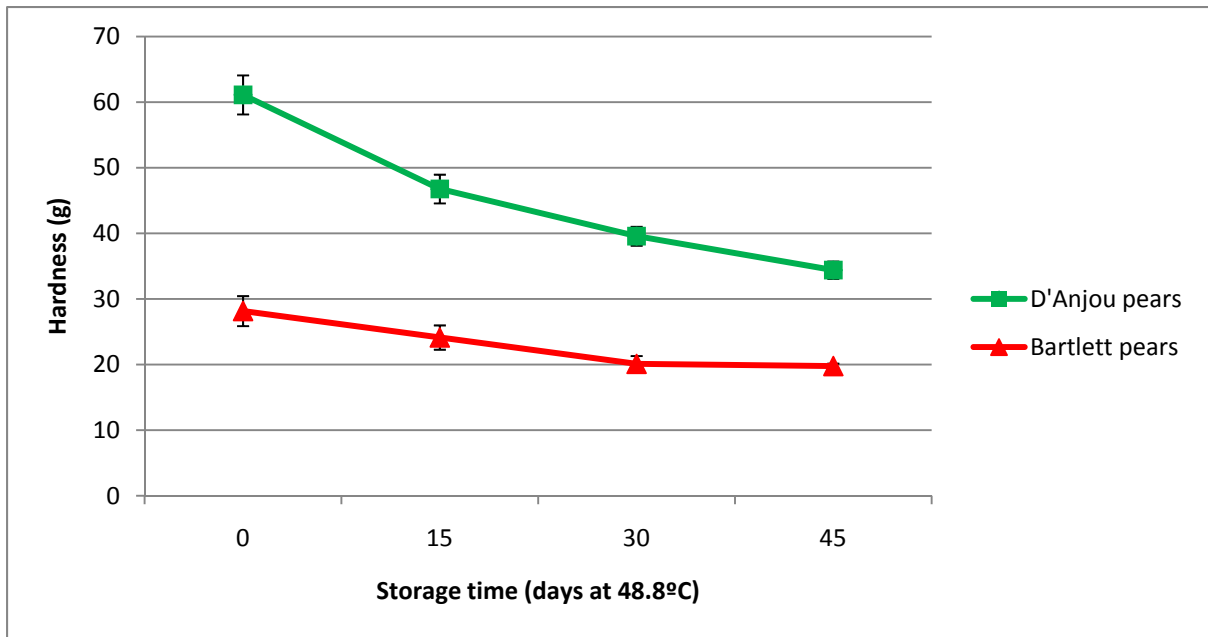

Figure 3.4: Texture results of MRE pear rations produced using fresh D'Anjou pears and canned Bartlett pears

Table 3.2: ANOVA for texture of MRE pears prepared with canned Bartlett pears or fresh

D'Anjou pears

Source	Sums of squares	D.F.	Mean Square	F value	Significance
Storage time	7147.02	3.00	2382.34	12.25	0.00
Pear type	33240.21	1.00	33240.21	170.85	0.00
Storage time * Pear type	1330.80	3.00	443.60	2.28	0.08
Error	38132.28	196.00	194.55		
Total	79794.15	203.00			

3.3 Modifications in Packaging and Thermal Processing

Experiments were carried out to determine if the MRE pouches had an effect on browning of pears compared to tin cans, since it is known that canned fruits have a very long shelf life. The color measurements of MRE pears and pears reformulated according to the MRE specifications and repacked in tin cans are shown in **Figure 3.5**; significant difference was found between pears packed in MRE pouches compared to tin cans, as shown in **Table 3.3**. Furthermore, the color of pears packed in lacquered cans and of MRE pears to which stannous chloride had been added degraded as fast as pears in MRE pouches (results not shown); however, pears in MRE pouches in which pieces of tin cans were placed inside did not degrade as fast as regular MRE pears (results not shown). Since the presence of metallic tin was the only difference between these treatments, and it is known that tin reacts with oxygen (Lee et. al., 2008; Eskin, 1990), we started working under the assumption that oxygen had to play a significant role in the browning reaction.
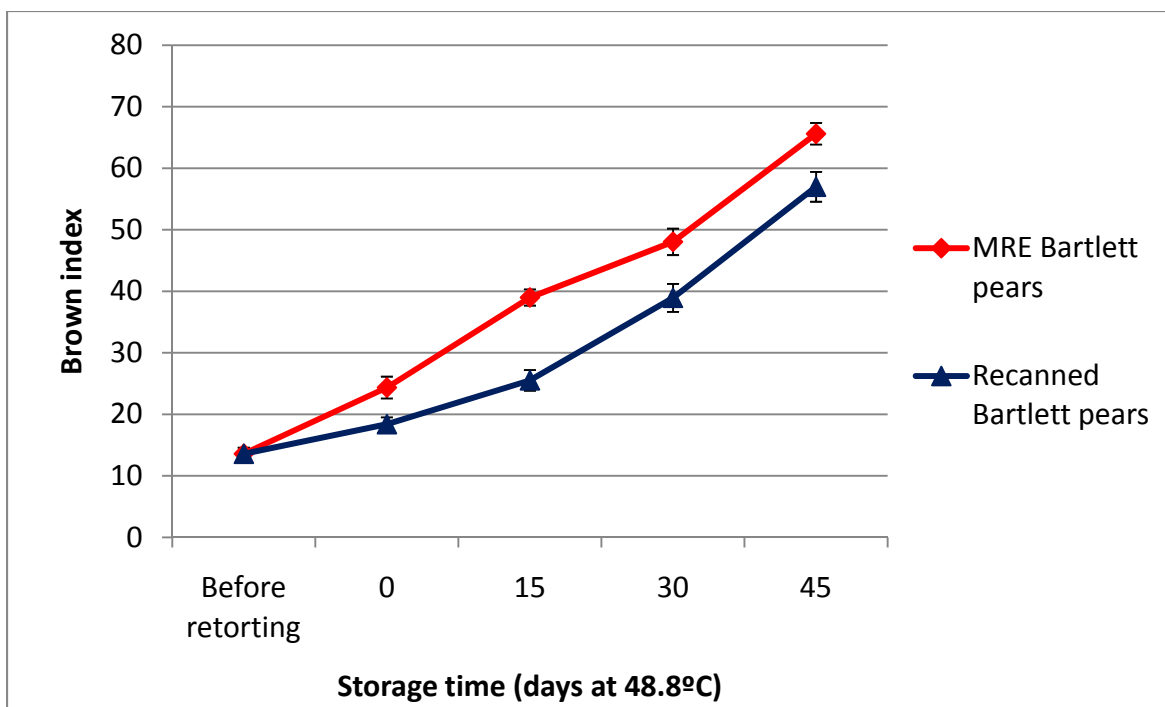
Figure 3.5: Browning of MRE pears compared to pears packed in tin cans

Table 3.3: ANOVA for pears packed in tin cans and MRE pouches

Source	Sums of squares	D.F.	Mean Square	F value	Significance
Storage time	34738.40	3.00	11579.47	162.77	0.00
Packaging	3501.38	1.00	3501.38	49.22	0.00
Storage time * Packaging	299.47	3.00	99.82	1.40	0.24
Error	11168.67	157.00	71.14		
Total	49777.35	164.00			

