Influence of Packaging Material and Storage Conditions on the Quality Attributes of Pressure-Assisted Thermally Processed Carrots

A Thesis

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Abstract

Pressure-assisted thermal processing (PATP) is an alternative sterilization technique where elevated pressures (500-700 MPa) and temperatures (90-120 °C) are used for a short time to sterilize low-acid foods. In this research, the influence of barrier properties of packaging materials and storage conditions on selected quality attributes of PATP-treated baby carrots were evaluated. Carrots were vacuum packaged in three different pouches (Nylon/EVOH/EVA, Nylon/EVA and metallized polyester). Pouches were preheated and then immediately processed at 600 MPa and 110 °C for 10 minutes using a pilot scale high pressure food processor. Processed pouches were stored at 25 and 37 °C and withdrawn over 12 weeks of storage on a periodical basis and analyzed for color, β-carotene, and total mesophilic aerobic count. Oxygen transmission rates (OTR), water vapor transmission rates (WTR), melting point and enthalpy of fusion of the packages were evaluated. Dissecting and scanning electron microscope pictures were utilized to document the impact of processing on the packages. Results indicated that chosen processing parameters resulted in shelf stability of processed samples during 12 weeks storage at 25 and 37 °C. Packaging type, storage time and temperature significantly influenced (p < 0.05) product color and β-carotene content. Nylon/EVOH/EVA laminate pouch was the best pouch in terms of preserving color and β-carotene content of the carrot samples. The metallized polyester pouches were damaged by the PATP treatment.
Following 12 weeks of storage increased the OTR of metallized polyester packages drastically (approximately 25 times at 25 °C and 45 times at 37 °C storage), which resulted in considerable changes (p < 0.05) in color and β-carotene content of the carrot samples. After 12 weeks of storage at 37 °C, Nylon/EVA, Nylon/EVOH/EVA and metallized polyester packages lost approximately 100, 36 and 100 % of β-carotene content, respectively. The red color of the carrot samples was reduced by 20, 87 and 72 % for Nylon/EVOH/EVA, Nylon/EVA and metallized polyester, respectively. There were slight changes in the melting points of the some polymers after PATP treatment. EVOH in Nylon/EVOH/EVA and polyethylene in metallized polyester pouch experienced significant decreases (p < 0.05) in the enthalpy of fusion values. Thermal analyses indicated a structural change in the polymers following PATP treatment. In summary, our study demonstrated the importance of utilizing high barrier packaging material for preserving quality attributes of PATP-treated carrots.
Dedication

Dedicated to my parents, brothers and my friends for their unconditional love and support.
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Chapter 1: Introduction

Thermal processing is the technology of choice for preserving shelf-stable low-acid foods. However, in recent years, consumers have shown preferences for minimally processed low-acid food products (Galotto et al 2008). The food industry has been exploring a number of alternative food preservation approaches such as irradiation, microwave sterilization and pressure-assisted thermal processing (PATP) for preserving high quality low-acid foods (Lopez-Rubio et al 2005).

Pressure applications (up to 700 MPa with or without addition of heat) can result in either pasteurization or sterilization of food products depending on the level of pressure and temperature used. When applied in range of 400 to 600 MPa at relatively low temperatures, high pressure processing can be used for pasteurizing food products. This process can decrease the microbial content remarkably. Depending upon the product, enzymes can be inactivated or activated. However, pressure itself at ambient or near temperature is not capable of inactivating spores in low-acid foods (pH> 4.6). For that reason, combining pressure with temperature is needed to achieve microbial inactivation (Galotto et al 2008).

Pressure-assisted thermal processing is essentially a combined process where elevated pressures (500-700 MPa) and process temperatures (90-120 °C) are applied to a preheated food product for a short holding time (Balasubramaniam and Farkas 2008).
2009, FDA issued a no objection letter to an industry petition for sterilization of a low-acid food product processed by PATP. This opens up opportunity for processing a variety of heat sensitive value added products such as soups, coffee, tea, meat entrees, egg products and mashed potatoes. Currently, there is no commercial food product produced with this treatment. However, recent research shows a promising potential for PATP to produce shelf-stable products.

Ninety percent of all high pressure applications use pre-packaged food products (Lambert et al 2000a). Therefore, packaging is very important for the success of the process. Packaging materials must have certain specific characteristics in order to be used in PATP. This includes the ability to withstand the pressure and heat treatment and maintain its integrity after treatment. Any damage or alteration in package as a result of PATP may facilitate a food safety risk (due to post process contamination) or product quality loss. This will also reduce the expected shelf-life of the processed products.

Limited studies have been conducted to investigate the effect of combined pressure-heat treatment on the barrier properties of packaging materials. It is also not well known how the barrier properties of the packages changes during extended storage time. Further, a limited number of studies have investigated how the barrier properties of PATP packaging materials influence the product’s quality during extended storage. Therefore, it is vital to see the effect of PATP on food quality, not only immediately after the process but also after extended storage times.
The main objective of this thesis was to investigate the influence of packaging material and storage conditions on quality attributes of pressure-assisted thermally processed carrots.

The specific objectives included:

- Document the combined pressure-heat effect on barrier properties, thermal properties and the physical structure of selected packaging materials
- Explore the effect of different storage temperatures and times on selected quality attributes of PATP-treated carrots and the barrier properties of chosen packaging materials.

The results generated from our research will be useful to food processors and packaging vendors in identifying suitable packaging materials which can be used for PATP.
Chapter 2: Literature Review

2.1 Pressure-Assisted Thermal Processing

2.1.1 What is High Pressure Processing?

High pressure technology has been successfully used in industries such as chemical, ceramic, plastic, metal forming and isostatic compression of advanced materials for a long time (Balasubramaniam et al 2004). However, its potential application in the food industry was discovered in the middle 1980s (Otero and Sanz 2003).

High pressure processing, also known as high hydrostatic pressure (HHP) or ultra high pressure (UHP) processing, is basically an application of high hydrostatic pressure in the range of 100 to 700 MPa for a period lasting from a few seconds up to several minutes at or near room temperature to the food products in order to inactivate microorganisms or alter product quality attributes. The process generally provides pasteurization type effect. Hydrostatic pressure is defined as isostatic (isobaric) pressure transferred by water. Since all parts of the food product are subjected to the same pressure at exactly the same time, high moisture foods are not generally distorted, damaged or deformed. This novel technique is used in the food industry for both solid and liquid food products to extend shelf-life while minimizing the loss of nutritional quality, texture and flavor (Schauwecker et al 2002). In North America (US, Canada and Mexico), Europe (Spain,
Italy, Portugal, France, UK and Germany), Asia (Japan, China and South Korea) and Australia, HPP has been commercialized for the processing food products (Balasubramaniam et al 2008).

Food products with high moisture content are generally more suited for HPP. On the other hand, foods with porous structure and air pockets such as strawberries and leafy vegetables are damaged by HPP because of difference in compressibility of air and food tissue (Balasubramaniam et al 2008).

Pressure applied during HPP has only a limited effect on covalent bonds and mainly acts on non-covalent bonds such as hydrogen, ionic bonds and hydrophobic interactions, which leads to less severe effect on the chemistry of the food. In addition to that, low molecular weight compounds in foods such as flavors, vitamins and pigments are affected very little by pressure treatment than thermal processing (Balasubramaniam et al 2008). Therefore, pressure treatment not only has the potential to preserve foods but also does not have a severe effect on the quality of the food products when compared with other food preservation methods such as thermal treatment and irradiation (Caner et al 2004)

Examples of commercial products processed by HPP include deli meat, sea food, juice, sauces etc. However, these products on the market are not shelf-stable and they need a secondary protection such as chilling and additional preservatives. This is because of the resistance of bacterial spores to commercially used pressure levels (Leadley et al 2008a). Bacterial spores can survive pressures above 1000 MPa at ambient temperatures (Balasubramaniam and Balasubramaniam 2003).
2.1.2 What is Pressure-Assisted Thermal Processing?

Pressure-assisted thermal processing (PATP), also known as pressure-assisted thermal sterilization (PATS), is a simultaneous application of elevated temperatures (90-120 °C) and pressures (500-700 MPa) to preheated low-acid food products for usually less than 10 minutes to sterilize them (Nguyen et al 2007).

By Feb 2009, FDA issued a no objection letter to the first petition for sterilization of a low-acid product processed by PATP. This opened up opportunities for processing several varieties of heat sensitive value added products such as soups, coffee, tea, meat entrees, egg products and mashed potatoes. Currently, there is no commercial food product produced by this treatment on the market.

Although research is still ongoing on this area, it has been shown that as long as appropriate process conditions such as pressure level, temperature and treatment time are chosen and applied along with expected rapid adiabatic temperature increase, inactivation of vegetative cells and spores are possible and shelf-stable products can be produced (Matser et al 2004). Many authors have proposed using preheating the food sample prior to pressurization to produce a commercially sterile product.

