The Effects of Pressure-Assisted Thermal Processing on the Quality Attributes of Black Beans (*Phaseolus vulgaris* L.)

THESIS

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Abstract

Pressure-assisted thermal processing (PATP) is an emerging alternative food processing technology that utilizes elevated pressure combined with heat to provide shelf-stable foods with superior quality attributes. This study examined the effect of PATP on texture, *in vitro* protein digestibility (IVPD), color, water imbibition and cooked volume of black beans (*Phaseolus vulgaris* L.). Beans were presoaked (15, 30, 60 and 120 min at 82°C, 24 hours at 23°C) and subjected to 600 MPa at 105°C for 1 second, 5, 10, 15 and 30 minutes. Pressure come-up time was 2 minutes and decompression time was 90 seconds. Thermally processed (TP; 105°C for 5 minutes) beans, using a still retort, were used as a control. PATP was found to significantly increase (p < 0.05) bean softening, IVPD and water absorption as compared to TP. Increasing pressure hold time during PATP generally increased bean softening. Changes in pressure holding time or soak time did not alter IVPD (=79%) of PATP treated samples. Beans soaked for 15, 30 and 60 min showed lower volume increases after 5 min PATP as compared to thermally processed beans. Neither soak time nor processing time were found to have any influence on bean
color. Within the range of process conditions of the study, PATP may be a viable alternative for preserving black beans.
Dedication

Dedicated to my parents, brother and my loving wife, for their encouraging work throughout my educational endeavors.
Acknowledgments

I would like to acknowledge my advisor Dr. V.M. Balasubramaniam for all his guidance throughout my years as a master's student, my committee members, Dr. Sheryl Barringer and Dr. Lynn Knipe, and my lab group whose help and advice made this work possible. I would also like to thank Dr. W. James Harper and Dr. David Min for their guidance and knowledge during my time as an undergraduate and graduate student at The Ohio State University.
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High pressure processing (HPP) is an emerging novel food processing technology that, despite having been first pioneered in the late 19\textsuperscript{th} century, has only begun to be used commercially during the last two decades. HPP has been described as one of the best innovations in food processing in 50 years (Dunne, 2005), and is an alternative processing method to traditional thermal processing that offers the ability for manufacturers to provide a cleaner ingredient label with fewer food additives (Balasubramaniam and Farkas, 2008).

While HPP involves elevated pressures (400-600 MPa or 58,000-87,000 psi) at room temperature, pressure-assisted thermal processing (PATP) combines the high pressures (500-900 MPa or 72,500 to 130,000 psi) and elevated temperatures (90-120°C) to produce shelf-stable, commercially sterile food products.

Many commercially available low-acid food products found on market shelves in cans are prepared with retort processing, however, a common disadvantage of this process is the long come-up time for the product to reach the desired temperature during which many quality attributes of the food (color, texture, flavor and
nutritional factors) may be degraded. Additionally, thick viscous products are prone to overcooking on the outsides of cans, leading to a lower quality product. PATP allows for rapid, uniform heating of the food product during compression, shorter processing times than traditional thermal processing, and rapid, uniform product cooling during decompression. As a result, PATP provides opportunities as an alternative to traditional thermal processing, providing less thermal degradation of key product quality attributes.

Common beans (Phaseolus vulgaris L.) are a good source of protein and in many developing countries are the primary protein source in starch rich diets (Bressani, 1994). As an economical source of protein (~18-25%), dietary fiber, vitamins and minerals, beans play an important role in the nutrition of low economic regions of the world (Swaminathan, 1974) where protein-energy malnutrition is the most common deficiency disease (FAO/WHO, 2001). The proteins in common beans provide arginine, aspartic acid, glutamic acid, leucine and lysine in significant quantities (Nielsen, 1991). However, the protein digestibility of raw beans is lower than that of animal and vegetable proteins (Nielsen, 1991; Bressani, 1994) and is limited in the presence of antinutritional factors (ANFs) including trypsin inhibitors (Gupta, 1987; Singh, 1988), tannins (Reddy et al., 1985), phytic acid (Urbano et al., 2000) and oligiosaccharides (Singh, 1988; Udensi et al., 2007). Despite this, proper cooking inactivates the ANFs and improves the nutritional
value of the proteins (Liener, 1989), while also softening the texture of the beans, and improving their flavor for human consumption.

Over the last two decades, dry bean consumption has gradually increased in the United States (Uebersax, 2006). Fifty-five percent of black beans consumed in the U.S. are consumed in southern regions of the country (Lucier et al., 2000). Canned bean products make up the largest sector in the market for bean products consumed in industrialized countries (Uebersax, 2006).

As of the 2002 Census of Agriculture, 8,647 farms in the United States produced dry edible beans over 1.7 million acres with consumer sales estimated to be $1.8 billion (USDA, 2005).

The objective of this study was to determine the effect of soak pretreatment and PATP and TP on texture, color, protein digestibility and water absorption of black beans.
2.1 Thermal Processing and Heat Sterilization

Heat treatment of food products is a common and convenient method for food preservation. To achieve commercial sterility of food products using traditional heat processing methods requires sufficient temperature combined with a long enough time to destroy pathogens and inactivate enzymes (Fellows, 2000). Heat inactivation of microorganisms occurs in part due to denaturation of proteins, resulting in the destruction of enzymes, leading to loss of metabolism. The reaction rate generally follows first-order kinetics, and provides for a logarithmic order of death when foods are sufficiently heated to a temperature that will destroy microorganisms (Fellows, 2000). Inadequate thermal processing can lead to multiple problems, including the growth of thermophilic microorganisms whose spores survived the heat processing, and potential growth of the pathogen *Clostridium botulinum* (Gavin and Weddig, 1995).
2.2 Retort Processing

Canning, or the retort process, is a common and effective means of a thermal process used to achieve commercial sterility. Foods are primarily packaged in metal cans, glass jars and bottles, flexible pouches, or rigid trays, and sterilized in package. These packages utilize a hermetic seal to protect the food from the outside environment and contamination. One type of retort process involves steam injection. The packages are placed in a vessel, known as a retort, and the vessel is sealed. The vessel, and thus product and food are then heated by injecting saturated steam into the retort, typically reaching a target temperature of 121°C for low-acid foods. As the steam collects on the outside of the containers and condenses, the heat is transferred to the container and its contents (Fellows, 2000, Gavin and Weddig, 1995).

Unfortunately, viscous and solid foods experience a slow rate of heat transfer to the center of the container, and result in over-processing of the food product near the edges of the container (Figure 2.1). This in turn lowers the sensory and nutritional quality of the food product, including, color, texture, flavor, and aroma. Once the process cycle is complete, cold water is typically sprayed onto the walls of the container to facilitate evaporative cooling; however, this is a slow cooling process and further enhances the thermal degradation of the product (Fellows, 2000).
Efforts over the last few decades have been made to develop new processing technologies that limit the disadvantages of traditional thermal processes, such as aseptic processing, ohmic heating, microwaves processing, irradiation and pressure-assisted thermal processing.
2.3 Aseptic Processing

Aseptic processing is a thermal processing method where temperatures higher than traditional retort processing are applied directly to unpackaged liquid and small particulate foods for shorter periods of time, and then packaged directly in pre-sterilized containers. The food is heated in thin layers in a continuous heat exchanger to the required sterilization temperature and held in holding tubes for the necessary calculated time. The temperature and time are precisely controlled to ensure proper sterilization and safety of the food product. Like retort processing, temperature and time are the two critical process parameters that govern the inactivation of pathogens and harmful bacterial spores. Once proper microbial inactivation has been achieved, the product is then rapidly cooled in another heat exchanger and packaged. This process allows for less thermal degradation due to the rapid heating and cooling of the product, and can be successfully used on many products such as milk, fruit juices, soups, tomato products and other foods with small particulate matter (Fellows, 2000).

2.4 Ohmic Heating

Ohmic heating is a form of aseptic processing that sterilizes foods containing water and ionic salts by subjecting the food product to direct current. The resistance of the food to the current, in turn, creates the necessary heat for the
process. Like traditional heat processing, temperature and time are two primary critical process parameters that govern the inactivation of harmful bacterial spores. Current research has not reported any additional lethal effects on microorganisms due to the exposure to electrical current. Unlike traditional direct heating methods, ohmic heating can be used on food products with larger particulates containing up to 60% solids, such as chunky soups. The process allows for uniform rapid heating of the food product (1°C s^{-1}), and temperature gradients can be minimized if the solid and liquid food parts have the same or similar resistances. The process does not use hot surfaces for heating as in conventional heat methods, therefore removing the problems of surface burning or thermal degradation of solid food products (Stirling, 1987; Palaniappan and Sastry, 1992; Reznick, 1996; Fellows, 2000).