Figure 3.6 and **Figure 3.7** show the evolution of oxygen and carbon dioxide on a

molar basis in MRE pouches. It was found that almost all the oxygen in the headspace of

the pouches was consumed during thermal processing, and that carbon dioxide was

formed throughout storage. Based on these findings and the literature on browning

reactions, we think that ascorbic acid degradation was involved in the browning of MRE

pears. The oxygen is consumed to form dehydroascorbic acid during thermal processing

at a very high rate due to the high temperature. Dehydroascorbic acid is degraded

during storage (Kacem et. al., 1987a) to form diketogulonic acid and carbon dioxide,

among other by-products, when reacting to form brown pigments.
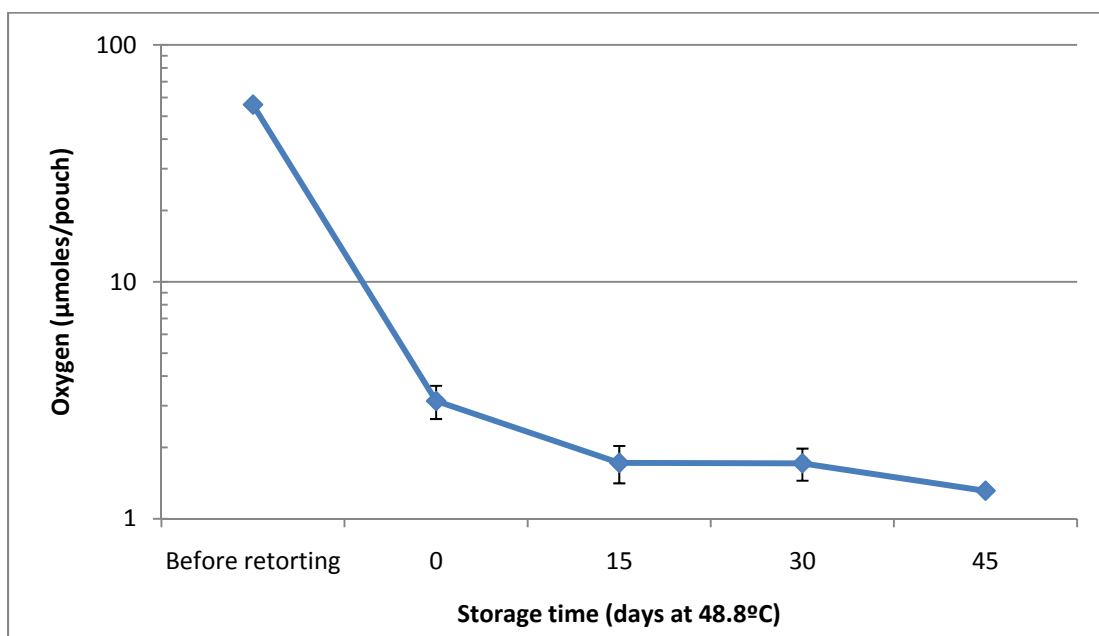
Figure 3.6: Oxygen content in the headspace of regular MRE pears
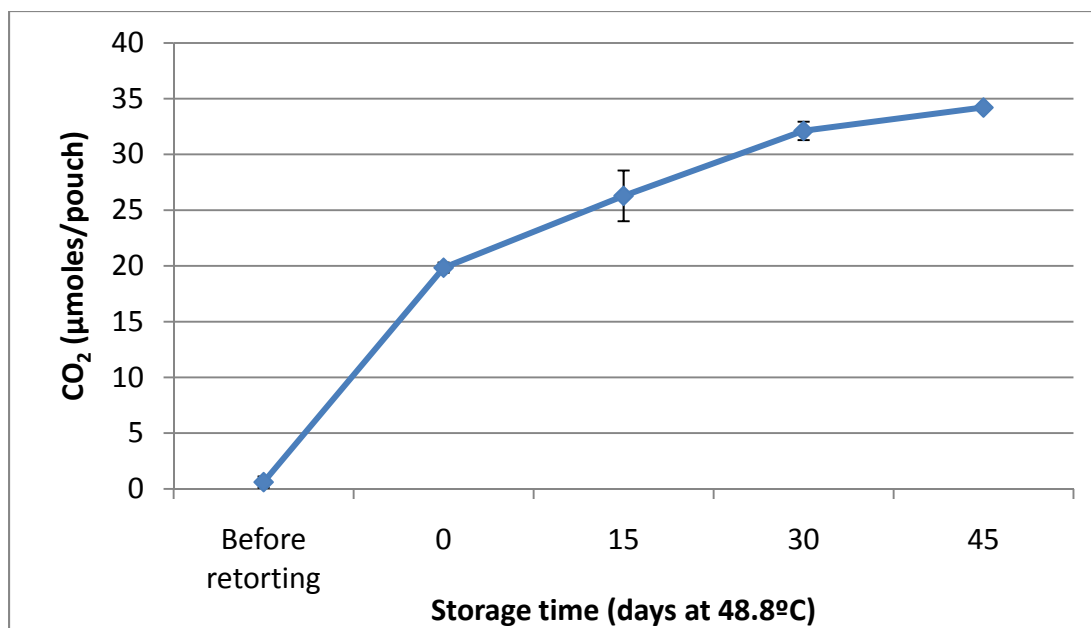
Figure 3.7: Carbon dioxide content in the headspace of regular MRE pears

Oxygen scavenger films were used in order to prevent the oxidation of ascorbic acid and therefore, reduce the formation of brown pigments. **Figure 3.8**, **Figure 3.9** and **Figure 3.10** show the results of three experiments using iron-based oxygen scavenger films. The first two experiments were done with Bartlett pears at the FMT facility, the color results between the pears in regular pouches and in oxygen scavenger pouches were significantly different as seen in **Tables 3.4** and **Table 3.5**, but still the pears in oxygen scavenger film were visually determined to be too dark to be acceptable. The third experiment was done with D'Anjou pears at Truitt Bros. which had pear flavor at 0.4% (which was previously determined not to influence the browning of the pears) besides the other ingredients. **Table 3.6** shows that no significant difference in browning was found in this experiment between pears packed in regular MRE pouches and pears packed in oxygen scavenger pouches. This result was confirmed by the sensory analysis

shown in **Figure 3.11**, in which no significant difference in preference of the appearance

of one sample was found (P<0.0001).
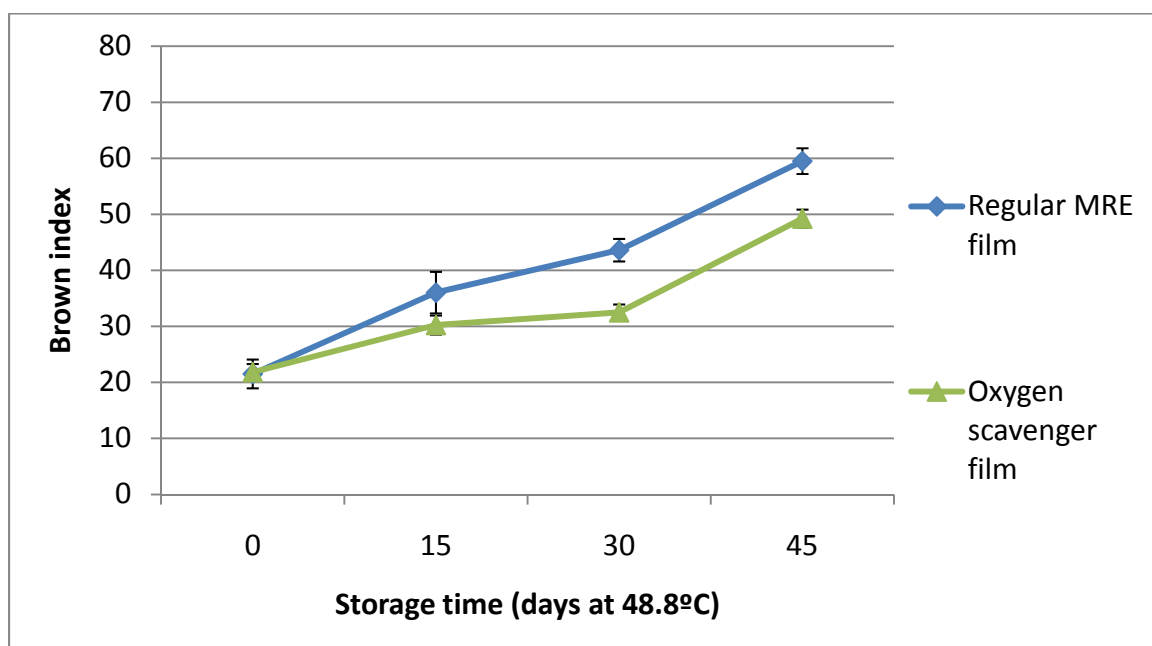
Figure 3.8: Color measurements of pears in regular MRE film and

oxygen scavenger film, first experiment

Table 3.4: ANOVA for color of pears packed in regular MRE film and oxygen scavenger

film, first experiment (at FMT Facility)