2.1.3 Advantages of Pressure-Assisted Thermal Processing

The main advantages of PATP over conventional heat sterilization are homogeneous heating of the food product caused by adiabatic heating, cooling upon depressurization and relatively shorter process time (faster come-up time and cooling rates). In conventional sterilization, slow heat penetration to the center of the product and
following slow cooling causes quality changes such as texture softening, change in color, vitamin degradation and off-flavors (Krebbers et al 2002a).

If the temperature profiles of PATP and conventional heat sterilization are compared, in PATP, the process time is shorter and the highest temperature level of the product is lower than that of conventional heat sterilization (Matser et al 2004).

2.1.4 Basic Principles of Pressure-Assisted Thermal Processing

A number of principles govern PATP. These include isostatic (Pascal’s principle), Le Chatelier’s and Arrhenious principles.

Isostatic pressure rule (Pascal’s principle) basically means that when pressure is applied through the food product, it can be applied quasi-instantaneous and uniform at the macroscopic level. Application of pressure distribution is also independent of product size and geometry (Cheftel 1995).

According to Le Chatelier’s principle, any physical or chemical change which causes volume decrease such as chemical reactions, phase transitions and change in molecular configurations is enhanced by pressure application and vice versa. In other words, reactions which cause a reduction in volume are encouraged by pressure, whereas the reactions cause an increase in volume are limited.

Arrhenius principle, which is another principle governing PATP states that the rate of reactions is temperature-dependent. Along with pressure, temperature is also a major parameter affecting the rate of reactions under pressure during PATP.
2.1.5 Compression Heating of Food Materials

Depending on the applied pressure level and the food product, there can be a decrease in the volume of processed product up to 10-15% caused by the physical product compression (Caner et al. 2004b). During high pressure processing of solid or liquid foods, there will be an increase in temperature due to adiabatic heating, which is dependent on the level of pressure applied and the composition of the food matrix. This phenomenon is called “heat of compression (δ)”. This temperature increase will be reversed once the decompression phase is reached (Morris et al. 2007).

While, water seems to have the lowest δ (3 °C / 100 MPa) under pressure, oils and fats have the highest δ (9 °C / 100 MPa) (Table 1) due to their molecular structure and phase transition characteristics. Moreover, most food products show similar properties to water under pressure.

Patazca et al. (2007) found that heat of compression values for water increased as initial temperature increased. According to their experiment, δ values of water are influenced by their initial temperature, while in the case of vegetable oil, the heat of compression values are independent of initial temperature (Patazca et al. 2007; Rasanayagam et al. 2003).

2.1.6 Equipment and Typical PATP Batch Procedure

PATP equipment is essentially a batch system. A typical system consists of pressure vessel, top and bottom closure, hydraulic pump, pressure intensifier, heating system and conditioning fluid, pressure and temperature monitoring and control systems and carrier baskets (Figure 1) (Balasubramaniam and Farkas 2008). To prevent corrosion, commercial pressure vessels generally have stainless steel liner.
In order to transmit pressure to food sample uniformly and instantaneously, pressure transmitting fluids are used. Some of the commonly used pressure transmitting fluids include glycol, glycol-water solution, sodium benzoate, and silicone oil (Schauwecker et al 2002).

**Table 1.** Heat of Compression values for different food components and products at 25°C

<table>
<thead>
<tr>
<th>Food Compound or Product</th>
<th>Heat of Compression (°C per 100 MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange juice, 2 % milk and other water like substances</td>
<td>3.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>2.6-3.6&lt;sup&gt;a,1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Proteins</td>
<td>2.7-3.3&lt;sup&gt;a,1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chicken fat</td>
<td>4.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Beef fat</td>
<td>6.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Olive oil</td>
<td>6.3-8.7&lt;sup&gt;a,1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mashed potato</td>
<td>3.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Honey</td>
<td>3.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tofu</td>
<td>3.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Substances show change in δ with increase in pressure
(Source: <sup>a</sup> Somerville 2009; <sup>b</sup> Nguyen 2009; Rasanayagam et al 2003)
Water is the most commonly used pressure transmitting fluid in high pressure pasteurization due to the similar δ to most food products. However, elevated temperature conditions encountered during PATP, prohibit the use of water as a pressure transmitting fluid of choice for PATP applications. Protecting inner vessel surface from corrosion during processing, ability to seal the vessel under pressure, incidental food contact properties, are some of the important points to be considered.

In PATP batch process, food material is first vacuum packaged in flexible, high-barrier containers. Vacuum packaging is done in part to minimize pressure come-up time as well as to reduce any oxidative reactions at elevated pressure-temperature conditions. Vacuum packaged food product is then preheated to certain initial temperature (70-95 °C). This preheating temperature is selected according to the target process temperature under pressure and desired magnitude of pressure. In other words, both preheating and the increase in temperature caused by adiabatic compression are used to reach the target temperature. The preheated product is placed into the pressure vessel and sealed. The wall of the pressure vessel is also preheated to the desired process temperature to minimize the heat exchange to the surrounding.

Preheated pressure transmitting fluid is pumped into the vessel and air in the system is removed. After air removal, vent valve is closed and pressure intensifier pumps the pressurization fluid until targeted pressure is reached. The time spent to increase the pressure from initial level (P₁) to target pressure (P₂) (Figure 2) is called “pressure come up time”. During this come up time, temperature of the preheated food sample increases to targeted temperature level with the contribution of compression heating.
Once the targeted pressure and temperature levels are reached, pressure and temperature
is maintained for a desired length of time, which is called “pressure holding time”.
After the holding time, pressure is released and temperature decreases to $T_f$ (Figure 2). If
the pressure vessel is insulated sufficiently, there will be no heat loss during holding time
and initial and final temperatures should be about the same. However, if pressure vessel
is not insulated well enough, heat lost from the food product to the vessel and
environment will cause the final temperature to be less than initial temperature.

Once the pressure in the vessel is decreased to atmospheric pressure level, the chamber is
opened and the food products are removed by using the basket. It is recommended to cool
down the food product quickly to prevent further thermal degradation.

In PATP, the heat of compression and final temperature during the process can be
calculated by using the following equation (Rasanayagam et al. 2003).

$$T_1 = T_2 - (CH \cdot \Delta P + \Delta T_h)$$  \hspace{2cm} (2.1)

$T_1$ = Initial temperature

$T_2$ = Target temperature under pressure

$CH$= Heat of compression value of the sample (defined as temperature increase per 100
MPa)

$\Delta P$= Process pressure

$\Delta T_h$= Temperature gained or lost by the test sample from the surrounding fluid bath
during pressure process time and early stages of pressure holding time (Nguyen et al.
2007).
Figure 1. Schematic diagram of pressure-assisted thermal processing equipment
2.2 Packaging

2.2.1 Packaging Requirements and Importance of Packaging for PATP

Packaging is a vital factor for any preservation methods to be successful since it protects the food products from adverse environmental conditions (Ozen et al 2001). For that reason, it is also very essential for the success of PATP. Packaging material needs to preserve the qualities of the treated product and maintain its mechanical performance,
consumer convenience and built-in features (Lambert et al 2000). Nature of the food products, type of the systems and process conditions are some key points for package selection (Somerville 2009).

Packaging requirement for HPP shows differences based on type of equipment, whether it is semi-continuous or batch operation. However, due to technical challenges, there is no semi-continuous system available for PATP, yet.

For semi-continuous operations, product is not packaged before the process, so package is not exposed to high pressure. In this case, after processing, food product is packaged aseptically to ensure shelf-life stability. On the other hand, PATP is essentially a batch process in which food material is packaged before the process to avoid any contamination from the pressure medium and to improve processing efficiency. Therefore, both product and package will be exposed to high pressure (Schauwecker et al 2002). Product is often vacuum packaged and packaged food is preheated to initial temperature. Then, the packaged product is subjected to combined pressure-heat treatment. Using proper packaging material with suitable barrier properties is very important to keep the product quality attributes during extended storage and distribution (Sorretino et al 2007).

One of the main packaging requirements for PATP is that package needs to endure the high pressure and high temperature levels that it is exposed to during the process and it also needs to sustain the physical integrity and sealing both during the process and shelf-life. Because of the requirements mentioned above, some packages such as metal cans and glass bottles are not suitable for the process. Metal cans collapse permanently and glass bottles are likely to break under high pressure. Moreover, packages which are made of paperboard are not suitable for PATP either, since they can degrade under pressure,
too (Caner et al. 2004a). Typically, high-barrier flexible pouch made of polymers or copolymers with at least one flexible side can be used for processing solid or liquid food products by batch PATP systems.