During a typical ohmic process, food products are pumped through vertical tubes containing electrodes. Here the product is heated to the process temperature by applying alternating current through the food from a 3-phase power supply. Similar to aseptic processing, ohmic heating systems use holding tubes to keep the food product at the required temperature and time to achieve pathogen inactivation, and it is important to ensure that the cold spots of the slowest heating particle of the product are properly processed (Fellows, 2000). The food product is then cooled using heat exchangers and aseptically packed into pre-sterilized containers.
2.5 Pressure-Assisted Thermal Processing

2.5.1 High Pressure Processing

High pressure processing (HPP) is an innovative emerging food processing technology that utilizes elevated pressures (400-600 MPa or 58,000-87,000 psi), with or without additional heat, as a method for food preservation. HPP has shown the ability to reduce the overall microbial load of food products, kill pathogens, and inactivate many deteriorative enzymes. However, pressure treatment conducted at ambient temperatures does not inactivate bacterial spores. HPP allows for an extension of product shelf life without degrading important quality attributes, such as texture, color, flavor and some nutritional factors (Balasubramaniam et al., 2008), as HPP does not break covalent bonds (Farkas and Hoover, 2000).

HPP, also commonly referred to as high hydrostatic pressure processing (HHP) and ultra high pressure processing (UHP), is suitable for both solid and liquid food products, and in turn, the nature of the food matrix is important to product suitability. High moisture content food products are best suited for HPP, as they are not severely deformed under pressure due to the compressibility of water under pressure (10% at 300MPa and 17% at 600MPa) (Farkas and Hoover, 2000). Products that contain high amounts of air or are porous in nature will be left
distorted after decompression, due to the high compressibility of air
(Balasubramaniam et al., 2008).

2.5.2 Pressure-Assisted Thermal Processing
Pressure-assisted thermal processing (PATP) is a technology whereby food
products are subjected to a combination of elevated pressure levels (500-900 MPa
or 72,500-130,000 psi) and applied heat (90-120°C) for several minutes to sterilize
low-acid foods (Ahn et al., 2007; De Heij et al., 2003; Matser et al., 2004; Rajan et
al., 2006). PATP offers commercial processors several advantages over thermal
processing. Faster thermal come-up and cool-down time allow for less thermal
degradation than traditional thermal processing, as pressure-induced heating and
cooling due to heat of compression (δ) is uniform throughout the product, unlike
conductive heating and cooling. Rapid heating and cooling of the product during
PATP could also have the potential to reduce processing times for the industry
(Leadley et al., 2008). Mashed potato, eggs, soups and tea are some of the
examples of products that can be preserved using PATP. As of 2009, the FDA has
issued no objection for a low-acid product processed by PATP, however, no PATP
products are currently available on the market.
2.5.3 Principles of PATP

During PATP, both pressure and temperature can influence the inactivation of microorganisms as well as various chemical reactions. Pressure-thermal interactions can be synergistic, additive or antagonistic, depending upon the type of reaction.

The two fundamental principles governing PATP are the isostatic pressure rule and Le Chatelier’s principle (Smelt, 1998). The isostatic pressure rule dictates that on a macroscopic level, an applied pressure is transmitted instantaneously throughout the system and the food product volume, and is independent of the product’s size and geometry (Cheftel, 1995). Le Chatelier’s principle states that an application of pressure shifts the equilibrium of the system to the state that occupies the smallest volume. Therefore, any physical or chemical change (phase transitions, chemical reactions, molecular configuration changes) that results in a volume decrease is enhanced by the application of pressure (Farkas and Hoover, 2000).

In PATP, food products are packaged in an appropriate container and submerged in a pressure transmitting fluid. Suitable packing requires a means of transmitting the applied pressure, therefore it should be flexible, or contain at least one flexible component. Products containing sufficient moisture will not be adversely damaged
during pressurization since the pressure is applied uniformly in all directions (Balasubramaniam et al., 2008).

2.5.4 Thermal Effects During PATP

As pressure is applied to the system, an accompanied increase in temperature occurs due to adiabatic heating. This phenomenon is referred to as heat of compression (δ), and may be different for each building block of food products (carbohydrates, proteins, fats, water) as they respond to the pressure and ergo, increase differently (Rasanyagam et al., 2003). The time from which the system pressure is increasing from initial pressure $P_i$ to target pressure $P_1$ (Figure 2.2, A) is referred to as "pressure come up time" (Farkas and Hoover, 2000), and is when the δ effect occurs. If the pressure vessel lacks sufficient insulation during the process hold time, a decrease in temperature from $T_i$ to $T_1$ (Figure 2.2) occurs due to heat loss from the product to the vessel and surrounding environment. During decompression ($P_2$ to $P_f$), expansion of the product results in a uniform temperature drop from $T_2$ to $T_f$. 
Figure 2.2. Sample Pressure-Temperature profile for HPP.
2.5.5 Process Uniformity

PATP treatments have better process uniformity than traditional retorted processes. During the pressure come up time, the uniform application of pressure produces a uniform temperature increase in products, and further a uniform and rapid temperature decrease during decompression, reducing the severity of thermal quality degradation. While this allows for improved process uniformity over traditional thermal processing methods, some process uniformity issues exist. Food matrices, pressure transmitting fluid, packaging material may experience temperature gradients resulting from different δ values (Nguyen and Balasubraminiam, 2009). This results in heat exchange between test samples, pressure transmitting fluid, and the environment. At the elevated temperatures used for PATP, it is necessary to ensure proper system and product insulation from the surrounding environment to minimize heat loss and ensure proper processing temperature is maintained to reach appropriate microbial lethality (Balasubramaniam and Farkas, 2008).

2.5.6 Packaging Requirements

PATP package requirements depend on the nature of the food product, system design and process conditions. Batch systems for solid or liquid food products are advised to use a high-barrier flexible pouch made of single or multi-layered
polymer, or a semi-rigid container with at least one flexible side, and be able to withstand the nature of the process (Balasubramaniam et al., 2008). The package should protect the food product from potential quality degradation during storage, so a film or package with light, oxygen, and water impermeability may be desired (Hogan et al., 2005).

It is also ideal to vacuum package or remove as much air as possible when packaging the product. Notably as the compressibility of air is much higher than other food products, energy will be wasted during compression, while oxygen related reactions such as lipid oxidation may be accelerated during the processing.

2.5.7 Typical Batch PATP Procedure

A PATP system requires several components to function (Figure 2.3); pressure vessel, top and bottom vessel closure, hydraulic pump, pressure intensifier, heating system and conditioning fluid, pressure and temperature monitoring and control systems, and a product handling system, often perforated baskets (Balasubramaniam and Farkas, 2008). Pressure vessels are often designed with a stainless steel liner to prevent corrosion. Water is often preferred for use as a pressure transmitting fluid as it has a similar δ to most food products, thus minimizing temperature gradients between food and pressure transmitting fluid, while also being cost effective and non-toxic (Nguyen and Balasubramaniam,
Pressure vessels used for PATP should also have a means of maintaining target temperature to minimize heat loss to the surrounding environment. This can be achieved by utilizing a jacketed vessel system with a temperature controlled conditioning fluid.

The prepackaged product is placed in the product handling system and preheated to the initial temperature \(T_i\), where it is then placed into the pressure vessel and sealed. Heated pressure transmitting fluid is pumped into the vessel, venting any remaining air through a vent valve, which is closed once air removal is complete. Once the vent valve is closed, pressure transmitting fluid continues to pump in backed by the pressure intensifier to create the target pressure, which is transmitted to the food. It is during this pressure come-up time where the \(\delta\) of the product and pressure transmitting fluid is observed (Figure 2.2, A). Pressure come-up time is dependent on the vessel size, horsepower of pressure pump and target pressure. Commercial scale high pressure systems are typically designed to reach 600 MPa in 2-3 min (Farkas and Hoover, 2000; Balasubramaniam et al., 2004).

Pressure and temperature is maintained for the desired holding time to inactivate pathogens. This is referred to as pressure hold time, and is defined as the time from which target pressure \(P_i\) is reached, until the decompression cycle is started (Figure 2.2, B). Pressure hold times of less than 10 min are typically desired to economically justify the process (Balasubramaniam et al., 2004).
Following the end of the pressure hold time, the system is decompressed and product temperature is rapidly cooled from $T_2$ to $T_f$ due to the release of pressure (Figure 2.2, C). Most systems allow for rapid decompression time, often just a few seconds, however, some food product may experience structural damage from dissolved gases during rapid decompression. To prevent undesirable decompression changes, many systems allow for extended decompression times (Nguyen and Balasubramaniam, 2009). Once the pressure inside the vessel has returned to atmospheric pressure, the chamber is opened and the product basket removed. It may then be desirable to cool the product to ambient temperature or below it to minimize further thermal degradation (Balasubramaniam and Farkas, 2008).

During the pressure hold time there maybe be a drop in temperature of the food product due to heat loss to the surrounding environment (Figure 2.2, $T_1$ to $T_2$). If this occurs upon decompression (Figure 2.2, $P_2$ to $P_f$), the resultant temperature decrease may bring about a final temperature ($T_f$) that is lower than the initial product temperature ($T_i$).

The total cycle time of the process will include time for preheating, vessel loading, vessel closing, compression, decompression, vessel opening and product unloading in addition to the pressure hold time. Package design should also account for a
means to maximize volumetric efficiency of the product basket (Nguyen and Balasubramaniam, 2009).