Source	Sums of squares	D.F.	Mean Square	F value	Significance
Storage time	18205.11	3.00	6068.37	90.74	0.00
Packaging	1472.28	1.00	1472.28	22.01	0.00
Storage time * Packaging	660.09	3.00	220.03	3.29	0.02
Error	8694.04	130.00	66.88		
Total	29173.38	137.00			
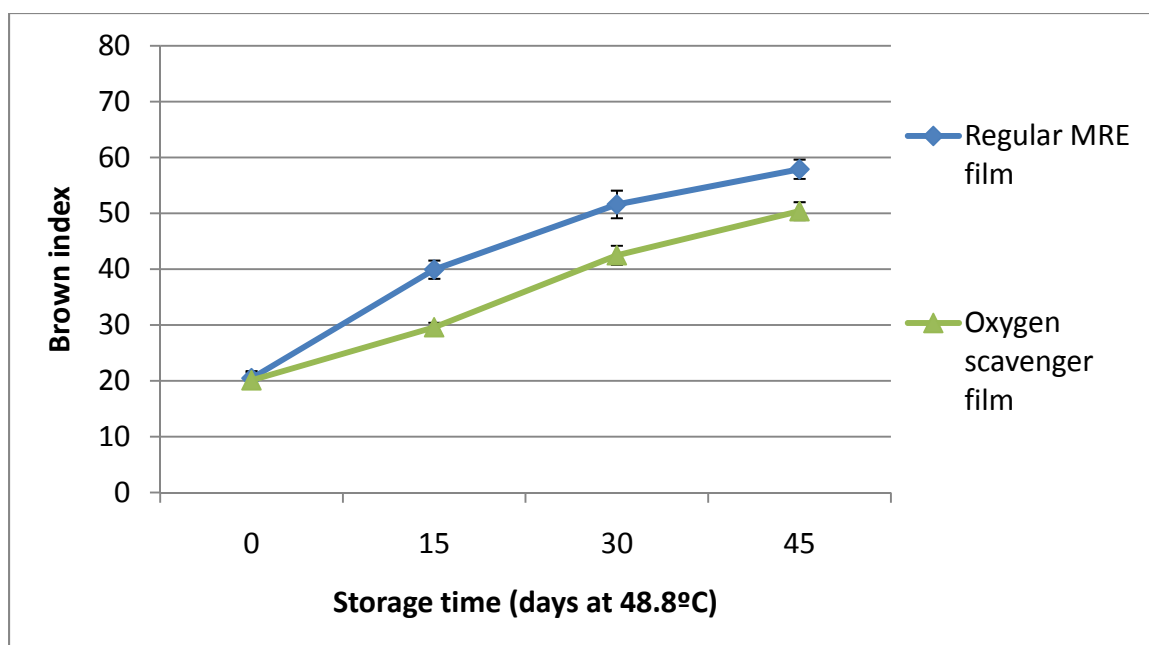
Figure 3.9: Color measurements of pears in regular MRE film and

oxygen scavenger film, second experiment

Table 3.5: ANOVA for color of pears packed in regular MRE film and oxygen scavenger

film, second experiment (at FMT facility)

Source	Sums of squares	D.F.	Mean Square	F value	Significance
Storage time	31333.26	3.00	10444.42	161.78	0.00
Packaging	2213.22	1.00	2213.22	34.28	0.00
Storage time * Packaging	717.09	3.00	239.03	3.70	0.01
Error	11491.68	178.00	64.56		
Total	45813.43	185.00			
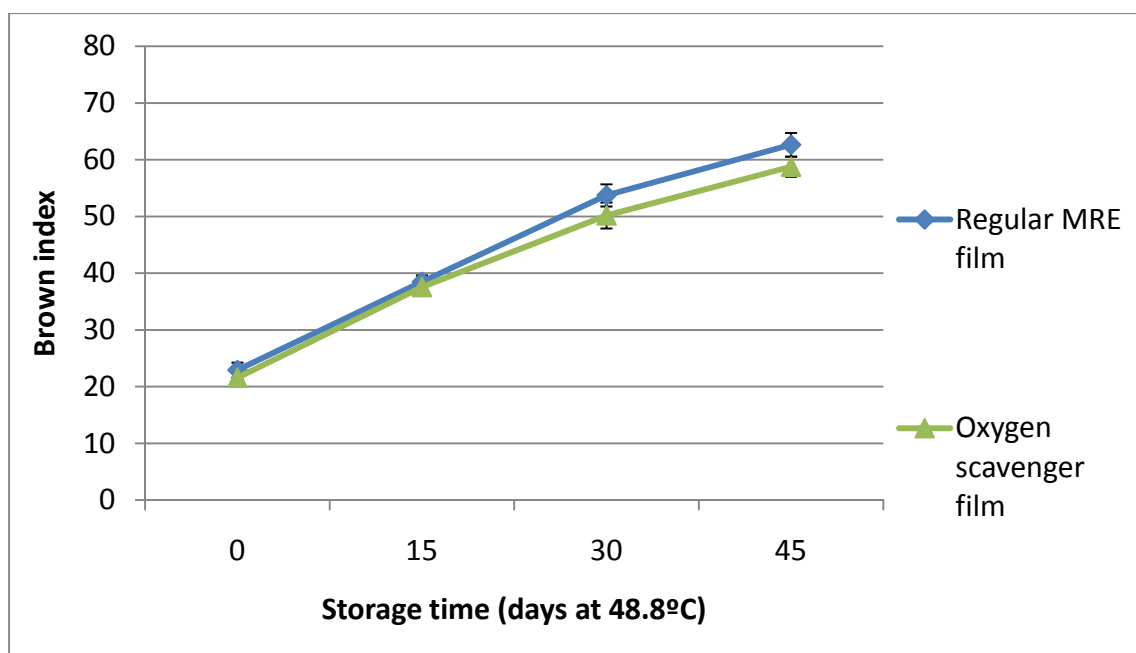
Figure 3.10: Color measurements of pears in regular MRE film and

oxygen scavenger film, third experiment

Table 3.6: ANOVA for color of pears packed in regular MRE film and oxygen scavenger

film, third experiment (at Truitt Bros. facility)

Source	Sums of squares	D.F.	Mean Square	F value	Significance
Storage time	14310.87	2.00	7155.43	94.78	0.00
Packaging	268.58	1.00	268.58	3.56	0.06
Storage time * Packaging	56.68	2.00	28.34	0.38	0.69
Error	11776.96	156.00	75.49		
Total	26413.09	161.00			
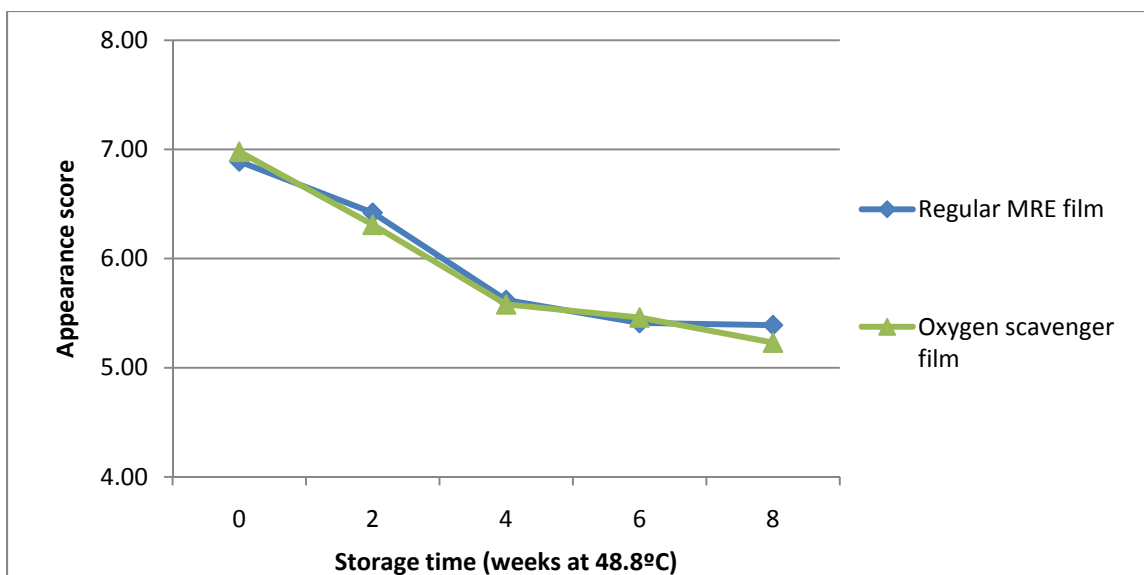
Figure 3.11: Sensory score for appearance of MRE pears packed in

MRE pouches and oxygen scavenger pouches

We think that the color results with oxygen scavenger films are not consistent

due to the positions and sizes of iron particles embedded in the film being not uniform,

shown by Gomes et al. (2009). This non uniformity would affect oxygen diffusion into

the pouch and its reaction rate with the iron particles as well as the leaching of iron

particles into the syrup during thermal processing, which would accelerate the oxidation

of ascorbic acid and reduce the effectiveness of the film. An alternate mechanism could

be iron leaching during thermal processing and/or storage and catalyzing Maillard

reaction, however it is unlikely as it would be too much coincidence that the browning

levels matched even though the mechanisms were different.