Furthermore, vacuum packaging is very significant for a uniform-treatment because air in the package has higher compressibility than food products and this will lead to non-uniform treatment and package deformation (Lopez-Rubio et al. 2005). Additionally, vacuum packaging can avoid oxygen related reactions including lipid oxidation during the processing. Minimizing the air in the package can also improve the loading factor which means more packaged food products can be processed at one run. Vacuum packaging of low-acid foods such as carrots has the advantage of reducing the total microbial load compared to non-vacuum packaged carrots due to microaerophilic and fermentative environment of the vacuum packaged food product (Orsat et al. 2001).

Package size and shape is critical in terms of maximizing the number of packages which can be fit in the chamber. Proper package design can contribute to processing economically (Nguyen, 2009).

Another importance of proper selection of materials and packaging technologies for the success of PATP is based on the properties of the package. It can be either possible to retain the quality and freshness of the product throughout the shelf life or not (Sorrentino et al. 2007). Although, most studies show that commonly used packaging materials are not affected significantly by high pressure alone, when it is combined with temperature, some changes will occur in the package, which can cause quality degradation on food products, particularly during storage.
2.2.2 Effect of Pressure on Package

Water and oxygen are two of the most important components in food which determine the quality and shelf life (Galotto et al 2008). For instance, foods that are sensitive to change in moisture can spoil quickly or lose its characteristics by either absorbing the water or losing the water and foods that are sensitive to change in oxygen presence can become rancid (Yoo et al 2009). Therefore, barrier properties of polymers are very vital considerations for shelf life and type of polymer chosen plays a crucial role at this point. Developing packaging material with improved water, oxygen and light impermeable properties is essential to ensure desired shelf life for PATP.

During the process, temperature increases and the volume of the package decreases because of the pressure applied. Normally, this decrease in volume is expected to be temporary and once the pressure is released, the package needs to recover its original condition. However, according to the research done in this area, this is not always true and particularly some composite flexible materials can be damaged by the process (Caner et al 2004). Depending on the film composition and the PATP conditions, some changes can be observed in the package (Caner et al 2000). For a successful process, barrier, mechanical and mass transfer properties of the package must be resistant to this changes occurred during the process. This is possible if only the thermo-mechanical stresses generated during combined pressure-heat treatment is within the limit that the package can go back to its original condition (Caner et al 2004b).

Polymers have repeating functional groups in their structures and these groups help the formation of crystalline regions. As opposed to amorphous regions (void spaces) in the
structure, atmospheric gases as well as organic compounds cannot penetrate from crystalline regions. Therefore, high crystallinity in a polymer can result in better barrier to oxygen, water vapor, carbon dioxide and organic compounds. Additionally, increase in crystallinity in a polymer improves its strength and stiffness (Schauwecker 2001). For instance, EVOH is known as high oxygen barrier and this property is a result of hydroxyl groups in its structure, which tend to make high degree of hydrogen bonds and reduce the free volume between the chains in the polymer. This results in higher barrier to gas exchange. However, these hydroxyl groups also make the copolymers sensitive to water. That is why, barrier properties of EVOH are weakened in high relative humidity (Lopez-Rubio 2005). Mertens (1993) applied high pressure on EVOH and observed 15 and 6 % reduction in OTR and WVTR, respectively. Similarly, Kovarskii (1994) evaluated the effect of HPP on PET and reported 70 and 25 % decreases in OTR and WVTR, respectively. Le-Bail (2006) tested LDPE, which is the packaging commonly used for HPP applications. They found that barrier properties were not significantly affected and even a little improved by HPP. Masuda et al (1992) reported no significant change in barrier properties of tested PP/EVOH/PP, OPP/EVOH/PE, PVDC-coated OPP/CPP at 400 and 600 MPa for 10 minutes. Similarly, Caner et al (2000) processed PET/SiO$_2$/LDPE, PET/Al$_2$O$_3$/LDPE, PET/PVDC/Nylon/PE, Met-PET/EVA/LLDPE, PP/Nylon/PP and PET/EVA/PET at 600 and 800 MPa for 5, 10 and 20 minutes. They found no statistical difference in barrier properties except Met-PET film. However, some recent studies have indicated that there can be some loss in barrier properties of flexible packaging materials. It was reported that metallized films were damaged by high pressure applications and their barrier properties were impaired. Galotto
et al (2003) reported a significant increase in OTR of metallized pouch and they reported that the increase in OTR was due to the damage in the metallized surface coating. Similarly, Caner et al (2003) also showed that there was up to 150 % barrier loss for MET-PET film after processing at 600 MPa and 45 °C.

According to the research conducted by Caner (2003), no major impact of pressure alone was found in tensile strength, elongation and modulus of elasticity for the tested packaging films. Mertens et al (1993) also evaluated the mechanical properties of LLDPE/EVA, EVOH/EVA/LLDPE and PET/Al foil/PP after high pressure applications at 400 MPa and 60 °C. They reported no change in mechanical properties including tensile strength and elongation between control and HPP-treated films. Moreover, there can be some increase in the tensile strength of the packages after high-pressure processing irrespective of the pressure level, the initial rigidity and the thickness of the package. This shows that the package becomes more rigid and less flexible (Lambert et al 2000a).

It was found that HPP has no significant effect on the diffusion of food components into a polymer packaging material (Kuebel et al 1996).

As summarized above, many articles showed that high pressure itself does not considerably affect the barrier properties of the packaging structures commonly used in food industry (Lopez-Rubio et al 2005). However, if the adhesion between the layers of multilayer structure of the packaging film is affected by processing, gaps may appear within the structure resulting in loss of integrity and therefore the safety problems for the packages made from this kind of films (Caner et al 2003).
Research related to the effects of high-pressure processing on food quality and package has been mostly conducted either at room or moderate temperatures (Wilson et al 2008). However, depending on the processing conditions, some properties of the packaging material can be changed and any change on the properties of the package can affect the quality of the food products (Lambert et al 2000b).

2.3 Quality of PATP Products

Among the preservation techniques, heat preservation is known as the oldest form which can reduce the number of microorganisms and inhibit enzymatic activity. However, thermal treatment alone may adversely affect quality properties of food products such as flavor, texture, color and nutritional quality (Orsat et al 2001). In traditional thermal processing such as sterilization, it takes a long time for heat to penetrate to the core of the product as well as extended time is necessary for product to cool down. Therefore, thermal processing provokes quality changes such as color, vitamin degradation, off-flavor formation and texture softening (Krebbers et al 2002b).

On the other hand, high pressure treatment alone is not capable of inactivating bacterial spores and some pressure-resistant enzymes. That is why for low-acid food products combination of pressure and other processing variables such as heat in the case of PATP is required.

In order to increase the shelf-life and salability of food products, storage conditions need to be chosen accurately (Orsat et al 2001). Storage conditions can affect the product differently. For instance, temperature can cause spoilage of the food product. High storage temperature can accelerate the interactions between the environment and
packaged food or it can speed up the reactions taking place in the product itself (e.g. β-carotene degradation), which shortens the shelf life of the food product.

2.3.1 Color

One of the major quality characteristics of fruits and vegetables is color. From consumers’ point of view, color is very important parameter which shows the product quality and it is extensively affected by thermal sterilization (Leadley et al 2008b).

Pigments responsible for the color of fruits and vegetables such as chlorophylls, carotenoids and anthocyananins are not significantly affected by high pressure (Oey et al 2008). Generally, green color of vegetables becomes stronger, most likely because of cell disruption occurred during high pressure processing, which causes chlorophyll to flow into intercellular space. However, during the storage, the green color of vegetables turns into a pale yellow color possibly due to chemical reactions such as oxidation (Islam, 2007). Chlorophyll shows extreme pressure stability. However, at temperature levels higher than 50 °C, increase in either pressure or temperature while one of them is kept constant, speeds up the degradation of chlorophyll a and b (Islam, 2007).

Apart from color pigments, browning can also cause discoloration of PATP-treated food products. According to a research conducted by Leadley (2008), the color of PATP-treated green bean samples were not better than traditionally sterilized samples and PATP samples were even darker and worse than canned samples in some cases.