2.5.8 Typical Semi-continuous PATP Procedure

Semi-continuous processing can be used for liquid foods, by utilizing two or more pressure vessels with free-floating pistons. The pressure vessel’s piston creates two separate chambers in each vessel; one for the liquid product, the other for the pressure transmitting fluid. During operation, the first chamber is filled with food product, then sealed by closing the filling valve. Pressure transmitting fluid is pumped into the second chamber, separated by the piston, which transfers pressure to the food product. A typical three vessel setup would allow for semi-continuous operation by having one vessel under pressure, a second vessel filling, and a third discharging. After the pressure cycle, the liquid food product is pumped into a sterile tank to await aseptic packaging (Farkas and Hoover, 2000). At this time there is no pilot or commercial scale semi-continuous equipment available for PATP treatment due to the technical challenges associated with maintaining sterility in the closed system.
Figure 2.3. Typical batch high pressure system diagram.
2.5.9 Important Factors for PATP Process Design

The composition of the food product plays an important role in the $\delta$ of the product, and in turn, the required initial temperature necessary to reach the target temperature is based on the desired process pressure. The primary building blocks of food matrices (water, lipid, protein, carbohydrates) react differently under applied high pressures, and in turn have different $\delta$ values (Table 2.1, 2.2). Since water is the often the main ingredient in most food products, the product $\delta$ generally follows a $\delta$ pattern similar to water. Of the main food constituents, water has the lowest $\delta$ value under pressure ($3^\circ$C per 100MPa at $25^\circ$C) (Table 2.2) and varies depending on the initial temperature when pressure is applied, where fats and oils containing long-chain fatty acids have higher $\delta$ values (up to $9^\circ$C per 100MPa) and are generally not affect by initial temperature (Table 2.1).
<table>
<thead>
<tr>
<th>Food Constituent or Product</th>
<th>Heat of compression (°C per 100 MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Short-chain fatty acids</strong></td>
<td></td>
</tr>
<tr>
<td>Acetic acid (CH₃COOH)</td>
<td>11.44±1.51a</td>
</tr>
<tr>
<td>Propionic acid (CH₃CH₂COOH)</td>
<td>6.66±0.04a</td>
</tr>
<tr>
<td>Butyric acid (CH₃CH₂CH₂COOH)</td>
<td>4.65±0.19a</td>
</tr>
<tr>
<td><strong>Long-chain fatty acids</strong></td>
<td></td>
</tr>
<tr>
<td>Oleic acid (C₁₂H₂₄O₂)</td>
<td>8.47±0.19a</td>
</tr>
<tr>
<td>Linoleic acid (C₁₈H₃₂O₂)</td>
<td>6.42±0.08a</td>
</tr>
<tr>
<td>Linolenic acid (C₁₈H₃₀O₂)</td>
<td>6.88±0.27a</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>2.6 - 3.6bc</td>
</tr>
<tr>
<td>Proteins</td>
<td>2.7 - 3.3bc</td>
</tr>
<tr>
<td>Chicken breast</td>
<td>3.2b</td>
</tr>
<tr>
<td>Chicken fat</td>
<td>4.5b</td>
</tr>
<tr>
<td>Beef fat</td>
<td>6.3b</td>
</tr>
<tr>
<td>Olive Oil</td>
<td>6.3 - 8.7bc</td>
</tr>
<tr>
<td>Soy Oil</td>
<td>6.2 - 9.1bc</td>
</tr>
<tr>
<td>Orange juice, 2% milk, other water like substances</td>
<td>3.0b</td>
</tr>
<tr>
<td>Cream cheese</td>
<td>3.0b</td>
</tr>
<tr>
<td>Tomato salsa</td>
<td>3.0b</td>
</tr>
<tr>
<td>Egg albumin</td>
<td>3.0b</td>
</tr>
<tr>
<td>Mashed potato</td>
<td>3.0b</td>
</tr>
<tr>
<td>Yoghurt</td>
<td>3.1b</td>
</tr>
<tr>
<td>Honey</td>
<td>3.2b</td>
</tr>
<tr>
<td>Tofu</td>
<td>3.1b</td>
</tr>
</tbody>
</table>

Table 2.1. Heat of compression of various food constituents and products at 25°C.

a Source: Ramaswamy, R., 2007

b Adapted from Otero and Sanz, 2000; Rasanayagam et al., 2003; Patazca, Koutchma, and Balasubramaniam, 2007, Kesavan et al., 2002

c Substances exhibited decreasing temperature rise with increase in pressure
Heat of compression can be theoretically estimated using the following equation

\[ \delta = \frac{dT}{dP} = \frac{T\alpha}{C_p\rho} \]  

(1)

where \(\alpha\), \(T\), \(\rho\), and \(C_p\) are respectively thermal expansion coefficient, temperature, density, and heat capacity at constant pressure, however, this is only applicable to small pressure changes (Otero et al., 2000). It is thus often easiest to estimate a product’s \(\delta\) experimentally by directly measuring temperature changes during compression (Otero et al., 2007; Rasanayagam et al., 2003; Patazca et al., 2007).

In order to measure \(\delta\), the high pressure system used should be properly equilibrated at the desired initial temperature, and a pressure transmitting fluid with a known \(\delta\) value similar to that of most food products should be used. This allows for the minimization of temperature gradients influencing the calculated \(\delta\).

<table>
<thead>
<tr>
<th>Initial Temperature (°C)</th>
<th>Heat of compression (°C per 100 MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.6</td>
</tr>
<tr>
<td>15</td>
<td>2.5</td>
</tr>
<tr>
<td>30</td>
<td>3.0</td>
</tr>
<tr>
<td>45</td>
<td>3.5</td>
</tr>
<tr>
<td>60</td>
<td>4.0</td>
</tr>
<tr>
<td>75</td>
<td>4.6</td>
</tr>
<tr>
<td>90</td>
<td>5.3</td>
</tr>
</tbody>
</table>

Table 2.2. Estimated compression heating factors (°C per 100 MPa of water at various initial temperatures (Source: Nguyen and Balasubramaniam, 2009).
value of the food product. Prior to the experiment, the food sample should be
equilibrated in a water bath to the desired initial temperature (T ± 0.5°C) and then
transferred to the pressure chamber. Temperature of the food sample should be
monitored with a thermocouple unaffected by the high pressures used, and placed
in the cold spot of the food product. Pressure should then be applied and held at
the selected target pressure to monitor temperature change, while keeping hold
times short enough to minimize heat loss to the surrounding environment
(Rasanayagam et al., 2003; Ramaswamy and Balasubramaniam, 2007). The heat of
compression of the food product can then be calculated by the following equation.

\[ \delta = \left( \frac{T_p - T_0}{\Delta P} \right) \times 100 \]  

(2)

Here, \( \delta \), \( T_p \), \( T_0 \) and \( P \) are \( \delta \) (°C per 100MPa), maximum sample temperature (°C) at
the applied pressure, initial sample temperature (°C) at atmospheric pressure, and
applied pressure in MPa, respectively (Ramaswamy and Balasubramaniam, 2007).

The pH of the food product is a very important factor in the design of the process
and is key in determining the required heat levels that assist the applied pressure.
Pathogens present in acidic foods (pH ≤ 4.6) can be inactivated with pressures of
600MPa combined with temperatures of 45-50°C, however, bacterial spores have
shown to be highly resistant to pressures at ambient temperature. Therefore, low-
acid foods (pH > 4.6) require combined heat (90-120°C) and pressures of 500-
900MPa to inactivate spore-forming pathogens such as Clostridium botulinum to
achieve commercial sterility (Rastogi et al., 2007). Under pressure water undergoes ionic dissociation, leading to a transient acidic pH shift (Farkas and Hoover, 2000), however, more research is needed to understand the pH shift of various food constituents under pressure.

The $a_w$ of the food product can also affect the efficacy of the PATP process. Though there have been limited studies on the affects of $a_w$ on the inactivation of bacterial spores during PATP, it has been reported that in general, reduced $a_w$ of a food system results in a reduction of bacterial spore inactivation (Ananta et al., 2001; Furukawa and Hayakawa, 2000; Raso et al., 1998).