Due to the failure of oxygen scavenger films as a solution to prevent browning in MRE pears, other options were explored. Full vacuum packaging is normally not used in pouches because it requires that high vacuum be applied for an extended period of time, which could puncture the film; however we found settings for the horizontal form fill sealer machine that allowed to remove almost all the headspace while at the same time preserving the integrity of the pears and the film. Leaving some headspace in products that are to be retorted is also convenient since the air bubble enhances heat transfer by convection. Agitated retorting is also not a standard practice by the Army contractors when processing pouches because the edges of the pouches suffer damage, however it was found that if the pouches are properly secured in trays they would not be damaged.

Figure 3.12 shows the heat penetration curves for a regular stationary process and an agitated one. Agitation fulfilled its purpose of reducing the come up time for the pear dices, which in turn reduced the overprocessing of the syrup. Since most of the ascorbic acid at that point is dissolved in the syrup, reducing overprocessing could reduce the oxidation of ascorbic acid during thermal processing. It was also found that the air bubble made no difference in thermal processing when agitation was used.
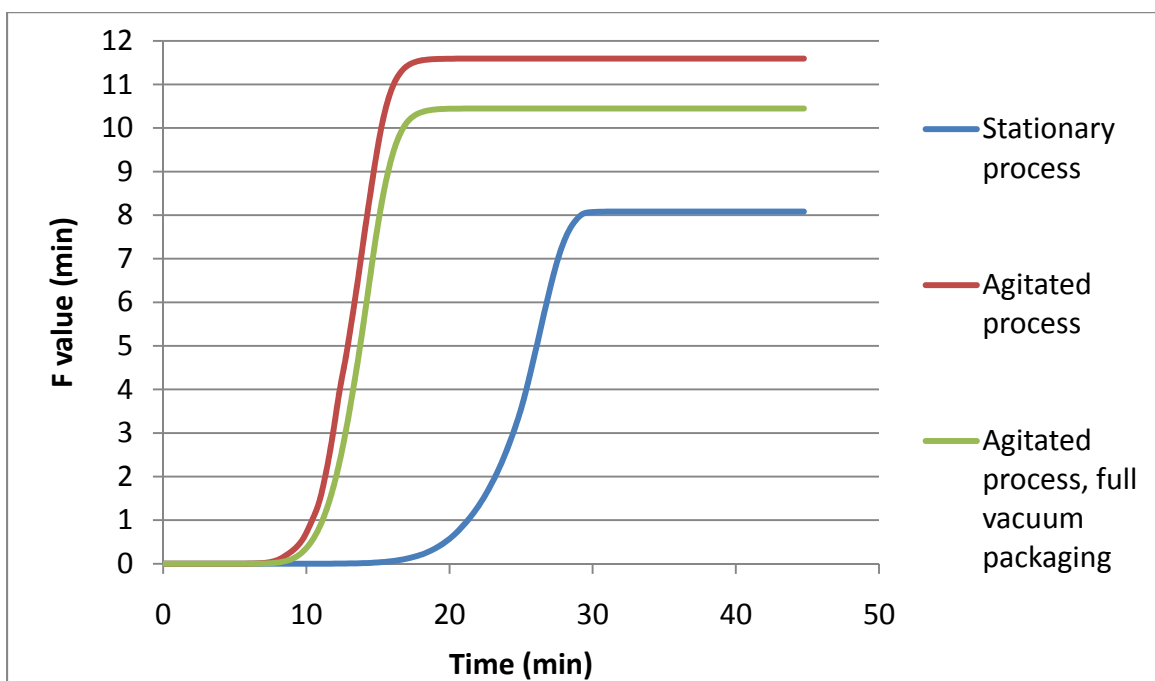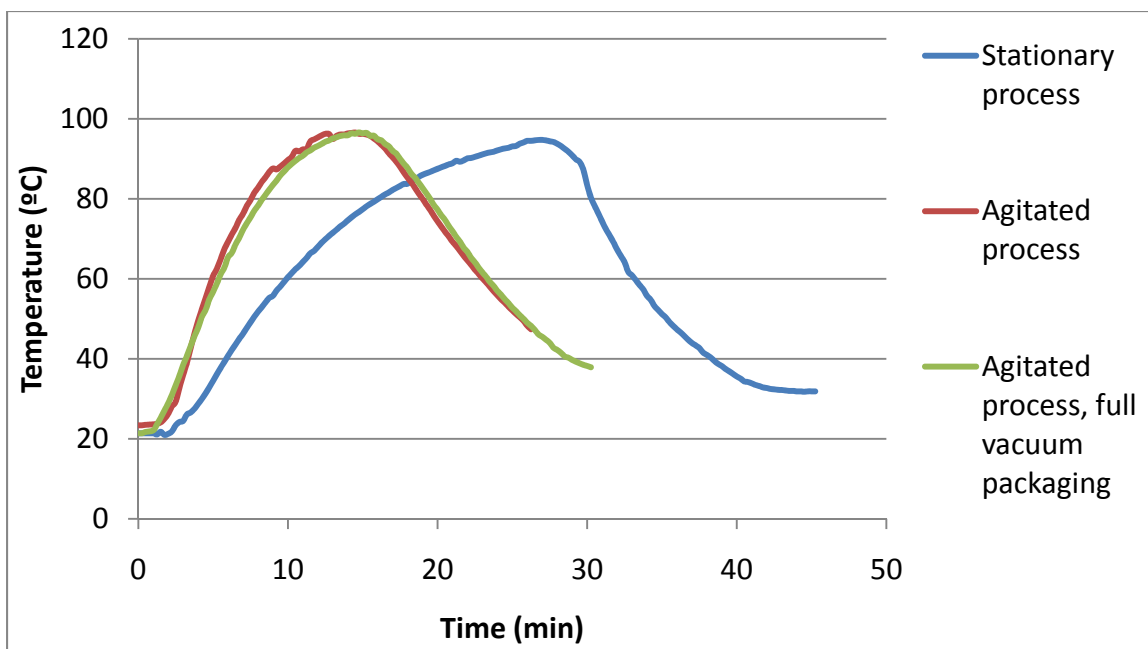
Figure 3.12: a) Temperature history for the retort processes.

b) F value for the retort processes.