Krebbers et al (2002) conducted a research and applied several preservation methods to green beans including high pressure processing and PATP to observe the change in color. They concluded that color of both heat sterilized and PATP-treated beans were stable during one month storage period.
2.3.2 Vitamin

Currently, it is known that high pressure application at moderate temperatures does not affect the vitamin content of fruits and vegetables remarkably but some degradation may occur in the case of PATP, where high temperature levels are involved (Islam, 2007). High temperature levels as well as high pressure can speed up the chemical reactions which affect the vitamin stability. There is limited number of studies available about the effect of high pressure on some vitamins, particularly about fat-soluble vitamins. Further research is needed to understand the mechanism of vitamin stability especially at elevated heat and pressure combinations (Oey et al 2008). Tauscher (1998) reported that during pressure treatment at 600 MPa and 75 °C for 40 minutes, carotene loss in carrots was low, whereas Nguyen et al (2007) reported that PATP treatment reduced the carotene content of carrot samples considerably at 105 °C at 500 and 700 MPa. Although, they reported that carotene retention was higher for PATP samples than thermally processed samples, there was still significant reduction in carotene content caused by PATP treatment. For PATP, different pressure and temperature combinations can be applied in order to have the desired effects on color and vitamin content of food products.

2.3.2 Texture

Texture is a very important parameter which shows product quality and it is significantly affected by thermal sterilization.

During ripening, processing and storage, texture is changed mainly because of the biochemical changes in pectin. The importance of pectin in terms of texture is that it is abundant in the plant middle lamella and help cell-cell adhesion. Furthermore, it can be
solubilized easily and it is more chemically reactive compared to other cell wall polymers, which causes the texture degradation (Trejo Araya et al 2007). If the forces maintaining the cell structure is stronger than the cell walls, there will be a cell breakage, damage or cell separation and vice versa (Roeck et al 2008).

According to Rastogi (2008), preheating the food product to a temperature between 50-90 °C prior to PATP, improves the texture of carrots. During processing, due to cell wall breakdown and loss of turgidity, tissue firmness is lost.

Krebbers et al (2002) found in their experiment that only 3 % of the original firmness of green beans remained after conventional sterilization; whereas, they found that 60 % of the original firmness was kept after high pressure pasteurization. It is probably due to the lower temperature and shorter time of processing, which also results in less β-elimination of pectin. In other word, β-elimination of pectin is temperature dependent and the higher the temperature, the more β-elimination of pectin will be observed and this will cause more softening in processed products.

Leadley et al (2007) conducted an experiment to compare the quality attributes of PATP-treated and thermally sterilized green beans. Their results showed a clear improvement in texture of PATP-treated green beans. PATP-treated beans were firmer than retorted samples both right after processing and 7 months storage. PATP-treated samples were nearly twice as firm as their retorted counterparts (Leadley et al 2008a).

Rastogi (2008) found in their experiment that relative hardness of PATP-treated carrots at 500 MPa and 105 °C was 16 %, whereas it was 4 % for thermal processing at 105 °C.

De Oreck et al (2008) stated in their experiments that PATP-treated samples did not undergo further softening as the treatment time increased as opposed to thermally treated
carrots, which experienced an increasing softening. This suggests that the combination of pressure and heat inhibits the beta-elimination of pectin.

Significantly different texture results can be obtained from various experiments depending on processing temperature and degree of β-elimination of pectin.

2.3.3 Microbiological Quality

Survival of bacterial spores will be the main concern if an alternative sterilization technique is used to produce shelf-stable food products. A number of researchers have shown that various bacterial spores including *Bacillus* spp. and *Clostridium* spp. are successfully inactivated by the simultaneous application of pressure (500-700 MPa) and heat (90-121 °C) for a short duration (≤ 10 min) (Rajan et al 2006; Ahn et al 2007; Ratphitagsanti et al 2009). In general, the level of spore inactivation increases with the increase of pressure, temperature, and holding time. Pressure pulsing has also been reported to enhance the PATP lethality (Ratphitagsanti et al 2009). Food composition and pH can also influence spore inactivation by PATP (Raso et al 1998; Roberts and Hoover 1996). Vegetative bacteria are easily inactivated by PATP even at less severe pressure-thermal conditions.
2.4 REFERENCES


Chapter 3: Influence of packaging material and storage conditions on quality attributes of pressure-assisted thermally processed carrots

3.1 Abstract

Pressure-assisted thermal processing (PATP) is an alternative sterilization technique where elevated pressures (500-700 MPa) and temperatures (90-120 °C) are used for a short time to sterilize low-acid foods. This study was conducted to evaluate the influence of barrier properties of packaging materials and storage conditions on selected quality attributes of carrot samples processed by PATP. Baby carrots were vacuum packaged in pouches made from three different film types (Nylon/EVOH/EVA, Nylon/EVA and metallized polyester). The pouches were preheated in 90 °C water kettle for 15 minutes and then immediately processed at 600 MPa and 110 °C for 10 minutes using a pilot scale high pressure food processor. Processed pouches were stored at 25 and 37 °C and withdrawn over 12 weeks of storage on a periodical basis and analyzed for color, β-carotene, and total mesophilic aerobic count. Oxygen transmission rates (OTR), water vapor transmission rates (WTR), melting point and enthalpy of fusion of the packages were also evaluated. Dissecting and scanning electron microscope pictures were utilized to document the impact of processing on the packages. Results indicated shelf stability of the processed samples during 12 weeks storage at 25 and 37 °C. Packaging type, storage temperature and time significantly influenced (p < 0.05) product color and β-carotene
content. Nylon/EVOH/EVA laminate pouch best preserved color and β-carotene. PATP increased OTR of metallized polyester packaging. This might have caused considerable change in color and β-carotene content of carrot samples after 12 weeks storage. After 12 weeks of storage at 37 °C, Nylon/EVA, Nylon/EVOH/EVA and metallized polyester packages lost 100, 36 and 100 % of β-carotene content respectively. The red color of carrot samples was reduced by 20, 87 and 72 % for Nylon/EVOH/EVA, Nylon/EVA and metallized polyester, respectively. Thermal analyses indicated a structural change in the packaging polymers following PATP treatment. In summary, our study demonstrated the importance of utilizing high barrier packaging material for preserving quality attributes of PATP-treated carrots.

3.2 Introduction

Thermal processing has been used for a long time to preserve low-acid food products. However, in recent years, consumers have shown preferences for minimally processed low-acid food products (Galotto et al 2008). The food industry has been exploring a number of alternative food preservation approaches such as irradiation, microwave sterilization, pressure-assisted thermal processing for preserving high quality low-acid foods (Lopez-Rubio et al 2005).

Pressure applications (up to 700 MPa with or without addition of heat) can result in either pasteurization or sterilization of food products depending on the level of pressure and temperature used (Balasubramaniam and Farkas 2008). When applied in the range of 400 to 600 MPa at relatively low temperatures, high pressure processing can be used to
pasteurize food products. This process can decrease the microbial content remarkably. Depending upon the product, enzymes can be inactivated or activated. However, pressure itself at or near ambient temperature is not capable of inactivating spores in low-acid foods (pH > 4.6). For that reason, combining pressure with elevated temperature is needed to achieve microbial inactivation (Galotto et al, 2008).

Pressure-assisted thermal processing (PATP) is essentially a combined process where elevated pressures (up to 700 MPa) and process temperatures (90-120 °C) are used for a short time to sterilize low-acid foods (Balasubramaniam and Farkas, 2008). In February 2009, FDA issued a no objection letter to an industry petition for sterilization of a low-acid food product processed by PATP. This opens up opportunity for processing a variety of heat sensitive value added products such as soups, coffee, tea, meat entrees, egg products and mashed potatoes. Currently, there is no commercial food product produced with this treatment.

Limited studies evaluated the impact of combined pressure heat treatment on barrier properties of the packaging material. It is further not well known how the barrier properties of the packages changes during extended storage period. Further, very limited studies investigated how the barrier properties of the PATP packaging material influence the product quality during extended storage. Therefore, it is vital to see the effect of PATP on food quality, not only immediately after the process but also after extended storage times.
The main objective of this thesis was to investigate the influence of packaging material and storage conditions on quality attributes of pressure-assisted thermally processed carrots.

The specific objectives included:

- Document the combined pressure-heat effect on barrier properties, thermal properties and physical structure of selected packaging materials.
- Explore the effect of different storage temperatures and storage times on selected quality attributes of PATP-treated carrots and barrier properties of the chosen packaging materials.

The results generated from our research will be useful for the food processors and packaging vendors in identifying suitable packaging material which can be used for PATP.

3.3 Materials and Methods

3.3.1 Samples

Baby carrots (*Daucus carota* L.) were sourced from a local grocery store in Columbus, Ohio and stored at refrigerated temperatures for up to three days before processing. All carrots were purchased at the same time and from the same store for the purpose of minimizing the variation in quality, source and age of the raw carrots. Samples were cleaned and undesired parts were removed.
3.3.2 Package Selection

Packaging materials used for the research were provided by a commercial packaging company (Appleton, Wisconsin). These packaging materials were (1) Nylon/EVOH/EVA (2) Nylon/EVA (3) Metalized polyester/Adhesive/Modified Polyethylene sealant. The film thicknesses were 3.0 mils for Nylon/EVOH/EVA and Nylon/EVA and 2.6 mils for metallized polyester. Water vapor transmission rates were reasonably similar for all three pouches tested whereas Nylon/EVOH/EVA and metallized polyester pouches had very low oxygen transmission rates.

