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CONTINUOUS STEAM STERILIZATION SEGMENTED FLOW ASEPTIC PROCESSING OF PARTICLE FOODS

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ABSTRACT

A continuous steam sterilization unit capable of producing shelf-stable aseptically processed particulate foods of high quality was developed. The system utilizes steam as the heating medium to achieve better heat transfer and segmented-flow technology to produce a smaller residence time distribution than pipe-flow aseptic processing.

A temperature penetration test was used as the foundation for developing a scheduled thermal process using microbiological kinetics. Most conservative process times of 11.04 and 3.74 minutes to achieve a 5D process for *Clostridium sporogenes* for whole and sliced mushrooms, respectively, were calculated from temperature penetration data. Aseptic processing times are less than would typically be encountered in conventional canning of mushrooms.

When compared to conventionally canned mushrooms, aseptically processed yield (weight basis) increased 6.1% (SD=2.9%) and 6.6% (SD=2.2%), whiteness (L) improved 3.1% (SD=1.9%) and 4.7% (SD=0.7%), color difference (ΔE) improved 6.0% (SD=1.3%) and 8.5% (SD=1.5%), and texture improved 3.9% (SD=1.7%) and 4.6% (SD=4.2%) for whole and sliced mushrooms, respectively. The segmented-flow processing system utilized high temperature, short time (HTST) processing conditions, eliminated a separate blanching step, eliminated the unnecessary packaging of water and promoted the use of bag-in-box and other versatile aseptic packaging methods.

Inoculated pack studies proved the development of a scheduled process based on temperature penetration data of the slowest heating particle lead to predictable destruction of *Clostridium sporogenes* spores. At an inoculation level of approximately 10^3 CFU/container, viable spores were eliminated after 9.3 minutes process time, indicating that 11.0 minutes of process time used in this study could be likely be reduced to approximately 10 minutes.

Following aseptic processing, mass had decreased 24.4% (SD=2.9%) and 55.9% (SD=3.1%), volume 51.8% (SD=1.9%) and 56.8% (SD=3.3%), and characteristic mushroom dimensions 4.7% (SD=5.8%) to 28.4% (SD=5.2%) and 9.0% (SD=3.8%) to 30.4% (SD=4.4%) for aseptically processed versus raw, and aseptically processed versus vacuum hydrated mushrooms, respectively. However, on average, length decreased 96.6% and 63.8% more than diameter. Vacuum hydration increased moisture content (dry basis) by 71.5% relative to raw mushrooms. Aseptic processing decreased moisture content (dry basis) by 27.4% and 57.7% relative to raw and vacuum hydrated mushrooms, respectively.

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I would also like to thank the faculty, staff and students of the Department of Agricultural and Biological Engineering at Penn State University for a superb education and many great friendships that will last a lifetime, Tom Rhodes for a steady supply of mushrooms from the Mushroom Test and Demonstration Facility at Penn State, and the Pennsylvania Department of Agriculture whose grant supported this project.

Chapter 1

Continuous Steam Sterilization Segmented-Flow Aseptic Processing of Particle Foods

Abstract

A continuous steam sterilization unit capable of producing shelf-stable aseptically processed particle foods (non-uniform in size and shape) over a wide time-temperature range was developed. The system utilizes pressurized steam as the heating medium to achieve better heat transfer and segmented-flow technology to produce a smaller residence time distribution than pipe-flow aseptic processing. Foods are processed only as long as needed to achieve the target microbiological lethality, and are not overcooked. The heating and holding processes occur on a 7 m flight conveyor with a bed width of 13.0 cm and height of 5.3 cm contained in a 0.2 m stainless steel tube. Conveyor speed was adjustable to provide a residence time in the range of 4.5 to 30.0 minutes and steam pressure was adjustable to provide a processing temperature in the range of 100°C to 130°C. Sterile cool water circulating at 0.06 to 0.32 L sec^{-1} cools particles in a direct-contact, gravitational counter-flow chilled water system. A direct-contact, gravitational counter-flow chilled water system aseptically cools particles following sterilization. The aseptically process particles are packed in glass jars, with or without liquid, and sealed in an aseptic glove box. Vacuum hydrated mushrooms were tested in the system, without using the separate

blanching step required for canning. When compared visually to canned mushrooms, aseptically processed mushrooms were of superior quality in general appearance and, particularly, were lighter colored and exhibited no mechanical damage. Sterile processing and packaging was demonstrated by storing jars of aseptically processed mushrooms for an extended period with no apparent storage.

Introduction

Continuous Aseptic Processing of Particulate Foods

Foods are thermally processed to inactivate pathogenic microorganisms, spoilage organisms and enzymes. The thermal death rate of microorganisms increases with temperature; hence, high temperatures will inactivate microorganisms more rapidly than lower temperatures. Therefore, if foods are heated to high temperatures, processing time can be short, commercial sterilization can be achieved, and quality degradation minimized. High temperature-short time (HTST) conditions are produced using aseptic processing systems. In these continuous thermal processing systems, foods are heated to a target sterilization temperature and passed through a long hold-tube where this temperature is maintained and lethality is accumulated. Upon exiting the hold-tube, the product is considered commercially sterile. After cooling, the product is packaged in a sterile container under aseptic conditions. HTST processes are

predominantly used to commercially sterilize liquid foods since it is relatively easy to predict the residence time distribution for liquids and, specifically, the minimum residence time of these homogeneous mixtures in the hold-tube. HTST processes are also used for commercial sterilization of high-acid products containing particles since the acidic nature of these products naturally controls pathogenic microorganisms; although, heat is still required to control spoilage organisms.

In contrast, within low-acid foods, pathogenic microorganisms must be thermally inactivated. Thus, accurate prediction of the minimum residence time for any differential volume of the food is critical. Residence time is defined as the time period for which a differential volume of food resides within the holding tube. Not all differential volumes have the same residence time, even for homogeneous liquids, and so the residence time is represented as a distribution of residence times. Residence time of particles is influenced by many factors: pipe size, pipe configuration, flow rate, fluid properties, and particle properties.

> "Residence time in the conventional tube is a relatively broad distribution with long tails in both directions, indicating that some particles would be in the system for a short time, most for a moderate time, and some for a long time. Minimum residence time is the processing time of the fastest particles which means that essentially 100% of the particles have a longer processing time. The conventional aseptic processing system must be designed (by adjusting tube length, temperature, and food flow) so that sterilization is achieved for particles with the minimum residence time. Since virtually all the particles have a longer residence time than the minimum, then virtually all the particles are overcooked… [as shown in Figure **1.1**]." (Walker, P. N. and R. B. Beelman, 2001)

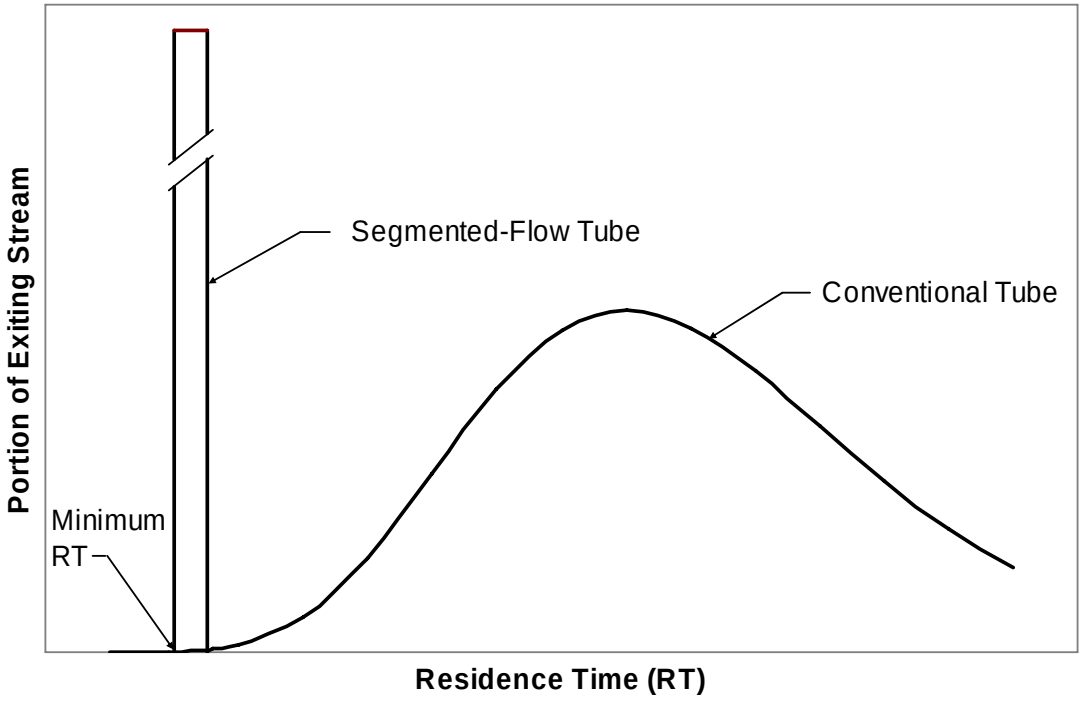

Figure **1.1**: Residence time distribution of particles in a conventional and segmented-flow hold tubes (Walker and Beelman, 2002).

The lack of practical technology for predicting or controlling the residence time of particles in the holding tube of aseptic processing systems has prevented continuous aseptic processing of low-acid particulate foods from being adopted in the U.S. (Walker and Beelman, 2002). Sandeep and Zuritz (1994, 1995), Tucker and Withers (1994), Abdelrahim et al. (1995); Baptista et al. (1995), Grabowski and Ramaswamy (1995), Abdelrahim et al. (1997), Alhamdan and Sastry (1997) attempted to predict the residence time distribution (RTD) of particles in conventional hold tubes, while Fan and Wu (1996), Lareo et al. (1997), Lareo and Fryer (1998) focused on determination of RTD in vertical hold tubes. Salengke and Sastry (1995, 1996) focused explicitly on curved sections

of piping, such as elbows and Sandeep et al. (1997) on helical hold tubes. These are but a few of the countless papers written on the topic of aseptic processing of particulate foods. Lewis and Heppell (2000) and Sastry and Cornelius (2002) have written excellent compilations of research related to this topic.

Segmented-flow (U.S. Patent No. 6,457,513) is a technology invented at Penn State (2002), specifically for the aseptic processing of low acid foods (pH greater than 4.6) with large particles, such as mushrooms, potato soup, green beans, beef stew, and macaroni and cheese. It provides precise control of residence time of particles in a continuous process that minimizes processing (Walker and Beelman, 2002).

Technical Advantages of Segmented-Flow

In segmented-flow, a series of barriers are introduced into the flow stream and particles are trapped between the barriers. Since the particles move through the tube at the same apparent speed as the barriers, residence time of the particles is controlled by the speed of the barriers (Walker and Beelman, 2002). Therefore, segmented flow can precisely control the residence time in the heating, holding and cooling sections of an aseptic processing operation (Figure 2) though most of the advantages still come from controlling the residence time in the hold tube (Walker and Beelman, 2002). Shown in Figure **1.1** "…is that, using segmented-flow, all particles are processed only for the minimum residence time,

thus avoiding overcooking (Walker and Beelman, 2002)." When used in aseptic processing, segmented flow results in a higher quality product (because all food particles are cooked for the minimum amount of time, assuming all particles have equal thermal properties) and the ability to process low-acid foods with large particles (because residence time of each particle is controlled) (Walker and Beelman, 2002). A large particle is defined as a particle larger than a grain of rice. "This advantage holds regardless of the type of food (Walker and Beelman, 2002)." In other words, uniform residence time is an advantage for liquid, particulate, or combination foods. Stephens (2003) attempted to model particle temperature throughout the heat-hold-cooling sections of a segmented flow system and to thermally validate microbial lethality; although, he used a substantially different segmented flow system than the one that is the subject of this paper.