Although applied pressure provides for a uniform increase in temperature of a homogeneous food product due to $\delta$, physical attributes of the food product such as viscosity and thermal conductivity are important to consider for product preheating. One primary advantage of PATP is reduced thermal degradation to food products when compared to traditional heat processes, it is important to design the preheating segment of the process such that it minimizes product come-up time and unwanted thermal effects. This can become a significant factor for food products that are heterogeneous, viscous, have low thermal conductivity, or are packaged in large volumes.
2.5.10 Quality of PATP

Color and texture are considered important quality factors of a product by consumers, and these are the two most changed during thermal sterilization (Leadley et al., 2008). Texture benefits of PATP on vegetables have been studied and shown in many cases to provide firmer, most fresh-like qualities, while often retaining colors much closer to their fresh state than traditional thermal processing. Leadley, Tucker, and Fryer (2008) compared the texture and color of green beans subjected to PATP with 86°C preheat and double pulse process of 2min 700MPa, 1 min ambient pressure and a second 2 min 700MPa hold (peak temperature 117°C); and a thermal process equivalent to F0 121.1°C 1 to 3 min at the end of cooling. Thermal processing temperature was 115°C. The texture of the PATP processed green beans was significantly firmer than the thermally processed beans, and held their firmness for up to 7 months (maximum force for cutting of bean 6.8 N and 2.5 N for PATP and 3 min retort processes respectively). Carrots subjected to PATP conditions of 700MPa at 105°C for 15 min showed significantly firmer texture than that of carrot samples processed at 105°C for 15 min (hardness of 14.08±3.28 and 4.36±1.24 PATP and thermal process respectively)(Rastogi et al., 2008).

Tomato puree has been shown to retain much of its fresh-like color and color degradation kinetics after subjection to 300-700MPa and 65°C for 60 min (Rodrigo, van Loey, and Hendrickx, 2007).
Vitamin retention can be significantly reduced by thermal processing. Kreebers, Matser, Koets, and van den Berg (2002) used pressure combined with 75°C preheat for 2 min and two pulses of 1000MPa and 105°C for 80s with 30s ambient pressure separating the pulses on green beans and noted that 76% of ascorbic acid present in raw bean samples was present in those treated with PATP.

2.6 Legumes

2.6.1 Importance as a World Crop

*Phaseolus vulgaris*, known as common beans, are the most widely cultivated and consumed legume in the world (FAO, 1995), and provide more than 20% of the total dietary protein for many populations in Latin America, Africa and many other tropical and subtropical regions (De León et al., 1992). Beans are an economical source of protein 18-25% (Swaminathan, 1974), dietary fiber 15-25% (Hughes et al., 1996), complex carbohydrates 60-67% (Guegen, 1983; Augustin and Klein, 1989), vitamins and minerals (1-3%), and are highly important in low economic regions of the world where protein-energy malnutrition is a significant problem (FAO/WHO, 2001).

In 1995 bean consumption in the United States was 7.6 lbs per capita, an increase of 31.0% over previous years (USDA, 1997). As of 2002, it has been reported by the Census of Agriculture that 8,647 Farms in the United States produce dry edible
beans, with 1.7 million acres of land committed to their cultivation and consumer sales reached $1.8 billion (USDA, 2005).

2.6.2 Nutritional Factors

Bean proteins contain significant quantities of arginine, aspartic acid, glutamic acid, leucine and lysine (Nielsen, 1991). Are a good source of niacin, thiamin, calcium, iron and potassium (Guegen, 1983). The high levels of lysine present make beans a suitable complementary crop to cereal grains, of which lysine is the limiting amino acid (Nielsen, 1991). However, beans are limited in sulfur containing amino acids, and the protein digestibility of raw beans is lower than animal and vegetable proteins, approximately 50 to 79%, but increases to 90% when cooked (Nielsen, 1991; Bressani, 1994).

Beans contain several anti-nutritional factors (ANFs), including trypsin inhibitors (Gupta, 1987; Singh, 1988), tannins (Nielsen, 1991; Reddy et al., 1985), phytic acid (Urbano et al, 2000) oligiosaccharides (Singh, 1988; Udensi et al., 2007), protein structure, protein-starch matrix, and hemicellulose. Tannins, lectins and tripsin inhibitors may bind with proteins or protein receptors (Jaffé, 1968; Nielsen 1991). These factors limit the nutritional value (Deshpande and Nielsen, 1987), but can be minimized with traditional processing methods of soaking, cooking, germination and fermentation (Yadav and Khetarpaul; 1994; Chau and Cheung, 1997; Trugo et
Adequate cooking inhibits the ANFs and unfolds the bean proteins, exposing sites susceptible to proteolysis (Liener, 1989; Carbonaro et al., 1997). However, prolonged cooking reduces protein quality and lysine retention (Bressani et al., 1963; Antunes and Sgarbieri, 1979).

2.7 Texture

2.7.1 Importance of Texture in Food

The texture of processed food products is considered a very important quality attribute (McKenna, 2003). Texture plays a key role in the mouthfeel of a food product (McKenna, 2003), and as noticed by Schiffman (1977; Schiffman et al., 1978), is a necessary component in identification of foods to the consumer.

Many food processes are developed specifically for the modification of food textures, such as grinding mechanisms of whole wheat to flour, to then produce breads and cereals, or complex food ingredient systems developed to achieve a desired texture affect (Bourne, 2002). However, in retrospect, some food processes develop undesirable textural changes, such as softening of fruits and vegetables through freezing, canning irradiation of fruits and vegetables (Bourne, 2002).
2.7.2 Texture of Beans

Water imbibition during soaking softens the texture of beans, and further reduces necessary cooking times (Quast and da Silva, 1977). Utilizing soaking temperatures greater that 30°C improves water imbibition and reduces soaking times (Abu-Ghannam and McKenna, 1997; Ramaswamy et al., 2005). Pressure treatment of navy beans shows significant improvement of water imbibition over thermal soaking alone (Ramaswamy et al., 2005). Pressure treatments applied to black beans decreased soak time to the saturation point by 50% compared to standard soaking, and reduced cooking times to achieve soft texture by 25 to 39% (Sangronis, 1999). Ibarz et al. (2004) reported applied elevated pressure to chickpeas at room temperature increased water absorption rates and decreased cooking time as compared to chickpeas soaked at room temperature for 23 h. It was also reported that pressures combined with heat could adequately soften bean texture comparable to that of beans soaked for 10 h at room temperature (Sangronis, 1999).

Processing and soaking effects on texture can be measured using automated equipment and data acquisition systems. Measuring bean hardness by analysis the peak force required to cut transversely through beans has been previously used with great success (Abu-Ghannam, 1998; Leadly et al., 2008).
2.8 Proteins

2.8.1 Protein Digestibility

The susceptibility of a protein to enzymatic hydrolysis in the digestive system determines its digestibility (Nielsen, 1991), and can be analyzed by both in vivo in animals and in vitro using a multi-enzymatic system of gastric enzymes designed to simulate proteolysis in the human stomach. In vivo methods take several days to complete and are costly, so in vitro methods are often preferred (Hsu et al., 1977). Exposed carboxy ends of amino acids due to the hydrolysis of proteins results in a pH drop which can be measured and correlated to traditional rat in vivo methods (Hsu et al., 1977; Rasco, 1994).

2.8.2 HPP effects on Proteins and Enzymes

It has been reported that HPP modifies the structure of proteins by ionizing acidic and basic amino acids, disrupting hydrogen and hydrophobic bonds, resulting in the formation of new bonds upon the release of pressure (Hoover et al., 1989; Johnston et al., 1992). Protein structures are affected by pressures greater than 100 to 200 MPa. At these elevated pressures oligomeric chains dissociate into subunits, monomeric chains experience partial unfolding and denaturation. This in turn can lead to protein aggregation and possible gelatinization; however, these effects are dependent on the temperature and pressure used during processing, and the pH of
the food system (Cheftel, 1995). Under pressures of 100-300 MPa the changes may be reversible, however pressures in excess of 300 MPa are irreversible (Thakur and Nelson, 1998). The temperature of denaturation for proteins is also affected by HPP. At pressures up to 100 MPa, the denaturation temperature generally increases, while at higher pressures the temperature decreases (Figure 2.4). As a result, under higher pressures proteins tend to denature at room temperature, rather than higher temperatures (Messens, Van Camp, and Huyghebaert, 1997).

Figure 2.4. Phase diagram for pressure-temperature of proteins (from Messens, Van Camp and Huyghebaert, 1997).
In Figure 2.4, Zone I indicates an increase in pressure results in an increase in denaturation temperature, Zone II shows that below the maximum phase transition temperature, higher pressures result in lower denaturation temperatures, where Zone III indicates below the maximum transition temperature, lower pressures and lower temperatures are required for protein denaturation (Messens, Van Camp, and Huyghebaert, 1997).

Enzymes can exhibit a loss or change of functionality through active site changes or protein denaturation (Tsou, 1986). HPP has shown both increase and decrease in enzyme activity depending on the enzyme, enzyme and substrate concentration, process pressure and temperature and food system pH (Butz et al., 1994; Gomes and Ledward, 1996; Hoover et al., 1989; Morild, 1981).
2.9 References


Chapter 3. The Effects of Pressure-Assisted Thermal Processing on the Texture, Color and \textit{in vitro} Protein Digestibility of Black Beans (\textit{Phaseolus vulgaris L.})

3.1 Abstract

Pressure-assisted thermal processing (PATP) is an emerging alternative food processing technology aiming to provide shelf-stable foods with better quality attribute retention than traditional thermal processing techniques. The effect of PATP on texture, \textit{in vitro} protein digestibility (IVPD), color, water imbibition and cooked volume of black beans (\textit{Phaseolus vulgaris L.}) was investigated. Beans were presoaked (15, 30, 60 and 120 min at 82°C, 24 h at 23°C) and subjected to 600 MPa at 105°C for 1 s, 5, 10, 15 and 30 min. To identify the effect of pressure on the selected factors, beans were thermally processed (TP) at 105°C for 5 min using a still retort. PATP was found to significantly increase bean softening, IVPD and water absorption as compared to thermal processing. Increasing pressure hold time during PATP generally increased bean softening. Changes in pressure holding time or soak time did not alter IVPD of PATP treated samples. Beans soaked for 15, 30 and 60 min showed lower volume increases after PATP of 5 min as compared
to thermally processed beans. Within the range of process conditions of the study, PATP appears a viable alternative for preserving black beans.