3.3.3 Sample Preparation

The packaging films were used to make pouches (6 × 8 inches dimension). Each sample pouch was filled with 200 g baby carrots (approximately 16 baby carrots) suspended in 1 % NaCl solution (2 g NaCl / 200 ml distilled water). This helped to minimize any loss of solids from the carrots to the covering liquid (verified by preliminary experiments). The ratio of sample to NaCl solution was 1:1. The pouches were sealed by a vacuum packaging machine (Ultravac, UV 250, Koch Supplies Inc., MO, USA). Processed samples were analyzed as outlined in Figure 3.

3.3.4 Pressure-Assisted Thermal Processing

Pressure-assisted thermal processing experiments were conducted using a 5-liter capacity High Pressure Food Processor (Iso-Lab FPG11500 Standsted Fluid Power Ltd, Essex, UK). Propylene glycol (Brenntag Mid-South, Inc., St. Louis, MO) was used as the pressure transmitting fluid.
Initially, vacuum packaged pouches were preheated to 86 ± 2 °C in a water kettle prior to processing. The initial temperature of the sample was adjusted taking the anticipated heat of compression into account. A K-type thermocouple (Omega Engineering, CT, USA) inserted into the geometric center of the baby carrots was used to monitor sample temperature during preheating. Carrot samples were placed in a preheated cylindrical stainless steel loading basket (11 cm × 65.5 cm) containing preheated USP kosher polypropylene glycol. The loading basket was placed in high pressure system and samples were treated. During the process, temperature was monitored at different locations (top, center and bottom) of the carrier basket by using T-type thermocouples (Omega engineering, CT, USA). Thermocouples were mounted in the carrier basket by using a C-5.2 stuffing box (Ecklund-Harrison Technologies, FL, USA). Pressure come-up time was approximately 2 minutes, holding time was 10 minutes at 110 °C and the decompression cycle was 90 seconds. After decompression, the basket was removed from the chamber and samples were suspended in an ice-water bath to cool them down to room temperature to avoid any further thermal degradation. The untreated pouches were used as control samples. The processed samples were immediately stored in the dark at two different temperatures (24 ± 1 °C and 37 ± 1 °C) and analyzed for color, β-carotene, microbial stability, oxygen and water vapor transmission rates, thermal properties of the packages and visible changes on the package surface during 12 weeks storage.

Additionally, in order to evaluate the effect of high pressure only on the thermal properties of the packaging materials, pouches were also processed at 600 MPa for 10 minutes at room temperature.
Figure 3. Experimental design for pressure-assisted thermal processing of carrots
3.3.5 Analysis

Color Measurement

The influence of PATP treatment, packaging material type, storage time and temperature on color of the samples were evaluated using a tristimulus colorimeter (CR-300, Minolta, Osaka, Japan). The colorimeter was calibrated by using the standard white tile (Y=92.6, X=0.3161, y=0.3321). Baby carrots were chosen randomly from the different pouches to minimize inherent sample-sample biological variations and the analyses were conducted in the same room and the same location every time to avoid variations. Each data point was an average of at least 30 measurements. After calibration, the baby carrots were placed on a white paper towel and each carrot sample was measured individually. Sufficiently large size carrot samples were used so that aperture of the calorimeter recorded only the color of the carrot samples. L, a, b and ΔE values were measured. L represents the lightness, +a represents the red direction, -a represents the green direction, +b represents the yellow direction and -b represents the blue direction. The overall change in color (ΔE) was calculated from the following formula (Avila and Silva 1999):

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$  \hspace{1cm} (3.1)

β-carotene Analyses

β-carotene analyses were performed using the samples collected for weeks 0, 1, 2, 4, 8 and 12 of storage as well as untreated raw carrots in duplicates. β-carotene content was determined by using the method described by Ferruzzi et al (1998). Five grams of blended baby carrots were put into a 150 mm beaker. One g calcium bicarbonate and 4.0
g celite were also added into the beaker to help filtration. Fifty ml methanol was added and the mixture was led to stand for around 1 minute. Then, the mixture was homogenized for approximately 1 minute. The sample was filtered using Whatman paper size #1 and #42. The filtrate was collected and filtrant was removed. A 50 ml aliquot of 1:1 acetone/hexane solution was added to the filtrate and the mixture was allowed to stand for 1 minute (at room temperature). Homogenizing, filtering and collecting filtrate were repeated 3 times. Then, the filtrate was transferred to a separatory funnel. The layers were washed with approximately 20 ml distilled water. Bottom layer was discarded and the top layer was filtered through glass wool containing sodium sulfate and rinsed with hexane into a 100 ml flask and filled to top with hexane. The concentration of β-carotene in the solution was determined spectrophotometrically (HP 8453 UV–Visible Spectrophotometer, Agilent Technologies, Palo Alto, CA) at 450 nm. The total carotenoid content determined as β-carotene standard.

**Microbial Plate Counting**

The total aerobic mesophilic counts of the raw and processed carrot samples were determined. Microbial analyses were performed using PATP-treated samples during the weeks 0, 1, 2, 4, 8 and 12 of storage in duplicates. Each pouch was opened under aseptic conditions. Two baby carrots were randomly taken from the pouch and weighed in a filtered stomacher bag. The same amount of covering solution was then added to the bag. The samples were then homogenized in a stomacher (Seward Lab Stomacher, Norfolk, UK) at 230 rpm for 2 min. Serial dilutions were prepared in 0.1 % (w/v) sterilized peptone water. The 0.1 ml aliquots of the appropriate dilutions were then spread-plated on duplicate trypticase soy agar (TSA) and the colonies of survivors were enumerated.
after incubation at 37 °C for 72 hours under aerobic conditions. The technique had a detection limit of 10 colony forming units (CFU) per gram.

**Oxygen Permeability Test**

Changes in oxygen permeability of unprocessed and processed pouches during extended storage time were analyzed. The oxygen transmission rate (OTR) was determined using an Ox-Tran 2/20 (Modern Control, Inc., Minneapolis, MN) according to the ASTM D3985 method (ASTM 2005). Two 50 cm² specimens were tested for each sample at 23 °C and 50 % RH. At least two pouches were analyzed for the same type of material for each week of storage. Average of the duplicates were calculated and reported.

**Water Vapor Permeability Test**

For water vapor permeability analyzes, pouches with no treatment, preheated only, PATP-treated and stored for 4 and 12 weeks were analyzed. The water vapor transmission rate (WVTR) was determined using a PermaTran 3/31 (Modern Control, Inc., Minneapolis, MN) following the ASTM F1249 method (ASTM 2006). Two 50 cm² specimens were tested for each sample at 37.8 °C and 90 % RH. At least two pouches were analyzed for the same type of material for each week of storage. Average of the duplicates were calculated and reported.

**Thermal Analysis**

A model 2920 TA Instruments Modulated Scanning Calorimeter (DSC) (New Castle, DE) was used to determine the effect of PATP and storage on thermal transition of Nylon / EVOH / EVA, Nylon / EVA and metallized polyester pouches. The analysis was conducted at a constant heating rate of 10 °C/min from -30 to 300 °C, modified from
ASTM E794-798 (ASTM 2006). Melting temperature (T\textsubscript{m}) and heat of fusion (ΔH) were calculated from the DSC thermograms using the software (TA Universal Analysis) from the instrument manufacturer. The results were the average of 4 replications.

**Dissecting Stereo Microscopy**

Olympus SZH dissecting stereo microscope with 2X objective was used to determine the overall impact of PATP and storage on the test films. Olympus Magnafire Digital Camera was also used to take the pictures of packaging films.

**Scanning Electron Microscopy (SEM)**

Packaging films for control (untreated) and PATP samples were analyzed using a Scanning Electron Microscope (Nova NanoSEM 400, FEI Company, Hillsboro, OR). The test films were prepared by cutting 2 cm × 3 cm strips from each pouch. Then, each strip was mounted in the microscope specimen holder in the chamber and covered with gold-palladium in the Cressington 108 Sputter Coater. The samples were observed at 5 kV voltages at magnifications from x300 to x10,000. A representative number of pictures was taken.