Others have recognized the benefits and have sought to control the residence time of aseptically processed particulate foods. Green, H. J. Heinz Company Limited (1977), and Hersom et al., Society d'Assistatnce Techique pour Produits Nestle S.A. (1980) respectively filed U. S. Patents 4,059,919 and 4,234,537, for aseptically processing particulate materials in batches. However, it is not practical to use batch systems in large scale production environments. Hay et al. (1998), FMC Corporation, filed U. S. Patent 5,802,961 for an aseptic processing system capable of continuous sterilization of diced tomatoes and likely capable of substantial output. This system employs an auger to convey the

particles at a specific rate up an inclined perforated tube. Understanding that residence time must be longer for larger particles, but can be shorter for smaller particles, Stork N. V. designed the Rota-Hold system. This device retains particles by interleaving a series of spiraling and stationary blades. The spacing of the blades controls the residence time of particles. When used in series, multiple vessels, with various blade spacing, accurately control the residence time of a wide range of particles and thereby minimize over processing of smaller particles. None of these rather complex mechanical systems has proven practical for commercial production.

Currently, only the time the food is in the holding tube can be counted toward the lethality for microorganisms in a conventional aseptic processing system, per the Food and Drug Administration (FDA). "The reason for not including the time in heat exchangers, especially scraped-surface heat exchangers, is clear—the residence times of both fluid and particle elements are highly variable in these units. However, segmented-flow controls the residence time in tubular heat exchangers as shown in Figure **1.2** and informal feedback from FDA indicates that using segmented-flow heating may allow the residence time in the heat exchangers to be counted toward microbial lethality. If so, this would further improve food quality by further shortening thermal treatment time while still achieving commercial sterility (Walker and Beelman, 2002)." It is unlikely that the time in the cooling heat exchangers, even with segmented flow, can be counted toward lethality because some differential volumes of liquid may

cool rapidly by contacting the cold exchanger wall and added lethality will therefore be negligible.

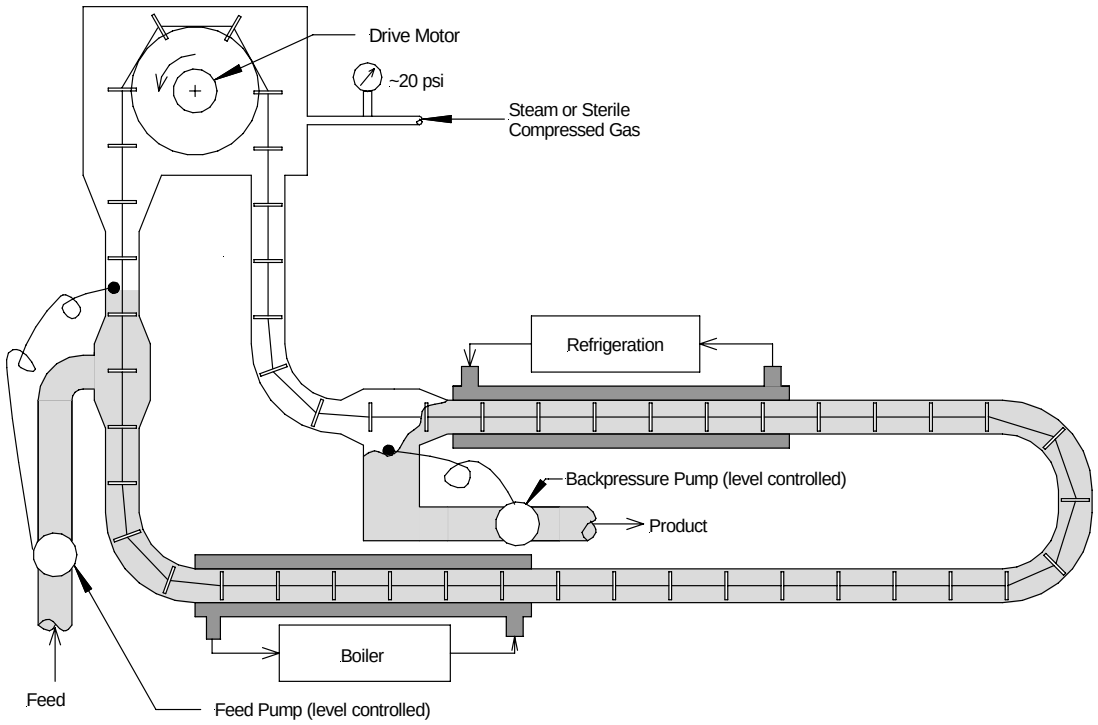

Figure **1.2**: Segmented-flow unit integrating heating, holding, and cooling (Walker and Beelman, 2002).

The benefits of segmented-flow aseptic processing are even greater when compared to conventional canning. In canning, mushrooms are packaged in a container, topped with brine and the container is sealed; and then, the container and its contents are sterilized. Package integrity is of concern in canning and processing temperature is often limited as a result. In aseptic processing the food is sterilized separately from the package. Because compromising package integrity during processing is of no concern in aseptic processing, higher temperatures are often used. Higher processing temperatures require shorter

processing times. Further, since only individual particles are heated in segmented-flow aseptic processing, compared to a can of many particles, heating time is significantly shorter and can be more precisely controlled. Reduced cooking in aseptic processing reduces shrinkage and weight loss during processing, which is considerable for products such as mushrooms. These factors combined significantly improve the quality and value of aseptically processed foods.

During conventional canning, many products are blanched prior to being sealed in containers for sterilization. Blanching pre-shrinks particles and inactivates enzymes that degrade quality. Particles are processed separately from the container in aseptic processing, so it is possible to eliminate the separate blanching step. Therefore, a single processing unit can replace multiple units required in canning. Since only a single processing unit is required, utility costs and water consumption are less in aseptic processing.

Containers larger than a No. 10 can (about 3.25 L) are impractical in conventional canning, because the long heating and cooling times required for commercial sterilization would result in severe overcooking. With segmented-flow aseptic processing, the problem is eliminated because the food and the container are sterilized separately. This profound difference from canning means that many packaging alternatives (plastic pouches, bag-in-box, and 500 kg totes) can be considered. In food service and further processing applications, where there are labor, disposal and safety issues with metal cans and glass jars, this is

a big benefit. Further, in canning, virtually all the voids between particles must be filled with liquid to maximixe heat transfer; however, in aseptic processing, much of this liquid can be recycled or discarded after processing to reduce package weight and minimize handling and shipping costs.

Objective

Configure a continuous processing system capable of aseptically processing and packaging mushrooms and similar particle materials easily susceptible to mechanical damage; the specific goal being to produce a mushroom product similar to canned mushrooms, but of significantly higher quality.

Methodology

After a design concept is developed, many engineering developments begin with a prototype. Prior to this research, a prototype segmented flow aseptic processing unit was developed; therefore, the researchers first evaluated the capability of the existing prototype to process sliced mushrooms and whole mushrooms. Based on the outcome of these early evaluations, the researchers made several observations that led to the design of new and modified systems. Design concepts were sketched, sketches were refined to detailed drawings, detailed drawings were used to produce and procure system components,

system components were assembled, and the final assembly was tested to see if the objective had been met. The mushroom used throughout this research was species *Agaricus bisporus.*

Results and Discussion

Preliminary Testing of an Existing Prototype Segmented Flow System

Preliminary testing using mushrooms in an existing prototype segmented flow system (Figure **1.2**) revealed that excessive physical damage (breakage, scuffing, bruising, and deformation) occurred as mushrooms rubbed against the 5 cm (nominal 2 inch) sanitary tube wall. In some instances, the mushrooms would break into pieces. When mushrooms were blanched prior to processing, damage was reduced, but mushrooms were considered visually unacceptable. It was judged that increasing the tube diameter alone would have substantially reduced, but not sufficiently mitigated this damage. Based on these initial observations, a conveying system that protected the mushrooms from contact with the tube wall was necessary.

The existing prototype aseptic processor used shell-in-tube heat exchangers to indirectly heat the product with steam. A liquid broth—in this case water—was therefore required both as a particle carrier and as a heat transfer medium. Though water has a high surface heat transfer coefficient (2.5 to14.7 W

$m^{-2}\,°C^{-1}$), the surface heat transfer coefficients for steam are typically 49 to147 W $m^{-2}\,°C^{-1}$ (Ling et al., 1974).

No aseptic packaging environment was developed in conjunction with the existing prototype system, but one was needed for this research.

System Design, Development and Specifications

Following preliminary testing and evaluation of the existing prototype system, a modified design concept was developed and is pictured in Figure **1.3**. This concept was further refined and detailed drawings created. A complete processing system was designed and is sketched in Figure **1.4**. The pilot-scale segmented flow aseptic processor is pictured in Figure **1.5**.

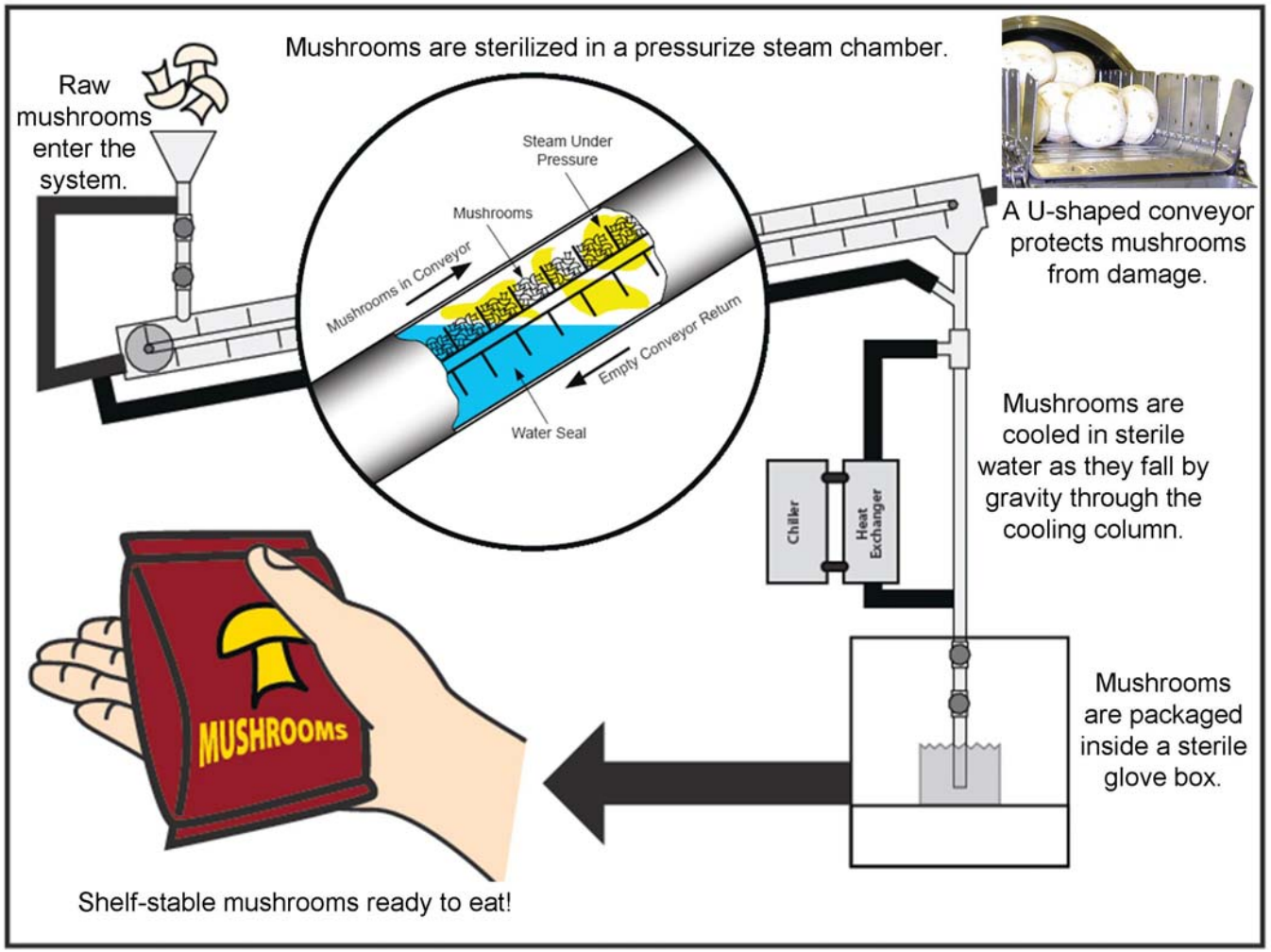

Figure **1.3**: Conceptual design of a steam sterilization segmented flow aseptic processing system.