The effects of full vacuum packaging and agitated thermal processing can be seen in **Figure 3.13**. Both treatments have a significant effect in reducing browning of MRE pears during storage (**Table 3.7**). Although browning was reduced, it wasn't fully prevented. This is because either anaerobic ascorbic acid degradation is still taking place or Maillard browning has also an effect on browning of MRE pears. Based on the brown index – appearance sensory score (**Figure 3.1**), we consider the decrease in browning achieved with full vacuum packaging and agitated retorting to be meaningful and to fulfill the objective of extending the MRE pear ration shelf life. The mean brown index after 30 days at 48.8ºC was 40.29, slightly lower than the mean brown index that would produce an appearance score of 6, the target at the end of shelf life. The brown indices achieved with the other treatments after 30 days at 48.8ºC were above the 95% confidence interval of 40.3 +/- 2.7, therefore it is expected that the panelists would notice them to be browner.
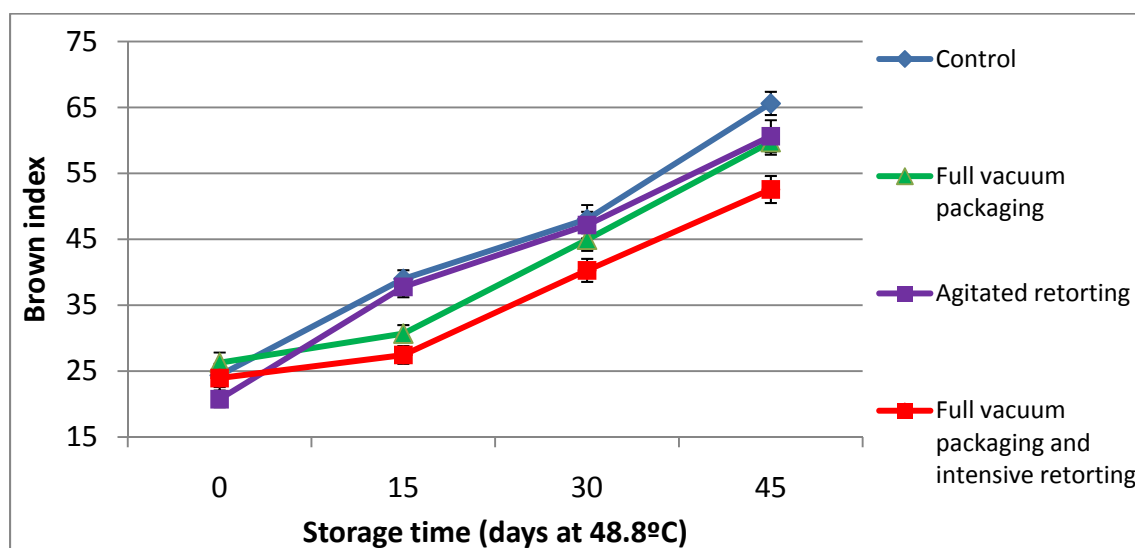
Figure 3.13: Effect of full vacuum packaging and agitated retorting on the formation of brown pigments in MRE pears.

Table 3.7: ANOVA of color measurements of pears after different types of processing

Source	Sums of squares	D.F.	Mean Square	F value	Significance
Storage time	65333.60	3.00	21777.87	301.04	0.00
Agitated thermal processing	1173.05	1.00	1173.05	16.22	0.00
Full vacuum packaging	2070.07	1.00	2070.07	28.62	0.00
Storage time * Rotation	211.30	3.00	70.43	0.97	0.41
Storage time * Full vacuum	1772.51	3.00	590.84	8.17	0.00
Agitation * Full vacuum	70.18	1.00	70.18	0.97	0.33
Error	26187.83	362.00	72.34		
Total	96376.28	374.00			

Figure 3.14 shows the effect of the changes of processing conditions on the retention of ascorbic acid. Similarly to brown color development, both intensity of vacuum packaging and type of thermal processing had a significant effect on the retention of ascorbic acid but there was no interaction between the treatments (**Table 3.8**). Ascorbic acid degradation continued during storage even though the oxygen was depleted, which confirmed that anaerobic ascorbic acid degradation was taking place, which could be contributing to browning. Moreover, ascorbic acid degradation rates during storage were similar regardless of the processing conditions, which was expected.
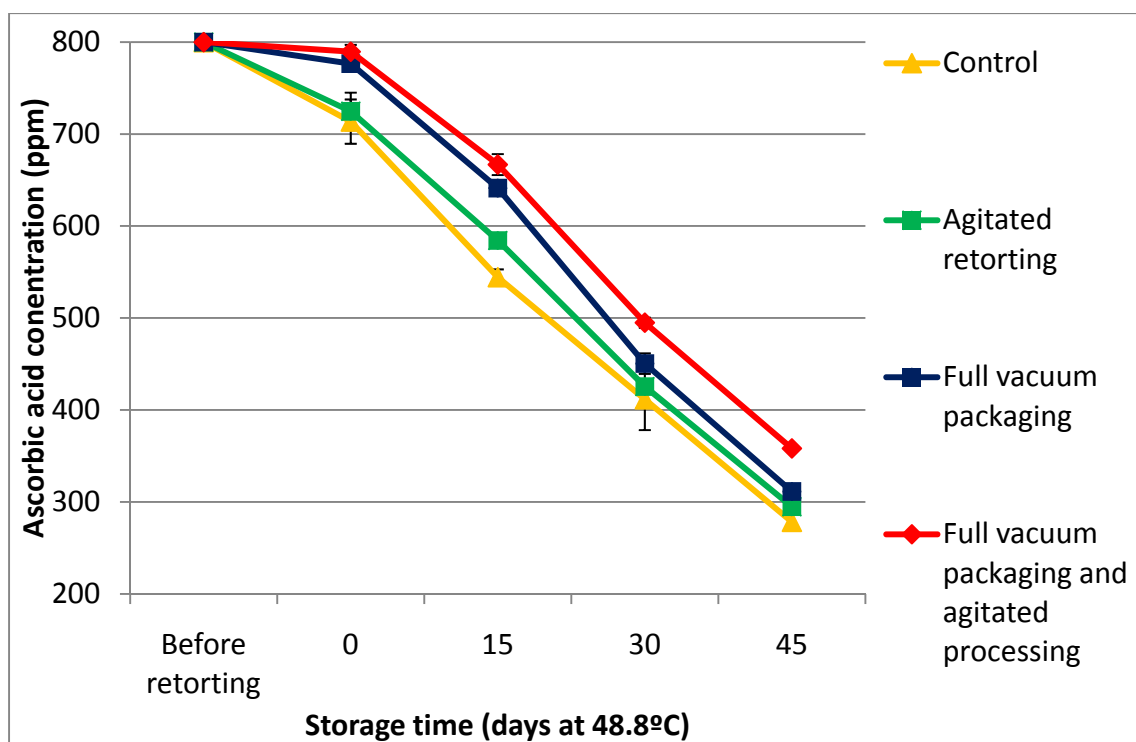
Figure 3.14: Effect of thermal processing and vacuum packaging on retention

of ascorbic acid in MRE pear rations

Table 3.8: ANOVA of ascorbic acid measurements of pears after different types of

processing

Source	Sums of squares	D.F.	Mean Square	F value	Significance
Storage time	883284.57	3.00	294428.19	905.37	0.00
Agitated thermal processing	5586.39	1.00	5586.39	17.18	0.00
Full vacuum packaging	32807.40	1.00	32807.40	100.88	0.00
Storage time * Rotation	564.72	3.00	188.24	0.58	0.64
Storage time * Full vacuum	2018.39	3.00	672.80	2.07	0.14
Agitation * Full vacuum	293.97	1.00	293.97	0.90	0.35
Error	6178.86	19.00	325.20		
Total	930734.30	31.00			

A possible explanation on the significantly high effect in ascorbic acid and color

retention of reducing the headspace from 4 - 7 mL of air to almost nothing is the

formation of hydrogen peroxide during ascorbic acid oxidation, which could be used by

any peroxidase enzyme that survived thermal processing or recovered activity during

storage. **Figure 3.15** shows the absorbance at 420 nm of samples prepared to measure

peroxidase activity; MRE pears packed in regular pouches, processed under regular

conditions (with headspace and stationary thermal processing), and stored for 45 days

at 48.8ºC were used. No peroxidase activity could be detected; moreover, the reagent

control showed higher activity than the sample. This indicates that not even the

spontaneous oxidation of o-phenylendiamine took place, very likely because the

hydrogen peroxide was degraded by metals present in the pears before it could be used

for any reaction. If this was the case then peroxidase enzymes could still remain active

in the pears however would not have any effect as any hydrogen peroxide formed

would be degraded before they could use it. We attempted to test this by using Chelex®