**Data analysis**

Statistical analysis of data was performed using Minitab Statistical software version 16 (Minitab, Inc., State College, PA). For the analyzes of lightness, a values, b values, β-carotene content of the carrots and oxygen and water vapor permeability of the pouches, the data was separated into two groups (before storage and during storage). Then,
General Linear Model (GLM) was at 95 % confidence was used for statistical analysis. The independent variables included treatment type (untreated, preheated only and PATP-treatment), pouch type (Nylon/EVOH/EVA, Nylon/EVA and Metalized Polyester), storage temperature (25 and 37 °C) and storage time (1, 2, 4, 8, 12 weeks). The change in color parameters (L, a, b and ΔE) and β-carotene of the carrots as well as OTR and WVTR of the packaging materials were the dependent variables. The influence of treatments, packaging materials, storage temperatures and time on selected quality attributes (color, β-carotene) of the carrot samples and permeability of packaging films (oxygen and water vapor) were investigated.

In addition, the influence of HPP and PATP on the melting points and heat of fusions (ΔH) of the polymers were analyzed using One-way ANOVA with Tukey’s test at 95 % confidence interval. Significance for all statistical analysis was defined as p < 0.05.

3.4 RESULTS AND DISCUSSION

3.4.1 Temperature and pressure history during PATP

Figure 4 represents the typical pressure-temperature history during preheating and pressure-assisted thermal processing of carrot samples. By preheating the pouches in 90 °C water kettle for 15 minutes, temperature of the carrots increased to 86 ± 2 °C. Following the preheating, carrot samples lost about 1 °C during transfer from water kettle to high pressure processor.

When pressure started to build on, carrot samples experienced a rapid increase in temperature during the pressurization as a result of heat of compression (Figure 4). It took approximately 2 min for carrots to reach the targeted processing conditions. In 2
min, pressure and temperature of the carrots reached to $600 \pm 5 \text{ MPa}$ and $110 \pm 2.5 \degree \text{C}$, respectively. During 10 min holding time, temperature and pressure were maintained at or slightly above of the targeted levels. During the decompression, temperatures of carrots quickly decreased to about $85 \degree \text{C}$ or slightly below. The slight decrease compared to initial temperature was due to the heat lost from carrots to the vessel and to environment.

**Figure 4.** Sample temperature-pressure history for carrot samples processed at $600 \text{ MPa}$, $110 \degree \text{C}$ for 10 min.
3.4.2 Color

The effects of preheating, PATP and storage on carrot color were determined for the carrot samples. Changes in lightness (L) (Figure 5), redness-greenness ($a$) (Figure 6), yellowness-blueness ($b$) (Figure 7) and overall change ($\Delta E$) (Figure 8) are shown.

Preheating and following PATP treatment caused statistically significant changes ($p < 0.05$) in lightness of the carrot samples (Figure 5). Additionally, pouch type, storage temperature and time also affected the lightness of the carrots significantly during storage ($p < 0.05$).

**Figure 5.** Lightness value for carrots as influenced by packaging type, process treatment and storage conditions. A: Nylon / EVOH / EVA; B: Nylon / EVA; C: Metallized polyester
Figure 6 illustrates that preheating the carrots prior to PATP caused a significant decrease (p < 0.05) in a values of carrots in all three types of packaging films tested at both storage temperatures. As expected, the level of decrease as a result of preheating and PATP treatments were quite similar among the pouches. There was a slight decrease in a value of preheated samples followed by PATP treatment.

When the effect of storage was analyzed, variations could be seen among the pouches in terms of preserving the red and yellow color of processed carrots. Nylon/EVOH/EVA laminate preserved the red color of the carrots, the most at either 25 or 37 °C for 12 weeks storage. On the other hand, Nylon/EVA and metallized polyester pouches experienced a rapid decrease in a values at 37 °C during the storage. For these two type of pouches, a values were more stable at 25 °C and there was no statistical difference (p > 0.05) compared to beginning of storage (week 0). It was also observed that between Nylon/EVA and metallized polyester pouches, the reduction in redness at 37 °C was more severe for Nylon/EVA laminate.
Figure 6. $a$ values for carrots in different pouches and storage temperature. A: Nylon / EVOH / EVA; B: Nylon / EVA; C: Metallized polyester

Figure 7 presents changes in the $b$ value of carrot samples as influenced by preheating, PATP treatment, packaging type and storage conditions. Preheating the samples significantly ($p < 0.05$) reduced the $b$ value for all pouches tested. During storage, Nylon/EVOH/EVA laminate pouch preserved the yellow color of carrots best at both 25 and 37 °C for 12 weeks storage. For Nylon/EVA and metallized polyester pouch, it was observed that both pouches experienced a decrease in the $b$ value and it was more severe at 37 °C.
Figure 7. $b$ values for carrots in different pouches and storage temperatures. A: Nylon / EVOH / EVA; B: Nylon / EVA; C: Metallized polyester

Total color change of carrots was also calculated. It should be noted that there was not a drastic change in color of the carrots in Nylon/EVOH/EVA laminated pouch at either 25 or 37 °C (Figure 8). However, more considerable change ($p < 0.05$) for the color of carrots in Nylon/EVA pouch was observed, especially at 37 °C during the storage. The same trend was seen for metallized polyester pouch. The change in color was significant ($p < 0.05$) at both 25 and 37 °C. However, total color change was more pronounced at 37 °C storage.
Figure 8. Total color change (ΔE) for carrots in different pouches and storage temperatures. A: Nylon / EVOH / EVA; B: Nylon / EVA; C: Metallized polyester

The overall visual color change of carrots in tested pouches after PATP treatment and following 12 weeks storage is presented in Figure 9. The results obtained for color were in accordance with previous research (Krebbers et al 2002; Nguyen et al 2007; Islam et al 2007; Leadley et al 2008; Gupta et al 2010). Gupta et al (2010) processed tomato juice at 600 MPa and 100 °C for 10 minutes and stored the samples for 52 weeks at 4, 25 and 37 °C. They observed a change in the color of PATP-treated tomato juice as a function of storage time and temperature. Bauernfeind et al (1981) also reported that isomerisation of carotenoids during heat treatment can reduce the color intensity.

Nguyen et al (2007) processed carrot slices at 500 and 700 MPa at 95, 105 and 121 °C for varying treatment times and observed a significant color change in PATP-treated carrot
slices. They also reported that as temperature increased from 95 to 121 °C, pressure lost its efficacy and temperature became more predominant. This resulted in more pronounced change in color of PATP-treated carrot slices.

3.4.3 β-carotene

In our research, it was observed that harsher preheating temperatures degraded β-carotene significantly (Figure 10). Additional β-carotene degradation occurred during PATP treatment. β-carotene is a fat-soluble, red-orange pigment that is highly unsaturated. Due to its high degree of unsaturation and natural tendency to quench free radicals, β-carotene is quite susceptible to oxidation. β-carotene is very sensitive to heat, oxygen and light (Clydesdale et al 1976). β-carotene in carrots occurs only as all-E (all-trans) isomers. When exposed to heat, it can be oxidized and isomerized (Bao and Chang 1994). Therefore, processed carrots can contain significant amounts of cis isomers, which are generated during processing. The extent of isomerisation varies depending on temperature. In our study, the carrot samples were preheated in a 90 °C water kettle for 15 minutes. However, isomerisation of β-carotene in our research was not investigated. Marx et al (2003) reported that blanching was a prerequisite for trans-cis isomerisation and high temperature levels (e.g. > 100 °C) are needed for solubilization of the crystalline structure of β-carotene (Marx et al 2003).

Similarly, Dietz et al (1988) found that blanching carrots at 95 °C caused 40 % β-carotene degradation. Von Doering et al (1995) reported that at temperatures below 100 °C, all trans-β-carotene resulted in formation of 13-15-cis-β-carotene while, the 9-cis isomer is
Figure 9. The color of carrots after PATP treatment and 12 weeks storage
(a) Nylon/EVOH/EVA at 25 °C; (b) Nylon/EVOH/EVA at 37 °C; (c) Nylon/EVA at 25 °C; (d) Nylon/EVA at 37 °C; (e) Metallized Polyester at 25 °C; (f) Metallized Polyester at 37 °C
formed above 100 °C. Nguyen et al (2007) also reported carotene degradation caused by PATP at 500 and 700 MPa at 105 and 121 °C.

During 12 weeks of storage, carrots in Nylon/EVA and metallized polyester pouches continued to experience significant decreases in β-carotene at both storage temperatures. The loss in β-carotene content was significantly more pronounced at 37 °C storage. At this temperature, β-carotene content of carrots in Nylon/EVA and metallized polyester pouches was almost completely lost. On the other hand, the Nylon/EVOH/EVA pouch was quite successful in maintaining the β-carotene content of carrots during 12 weeks at either 25 or 37 °C storage. Lin et al (2005) also found that higher storage temperatures caused greater losses of β-carotene. They also reported that both isomerisation and degradation of β-carotene could occur simultaneously.