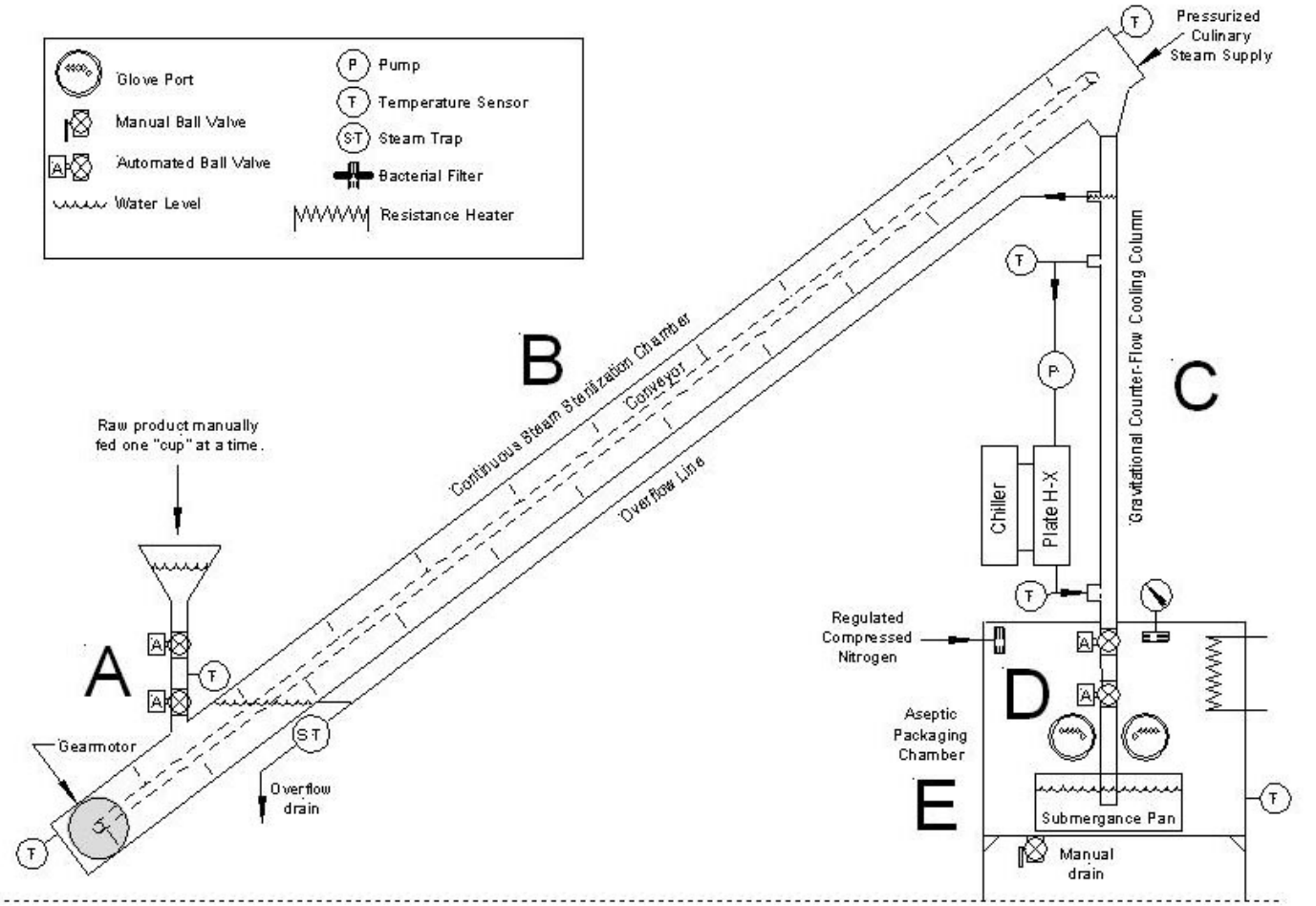

Figure **1.4**: Conceptual design of aseptic mushroom processing system capable of processing particle foods.

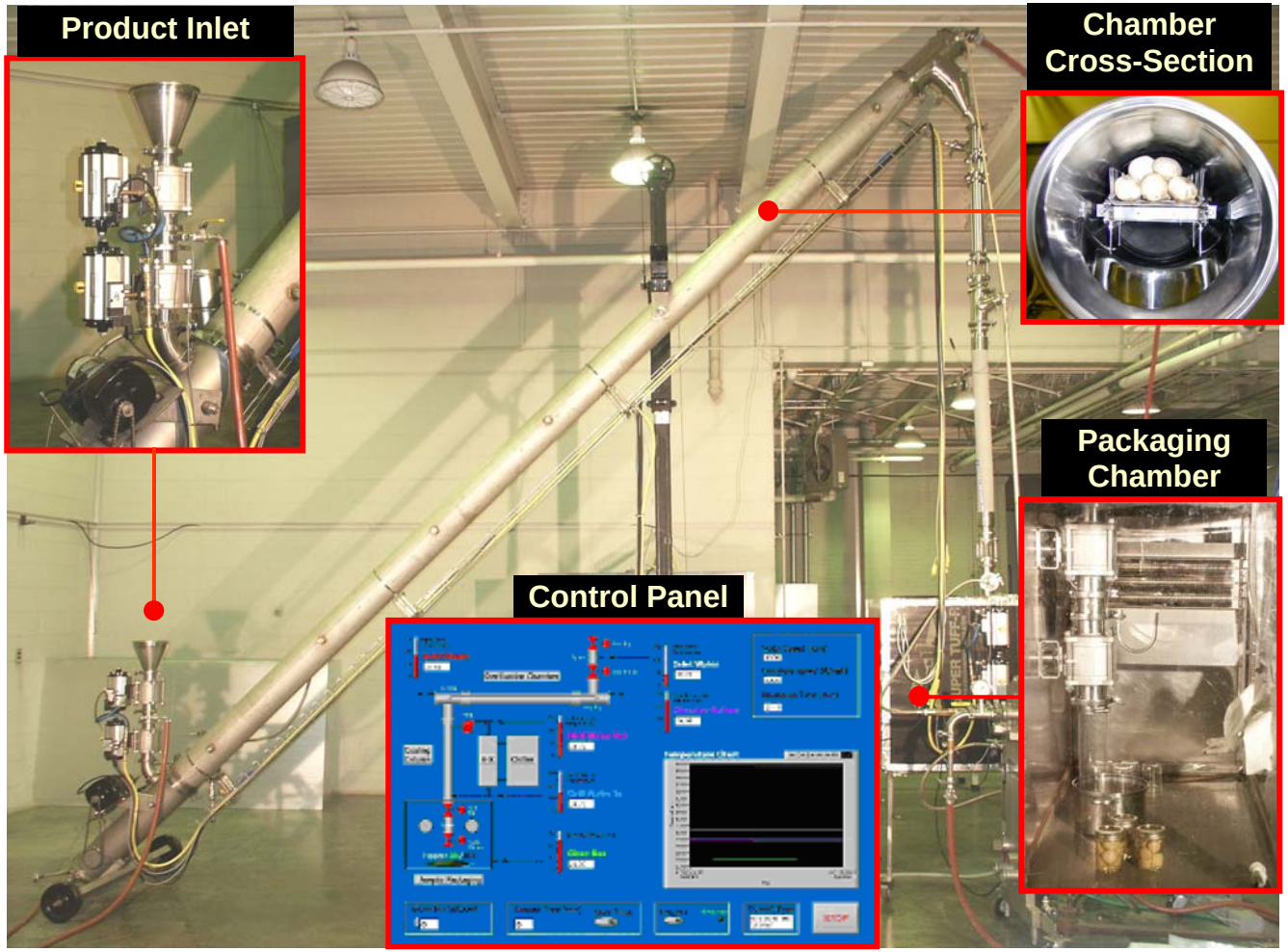

Figure **1.5**: Pilot-Scale Segmented-Flow Aseptic Processor

Vacuum hydrated mushrooms entered the sterilization chamber gently through a column of water that prohibited air from entering the system. To prohibit steam from entering the inlet water column, the water level inside the sterilization chamber was maintained at the approximate level of the lower of two pneumatically actuated ball valves that act as a pressure lock (**A**, Figure **1.4**).

Three-inch sanitary ball valves equipped with air-open, air-close pneumatic actuators were coupled to form the product inlet pressure lock (Figure **1.5**). Computer controls cyclically opened and closed the valves to feed and discharge the mushrooms; alternatively, the operator triggered a valve by selecting the appropriate valve icon on the processor control panel (Figure **1.5**). One complete cycle of the double-valve pressure lock took approximately 30 seconds to complete in the pilot-scale machine. To speed this process in a full size commercial system, the double-valve system could be replaced by a pump or continuously rotating pressure lock system. A double-valve system was used in the pilot-scale processor because no pump is available that will pump large particles (the mushrooms) without damage at the low volumetric rate demanded by this pilot-scale system.

Once mushrooms exited the water at the lower portion of the inclined sterilization chamber, they were heated under pressure directly with condensing culinary steam (Figure **1.3**). Condensing steam has a higher heat transfer rate than liquid water and also ensures a uniform temperature within the entire sterilization section (Ling et al., 1974). The system was typically operated at a processing temperature of 130°C and 270 kPa. The processor was capable of

operating at lower or higher processing temperatures; however, the supply of steam available in the laboratory was limited and temperatures above 131°C could not be consistently achieved.

Thermistor probes were used to monitor temperature at six key points in the processing system (as identified by the symbol Ⓣ in Figure **1.4**). Temperatures were displayed as a numeric and were charted with time on the control panel shown in Figure **1.5**.

Heating—including blanching—and holding of the mushrooms took place on the inclined conveyor (**B**, Figure **1.4**) inside the sterilization chamber. A U-shaped cross-section conveyor was designed to support mushrooms from the bottom and sides and prevent the mushrooms from contacting the tube wall (Figure **1.3**). Figure **1.6** illustrates mushrooms being conveyed on the U-shaped conveyor passing through the sterilization chamber constructed of 20 cm (nominal 8 inch) diameter sanitary tube, 7 m in length. Enough clearance was present above the conveyor bed to minimize the chance of mushrooms coming in contact with the tube and being mechanically damaged. The incline (approximately 45°) of the conveyor and the presence of Teflon partitions (or flights), placed approximately every 40 cm along the conveyor belt (Figure **1.3**), doubly ensured that no particle advanced faster—or slower—than the conveyor itself. Mushrooms moved at a fixed speed on the conveyor belt which serves both as the heating and holding sections; the steam temperature and belt speed are adjusted appropriately to render mushrooms commercially sterile.