3.2 Introduction

Pressure-assisted thermal processing (PATP) is an alternative technology to traditional thermal processing, that utilizes elevated pressures of 500 to 900MPa (72,500 psi to 130,000 psi) combined with temperatures of 90 to 120°C to produce shelf-stable, commercially sterile, low-acid food products while minimizing thermal degradation of important quality attributes (texture, color, flavor, aroma, nutritional value) (Balasubramaniam and Farkas, 2008; Balasubramaniam et al., 2008; Leadley et al., 2008; Rastogi et al., 2008).

In traditional thermal processing of low-acid food, products typically undergo severe thermal treatments in the retort process. Although the retort (or canning) has been used for food preservation since the late 19th century, it can be detrimental to quality attributes of food that are susceptible to thermal degradation. This can be particularly troublesome for thick viscous products or those products packaged in large volume containers, as slow temperature come-up and post processing cooling can lead to over-processing of the food product near the walls of the container (Fellows, 2000).
Under high pressures, food products experience a uniform increase in temperature that occurs almost instantly due to heat of compression (δ). Heat of compression during PATP allows for rapid heating to final processing temperatures of pre-heated food products, and further, rapid cooling following processing times, minimizing detrimental thermal processing effects (Nguyen and Balasubramaniam, 2009).

Common beans (Phaseolus vulgaris L.) are an important world crop, providing many developing countries with their primary source of proteins (Bressani, 1994), and contain high levels of lysine, the limiting amino acid in many cereal crops (Nielsen, 1991). Beans continue to play an important role in the nutrition of low economic societies, containing approximately 18 to 25% protein, along with other important nutritional components such as dietary fiber, vitamins and minerals (Swaminathan, 1974). However, beans in their raw form contain several antinutritional factors (ANFs) that limit the ability for the human body to digest their protein (Gupta, 1987; Singh, 1988; Nielsen, 1991; Bressani, 1994).

Traditional processing methods such as soaking, cooking, germination and fermentation can inhibit the effects of the ANFs in beans (Yadav and Khetarpaul, 1994; Chau and Cheung, 1997; Trugo et al., 2000), and unfold the bean proteins, allowing for better digestion due to proteolysis of exposed susceptible sites (Liener, 1989; Carbonaro et al., 1997). Lysine is, however, susceptible to heat and
prolonged cooking times can reduce lysine retention and protein quality (Bressani et al., 1963; Antunes and Sgarbieri, 1979).

The effects of pressure on beans have been previously studied by Ramaswamy et al. (2005) and Sangronis (1999). Pressures of 400 and 700 MPa at 55°C were applied to navy beans. The treatment showed an increase water imbibition rates as compared to thermal soaking without applied pressure (Ramaswamy et al., 2005). Pressures of 275, 410, 550 and 690 MPa at 25°C for 5 min were applied to black beans. The black beans reached water saturation 50% faster than untreated beans, and showed reduced cooking times by 25 to 39% (Sangronis, 1999). The effect of pressure treatment on black bean texture and protein digestibility was also investigated by Sangronis (1999). Pressures were applied at 275, 413, 550 and 690 MPa at temperatures of 25, 45, 65 and 85°C for 5, 10 and 15 min. It was reported that an increase in \textit{in vitro} protein digestibility (IVPD) was observed for treatments at pressures greater than 410 MPa as compared to raw beans. The IVPD was independent of temperature and time of processing, but dependent on the pressure applied. Pressure treatments softened the texture of black beans, but the effects were independent of pressure and dependent on temperature.

While these previous studies showed promise for the application of pressure and temperature as a processing method for beans, the potential use as a complete alternative processing step utilizing elevated pressure combined with high
temperature to produce a shelf-stable low-acid food product was not investigated. Further, the soaking of beans prior to processing is common in the processing of beans. The interactive effect of soaking techniques combined with PATP has not been previously investigated.

In this study, PATP is being investigated to determine its effect on texture, color, protein digestibility and water absorption of black beans, and compare these results to a thermal treatment.

3.3 Materials and Methods

3.3.1 Samples

Common beans (*Phaseolus vulgaris* L.) or cultivar Black Beans were acquired from a local market in Columbus, Ohio. Prior to processing, the beans were stored in a dry environment at a room temperature of 23 to 25°C.

3.3.2 Heat of Compression Determination

The heat of compression (δ) for soaked black beans (24 hrs at 23°C) was experimentally calculated in order to determine the proper initial temperature for PATP processing. Sixty grams of dry, sorted and rinsed black beans were soaked in deionized, demineralized water (1:3 w/w) for 24 hrs at 23°C to allow for complete
hydration. The beans were drained and filled into a 10 ml plastic syringe (internal dimensions 1.4 cm x 6.5 cm, wall thickness .1 cm; 14-823-2A; Fisher Scientific Co., IL) that was insulated with three layers of sports tape (CVS/Pharmacy, Columbus, OH). To remove void space between beans, the plunger was inserted into the syringe to compress the beans, then removed and additional beans added. This was repeated until the syringe was compacted with beans and full. A K type thermocouple probe (SCASS-062U-7, Omega Engineering, Inc, Stamford, CT) attached to the top closure of the pressure chamber was placed inside the syringe and into the beans at the geometric center of the syringe. The bean sample was placed in a water bath (Iso Temp 128, Fisher Scientific Co., IL) set at 60.5°C and allowed to equilibrate with water bath temperature. The bean sample was then placed in a 54 ml vertical stainless steel pressure chamber (2 cm internal diameter) of a custom-made lab scale high pressure kinetic tester (PT-1, Avure Technologies, Inc., South Kent, WA, USA). The pressure chamber was immersed in a temperature controlled bath consisting of propylene glycol (Houghto-safe 620TY, Houghton International Inc., PA; δ = 4.2 to 4.7 °C per 100MPa) and a recirculation heater (Iso Temp 2150, Fisher Scientific Co., IL) at 60.5°C to maintain isothermal conditions. The system was pressurized to 700 MPa (come-up time approx. 37 s) using propylene glycol (Houghto-safe 620TY, Houghton International Inc., PA; δ = 4.2 to 4.7 °C per 100MPa) as the pressure transmitting fluid, maintained for 30 s, and was depressurized. Sample temperature and pressure during the process,
along with bath temperature was recorded using a data acquisition system (Daq-
Board/2000, IOtech, Inc., Cleveland, OH; DasyLab 8.0, National Instruments
Corp., Austin, TX). The experiment was repeated in triplicate using fresh samples
for each iteration.

The $\delta$ of the black bean samples was calculated as the difference between the
initial temperature before pressurization and the maximum temperature of the
sample at the target pressure of 700 MPa.

$$\delta = \left(\frac{T_p - T_o}{\Delta P}\right) \times 100$$

In this equation, $\delta$, $T_p$, $T_o$ and $P$ are $\delta$ (°C per 100MPa), maximum sample
temperature (°C) at the applied pressure, initial sample temperature (°C) at
atmospheric pressure, and applied pressure in MPa, respectively (Ramaswamy and
Balasubramaniam, 2007).

3.3.3 Experimental design for bean processing

Two factors in a 5x5 model were used in this experiment; soak time and processing
time. Black beans were soaked at 23°C for 24 hrs, and 82°C for 15, 30, 60 and 120
min, then treated at 105°C under 600 MPa of pressure for processing times of 1 sec,
5, 10, 15 and 30 min. For comparison, black beans were also soaked and treated
under thermal conditions at 105°C; another set of samples were prepared for soak only comparative analysis. All sample sets included two replications.