The β-carotene results obtained correlated with the change in color of the carrot samples. This finding was in agreement with the previous research (Marx et al 2003; Dietz et al 1998; Von Doering et al 1995; Munch et al 1983). Munsch et al (1983) reported that color change during the heat treatment of carrot juice could be correlated to total carotenoid content and cis carotenoid isomer content. Bauernfeind et al (1981) also reported that isomerisation of carotenoids during heat treatment can reduce the color intensity. Gupta et al (2010) stated that conversion of lycopene from trans to cis as well as degradation of lycopene resulted in loss in red color of the PATP-treated tomato juices. Gupta et al (2010) also found reasonably high correlation ($R^2 > 0.85$) between the color change in PATP-treated tomato juice and degradation of lycopene.
**Figure 10.** β-carotene content of raw, preheated, PATP-treated carrots in different pouches and storage temperatures A: Nylon / EVOH / EVA; B:Nylon / EVA; C: Metallized polyester

### 3.4.4 Total Plate Count

Figure 11 shows the results of the reduction in aerobic mesophilic microflora of baby carrots caused by PATP treatment. PATP conditions applied (600 MPa and 110 °C for 10 minutes) reduced the total microflora to below the detection limit (10 CFU per g of the product). The total count remained below the detection limit over 12 weeks storage at 25 and 37 °C (data not shown). Data indicated that the chosen process conditions resulted in shelf-stability of the processed carrot samples for 12 weeks storage at either 25 or 37 °C. Krebbers et al (2003) also reported similar results for microbial inactivation by PATP (90 °C-700 MPa) in tomato puree. Koutchma et al (2005) obtained 6-log reduction of *Geobacillus stearothermophilus* in egg patties when processed at 700 MPa and 105 °C.
for 5 minutes. Moreover, Ahn et al (2007) applied the combined heat and pressure (700 MPa and 121 °C for less than 1 min) on some *Clostridium* and *Bacillus* surrogate spores such as *B. amyloliquefaciens* and achieved 7 to 8 log reduction.

![Figure 11](image.png)

**Figure 11.** Total aerobic mesophilic count of raw, PATP-treated and stored carrots

### 3.4.5 Oxygen Transmission Rate (OTR)

The results of oxygen transmission rates for control, preheated and PATP-treated pouches are presented in Figure 12. It can be seen that Nylon/EVOH/EVA and Nylon/EVA pouches followed a similar trend except the magnitudes of the transmissions were much higher for Nylon/EVA pouch. In the case of Nylon/EVOH/EVA pouch, although there was a significant increase (p < 0.05) in the oxygen transmission rate caused by PATP, it was still very low compared to the transmission rates of other two pouches. For Nylon/EVA pouch, the oxygen transmission rate was not significantly different (p >
0.05) for either preheated or PATP-treated and stored pouches. For metallized pouch, even preheating the pouches only without further processing increased the oxygen transmission rates as opposed to other two types of pouches analyzed. Moreover, PATP application and 4 week storage also caused a significant increase (p < 0.05) in the OTR of the metallized pouch significantly at either 25 or 37 °C storage. A remarkably higher increase (p < 0.05) was observed at 37 °C storage. The results for metallized pouch are in agreement with the literature. Galotto et al (2009) reported a significant increase in OTR of metallized pouch and they reported that the increase was due to the damage in the metallized surface coating. Caner et al (2003) also showed that there was up to 150 % barrier loss for MET-PET film after processing at 45 °C and 600 MPa.

**Figure 12.** Oxygen transmission rates of control, preheated and PATP-treated and stored films
3.4.6 Water Vapor Transmission Rate (WVTR)

Water vapor transmission rate (WVTR) of tested pouches did not change significantly ($p > 0.05$) after preheating or PATP application (Figure 13). However, metallized polyester pouch experienced more considerable increase in WVTR after preheating and PATP compared to control pouch. This data was in agreement with the previous research. Halim et al (2009) processed several co-extruded packaging materials at 800 MPa and 70 °C for 10 minutes. They reported that no significant difference caused by processing was observed in WVTR of any tested co-extruded films including Nylon6/EVOH. Caner et al (2000) found that WVTR of MET-PET was affected by HPP applications at 600 and 800 MPa for 10 and 20 minutes.

Figure 13. Water vapor transmission rates of control, preheated and PATP-treated and stored films
3.4.7 Thermal Analysis

Table 2 summarizes the thermal analysis results showing the influence of high pressure processing only and combined pressure-heat treatment on three different pouches using differential scanning calorimeter.

It was observed that some of the polymers experienced slight changes in their typical melting points because of HPP and PATP-treatment. It was worth noting that depending upon the composition of the films, they exhibited characteristic peaks at approximately 104 °C for EVA, 112 °C for polyethylene sealant in metallized pouch, 170 °C for EVOH, 218 °C for Nylon and 254 °C for polyester. However, because of the changes in the structure of the melting peaks, (Figure 14, Figure 15, Figure 16), some changes were observed in the enthalpy of fusion values for PATP-treated pouches. There was a slight but not significant decrease (p > 0.05) in the enthalpy for EVA polymer in both Nylon/EVOH/EVA and Nylon/EVA laminates after PATP. Although Nylon in Nylon/EVOH/EVA and Nylon/EVA pouches experienced slight decreases in the enthalpy of fusion, they were not statistically significant (p > 0.05). However, PATP treatment caused a significant decrease (p < 0.05) in the ΔH of EVOH. There was a contradiction in the literature about the effect of PATP on EVOH (Ozen et al 2001; Schauwecker et al 2002). Authors reported that there was no change in T_m and ΔH after PATP for EVOH polymers as opposed to Galotto et al (2008) who reported slight decrease in T_m and ΔH of EVOH polymers.

On the other hand, for metallized polyester pouch, PATP treatment caused a significant decrease (p < 0.05) caused by PATP in the enthalpy of fusion for polyethylene sealant.
For polyester in this type of pouch, no statistical change (p > 0.05) in ΔH was observed. Additionally, no difference in ΔH values was observed between untreated and HPP-treated metallized polyester pouch.

Combined pressure-heat treatment caused a similar change in the shape of the melting peaks of EVA in both Nylon/EVOH/EVA and Nylon/EVA pouches and Polyethylene in metallized polyester polymers (Figure 14, 15, 16). The difference in the shape of the melting peaks could be caused by a change in the structure (e.g. crystallinity) of polymers as a result of PATP treatment.

![DSC thermogram of control, HPP and PATP-treated Nylon/EVOH/EVA pouch](image)

**Figure 14.** DSC thermogram of control, HPP and PATP-treated Nylon/EVOH/EVA pouch
Figure 15. DSC thermogram of control, HPP and PATP-treated Nylon/EVA pouch

Figure 16. DSC thermogram of control, HPP and PATP-treated Metallized Polyester pouch
Table 2. Thermal analysis results for control, HPP and PATP-treated packaging films

<table>
<thead>
<tr>
<th>Film Structure</th>
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<th>$\Delta H$ (J/g)</th>
<th>$T_m$ ($^\circ$C)</th>
<th>$\Delta H$ (J/g)</th>
<th>$T_m$ ($^\circ$C)</th>
<th>$\Delta H$ (J/g)</th>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NYLON/EVOH/EVA</td>
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<td>104.7 ±0.3</td>
<td>34.5 ±13.4</td>
<td>170.1 ±0.3</td>
<td>9.2 ±0.8</td>
<td>218.3 ±0.4</td>
<td>10.2 ±1.7</td>
</tr>
<tr>
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<td>HPP-treated</td>
<td>104.2 ±0.02</td>
<td>20.6 ±1.9</td>
<td>169.8 ±0.02</td>
<td>7.98 ±0.5</td>
<td>218.0 ±0.01</td>
<td>11.7 ±0.07</td>
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<td>20.9 ±1.0</td>
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<td>216.3 ±2.1</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Nylon/EVA</td>
<td>Control</td>
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<td>-</td>
<td>218.9 ±0.1</td>
<td>10.3 ±1.0</td>
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<td>-</td>
<td>218.8 ±0.3</td>
<td>10.0 ±0.6</td>
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<tr>
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<td>PATP-treated</td>
<td>105.0 ±0.1</td>
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<td>217.6 ±0.7</td>
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<tr>
<td>Metallized Polyester</td>
<td>Control</td>
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<td>72.0 ±6.0</td>
<td>253.6 ±0.3</td>
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<tr>
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<td>253.9 ±0.5</td>
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3.4.8 Dissecting Stereo Microscopy

Appendix A present the dissecting microscopic pictures of different packaging films that were PATP-treated and stored at 25 and 37 °C for 12 weeks. Dissecting microscopic pictures were taken to pre-evaluate the surface of pouches for SEM analysis. Some visible changes on the surface of Nylon/EVA and metallized polyester pouches were observed.