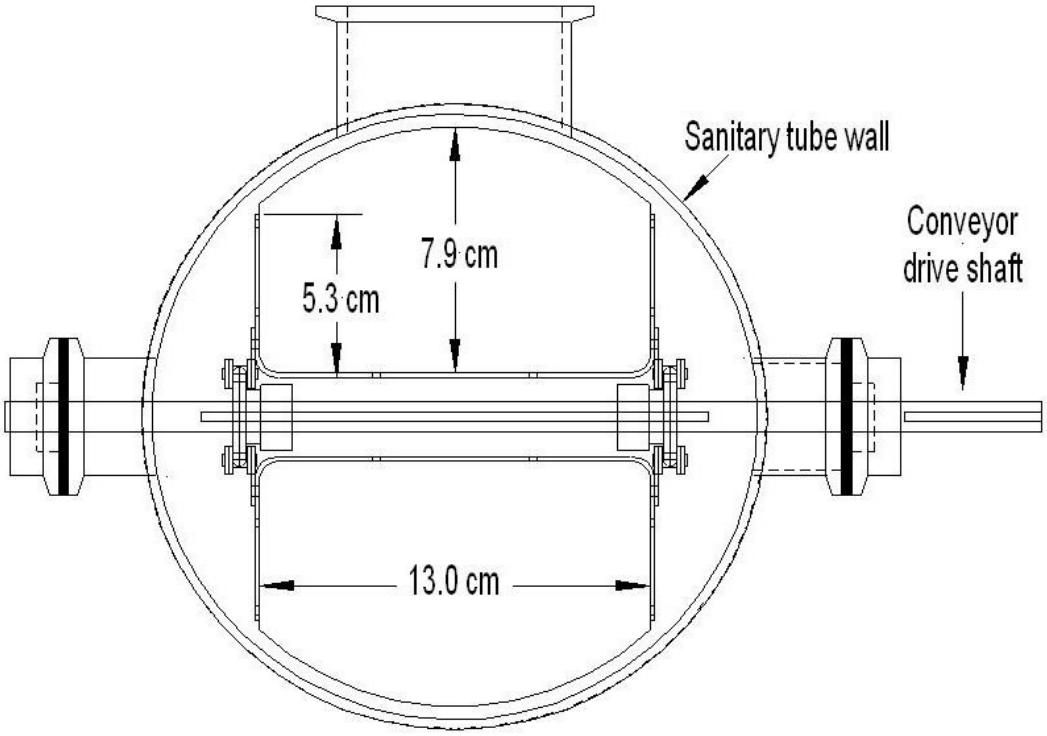

Figure **1.6**: Cross-section view of U-shaped conveyor and housing.

A variable speed, 187 W (¼ hp), DC motor (1750 rpm) coupled with a 41:1 gear reducer and a chain drive powered the conveyor. A 12-tooth driver sprocket and 35-tooth driven sprocket further reduced the speed. Given the current sprocket configuration, conveyor speed can be set anywhere in the range of 0.21-1.45 m per minute simply by controlling motor speed, yielding 4.4-30 min residence time through the steam environment portion of the processor. Motor speed, conveyor speed and residence time are displayed on the control panel shown in Figure **1.5** . Other gear configurations could be used to speed or slow the conveyor to obtain the optimum residence time for any material. At these speeds, the processor was capable of processing 0.0016-0.0107 m^3 per min of material. However, the current double-valve feed system, which has a slow cycle

time of approximately 30 seconds, prevented these maximum feed rates from being achieved.

Mushrooms fall from the conveyor into the counter-flow, direct-contact water cooling section (**C**, Figure **1.4**)—a pressurized, 7.62 cm (nominal 3 in) diameter, vertical column of chilled water. Mushrooms descend through the cooling unit by gravity. Cool, sterile water was circulated through the gravitational cooling column by a 15 W (1/50 hp) AC, magnetic drive, centrifugal pump. Flow rate, within a range of 0.063-0.315 L per sec, was controlled using a variable frequency AC drive. In this flow rate range, particles, such as mushrooms, can be suspended indefinitely in the cooling water or allowed to gently fall through the water. Small, potentially clogging particles were removed using filters with a No. 40 mesh size (382 micron nominal retention). These filters are not illustrated but were located both before the cooling water pump and before the steam trap shown in Figure **1.4**.

A double-valve system, identical to that used for product entry, were used to transfer cool, processed mushrooms from the pressurized cooling column into the sterile glove box at atmospheric pressure (**D**, Figure **1.4**). As was the case for the double-valve system used for product entry, this double-valve system could be replaced by a continuous pressure lock in a commercial system.

The aseptic packaging chamber (**E**, Figure **1.4**) provided an environment for effectively packaging low volumes of product aseptically. During sterilization the chamber was first flushed to remove air and heated to the saturation temperature using culinary steam at a high flow. The chamber outlet was closed

and the steam flow rate was reduced, so that a minimum pressure was maintained in the chamber. An electric resistance heater was then used to superheat the steam *in situ*, and thereby sterilize the glove box. During sterilization, the glove box temperature was monitored and automatically controlled by a set point temperature (typically 120°C for 30 minutes). Following sterilization, a supply of filter-sterilized nitrogen was used to pressurize the glove box and provide a sterile, anaerobic packaging environment in the glove box. The end of the cooling column was submerged in water to prevent atmospheric gases (nitrogen gas in this case) from entering the column; therefore, no net exchange of water occurs when mushrooms are removed from the system. Mushrooms were removed from the submergence water pan, drained, placed in sterile containers and hermetically sealed within the sterile environment of the glove box interior (Figure **1.5**). Manual aseptic packaging could be replaced by automated aseptic packaging in a commercial system.

The mechanical systems (conveyor drive motor, circulating pump, automatic valves, and temperature sensors) of the processing system are monitored and controlled with a computer using LabView software (National Instruments, Austin, TX). The system control panel is shown in Figure **1.5**.

Evaluation

Vacuum-hydrated raw mushrooms were processed in the system at 130°C for 11.0 minutes to achieve a F_o (121°C reference temperature, z =10°C) value of

7 minutes without the separate blanching step required in conventional canning. Control mushrooms taken from the same vacuum hydration batch were canned in a still retort. When compared visually, the aseptically processed mushrooms were of superior quality in general appearance and, particularly, were lighter colored and exhibited no mechanical damage. The aseptically processed mushrooms were packed in glass jars, sealed and stored for several months, never showing signs of spoilage, thereby demonstrating sterile processing and packaging was achieved.

Conclusion

A continuous steam sterilization unit capable of producing shelf-stable aseptically processed particle foods (non-uniform in size and shape) over a wide time-temperature range was developed. The system utilizes steam as the heating medium to achieve better heat transfer and segmented-flow technology to produce a smaller residence time distribution than pipe-flow aseptic processing. Foods are processed only as long as needed to achieve the target microbiological lethality, and are not overcooked. A direct-contact, gravitational counter-flow chilled water system aseptically cools particles following sterilization. The aseptically process particles are packed in glass jars, with or without liquid, and sealed in an aseptic glove box. Vacuum hydrated mushrooms were tested in the system, without using the separate blanching step required for canning. When compared visually to canned mushrooms, aseptically processed

mushrooms were of superior quality in general appearance and, particularly, were lighter colored and exhibited no mechanical damage. Sterile processing and packaging was demonstrated by storing jars of aseptically processed mushrooms for an extended period at normal room conditions with no apparent spoilage.

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Chapter 2

Protocol for Developing a Scheduled Process in a Continuous Steam Sterilization Segmented-Flow Aseptic Processing System

Abstract

Steam provides a uniform heating medium and temperature distribution within the sterilization chamber of the segmented-flow aseptic processing system, and the process was validated mathematically for mushrooms. A temperature penetration test, adapted from conventional canning process development, was used as the foundation for developing a scheduled thermal process using microbiological kinetics. A sight-glass assembly provided an effective apparatus for conducting viewable temperature penetration tests in a pressurized steam environment. Process time was not significantly impacted by the depth of the mushroom bed on the processing conveyor and most conservative [mean (μ) plus three standard deviations (σ)] process times of 11.0 minutes for whole mushrooms (μ = 9.2 min, σ = 0.6 min) and 3.6 minutes for sliced mushrooms (μ = 2.1 min, σ = 0.5 min) were calculated from temperature penetration data. Both aseptic processing times are much less than would typically be encountered in conventional canning of mushrooms, even for small cans, which require approximately 20 minutes of process time; larger cans require additional process time.

Introduction

Steam sterilization segmented-flow technology provided a practical method for aseptically processing particle foods (Chapter 1). However, in order for this technology to be adopted by industry and approved by federal regulators, a practical means for developing a scheduled thermal process must also be developed.

Studies of internal container temperature, commonly referred to as temperature penetration studies, are widely accepted in the food industry for establishing scheduled processes for canned foods. (Since temperature is measured, and not heat, during penetration studies, these studies will be referred to as temperature penetration studies throughout this document.) Temperature sensor position, initial temperature, processing temperature, time steam turned on, time to reach processing temperature, and container size are several important factors to monitor during temperature penetration tests (National Canners Association, Vol. 1, 1968). Retort temperature and the center temperature of the "largest particle" at the "cold spot" of the can are monitored throughout the heating and cooling processes. A scheduled process is established based on the time-temperature relationship inside the can and target lethality. Target lethality is dependent on the microbial kinetics of the target microorganism likely present in the food to be canned.

Mushrooms, pH 6.0 to 7.0 typical, are considered a low-acid, pH > 4.6, food. *Clostridium botulinum* types A and B, typically associated with soil, occur

naturally on fresh mushrooms at an estimated incidence of 0.08 to 0.16 CFU per 100 g of mushrooms (Notermans et al., 1989). The pathogenic nature of *C. botulinum* toxin is of great concern to the processors of low-acid foods since the organism thrives in anaerobic conditions and at pH>4.6. The $D_{121\,^{\circ}C}$ for *Clostridium botulinum* spores is 0.10 to 0.21 minutes (Jay, 2000). A typical 12D process would therefore suggest an $F_{121\,^{\circ}C}$ = 2.52 minutes; however, several outbreaks of botulism and the presence of *Clostridium Sporogenes*, a thermophile with a higher $D_{121^{\circ}C}$ (typically 1.0 minute) that may cause economic spoilage, in canned mushrooms have led to more conservative processing regimens. The Food Processors Association (Deniston, 2003) suggests a reference thermal death time (thermal resistance, z = 10 °C; temperature, T = 121 °C), F_o = 7 minutes, be used to ensure commercial sterility of canned mushrooms packaged in brine in a U.S. standard 211 x 212 can, with diameter 6.8 cm (2-11/16 inches) and height 7.0 cm (2-12/16 inches), and processed in a still retort. "Experimental tests have shown that approximately 20 to 40% greater lethality is required for No. 10 cans than for No. 2 cans to destroy the same, per unit volume, concentration of spores (National Canners Association, 1968)." A 211 x 212 can is 65% smaller than a No. 2 can and 600% smaller than a No. 10 can.

In commercial canning operations, mushrooms are typically transported to the processor via refrigerated trucks within 24 hrs of harvest. At the processing plant, mushrooms are washed and stored at 4˚C for 18-24 hours. Mushrooms are removed from cold storage and vacuum hydrated using a similar procedure

to that outlined by McArdle et al. (1974). During the vacuum hydration process, mushrooms are submerged in water and subjected to approximately 94 kPa Hg vacuum to remove any air trapped in the intercellular spaces of the mushroom tissue. After a period of five minutes, the vacuum is released. Mushrooms are held, still submerged in water, for an additional 5 minutes at atmospheric pressure to maximize water retention; however, most commercial operations use continuous vacuum hydration processes. Vacuum hydration improved yield (Anantheswaren et al., 1986) and decreased enzymatic browning reactions catalyzed by the presence of oxygen and hastened by exposure to temperature during blanching (Jolivet et al., 1998; Wills, et al., 1998).

Following vacuum hydration, mushrooms are blanched in hot water, typically 190˚C, for 3-5 minutes. Blanching pre-shrinks the mushrooms and inactivates enzymes prior to commercial sterilization in a retort. Mushrooms are then packed in cans and topped with brine. Cans are hermetically sealed and thermally processed in retorts according to a scheduled process aimed at producing a commercially sterile, shelf-stable product.

Objective

Develop a scheduled process, i.e., determine operating conditions (time, temperature, feed rate, etc.) to achieve commercial sterility, for whole and sliced mushrooms processed individually in a steam sterilization segmented flow aseptic processing system.