3.3.4 Soak treatments
Ten grams of dry unsoaked black beans were rinsed and sorted to remove debris and broken beans. Beans were placed in ethylene vinyl alcohol (EVOH) bags (7 cm x 10 cm; Thompson Equipment & Supply, Cincinnati, OH) with deionized and demineralized water (1:3 w/w) and heat sealed, removing as much air as possible. Samples prepared for the 24 hr soaking period were placed in temperature controlled room at 23°C and allowed to hydrate for 24 hrs. The remaining samples were placed in an 82°C water bath (Iso Temp 128, Fisher Scientific Co., IL) and maintained at 82°C until processing for 15, 30, 60 and 120 min. The 24 hr soak beans were placed in the 82°C water bath for the final 15 min of their soak period to facilitate the preheating step of the PATP treatment. A zero soak treatment was not possible due to the need for the preheating step of the PATP process to ensure the product sample reached the required minimum initial temperature. Eighty-two degrees Celsius was chosen as the soak temperature based on the calculated $T_i$ needed to achieve 105°C processing temperature at 600 MPa at the experimentally determined δ of the black beans.
3.3.5 Pressure-assisted thermal processing treatment

Preheated, packaged bean samples were placed in a preheated cylindrical stainless steel loading basket (11 cm x 65.5 cm) containing 80°C USP kosher polypropylene glycol (Brenntag Mid-South, Inc., St. Louis, MO). The loading basket was insulated with 0.5 cm polytetrafluoroethylene (PTFE) to help minimize heat loss to surrounding environment. The loading basket was placed in a 5L, high pressure system (Iso-Lab FPG11500, Stansted Fluid Power LTD, Stansted, Essex, UK) with the system heating jacket maintained at 105°C. Polypropylene glycol (Brenntag Mid-South, Inc., St. Louis, MO) was used as the pressure transmitting fluid, and was heated to 77°C through temperature controlled tube-in-tube heat exchangers prior to entering the system. Samples were treated at 600MPa (87,000 psi) (pressure come-up time approx. 1.5 - 2 min) and 105°C for hold times of 1 sec, 5, 10, 15 and 30 min. After completion of hold time and the decompression cycle (90 s), the load basket was removed from the chamber, and samples were placed in an ice bath until cooled to ambient room temperature to minimize further cooking from residual heat. All samples were analyzed within 4 hours after processing.

3.3.6 Thermal Treatment

Preheated bean samples were placed in a still retort (Loveless Manufacturing, Tulsa, OK) and processed for 5 min at 105°C. Retort temperature was set for 105°C
and the bean sample temperature was monitored at the package cold spot using a T-type thermo couple and transducer (Omega Engineering, Inc., Stamford, CT). Process timing was started once internal temperature reached 105°C. The come-up time to 105°C was 6 min. Following process hold time, steam pressure was released, and samples cooled using cold water to 77°C over 3 min, then further cooled using an ice water bath to ambient temperature. Samples were analyzed immediately after processing.

3.3.7 Color Analysis
Bean sample color was measured using a Minolta CR-300 (Minolta Co., Ltd., Tokyo, Japan) hand-held colorimeter, using the Hunter L a b scale with a D$_{65}$ illuminant. The instrument was calibrated prior to sample measurement using a standard calibration plate supplied by Minolta for the instrument. Bean samples were removed from processed package and sealed in new, untreated EVOH bags prior to color measurement to prevent any color discrepancies from processing effects on packaging material. Beans were pressed together (without rupturing) by hand to remove dead space between adjacent beans for color measurement, to ensure bean skin was measured, not empty space. Color measurement values taken in triplicate.
3.3.8 Texture Analysis

Texture analysis of bean samples was performed on a Texture Analyzer TA-XT²® (Stable Micro Systems Ltd., Sodalming, Surrey, UK) with a 7 cm wide cutting blade on a stainless steel platform. The force (Newton) required to cut the individual bean was recorded. Cutting distance was set at 4 mm, and was run at 1mm/sec with a load cell of 25 kg. Four mm was selected as a cutting distance based on the 75% of the average thickness of 20 selected beans. Beans were positioned under the blade, laying flat and were cut transversely. Twenty beans were analyzed for each treatment. Peak force and force per distance displacement were recorded using Texture Expert Exceed v2.64 (Stable Micro Systems Ltd., Sodalming, Surrey, UK) (Abu-Ghannam, 1998; Leadley et al., 2008).

3.3.9 In vitro Protein Digestibility

The effects of two PATP processing conditions on the in vitro protein digestibility (IVPD) of black beans were investigated. Bean samples soaked at 15, 30, 60 and 120 min were processed at 105°C, 600 MPa for 5 and 10 min. For comparison, the IVPD of raw beans and beans cooked in boiling water for 60 min was also investigated. The cooked beans were prepared by sorting and rinsing dry black beans, then placing in a 500 ml beaker containing 50 g beans and deionized, demineralized water (1:3 w/w). The beans were boiled on a hot plate for 60 min once boiling...
conditions were reached. After 60 min, the beans were drained and cooled in an ice bath to stop the cooking process.

Bean samples used for in vitro protein digestibility were ground to pass through a 50 mesh sieve. A 50 ml aqueous suspension of bean protein was prepared with deionized demineralized water (6.25 mg protein / ml) and adjusted to pH 8.0 with 0.1 N HCl/NaOH as needed in a 37°C water bath (Fisher Scientific IsoTemp 128). A multi-enzyme aqueous solution of trypsin, chymotrypsin and peptidase (1.6 mg trypsin, 3.1 mg chymotrypsin and 1.3 mg peptidase / ml) was made fresh and maintained in an ice bath, with pH adjusted to 8.0 using 0.1 N HCl/NaOH. Five milliliters of the multi-enzyme solution was added to the bean protein suspension, and the pH was recorded after 10 min to determine the extent of hydrolysis (Hsu et al., 1977). Protein digestibility was determined using the regression equation \( Y = 210.46 - 18.10 X \), where \( X \) is the pH at 10 min (Hsu et al., 1977; Sangronis, 1999; Han et al., 2007; Khattab et al., 2009).

3.3.10 Water Absorption and Volume Change Analysis

Ten grams of beans were sorted and rinsed using only whole beans of average size. Residual water was dried using paper towels, and the beans were allowed to air dry for up to 1 h. Bean weights were recorded and then the beans were added to a 30 ml graduated cylinder contained 10 ml of deionized demineralized water. The
combined bean and water volume was recorded and the initial water volume subtracted to determine the volume of the beans from their displacement. The beans were then placed in EVOH bags (7 cm x 10 cm; Thompson Equipment & Supply, Cincinnati, OH) with an additional 20 ml deionized demineralized water and heat sealed. The beans were soaked in an 82°C water bath (Iso Temp 128, Fisher Scientific Co., IL) for 15, 30, 60 and 120 min. Following soak treatment beans were placed in an ice water bath to stop the heated soaking process for 5 min and then removed from bags, rinsed and dried by blotting with paper towels. Bean weights were recorded and the water displacement procedure was repeated to attain bean volume increase. Beans were then placed in new EVOH bags with 30 ml deionized demineralized and processed at 600 MPa 105°C for 5 min. Following the PATP process, beans were rinsed, dried, weighed. Volume change was calculated using the water displacement method. Correction for loss of solids during soaking and PATP treatment was not considered.

3.3.11 Statistical Analysis
Statistical analysis of data was performed using Minitab® Statistical software version 15.1.30.0 (Minitab, Inc., State College, PA). Two-way analysis of variance (ANOVA) at 95% confidence was used to analyze the significance of soak time, processing treatment and their interactive effect on bean texture softening, protein
digestibility and color. One-way ANOVA with Tukey’s test was used to analyze the significance of the individual factors of soak time and treatment on bean texture softening, IVPD, color, water absorption and volume change. Significance for all statistical analysis was defined as \( p < 0.05 \).

3.4 Results and Discussion

3.4.1 Heat of Compression

The heat of compression for the black beans was determined to be \( 3.85 \pm 0.12 \) °C/100 MPa, and was used to identify the \( T_i \) for PATP processing and soaking temperature (Figure 3.1). At 600 MPa the needed \( T_i \) for the black beans to reach 105°C is 82°C.
3.4.2 Temperature and pressure history during PATP and TP

Bean samples processed with PATP experienced a rapid increase in temperature during pressurization due to δ (Figure 3.2). Pressure come-up time was approximately 2 min for each run and during this time sample temperatures rose from 82°C to 107°C. The target temperature of 105°C was slightly exceeded during the first few minutes of processing, due to heat transfer from the heated vessel.
(maintained at 105°C) to the product basket and samples during compression. During the course of the pressure hold time, sample temperatures slowly approached 105°C due to thermal loss to the surrounding environment. During decompression, sample temperatures rapidly returned to 82°C over the 90 s required to reach atmospheric pressure.

Bean samples processed with TP experienced a slower temperature come-up time to 105°C (6 min) than the PATP samples (Figure 3.3). During the process hold time, sample temperature continued to rise to slightly above the target temperature. Cooling time to initial temperature was 3 min as opposed to 1.5 min for PATP. Longer come-up and cool-down times for TP created a longer overall cycle time for the process, leading to longer times exposed to higher temperatures.
Figure 3.2. Sample PATP temperature-pressure history for black bean samples processed at 600 MPa, 105°C 15 min.
3.4.3 Color

Two-way ANOVA indicated that both PATP and TP had a significant effect on the color of the beans \((p < 0.05)\), affecting lightness \((L)\) (Figure 3.4), red-green \((a)\) (Figure 3.5) and yellow-blue \((b)\) color attributes (Figure 3.6). Various soaking conditions only had significant effect on \(a\) and \(b\). An interactive effect between soak time and processing condition was observed for each color attribute \((p < 0.05)\), however, there was no noticeable trend observed between increase in PATP processing time and color values, nor soak time and color values.
Figure 3.4. Lightness value for soaked and processed beans.