3.4.9 Scanning Electron Microscopy

The changes in the structures of the pouches were further analyzed by using scanning electron microscopy. No significant difference on the surface of Nylon/EVOH/EVA pouch was observed between untreated and PATP-treated pouch (data not shown). These observations were consistent with Schauwecker et al (2002), who reported that HPP caused no change in the structure of EVOH film. On the other hand, PATP treatment created bubble look like structures in the structure of Nylon/EVA pouch (Figure 17). When different spots on the surface were analyzed, it was observed that the Nylon/EVA pouch had mixture of both slightly broken and non-broken bubble look like structures, whereas after 12 week of storage following PATP treatment, all these structures were completely broken and open.
Figure 17. Scanning electron micrographs of Nylon/EVA pouch illustrating the pinholes (magnification x400) a) PATP-treated only with no storage b) PATP-treated and stored at 25 °C for 12 weeks

SEM pictures of metallized polyester pouch revealed that PATP treatment damaged the surface of the film (Figure 18). Caner et al (2003); Galotto et al (2008); also reported that SEM can be used to locate damage on the surface of high pressure processed films. They also reported that multilayer films could be affected by high pressure depending on the type of films and processing conditions. It was consistent with the literature. Caner et al (2003) reported that high pressure processing at 45 °C and 600 MPa caused damage on MET-PET film. Galotto et al (2008) also reported wrinkles and delamination in tested metallized polymers at 400 MPa and 60 °C processing conditions. Schauwecker et al (2002) also showed delamination occurred in the MRE films at ≥ 90 °C and ≥ 200MPa for 10 min and they observed no delamination in the HPP-treated Nylon/EVOH/PE films.
Figure 18. Scanning electron micrographs of Metallized Polyester pouch illustrating the damage caused by PATP treatment a-) Control b-) and c-) PATP-treated (magnification x1000)

3.4.10 Conclusions

Combined pressure and heat treatment caused significant $\beta$-carotene degradation and change in color of carrot samples. Packaging type (particularly oxygen transmission rate of the package) and storage time and temperature also affected the color change and $\beta$-carotene content of carrots remarkably ($p < 0.05$). Nylon/EVOH/EVA laminate pouch was the best pouch in terms of preserving the color and $\beta$-carotene content after PATP treatment and following storage. Metallized polyester pouch was damaged by the processing and its’ OTR increased drastically. This resulted in change in color and $\beta$-carotene content of carrot samples in this pouch. Nylon/EVA pouch which was the lowest
barrier pouch tested, also could not preserve the color and β-carotene content of carrots. Additionally, the process conditions chosen resulted in shelf-stability of the processed carrot samples and no microbial growth was observed in any types of the pouches tested during 12 weeks storage at either 25 or 37 °C.

Dissecting microscopy and scanning electron microscopy pictures further confirmed the physical changes on the surface of Nylon/EVOH and metallized polyester pouches. Thermal analyses also implied that there was a change in the structure of the polymers tested after combined pressure and heat treatment.

In summary, the study demonstrated that the barrier properties of the packaging materials has significant role in preserving quality attributes of PATP processed carrot samples and high barrier pouches are required to preserve the quality attributes of PATP-treated products during extended storage.
3.4.11 References


Chapter 4. Conclusions and Future Recommendations

4.1 Summary and Conclusions

- Elevated pressure (600 MPa) combined with heat (110 °C) for 10 min holding time was applied to carrot samples packed in three different packaging materials and stored in dark at 25 and 37 °C for 12 weeks.

- Preheating and PATP treatments caused significant degradation \((p < 0.05)\) to the \(\beta\)-carotene content and change in the color of carrot samples. This degradation continued during the storage. The magnitude of the degradation was more pronounced at 37 °C for Nylon/EVA and metallized polyester pouches. Nylon/EVOH/EVA laminate pouch was not affected by combined heat and pressure treatment.

- Nylon/EVOH/EVA was the best pouch in terms of preserving the \(\beta\)-carotene content of processed carrots. However, metallized polyester pouch was damaged by the processing.

- Drastic increase in the OTR of metallized polyester pouch was determined after processing and during storage, which also resulted in considerable \(\beta\)-carotene degradation and color change for the carrots packed in this pouch. Nylon/EVA pouch also could not preserve color and \(\beta\)-carotene content of carrot samples.

- Change in color of carrots could be correlated to change in \(\beta\)-carotene content.
• Thermal analyses also implied that there was a change in the structure of polymers caused by PATP. PATP treatment caused a decrease in ΔH value of from 9.2 to 7.2 J/g for EVOH and from 72 to 45.5 J/g for polyethylene.

• The chosen process parameters resulted in shelf-stability of processed carrot samples during 12 weeks storage at either 25 or 37 °C.

4.2 Future Recommendations

• Investigate different techniques for preheating the samples rapidly prior to PATP to reduce the thermal degradation caused by preheating

• Investigate the kinetics of change in color and β-carotene content of PATP-treated carrots

• Investigate the usage of nanocomposite materials to improve the barrier and mechanical properties for commonly used packaging materials and evaluate their suitability for PATP applications

• Conduct sensory analysis on PATP-treated carrots to determine consumer acceptability.


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Appendix A. Dissecting Microscope Pictures
Figure 19. Dissecting microscopy pictures taken from surface of control and PATP-treated and stored Nylon/EVOH/EVA pouch (magnification x20), a) Control b) After 12 weeks storage at 25 °C c) After 12 weeks storage at 37 °C
Figure 20. Dissecting microscopy pictures taken from surface of control and PATP-treated and stored Nylon/EVA pouch (magnification x15), a) Control b) After 12 weeks storage at 25 °C c) After 12 weeks storage at 37 °C
Figure 21. Dissecting microscopy pictures taken from surface of control and PATP-treated and stored Metallized Polyester pouch (magnification x12), a) Control b) After 12 weeks storage at 25 °C c) After 12 weeks storage at 37 °C
Figure 22. Some of the pin holes observed in PATP-treated and stored Nylon / EVA a) (Magnification x88) b) (Magnification 38) c) (Magnification x88)
Appendix B. Statistical Analysis Results
**Table 3.** The influence of treatments (preheating and pressure-assisted thermal processing) on quality of the carrot samples (Preheating was at 90 °C water for 15 min and PATP was at 600 MPa and 110 °C for 10 min)

<table>
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<td>“a” value</td>
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</tr>
<tr>
<td>“b” value</td>
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</tr>
<tr>
<td>Total color change</td>
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<tr>
<td>β-carotene content</td>
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</tbody>
</table>

**Table 4.** The influence of treatments (preheating and pressure-assisted thermal processing), packaging material (Nylon/EVOH/EVA, Nylon/EVA and metalized polyester) on barrier properties of the packaging films (Preheating was at 90 °C water for 15 min and PATP was at 600 MPa and 110 °C for 10 min)

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Table 5. The influence of packaging material (Nylon/EVOH/EVA, Nylon/EVA and metalized polyester), storage temperature (25 and 37 °C) and time on quality of the carrot samples (color and β-carotene) and barrier properties (OTR and WVTR) of the packaging materials (Preheating was at 90 °C water for 15 min and PATP was at 600 MPa and 110 °C for 10 min)

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Table B3 continued

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**Oxygen transmission rate**

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Table 6. The influence of treatments (preheating and pressure-assisted thermal processing) on melting points of polymers (Nylon/EVOH/EVA, Nylon/EVA and metalized polyester) (Preheating was at 90 °C water for 15 min and PATP was at 600 MPa and 110 °C for 10 min)

<table>
<thead>
<tr>
<th>Polymer type</th>
<th>Pouch type</th>
<th>Treatments</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>EVA</td>
<td>Nylon/EVOH/EVA</td>
<td>Untreated-HPP</td>
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<td></td>
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<td>PATP-HPP</td>
<td>0.8514</td>
</tr>
<tr>
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<td>Nylon/EVOH/EVA</td>
<td>Untreated-PATP</td>
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<td>Nylon/EVA</td>
<td>HPP-Utreated</td>
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<td>HPP-PATP</td>
<td>0.5751</td>
</tr>
<tr>
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<td></td>
<td>PATP-Utreated</td>
<td>0.8913</td>
</tr>
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<td>Nylon/EVA</td>
<td>HPP-PATP</td>
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Table 7. The influence of treatments (preheating and pressure-assisted thermal processing) on heat of enthality (ΔH) of polymers (Nylon/EVOH/EVA, Nylon/EVA and metalized polyester) (Preheating was at 90 °C water for 15 min and PATP was at 600 MPa and 110 °C for 10 min)

<table>
<thead>
<tr>
<th>Polymer type</th>
<th>Pouch type</th>
<th>Treatments</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>EVA</td>
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<td>Untreated-PATP</td>
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<td>HPP-Untreated</td>
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<td>HPP-Untreated</td>
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