Procedure

Since the method of developing the scheduled process for conventionally canned mushrooms is widely understood and accepted, a similar protocol was used to develop a scheduled process for aseptically processed mushrooms. There were two key components of this protocol: a temperature distribution study and a temperature penetration study.

Temperature Distribution Study

Prior to conducting temperature penetration studies in any retort, an analysis of temperature distribution within that retort must be conducted. Therefore, the first step was to conduct a temperature distribution study on the sterilization section of the segmented-flow system. To characterize the temperature distribution under normal operating conditions, five model P60DB103M glass encapsulated thermistor probes with a temperature range of -60°C to 300°C and accuracy of ±0.05°C over the range 25°C to 125°C (Thermometrics, Edison, NJ) were placed at the center of the conveyor inside the sterilization chamber at positions denoted by locations **A**, **B**, **C**, **D**, and **E** in Figure **2.1**. A sixth, permanently installed reference thermistor probe, denoted by location **F**, inserted at the upper end-cap of the chamber, denoted by **F**, also measured the chamber temperature. The reference probe was used to measure the steam temperature of the sterilization chamber during actual processing in

future experiments and was calibrated according to the results of the temperature distribution test.

As would be the case under normal processing conditions, the segmented flow aseptic processing system was turned on and the temperature inside the sterilization chamber was allowed to equilibrate to a set-point, 117°C in this first case. Once steam temperature was stable at the set-point, temperature data from each of the six sensors was simultaneously monitored for 60 seconds at the temperature set-point. Steam pressure was increased and temperature was allowed to equilibrate at a second set-point—123°C. Steam temperature within the sterilization chamber was monitored for 60 seconds at the new temperature set-point.

The average temperature of each sensor position over the 60 second sampling period was then compared for each temperature set-point. The lowest, and therefore most conservative, temperature reading was used as baseline to calibrate the permanent reference probe (**F**) in the system that was used to monitor the sterilization chamber temperature during future experimentation.

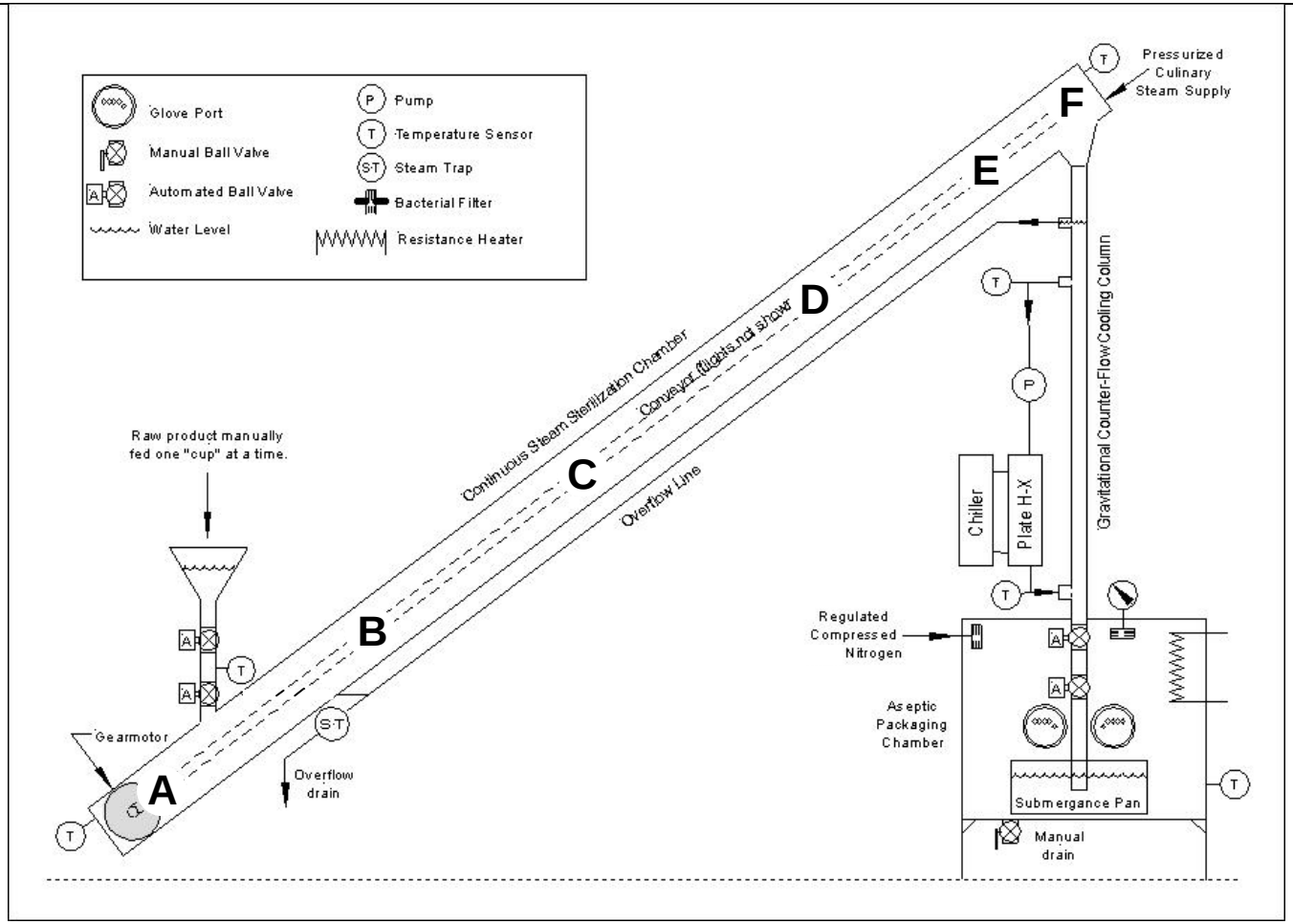

Figure **2.1**: Temperature sensors were positioned at six locations (A-F) in the Continuous Steam Sterilization Segmented-Flow Aseptic Processor during temperature distribution tests.

Temperature Penetration Studies

A detailed thermal analysis of a food inside a pressurized continuous processing system is a very difficult prospect, because sensors must enter and leave the pressurize system without damage and the sensors must travel with the food, all the while, accurately recording particle temperature. In this study, the segmented flow system was operated at pressures above 273 kPa absolute (40 psia), and temperatures in excess of 130 °C, which only compounded the difficulty of the effort.

Particle center temperature was measured based on the fact that the newly designed segmented flow system is analogous to a steam-processing retort. In retorts, temperature sensors, typically thermocouples, are placed in the "cold spot" of the can and the temperature is recorded during the entire thermal process (National Canners Association, Vol. 1, 1968). Once mushrooms entered the segmented flow system and position themselves on the conveyor, the mushrooms behaved similarly to cans in a retort.

Ideally, mushrooms with sensors attached would have been directly inserted into the working process system; however, mushroom and sensor positions were impossible to view in the interior of the stainless steel chamber. Therefore, static temperature penetration studies of mushrooms were conducted in a viewable and more easily accessible pressure vessel--a nominal three inch diameter sanitary sight glass attached to the inlet of the segmented-flow system

(Figure 2.2). The sight glass assembly provided similar processing conditions and a clear view of mushrooms and sensors during temperature penetration studies.

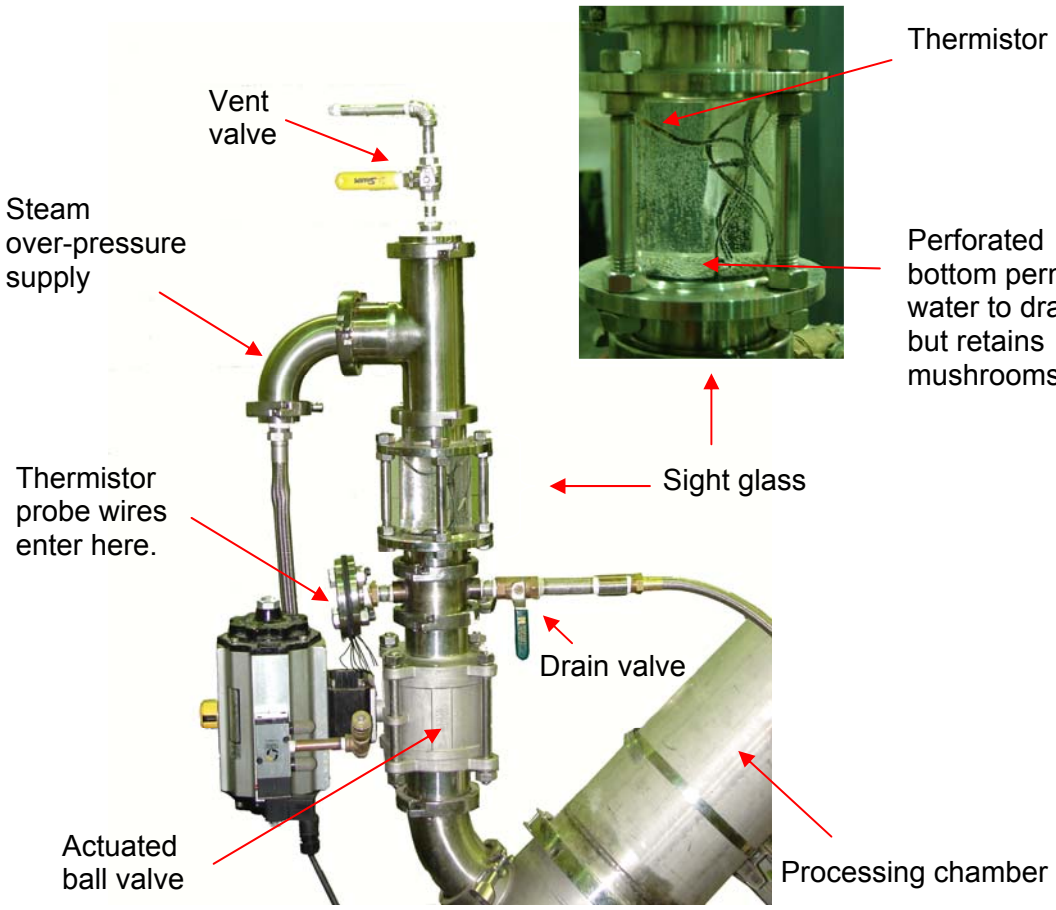

Figure 2.2: Temperature penetration experimental set-up.

The depth and arrangement of mushrooms on the conveyor bed—bed depth—were potentially the most important factors in the temperature penetration study. It was thought these factors may hinder the flow of steam to the center of the mushroom bed. Therefore, several thermistors were employed during each test to enable monitoring, inside the sight glass assembly, of the environmental

temperature and the center temperatures of three mushrooms at the bottom, center and top of the mushroom bed. The sight glass assembly was capable of holding a 9 cm bed depth sample of mushrooms; whereas, the conveyor in the actual processing system was only capable of holding an 8 cm bed depth. By conducting studies with a mushroom bed of depth greater than encountered under normal processing conditions, more conservative measurements were made.

Though mushrooms with 2.5 to 4.0 cm nominal cap diameters were to be processed during normal operating conditions, mushrooms selected for temperature penetration were a nominal 4.0 cm cap diameter, so as to develop a "most conservative" process based on the "largest particle" processed.

The mushroom, species *Agaricus bisporus,* used in this project were small whole mushrooms (2.5 to 4.0 cm cap diameter) and sliced mushrooms (7 mm thickness, 2.5 to 4.0 cm cap diameter). Raw mushrooms, obtained from the Mushroom Test Demonstration Facility (MTDF), Penn State University (University Park, PA), were of the same commercial quality typical for canned mushrooms. Mushrooms were washed in cold tap water and stored for 18-24 hours at 4˚C and vacuum hydrated prior to processing. Sliced mushrooms were prepared on a mandolin by cutting to 7 mm thickness. Only slices cut from the center of the mushroom were used in quality studies; pieces were discarded. Mushrooms were individually marked for identification and vacuum hydrated.