Figure 3.5. a value for soaked and processed beans.
3.4.4 Texture

The peak force (N) required to cut through 50-75% of the black beans was used as an indicator of the softening of bean texture. PATP treatments provided significantly softer texture compared to thermally treated and soaked only bean samples (Figure 3.7, Figure 3.8, Figure 3.9). In general, it was noted that increasing the pressure hold time during PATP increased the amount of bean softening. Further, increased soaking time at 82°C showed increased bean softening for PATP and thermal processing (Figure 3.8, Figure 3.9).
Two-way ANOVA indicated significant effects of soak time ($p < 0.05$), processing treatment ($p < 0.05$) and their interaction ($p < 0.05$) on the softening of black bean texture.

The peak force required to cut beans soaked at 82°C for 15, 30 and 60 min were not significantly different; however beans soaked for 120 min were significantly softer than all other soak treatments. Beans soaked under the 24 h at room temperature showed similar softening, compared to 30 and 60 min soak treatments at 82°C (Figure 3.7).

Thermally treated beans showed similar softening at soak treatments of 15 and 30 min (82°C). Beans soaked at 60 min (82°C) and 24 hours (room temp.) showed similar softening, but were significantly softer than those soaked at 15 and 30 min (82°C). The soak treatment of 120 min (82°C) provided significantly softer texture than other soak treatments subjected to retort processing (Figure 3.8).

Increased bean softening was observed with an increase in PATP processing time at each soaking condition (Figure 3.9). However, PATP samples at 5 and 10 min pressure holding time were not significantly different at 15, 60 and 120 min soak treatments. PATP samples soaked at 120 min did not show any significant difference in softening at 5, 10 and 15 min pressure holding time. Samples soaked at 30 min showed significant softening between each pressure hold time.
PATP at 5 min pressure holding time provided significantly softer texture for each soak treatment as compared to 5 min thermal processing at 105°C (Figure 3.10), indicating that pressure enhances bean softening under similar thermal treatment, despite shorter overall processing time as the thermally treated bean samples experienced longer come-up time (6 min thermal, 2 min PATP) and longer cooling time (3 min thermal, 1.5 min PATP).

Under longer pressure holding times (15 and 30 min) no significant difference in bean softening was observed at 30, 60 and 120 min. Several process and soak times combined to provide similar bean softening results; 15 min pressure hold at 30, 60 and 120 min; 120 min soak at 5 10 and 15 min pressure hold time all provided similar bean texture (Figure 3.9).
Figure 3.7. Bean texture softening under various soaking treatments (82°C for 15, 30, 60 or 120 min and 23°C for 24 h).
Figure 3.8. Texture softening of soaked (82°C for 15, 30, 60 or 120 min and 23°C for 24 h) and thermally processed (105°C, 5 min) beans
Figure 3.9. Texture softening of soaked (82°C for 15, 30, 60 or 120 min and 23°C for 24 h) and PATP (600 MPa, 105°C, 1s, 5, 10, 15 and 30 min hold time) beans
Sangronis (1999) previously reported that pressure alone was not an effective means of softening black beans and that temperature was required. Sangronis also reported that increasing pressure hold time did not increase bean softening. However, in this previous work, the bean samples were not subjected to soaking pretreatments. Sangronis also demonstrated that under 10 min pressure hold time...
there was no significant difference in bean softening during increased pressure and temperatures (275, 410, 550 and 690 MPa; 25, 45, 65 and 85°C).

3.4.5 *In vitro* Protein Digestibility

The IVPD of raw black beans was found to be 73%. The processing treatments used in IVPD evaluation showed significant increases (p < 0.05) in protein digestibility. Cooking beans in boiling water for 1 hour increased the IVPD to 76%, retort processing showed an increase to 75-76% and PATP increased IVPD to 79% (Table 3.1).

Two-way ANOVA analysis on the PATP treatments indicated that there was no significant difference between process (p = 0.640) or soak times (p = 0.517), nor was there any interaction between the factors (p = 0.413), indicating an increase in process time did not significantly affect IVPD. Further, there was no significant increase in IVPD by increasing the time of soaking prior to PATP.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soak Time</th>
<th>Protein Digestibility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>n/a</td>
<td>72.7 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cooked (boiled for 1 h)</td>
<td>n/a</td>
<td>76.1 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Retort 5 min</td>
<td>15 min</td>
<td>75.2 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>75.4 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>75.9 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>120 min</td>
<td>76.0 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>75.4 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>PATP 5 min</td>
<td>15 min</td>
<td>79.1 ± 0.2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>79.1 ± 0.3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>79.0 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>120 min</td>
<td>79.3 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>79.1 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>PATP 15 min</td>
<td>15 min</td>
<td>79.2 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>79.2 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>79.1 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>120 min</td>
<td>79.1 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>79.1 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table 3.1. *In vitro* protein digestibility of raw, cooked, retorted (105°C, 0.2 MPa) and PATP (600 MPa, 105°C) treated black beans. Values in same column with similar letters not significantly different at p ≤ 0.05. Means ± standard deviation.

No significant difference in IVPD was observed between retort samples soaked for 15, 30 min at 82°C and 24 h at 23°C. Retort samples soaked for 60 and 120 min prior to processing showed a significant increase in IVPD compared to 15, 30 min and 24 h soak treatments, but there was no further increase in IVPD observed by increasing soak time from 60 to 120 min.
IVPD values for PATP samples processed at 105°C 600MPa for 5 min (79%) were significantly greater than the samples processed with retort at 105°C for 5 min (75 - 76%) (p < 0.05) indicating that the elevated pressure used in this study increased protein digestibility of the beans over heat treatment alone.

An increase in IVPD due to heat and pressure treatments is expected. Physical factors such as soaking, heat, and pressure increase protein susceptibility to multi-enzyme digestion by disrupting the secondary, tertiary or quaternary protein structure (Nielsen, 1991; Carbonaro et al., 1997; Carbonaro et al, 2000). Han, Swanson and Baik (2007) reported that heating non-presoaked legumes (chickpea, pea, soybean, lentil) for 30 min in 98°C distilled water significantly increased IVPD of each legume tested, while soaking legumes in distilled water at 627 MPa for 0.5 and 1 hr also significantly increased IVPD from the raw state. Han et al. (2007) also reported that legumes soaked under HPP (627 MPa, 0.5 h, 1 h) then heat treated at 98°C for 30 min in distilled water showed significant IVPD increases as compared to raw legumes heated at 98°C for 30 min in distilled water.

In the previous work of Sangronis (1999) it was reported that HPP with pressures greater than 410 MPa combined with temperatures between 45 and 65°C of black beans significantly increased the IVPD (72 - 79%) from raw (63%) and soaked (71.8%) beans. Sangronis utilized varying pressure, temperature and holding time combinations (275, 410, 550, 690 MPa; 25, 45, 65, 85°C; 5, 10 , 15min) and noted that
protein digestibility was dependent on the pressure applied, but independent of temperature and time, though there was an interactive effect of pressure and temperature, but no significant interaction between temperature and time. Maximum protein digestibility occurred at 690 MPa at 85°C for 10 min (79%).

3.4.6 Water Absorption and Bean Volume

The effect of applied pressure during PATP on bean water absorption and bean volume as compared to a thermal treatment was examined (Figure 3.11). Water mass gained by 10 g of dry unsoaked black beans showed significant increases for increased soaking time (15, 30, 60, 120 min at 82°C; 24 h at 23°C). PATP provided a significant increase in water absorption from the soaking stage (3 - 5 g) and significantly greater water absorption as compared to thermally treated beans. Thermally treated beans showed a significant increase in water absorption for each soaking treatment. Total water gained after PATP treatment of 15, 30 and 60 were significantly different (p < 0.05), with 30 min having the greatest water gain after PATP (12.5 g). Beans soaked for 60, 120 min and 24 h were similar in water gain (12 g) after PATP treatment. After thermal treatment, beans soaked for 30, 60 and 120 min did not show any difference in water absorption (9 g).
Figure 3.11. Water gained under soaking (82°C 15, 30, 60, 120 min; 23°C 24hr), PATP (600 MPa 105°C 5 min), and thermal processing (105°C, 0.2 MPa, 5 min). Initial dry bean mass was 10 g.

Results indicate that the application of pressure (600 MPa) combined with heat (105°C) provides greater water absorption than heat (105°C) alone. Ramaswamy et al. (2005) reported that the application of pressure and heat (400, 700 MPa at 55°C) increased the initial water absorption rate of dry navy beans as compared to beans soaked under thermal conditions (55°C), though during extended soaking at room temperature following pressure treatment absorption rates slowed. Sangronis (1999) reported pressure treatment (275, 410, 550, 690 MPa at 25°C) of
black beans (*Phaseolus vulgaris*) increased water imbibition rates, allowing for saturation to be reached 50% faster than untreated beans. Further cooking times were reduced by 25 to 39% after pressure treatment. Ibarz et al. (2004) reported success in increasing water absorption rates fivefold and reduced cooking time of chickpeas subjected to pressure treatments (275, 410, 550, 690MPa) at room temperature for 5 min as compared to raw samples.