100 resin to remove metals; however the resin was no effective as the results obtained

were similar to the ones shown in **Figure 3.15**.
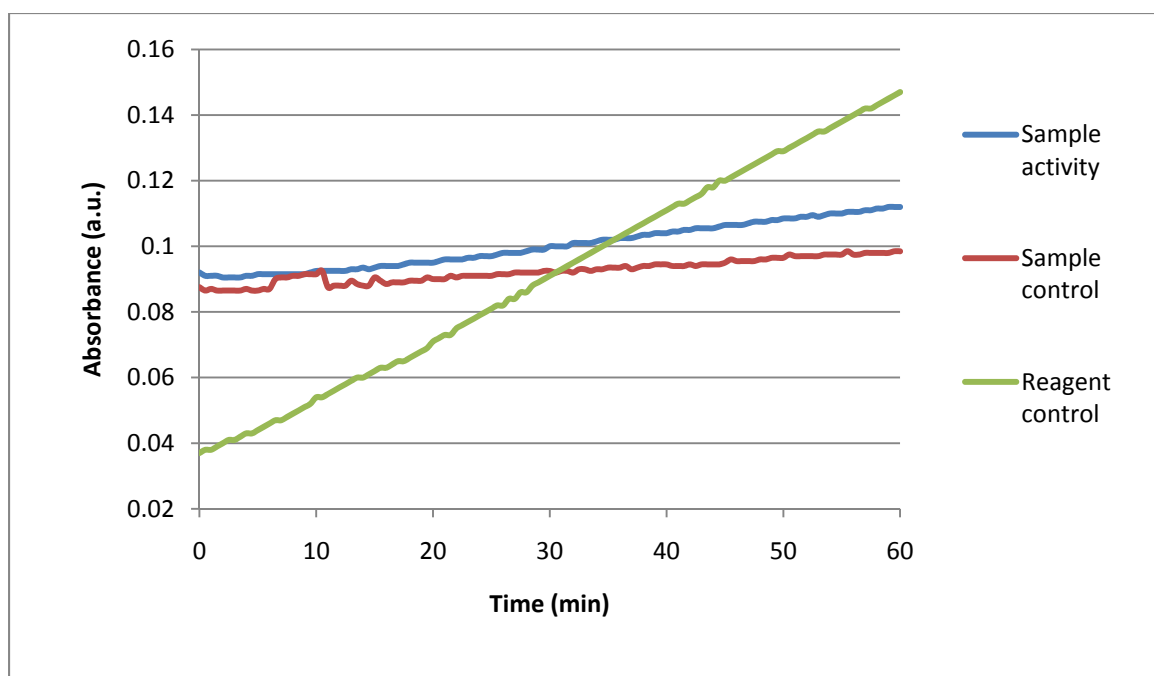
Figure 3.15: Peroxidase activity in MRE pears

Maillard reaction wasn't ruled out in this study as a mechanism for browning of MRE pears, however based on the literature on the reaction and the product parameters and processing conditions, it is unlikely that it has a significant role. Maillard browning is favored by high pH and intermediate water activity, which this product did not have (the water activity was 0.92). The product conditions do favor ascorbic acid degradation, as a significant proportion of ascorbic acid is in monoionic form (pKa is 4.1 while pH of the product was between 3.85 and 4.15), and catalyzers for the reactions, such as copper and fructose, were present.

3.4   Degradation of ascorbic acid

Experiments done on the effect of oxygen scavenger pouches on ascorbic acid degradation confirm that these do not fully protect ascorbic acid from oxidation. **Figure 3.16** shows the result of ascorbic acid measurements on MRE pears using regular pouches and oxygen scavenger pouches, both packed with regular vacuum packaging and retorted under stationary, and compares them with MRE pears in regular pouches with full vacuum packaging and agitated thermal processing. Although the oxygen scavenger pouch does increase the retention of ascorbic acid compared to the regular pouch, the best results were achieved with full vacuum packaging. **Figure 3.17** shows the results of a Tukey test at 0.05 confidence level. Similar effects of increased ascorbic acid retention in tin cans compared to lacquered cans or glass bottles were described by Nagy (1980). Although no difference in the rate of anaerobic degradation of ascorbic acid during storage could be appreciated, we think that the better preservation of color achieved with full vacuum packaging and agitated retorting is due to a decreased degradation of ascorbic acid during retorting, which would delay the formation of brown pigments. Stoichiometric analysis shows that there is a significant time between the oxidation of ascorbic acid and the formation of brown pigments. After retorting the ascorbic acid concentration decreases by 120 ppm approximately, which corresponds to 97 µmoles per pouch; however only 20 µmoles of carbon dioxide were formed (**Figure 3.7**). Ranganna and Setty (1974) showed that in aqueous medium most of the ascorbic acid forms carbon dioxide when degrading. The same analysis shows that anaerobic

degradation also starts during retorting, as the amount of oxygen consumed is not enough to justify the ascorbic acid loss (**Figure 3.6**).
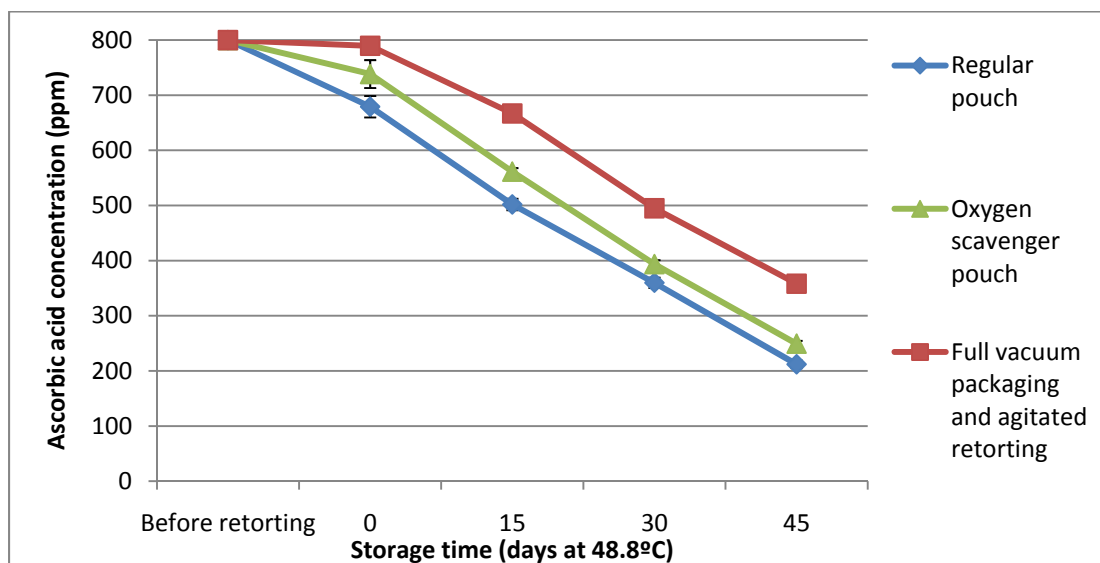
Figure 3.16: Ascorbic acid retention with oxygen scavenger films
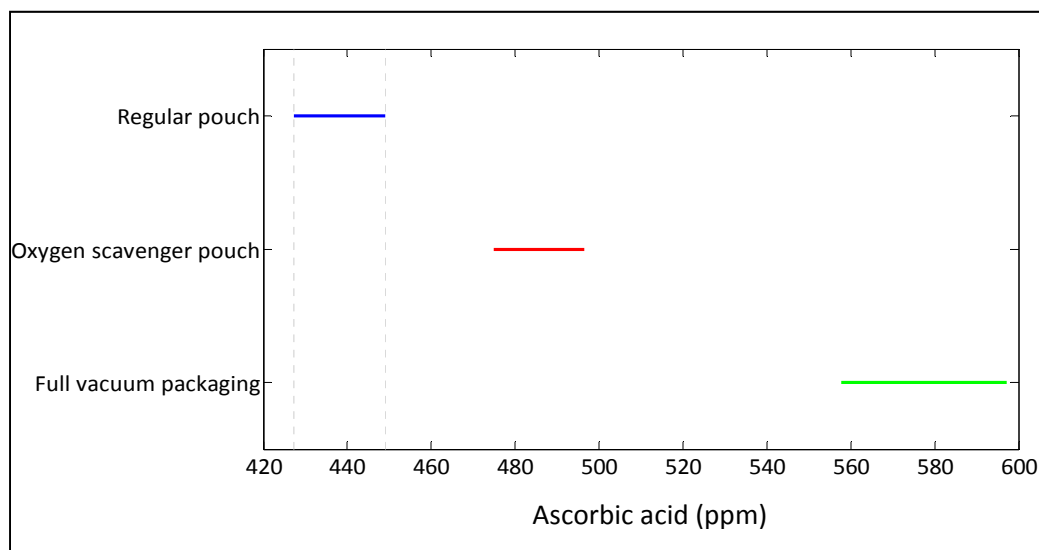
Figure 3.17: Tukey test for ascorbic acid retention with different packaging options (α=0.05)

Additional studies, such as leaching of iron to the syrup and oxidation of iron particles in the film during thermal processing, would be needed to determine if oxygen scavenger pouches could be optimized by narrowing the size distribution and position of the iron particles so they became a viable solution to browning problems in MRE pears. From a processor point of view it could be more convenient to use an oxygen scavenger film instead of full vacuum packaging, since the second option requires more time to pull the vacuum and therefore reduces the output of the production line.