Model P60DB103M (Thermometrics, Edison, NJ) glass encapsulated thermistor probes were carefully inserted longitudinally through the stem base to

the geometric center of the mushrooms as marked in Figure 2.3. A small nylon cable tie was carefully tightened around the stem of whole mushrooms to secure the sensor in its position. Three of these “largest particle” mushrooms, equipped with temperature sensors were added to the sight glass assembly for each test. One sensor equipped mushroom was placed at the bottom of the sight glass assembly (Figure 2.2). Then additional mushrooms, 2.5 to 4.0 cm cap diameter, were added until the sight glass assembly was loosely filled with mushrooms to approximately the half-way point. A second sensor equipped mushroom was added and then more mushrooms were added until the sight glass assembly was approximately filled to the top of the sight glass. Then, the third, and last, sensor equipped mushroom was added. Approximately 20 whole or 100 sliced mushrooms total were used during each test. Environment temperature was measured inside the sight glass assembly using a fourth sensor as a reference probe.

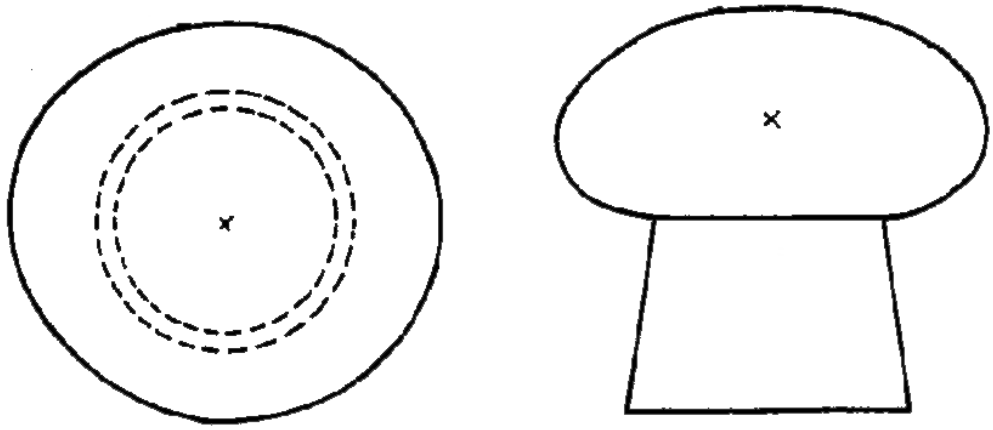

Figure 2.3: Temperature sensor placement in mushroom center during temperature penetration. The **X** marks the geometric center of the mushroom (Adapted from Sastry et al., 1985).

Water, approximately 25°C, was added, filling the chamber to the level of the steam over-pressure supply (Figure **2.2**). A cap was applied and tightly secured to the top of the sight glass assembly and the vent valve opened (Figure **2.2**) to begin the experiment process.

Steam was first applied via the steam overpressure port. Steam overpressure was used in the sight glass assembly to vent any air from the assembly out through the vent valve and to minimize water hammer created when the actuated valve (Figure **2.2**) was opened and the cool water initially surrounding the mushrooms rapidly collapsed pockets of saturated steam rushing into the chamber from the processor below. Without steam overpressure, water hammer caused violent pressure surges within the sight glass assembly that resulted in significant physical damage to the mushrooms and sensor placement.

The gas pocket above the mushrooms in the sight glass assembly was flushed with steam for approximately 30 seconds and then the vent valve was closed pressurizing the gas pocket with steam. The large ball valve between the sight glass assembly and the processing chamber (Figure **2.2**) was opened. The processor thus became the primary steam supply to the test chamber. A screen at the bottom of the sight glass (Figure **2.2**) held mushrooms in the chamber, but permitted water to drain away from the mushrooms directly exposing the mushrooms to steam. As a result, conditions inside the test chamber closely matched actual processing conditions inside the processor.

Temperature measurements were made once per second, recording the temperature in each of the three mushrooms and the environment temperature inside the sight glass assembly. Microbial kinetics calculations were used to convert the temperatures to lethality values. Processing time was considered to be the total time from when the sight glass assembly environment reached the processing temperature set-point of 130°C, until the target sterilization value, F_o = 7 minutes, was reached based on the recorded center temperature measurements of the "coldest" mushroom. The same steps were used in conducting temperature penetration studies on sliced mushrooms.

Fifteen "sample" mushrooms were used in temperature penetration studies of whole and sliced. A Randomized Complete Block Design (RCBD) was utilized such that the mushrooms were grouped into five blocks of three mushrooms. Three mushrooms (one block) were tested per trial, each at a different bed position (treatment), i.e., the top, middle or bottom of the test chamber. The procedure was repeated for each of the five blocks of mushrooms. Analysis of Variance (ANOVA) was used to determine the effects of bed position and mushroom block on processing time. The ANOVA General Linear Model used in statistical analysis is given in Equation **2.1**, where Y_{ij} is the random variable representing the response for the j^{th} bed position observed in mushroom block i, μ is the overall mean process time, α_i is the effect of the ith block (i = 1,2,…,5), β_j is the effect of the jth bed position (j = 1,2,3), and ε_{ij} is the random experimental error for the j^{th} bed position in the i^{th} block.

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$ (Kuehl, 2000) **2.1**

To test the equality of the treatment (bed position) means, the null hypothesis, H_O: $\mu_1 = \mu_2 = \mu_3$, i.e., all treatments give the same mean process time, was tested against the alternative hypothesis, H_A: $\mu_1 \neq \mu_2$ for at least one pair i,j.

The same RCBD ANOVA General Linear Model given in Equation 2 was used, but with cap diameter as the response variable, to determine the effects of mushroom block and bed location on cap diameter.

Tukey's Least Significant Difference test was used to confirm the findings of the ANOVA tests.

Results and Discussion

Temperature Distribution Study

Steam was very suitable for use as the heating medium in the sterilization chamber, because steam is a very uniform heating medium. This point was exemplified by the thermal conditions monitored during the temperature distribution study of the segmented flow aseptic processing unit. Close agreement between sensors existed (Table **2.1**). At a nominal set-point of 117°C, the average temperature of sensors **B**, **D** and **F** (the Reference Probe) was 117.6 with a standard deviation of 0.03, 0.04 and 0.05°C, respectively. The average temperature of sensor **A**, the furthest from the steam supply, was 117.3°C and had a standard deviation of 0.04°C. It was difficult to position the

fragile thermistor probes throughout the length of the conveyor without damage. Sensors **C** and **E** were damaged, but could not be replaced without risking subsequent damage to the undamaged sensors. Temperature data collected from these sensors was not used in evaluating the temperature distribution within the processing unit.

Table **2.1**: Mean temperature readings obtained in the temperature distribution study.

	117 ºC			123 ºC		
Sensor	Mean	St. Dev.	Offset	Mean	St. Dev.	Offset
F	117.6	0.03	N/A	123.5	0.03	N/A
A	117.3	0.04	-0.3	123.3	0.03	-0.2
B	117.6	0.04	0.0	123.5	0.03	0.0
D	117.6	0.05	0.0	123.5	0.04	0.0

Similar results were obtained at a set-point of 123°C (Table **2.1**). The average temperature of sensors **B**, **D** and **F** (the Reference Probe) was 123.5°C with standard deviations of 0.03, 0.03 and 0.04°C, respectively. The average temperature of sensor **A**, the furthest from the steam supply, was 123.3°C and had a standard deviation of 0.03°C. Temperature data collected from sensors **C** and **E** were not used due to malfunctions.

In both temperature distribution tests, the sensors were in close agreement indicating that steam temperature was uniform throughout the length of the sterilization chamber. Temperatures obtained from sensor **A** differed from sensors **B**, **D** and **F** by -0.3°C and -0.2°C at nominal set-points of 117°C and 123°C. This subtle difference in temperature was likely due to the fact that sensor **A** was located near the lowest point of the sterilization chamber. As steam cooled on the chamber surfaces, condensate formed and collected at the chamber bottom due to gravity. It was likely that the relatively cooler condensate formed a small region in close proximity to sensor **A** that was slightly cooler than rest of the chamber.

To ensure that future tests experiments were conducted using a most conservative approach, sensor **F**, the permanent reference probe, was adjusted to compensate for the greatest temperature difference, -0.3°C.

Since close agreement between sensors the length of the sterilization chamber existed, the temperature distribution tests were not repeated with working sensors at locations **C** and **E.**

Temperature Penetration Study

The sight glass assembly illustrated in Figure **2.2** provided a clear view of mushrooms and sensors during temperature penetration studies. Three mushrooms were tested during each of five repetitions. During temperature penetration tests, significant shrinkage of whole and sliced mushrooms was

visually observed. Shrinkage caused dramatic reduction in mushroom volume that resulted in some shifting of mushrooms. Volume reductions of individual mushrooms lead to an overall decrease in bed depth by approximately one-third for whole mushrooms and nearly one-half for sliced mushrooms. Substantial void space between mushrooms remained following the shrinkage phase. Some sliced mushrooms did stack, which had the potential to increase the effective slice thickness, but during shrinkage, the flat surfaces were constantly shifting against each other, helping to mitigate stacking effects.

Though shrinkage was observed, it did not appear to have an adverse effect on measuring mushroom center temperature. Sensors appeared to stay in place and shrinkage likely helped to create a tight fit between temperature sensors and mushrooms, as temperature leakage between the sensor body and mushroom tissue was not readily detectable in the temperature data. Also, the sensor tips did not appear to migrate from the initial position at the mushroom center.

Figure **2.4** and Figure **2.5** are the temperature penetration and accumulated lethality curves for a typical whole mushroom (cap diameter nominal 4.0 cm) and sliced mushroom (slice thickness 7 mm), respectively, evaluated during a temperature penetration test. The chamber temperature curve indicated the initial temperature of the water and the process temperature of the steam inside the test chamber. From this curve, it can be seen that once the ball valve at the chamber bottom was opened, chamber temperature rapidly reached the process temperature, closely mimicking actual processing conditions

when mushrooms enter the processing steam environment by being elevated out of the water at the bottom of the processor.

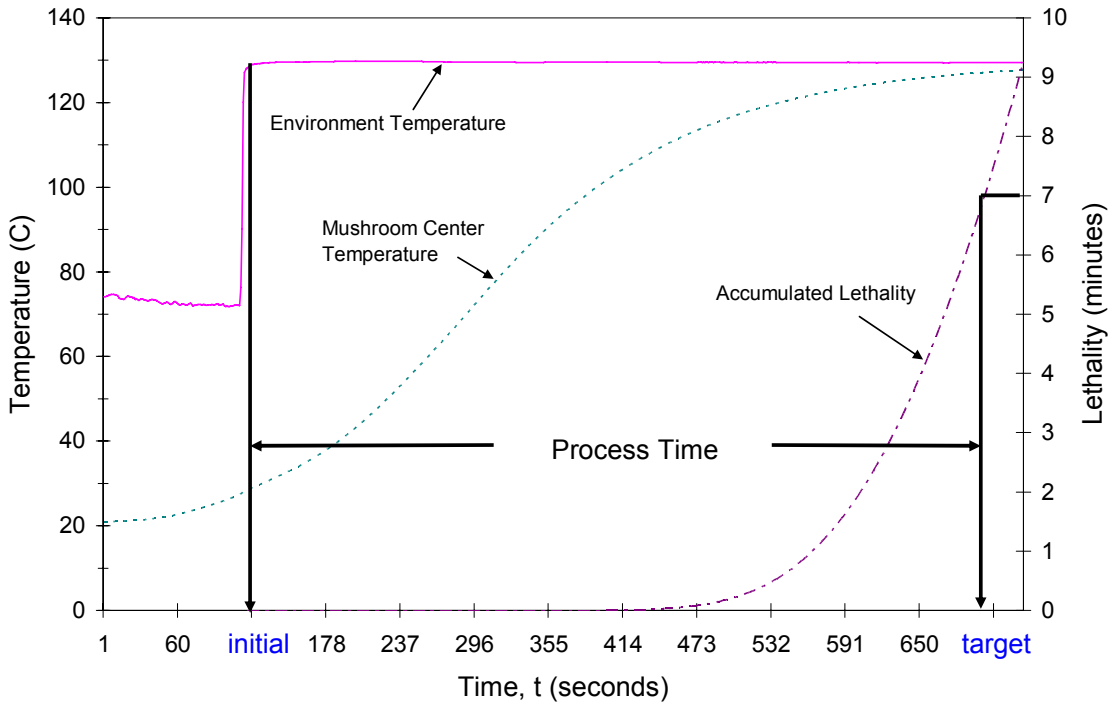

Figure **2.4**: Typical temperature penetration and accumulated lethality curves for a whole mushroom. Process time was found to be 10.1 minutes at a set-point of 130°C.