The increase in water absorption noted in PATP samples may be attributed to cracked seed coats from the applied pressure, damaging the bean structure and cellular protein bodies (Kaijiyama et al., 1995). Further, pressure may contribute to increased soluble material extraction, which results in greater water infusion (Zhang et al., 2004).

The effect of soaking, PATP and thermal treatment of black bean volume was investigated (Figure 3.12). Increasing the soak time at 82°C showed significant volume increase 15, 30, 60 min (p <0.05) but similar volume increases at 60, 120 min (82°C) and 24 h (23°C). PATP following soaking showed significant volume increases for each soaking condition as compared to soaking only. Thermal treatments also showed significant volume increase after soaking for each soaking treatment. A general trend of longer soaking times showed better bean volume increases for each processing condition, though 120 min and 24 h did not show significant differences under thermal or PATP treatments. Twenty-four hour
soaking treatment volume increase was also similar to 60 and 120 min for thermally treated beans.

Beans soaked for 15, 30 and 60 min showed less volume increase under PATP than thermal treatment; however each soak treatment showed significantly more water absorption under PATP than thermal treatments. This may indicate that at these particular soak treatments, the applied pressure compacted the beans.

Figure 3.12. Bean volume increase under soaking (82°C 15, 30, 60, 120 min; 23°C 24hr), PATP (600 MPa 105°C 5 min), and thermal processing (105°C, 0.2 MPa, 5 min). Initial volume 8 ml for 10 g dry beans.
3.5 Conclusions

PATP combined with presoaking of black beans provided significantly softer bean texture than thermal treatment alone. The application of pressure for only 1 s provided a 20 fold increase in bean softening compared to soaked only beans at 15, 30 and 60 min, with greater softening observed as pressure hold time was increased (up to 100 fold increase). PATP treatments also increased the \textit{in vitro} protein digestibility of the beans from the raw state and the application of pressure showed an increase in IVPD as compared to thermal treatment alone. The increase in IVPD was independent of the PATP pressure hold time and presoaking conditions. While no clear trend was observed in the color change of the beans due to increased PATP pressure holding time, samples soaked at 82°C were generally lighter in color after PATP with a shift toward blue (\(-b\)) at pressure hold times 1 s, 5, 10 and 15 min. PATP showed greater water absorption than thermal treatment but shorter pressure times displaced less volume increase than thermal treatment.

The use of PATP combined with bean presoaking is a viable alternative process to traditional thermal processing.
3.6 References


Chapter 4. Conclusions and Future Recommendations

4.1 Summary and Conclusions

Elevated pressure (600 MPa) combined with heat (105°C) held for 1 s, 5, 10, 15, 30 min was applied to black beans presoaked for 15, 30, 60 and 120 min at 82°C and 24 h at 23°C. These pressure-assisted thermal processing (PATP) treatments provided for significantly increased bean softening as compared to soaked only bean samples, and samples treated with heat only (105°C, 0.2 MPa for 5 min). In general, as pressure hold time was increased, bean softening increased for a given soak time. Further, in general, for a given pressure hold time, longer soak times at 82°C provided for increased bean softening. Two-way ANOVA analysis provided evidence for an interactive effect of bean soaking treatment and process treatment.

PATP increased in vitro protein digestibility (IVPD) of the beans as compared to their raw state and thermally processed state (105°C for 5 min). IVPD was independent of PATP pressure hold time and bean soaking treatment.

Different PATP pressure hold times and soaking times provided differences in bean color, however, no identifiable trend was observed in relation to increased
pressure hold times or increased soaking times. In general, PATP processed beans showed a slight increase in lightness (L) compared to their soaked only state, with shorter PATP processes (less than 30 min) displaying a slight blue shift.

PATP (5 min pressure hold) provided increased water absorption after soaking treatments, and greater water absorption compared to thermally treated beans. The volume increase of the beans was significantly greater after both PATP and thermal treatments when compared to soaking only. Beans soaked for 15, 30 and 60 min showed significantly less volume increase after PATP than with thermal treatment, indicating that slight compression of the bean material may occur at these conditions.

4.2 Future Recommendations

- Determine the shelf stability and microbial safety of PATP treatment of black beans processed at different hold times
- Conduct sensory analysis on samples to determine consumer acceptability of beans processed by PATP and compare against that to commercially available black bean products.
- Investigate the effect of PATP on trypsin inhibitors present in raw bean samples.
- Investigate the effect of PATP on black bean starch granules.
Appendix A. Texture of Black Beans
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Process Time</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soak Only</td>
<td></td>
<td>221.99 ± 69.67 a</td>
<td>200.14 ± 60.12 a</td>
<td>196.45 ± 55.55 a</td>
<td>69.35 ± 28.20 a</td>
<td>171.93 ± 51.52 a</td>
</tr>
<tr>
<td>Retort 105°C</td>
<td>5 min</td>
<td>24.44 ± 8.11 b</td>
<td>21.63 ± 4.72 b</td>
<td>16.22 ± 4.75 b</td>
<td>10.93 ± 3.37 b</td>
<td>15.68 ± 5.94 b</td>
</tr>
<tr>
<td>PATP</td>
<td>1 s</td>
<td>10.19 ± 1.77 c</td>
<td>9.82 ± 1.80 c</td>
<td>9.31 ± 1.71 c</td>
<td>7.41 ± 0.76 c</td>
<td>11.29 ± 2.09 c</td>
</tr>
<tr>
<td>PATP</td>
<td>5 min</td>
<td>7.00 ± 1.74 d</td>
<td>6.67 ± 1.69 d</td>
<td>5.01 ± 1.04 d</td>
<td>3.57 ± 1.25 d</td>
<td>8.54 ± 1.13 d</td>
</tr>
<tr>
<td>PATP</td>
<td>10 min</td>
<td>7.08 ± 1.47 d</td>
<td>5.23 ± 1.38 e</td>
<td>4.84 ± 1.17 d</td>
<td>3.11 ± 0.81 d</td>
<td>5.64 ± 0.99 e</td>
</tr>
<tr>
<td>PATP</td>
<td>15 min</td>
<td>4.52 ± 0.88 e</td>
<td>3.45 ± 1.07 f</td>
<td>3.31 ± 0.95 e</td>
<td>2.81 ± 0.88 d</td>
<td>3.48 ± 0.76 f</td>
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<tr>
<td>PATP</td>
<td>30 min</td>
<td>4.24 ± 0.56 e</td>
<td>2.35 ± 0.70 g</td>
<td>2.36 ± 0.51 f</td>
<td>1.98 ± 0.38 e</td>
<td>3.22 ± 0.78 f</td>
</tr>
</tbody>
</table>

Table A.1. Peak force (N) required to cut black bean after various processing treatments. Values in same column with similar letters no significantly different at p ≤ 0.05. Means ± standard deviation.
Soak Time Process Time Retort 105°C Soak Only

<table>
<thead>
<tr>
<th>Soak Time</th>
<th>1 s</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>30 min</th>
<th>5 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>10.19 ± 1.77 a c</td>
<td>7.00 ± 1.74 a</td>
<td>7.08 ± 1.47 a</td>
<td>4.52 ± 0.88 a</td>
<td>4.24 ± 0.56 a</td>
<td>24.44 ± 8.11 a</td>
</tr>
<tr>
<td>30 min</td>
<td>9.82 ± 1.80 a c</td>
<td>6.67 ± 1.69 a</td>
<td>5.23 ± 1.38 b</td>
<td>3.45 ± 1.07 b</td>
<td>2.35 ± 0.70 b</td>
<td>21.63 ± 4.72 a</td>
</tr>
<tr>
<td>60 min</td>
<td>9.31 ± 1.71 a b</td>
<td>5.01 ± 1.04 b</td>
<td>4.84 ± 1.17 b</td>
<td>3.31 ± 0.95 b</td>
<td>2.36 ± 0.51 b</td>
<td>16.22 ± 4.75 c</td>
</tr>
<tr>
<td>120 min</td>
<td>7.41 ± 0.76 b</td>
<td>3.57 ± 1.25 c</td>
<td>3.11 ± 0.81 c</td>
<td>2.81 ± 0.88 b</td>
<td>1.98 ± 0.38 b</td>
<td>10.93 ± 3.37 d</td>
</tr>
<tr>
<td>24 h</td>
<td>11.29 ± 2.09 c a</td>
<td>8.54 ± 1.13 d</td>
<td>5.64 ± 0.99 b</td>
<td>3.48 ± 0.76 b</td>
<td>3.22 ± 0.78 c</td>
<td>15.68 ± 5.94 c</td>
</tr>
</tbody>
</table>

Table A.2. Peak force (N) required to cut black bean after various processing treatments. Values in same column with similar letters no significantly different at p ≤ 0.05. Means ± standard deviation.
Figure A.1. Interactive effect of soak time and PATP process time on bean texture.

Peak force (N) required for cutting bean.
Bibliography


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