Our study shows that the degradation rate of ascorbic acid in MRE pears increases with increasing concentration of ascorbic acid before retorting, but remains constant during storage (zero-order kinetics, dAA/dt = k). This contradicts Nagy and Smoot (1977), who observed first-order kinetics at temperatures up to 30ºC in canned orange juice, and polynomial kinetics above those temperatures. Johnson et al. (1995) observed a first-order reaction for anaerobic ascorbic acid degradation in orange juice serum at 91.1ºC. Riemer and Karel (1978) also observed exponential decay for anaerobic ascorbic acid degradation on dehydrated tomato juice.

Figure 3.18 shows the results of initial concentrations of 800 ppm and 400 pmm of ascorbic acid; these rations were packed in regular film and processed under regular conditions. Additionally, we studied samples of MRE pears prepared by the current Army contractor, these came from a lot made with canned Bartlett pears but packed in vertical pouches using steam flush to generate the vacuum; almost no headspace was

left in the pouches and the ascorbic acid concentration before retorting was unknown. It

is important to notice that the second to last data point of the MRE pears made by the

Army contractor reaches the level of 800 ppm ascorbic acid MRE pears and yet the

ascorbic acid retention remains linear, which proves that it is not a first order reaction

with low reaction rate. It is possible that anaerobic degradation is enhanced by by-

products of the reaction, however determining the actual causes for this behavior would

require further studies. Analysis of the degradation rates with the initial concentration

suggest that the rate might reach a maximum value at an initial concentration between

800 ppm and 1,200 ppm of ascorbic acid, however the data available from our

experiments are not enough to reach a conclusion.
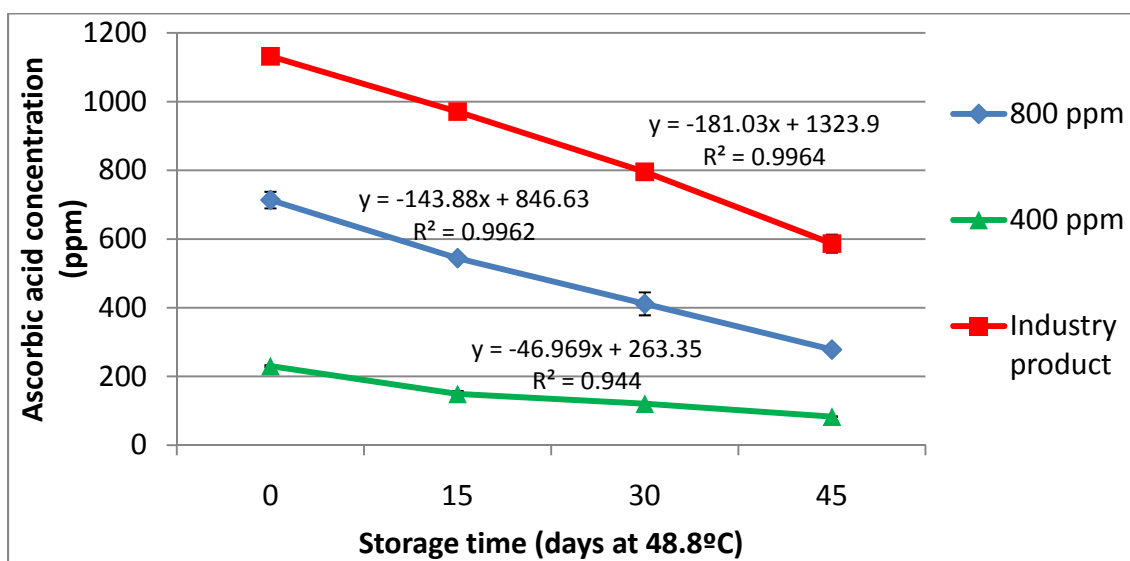
Figure 3.18: Anaerobic ascorbic acid degradation with time as function of

initial concentration of ascorbic acid

Carbon dioxide was monitored during storage of MRE rations packed in regular pouches and oxygen scavenger pouches. As mentioned before, carbon dioxide was generated during storage of pears in regular pouches due to ascorbic acid degradation, however almost no carbon dioxide was produced in oxygen scavenger pouches, as seen in **Figure 3.19**. Since ascorbic acid degradation was proven to take place in oxygen scavenger pouches, we think that leached iron ions altered the pathway of ascorbic acid degradation in favor of the formation of organic acids instead of carbon dioxide when diketogulonic acid is degraded. Ranganna and Setty (1974) have shown that switching from aqueous to alcoholic medium can alter the pathway of aerobic ascorbic acid degradation to favor the formation of acetaldehyde instead of carbon dioxide.
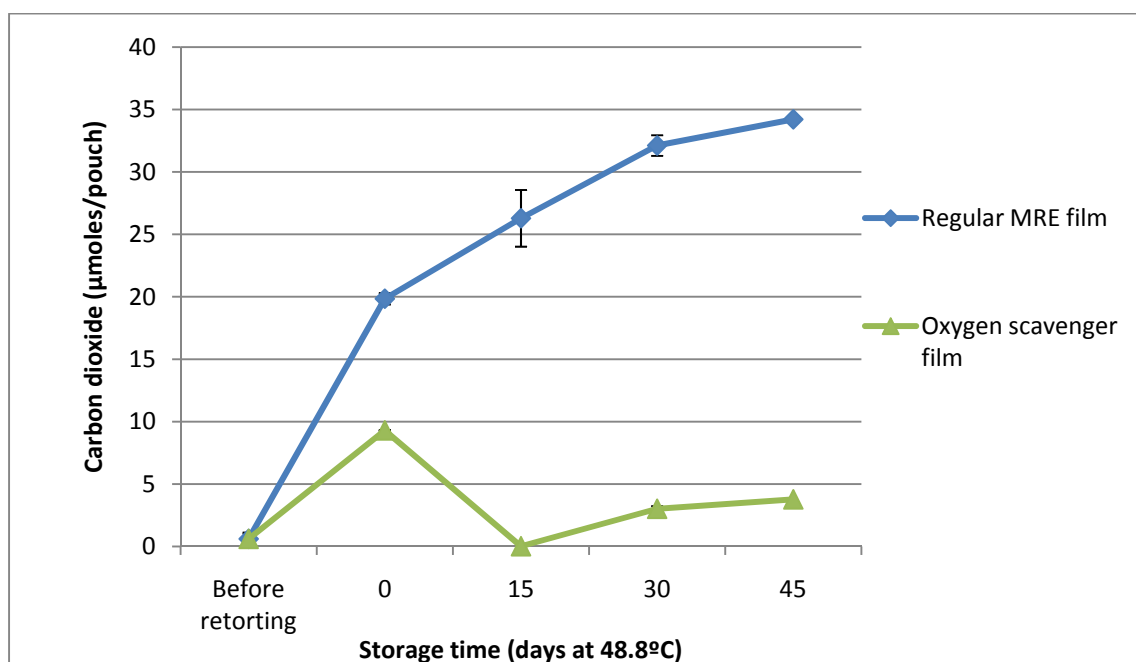
Figure 3.19: Effect of oxygen scavenger pouches