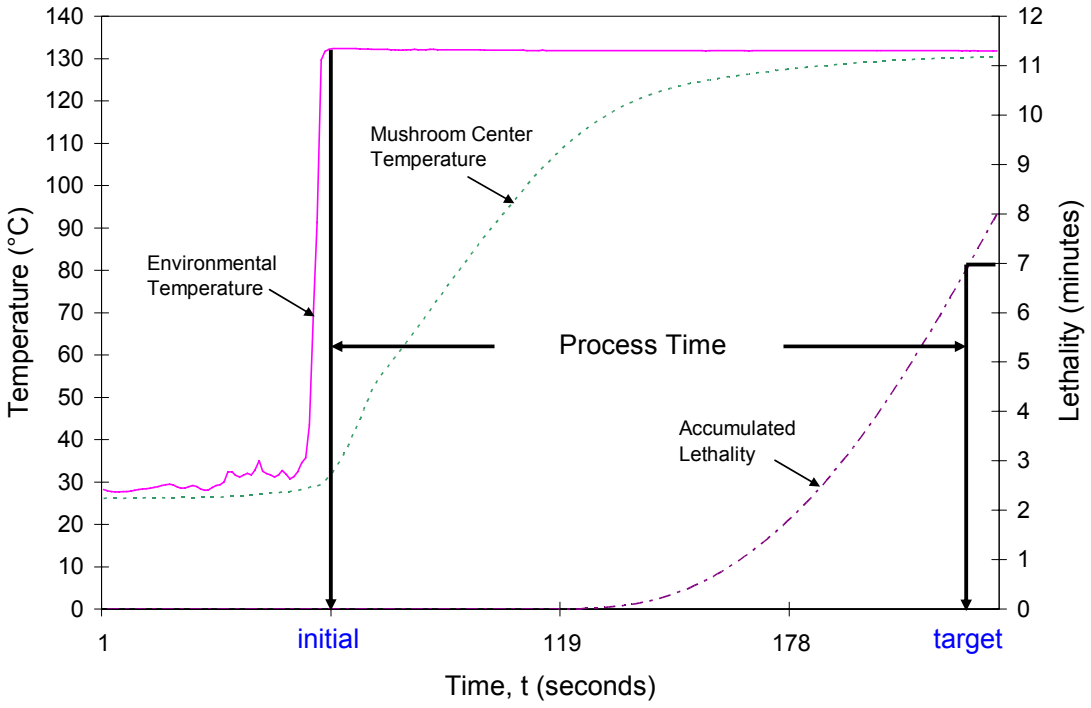

Figure **2.5**: Typical temperature penetration and accumulated lethality curves for a sliced mushroom. Process time was found to be 3.3 minutes at a set-point of 130°C.

Mushroom center temperature gradually increased from the initial whole mushroom temperature of 21°C (Figure **2.4**) and initial sliced mushroom temperature of 27°C (Figure **2.5**) to 45°C, where mushroom shrinkage begins (Biekman et al., 1997). From 45°C to 85°C, the temperature range where mushroom shrinkage is said to occur, rapid temperature increase was exhibited (Figures **2.4** and **2.5**), which agreed with the findings of Biekman, et al. (1997). Above 85°C, rate of temperature increase slowed as mushroom center temperature approached the surrounding steam temperature. Initial test

conditions for temperature penetrations tests of whole and sliced mushrooms are shown in Table 2.2.

Table 2.2:Initial conditions for temperature penetration tests

	Water Temperature (°C)		Mushroom Temperature (°C)	
	Whole	Sliced	Whole	Sliced
Minimum	71	27	19	21
Maximum	75	37	22	31

Accumulated lethality was tracked from the time, $t_{initial}$, at which chamber temperature reached the target process temperature, of 130°C (Figure 2.4 and 2.5). Accumulated lethality, F, was calculated using stepwise numerical integration of Equation 2.2 (Earle, 1983); of course, destruction of bacterial spores did not effectively commence until elevated temperatures were reached (Figures 2.4 and 2.5). Recall the target lethality, F_0, was equal to 7 minutes; therefore, the process was considered adequate when accumulated lethality totaled 7 minutes at time t_{target} (Figures 2.4 and 2.5). Process time (Figures 2.4 and 2.5), $t_{process}$, was calculated using Equation 2.3.

$$F = \int_{t_{initial}}^{t_{target}} 10^{-(121°C - T)/z}\, dt \qquad 2.2$$

$$t_{process} = t_{target} - t_{initial} \qquad 2.3$$

The trial number (block), bed position (treatment), cap diameter for whole mushrooms or slice thickness for sliced mushrooms, and process time in five trials of temperature penetration studies are shown in Table 2.3 and Table 2.4, respectively. The longest process time observed was 10.1 minutes at an average

process temperature of 130.2°C for a whole mushroom with cap diameter 39.6 mm and 3.3 minutes at an average process temperature of 131.7°C for a sliced mushroom with thickness of 7.0 mm. Note, one observation (block 1, treatment 1) in Table **2.4** is missing. In this particular trial, the temperature sensor did not stay in place for the duration of the test, rendering the measurement invalid.

Table **2.3**: Cap diameter, process time, and bed position (top, middle, bottom) in the sight glass assembly of whole mushrooms used in temperature penetration studies.

	Cap Diameter (mm)[a]			Process Time (minutes)[b]		
Trial (Block)	Top (Trt. 1)	Middle (Trt. 2)	Bottom (Trt. 3)	Top (Trt. 1)	Middle (Trt. 2)	Bottom (Trt. 3)
1	41.8	41.0	40.1	9.7	8.1	8.6
2	41.4	42.0	39.7	9.6	9.9	9.0
3	40.1	40.3	43.4	8.7	9.6	10.1
4	38.9	40.0	38.6	8.3	9.4	9.0
5	41.0	39.8	39.6	8.6	9.0	9.9

a Since P-value > 0.05, there was not sufficient evidence to support the null hypothesis that the mean cap diameter for the three bed positions differ.

b Since P-value > 0.05, there was not sufficient evidence to support the null hypothesis that the mean process time for the three bed positions differ.

Table 2.4: Slice thickness, bed position in the chamber, and process time of sliced mushrooms used in temperature penetration studies.

	Slice Thickness (mm)[a]			Process Time (minutes)[b]		
Trial (Block)	Top (Trt. 1)	Middle (Trt. 2)	Bottom (Trt.3)	Top (Trt. 1)	Middle (Trt. 2)	Bottom (Trt.3)
1	7.2	7.3	7.0	1.9	1.4	3.3
2	7.2	6.6	6.5	2.5	1.6	2.8
3	6.4	6.5	7.0	1.9	2.1	1.7
4	7.0	6.6	6.5	1.9	2.8	2.3
5	6.6	7.1	6.8	*	1.7	1.9

a Since P-value > 0.05, there was not sufficient evidence to support the null hypothesis that the mean cap diameter for the three bed positions differ.

b Since P-value > 0.05, there was not sufficient evidence to support the null hypothesis that the mean process time for the three bed positions differ.

ANOVA and Tukey LSD tests indicated that process time was not significantly influenced by bed position, mushroom block, and cap diameter or slice thickness at the 95% confidence level (See the footnotes in Table 2.3 and Table 2.4). Detailed discussion and presentation of findings of ANOVA and Tukey LSD tests to determine the effects of bed position and mushroom block on process time and cap diameter for temperature penetration studies of whole mushrooms and the effects of bed position on process time and slice thickness of sliced mushrooms can be found in Appendix A.

Though the mean process times were not statistically different, some statistical variation in the temperature penetration studies did exist. Since the temperature penetration data exhibited approximately normal distributions, it was assumed the most extreme, and therefore most conservative, process time lay three standard deviations from the mean accounting for 99.7% of the statistical

variation of the data set (National Canners Association, 1968). Though, the longest process time exhibited by the whole mushrooms was 10.1 minutes, the data for whole mushrooms had a mean process time of 9.2 minutes and a standard deviation 0.6 minutes. A most conservative process time for whole mushrooms would therefore be 11.0 minutes. Similarly, the longest process time exhibited by the sliced mushroom data set was 3.3 minutes; however, the mean was 2.1 minutes and the standard deviation 0.5 minutes. Therefore, the most conservative process time (three standard deviations from the mean) for sliced mushrooms would be 3.6 minutes. It was these most conservative process times that were considered the appropriate scheduled process to achieve commercial sterility. Temperature penetration tests were conducted on whole and sliced mushrooms canned in 237 ml (nominal 8 oz) glass jars following the procedure given by the NCA (1968). The scheduled process times for aseptic processing are considerably less than the 21 and 18 minute process developed for whole and sliced mushrooms, respectively.

Conclusion

Steam provided a uniform heating medium and temperature distribution within the sterilization chamber of the segmented-flow aseptic processing system, which was easily validated using a temperature distribution test.

A temperature penetration test, adapted from conventional canning process development, was used as the foundation for developing a scheduled

thermal process using microbiological kinetics. A sight-glass assembly provided an effective apparatus for conducting viewable temperature penetration tests in a pressurized steam environment. Process time was not significantly impacted by bed depth, mushroom cap diameter or slice thickness for the narrow range of sizes studied. Most conservative process times of 11.0 minutes for whole mushrooms and 3.6 minutes for sliced mushrooms for a process steam temperature of 130°C were calculated from temperature penetration data. Aseptic processing times for whole and sliced mushrooms are significantly less than would typically be encountered in conventional canning of mushrooms, even for small cans, which require approximately 20 minutes of process time; larger cans require additional process time.

It was noted that this research was conducted under very controlled conditions, with limited throughput and total material processed. Under commercial conditions, the accumulation of fines, particle arrangement, density, etc. will be more variable and significant, and therefore, need to be considered.

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Chapter 3

Aseptic vs. Canned: Evaluation of Processed Mushroom Quality

Abstract

A continuous steam sterilization segmented-flow aseptic processing unit produced shelf-stable aseptically processed mushrooms of superior quality to conventionally canned mushrooms. When compared to conventionally canned mushrooms, aseptically processed yield (weight basis) increased 6.1% (SD=2.9%) and 6.6% (SD=2.2%), whiteness (L) improved 3.1% (SD=1.9%) and 4.7% (SD=0.7%), color difference (ΔE) improved 6.0% (SD=1.3%) and 8.5% (SD=1.5%), and texture improved 3.9% (SD=1.7%) and 4.6% (SD=4.2%), for whole and sliced mushrooms, respectively. The segmented-flow processing system utilized high temperature, short time (HTST) processing conditions, eliminated a separate blanching step, eliminated the unnecessary packaging of water and promoted the use of bag-in-box and other versatile aseptic packaging methods.

Introduction

Global mushroom production in 2002 totaled nearly 3 million metric tons. The U.S. accounted for 13% of total global production and of that, Pennsylvania produced more than half. As of 1991, approximately 16% of all mushrooms

produced world wide were canned (Food and Agriculture Organization, United Nations and USDA, 1991). Excessive time and temperature abuse occurs during preservation by conventional canning resulting in over-processed mushrooms of low quality. Given the demand for shelf-stable mushrooms and their sensitivity to processing, an improved method of processing mushrooms is desired by the mushroom processing industry.