on the formation of carbon dioxide

The effect of the initial concentration of ascorbic acid on the browning of the pears was also studied. Since ascorbic acid degradation plays a significant role in the browning of the product, and it was shown that its degradation rate increases with increasing initial concentration, it would be expected that a lower concentration of ascorbic acid would decrease browning of the product. **Figure 3.20** shows the browning of MRE pears packed in regular pouches and processed under regular conditions but with different initial concentrations of ascorbic acid: 800 ppm, 400 ppm, and approximately 50 ppm (no ascorbic acid added). Additionally, we included the results from the product manufactured by the current Army contractor shown in the previous section (higher initial concentration of ascorbic acid than the specifications but packed with no headspace). **Figure 3.21** shows that there was no significant difference in browning between MRE pears with 800 ppm and 400 ppm of initial concentration of ascorbic acid; MRE pears with no ascorbic acid added show slight significant difference, which vanishes at $\alpha=0.1$. The product manufactured by the current Army contractor was not included in the statistical analysis because it was packed under full vacuum.
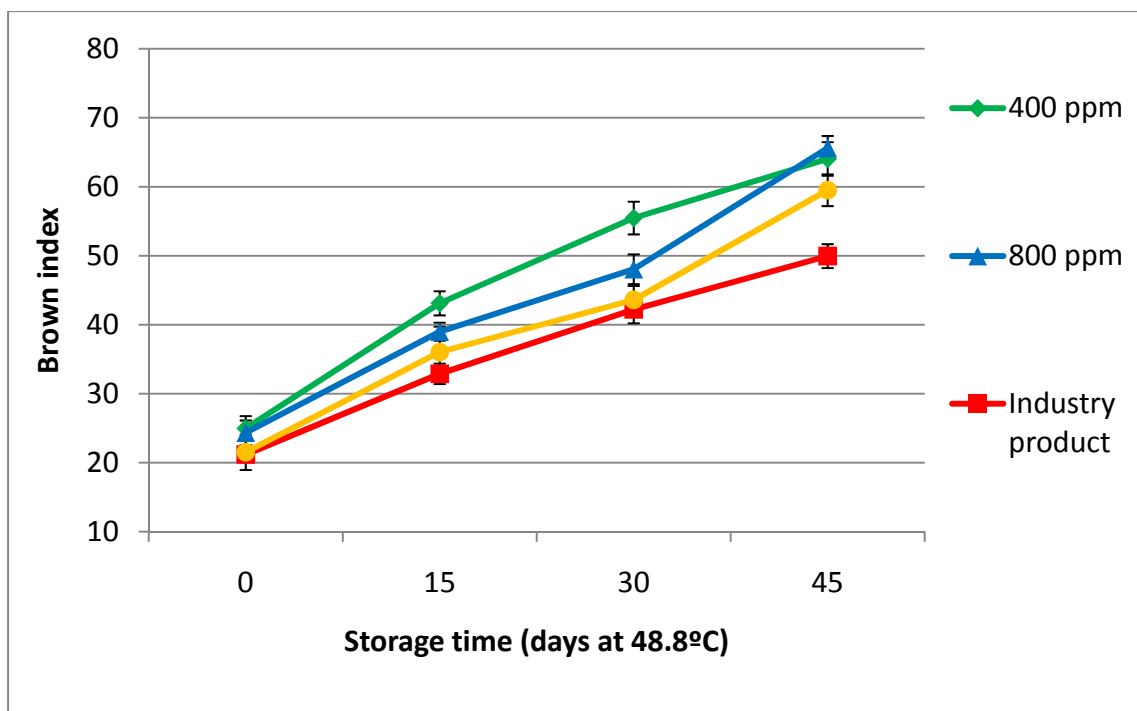
Figure 3.20: Effect of initial concentration of ascorbic acid in browning of MRE pears
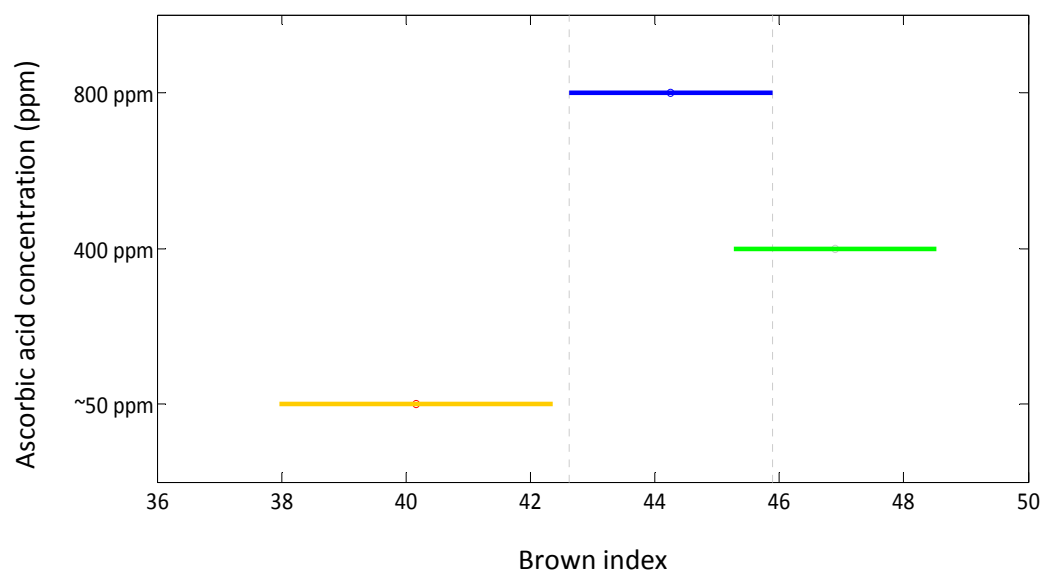
Figure 3.21: Paired comparison of effect of initial concentration of ascorbic acid

on browning

We think that there is no linear correlation between the amount of brown pigments and the browning of the product, since no difference was observed between the samples processed with 800 ppm of ascorbic acid (in which 500 ppm were lost after 45 days) and samples processed with 400 ppm (in which 300 ppm were lost after the same time). Even though the sample processed with no additional ascorbic acid only had 50 ppm at the beginning, the difference in browning was not enough to explain a difference of one order of magnitude in the degradation of ascorbic acid at the end of shelf life. Another possible explanation is the delay in the formation of brown pigments; however that cannot fully explain the small difference in browning of the pears with no additional ascorbic acid and the other samples when the differences in the amount of ascorbic acid degraded during the first 15 days are considered.

4.   Summary and Conclusions

The purpose of this project was to delay the browning and increase the hardness of MRE rations of pears in syrup so they comply with the required shelf life. Headspace analysis showed that most of the oxygen was consumed during thermal processing, and that carbon dioxide was formed throughout the shelf life. Ascorbic acid decreased sharply after thermal processing, and continued to decrease throughout the shelf life due to anaerobic degradation. Oxygen scavenger films, full vacuum packaging and agitated retorting were used as strategies to delay browning.

Increasing the intensity of vacuum packing so that almost all the oxygen was removed from the pouches was the best strategy to reduce the brown color of the MRE rations to acceptable levels at the end of shelf life. Although it did not affect the rate of ascorbic acid degradation during storage, which is an anaerobic process, it reduced the ascorbic acid degradation during retorting as no oxygen was available to react. This delayed the formation of brown pigments long enough so browning of the pears during shelf life was significantly reduced. Oxygen scavenger films did not give consistent results in preserving the color of the pears, and also underperformed full vacuum packaging in protecting ascorbic acid.

Changing the starting material from canned Bartlett pears to fresh D'Anjou pears significantly increased the hardness of the MRE pears. Although no correlation between

hardness and acceptability of the MRE pears could be determined, it was found that

MRE pears with very low hardness values achieved the lowest scores on the sensory

analysis.

5.   Future Work

Although shelf life of MRE pears was improved by prevent aerobic degradation of ascorbic acid, further shelf life extensions would require that anaerobic degradation be also inhibited. Very few studies were available on the anaerobic degradation of ascorbic acid, and they only mentioned that fructose was a promoter. Since fructose is a sugar naturally found in pears and most fruits, a strategy to counter its promoting effects should be identified.

No studies were done to determine the role of Maillard browning. One method to do this could be setting up a model system with the same carbohydrates and amino acids of pears, and at the same water activity and pH levels, but without ascorbic acid. Measurements of hydroxymethylfurfural, hydroxyfurfural and furfural could also give insight on the role of Maillard browning in the overall browning of MRE pears.

The kinetics of ascorbic acid degradation found in this study contradicts all previous studies that were referred. The causes for this are unknown at this point, as the ascorbic acid levels and other conditions were not very different between our study and the previous studies. Degradation kinetics of ascorbic acid in MRE pears should be studied at several initial concentrations in order to better describe its behavior.

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