A Continuous Steam Sterilization Segmented-Flow Aseptic Processor capable of processing mushrooms was developed (Chapter 1 contains a detailed description of the system.). In preliminary trials, the segmented-flow processing system produced foods of high quality, eliminated blanching or similar processing steps, eliminated the unnecessary packaging of water and promoted the use of bag-in-box and other versatile aseptic packaging methods.

Scheduled processes for aseptically processed whole and sliced mushrooms were developed using heat penetration studies (Chapter 2 contains a detailed description of the method and results for developing a scheduled process.). The scheduled processes developed (target F_o = 7 minutes) were 11.0 and 3.7 minutes for whole and sliced mushrooms, respectively.

Quality degradation of mushrooms

Mushroom quality is very sensitive to processing. Color attributes, textural characteristics, and yield decrease with increased processing time at temperatures typical for thermal sterilization (Anantheswaran et al., 1986).

Quality degradation from processing is due predominantly to disruption or destruction of cells within mushrooms. Though not a mushroom cell, Figure **3.1** is a diagrammatic representation of a typical plant cell and its constituent organelles. One distinct difference between fungal and plant cells is that the cell wall of fungal cells are comprised predominantly of chitin; whereas, the cell wall of plant cells are primarily cellulose.

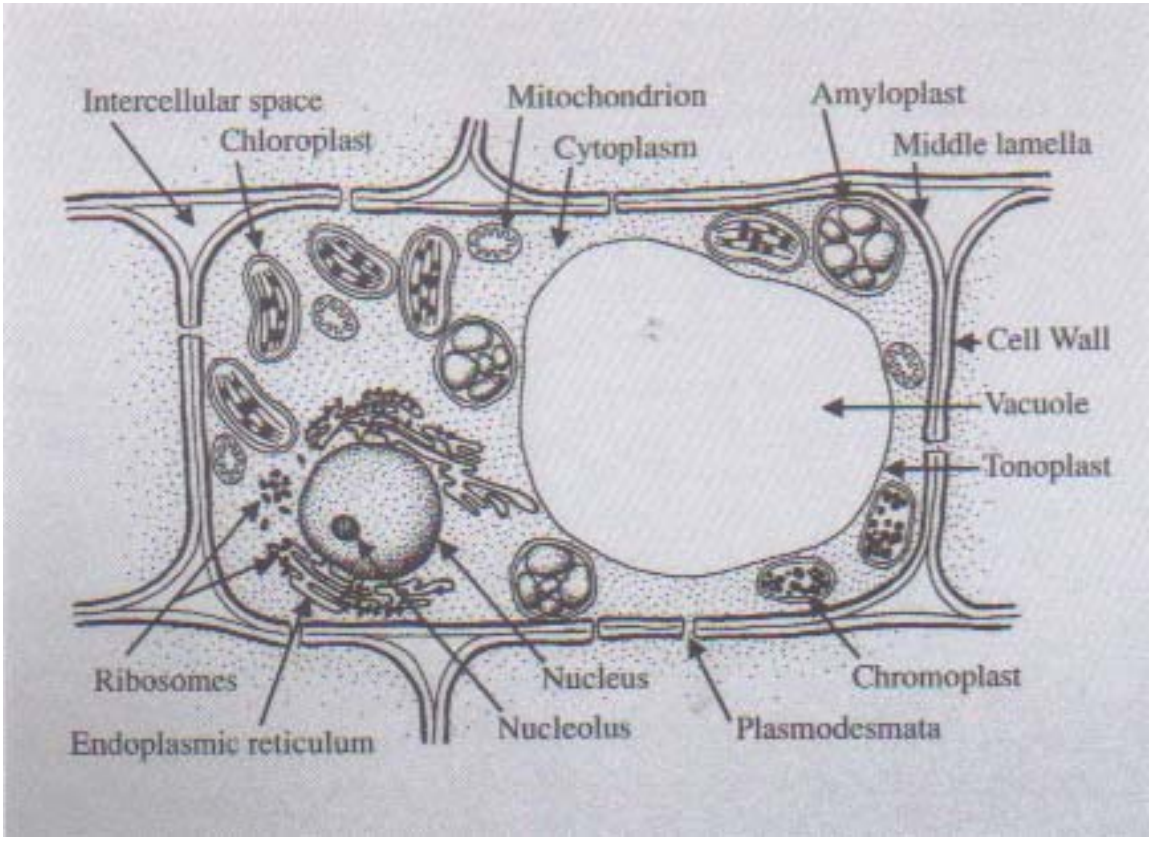

Figure **3.1**: Diagrammatic representation of a typical plant cell (Wills et al., 1998).

Texture

Fungus cells contain selectively permeable membranes. Due to the permeable nature of these membranes, water collects in the vacuole by osmosis and pushes the protoplast against the cell wall (Figure **3.1**). This pressure results in a firm, crisp, turgid texture in the mushroom. When exposed to extreme heat, membranes are destroyed and so too is the firm texture of a fresh mushroom (Wills et al., 1998; Beelman, 2002).

Anantheswaran et al. (1986) evaluated mushroom texture of various treatments of canned mushrooms, following blanching, before retorting, and after retorting, by measuring the peak force required to shear randomly-oriented sliced mushrooms using a Kramer shear compression cell. McGarry and Burton (1994) found that the stipe (stem) and pileus (cap) texture was not homogeneous when small, uniform tissue blocks were cut from raw mushrooms and subjected to a standard compression test.

Color

Browning is a common phenomenon in thermally processed mushrooms. Enzymatic browning is the consequence of the enzyme-catalysed oxidation of phenolic substrates into quinones (Jolivet et al., 1998). An illustration of the enzymatic browning reaction is given in Figure **3.2** . “Subsequent stages of this reaction, lead to the development of high molecular mass, dark pigments called

melanins (Jolivet et al., 1998)", such that a once white mushroom will appear brown and darkened.

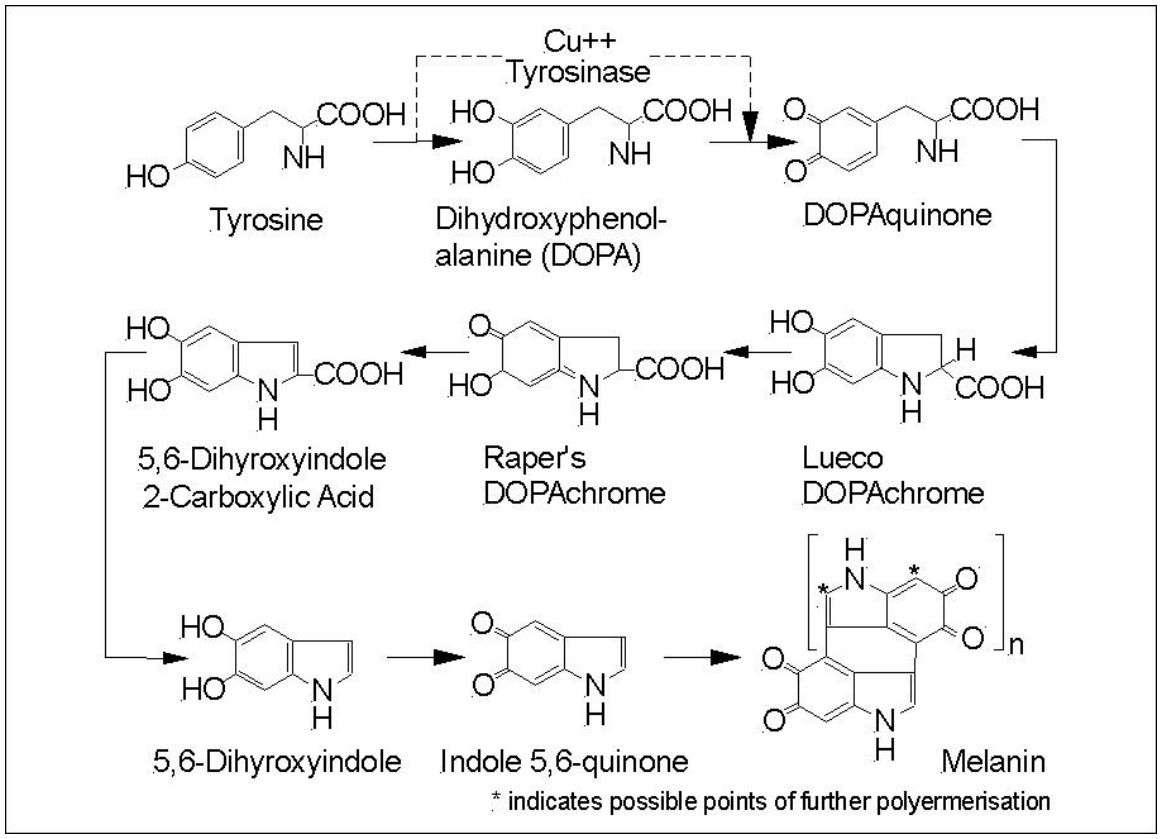

Figure **3.2**: Diagrammatic representation of enzymatic browning reactions (Adapted from Raper, 1928 and Beelman, 2002).

The polyphenol oxidase (PPO) tyrosinase is the most important phenolic enzyme involved in enzymatic browning. In a healthy, compartmentalized cell, tyrosinase and tyrosine are held separate from each other (Figure **3.1**). When cell destruction occurs and intercellular membranes break down, these compounds mix and enzymatic browning results (Jolivet et al., 1998; Beelman, 2002).

Blanching is an effective method of reducing enzymatic browning because it destroys the tyrosinase enzyme. Tyrosinase denatures at temperatures greater than 76°C (Beelman, 2002). Once denaturation of the enzyme occurs, tyrosinase cannot contribute further to enzymatic browning of mushrooms. Since mild heat encourages enzymatic activity, some browning will occur during blanching prior to denaturation of the enzyme due to excessive heat. Mushrooms are typically blanched in hot water for approximately 5 minutes at 95°C to achieve a center temperature greater than 80°C (Beelman, 2002).

Oxygen must be present for the enzymatic browning reaction to occur. Therefore, if the air trapped in the intercellular spaces of mushrooms is reduced or eliminated, the potential for the enzymatic browning reaction to occur is reduced. Vacuum hydration of mushrooms prior to processing is an effective means of reducing air trapped in the intercellular space of mushroom cells. Vacuum hydration is accomplished by drawing a vacuum on fresh mushrooms submerged in water (McCardle et al., 1974). Once the vacuum pulls the trapped air from the intercellular spaces of mushrooms, the vacuum is released and water is drawn into the now empty intercellular spaces, thus the amount of air contained in the mushrooms significantly reduced. Anantheswaran et al. (1986) showed that vacuum hydration of mushrooms caused color, texture and yield to be less sensitive to process temperature changes.

Yield

Significant reduction in volume and weight loss occurs in mushrooms during thermal processing. Shrinkage of mushrooms during processing, most of which occurs during blanching, has been reported to be in the range of 17% to 40% (weight basis), the average being approximately 30% (McCardle et al., 1974; Jasinski et al., 1984; Devece et al., 1999). Okereke and Beelman (1990) characterized yield by marking and weighing raw mushrooms before processing and measured the drained weight of the same mushrooms after processing. Yield was said to be less sensitive to process temperature changes when mushrooms were vacuum hydrated prior to processing (Anantheswaran et al., 1986). One reason could be that non-condensable gases (oxygen, nitrogen, etc.) contained within the tissue of vegetables can compromise the high rate of heat transfer associated with steam heating (Ling et al., 1974). As the gas flows out of the food during heating, it mixes with the steam at the sample surface and reduces the partial pressure of the steam and therefore the condensing temperature (Ling et al., 1974). When air has not been evacuated from vegetables the effect of this mixing can be so significant that the steam heat transfer coefficient can be lower than that of hot water (Melnick et al., 1944). Ling et al., (1974) reported the center temperature of vacuum evacuated mushroom samples had a faster rate of temperature change than samples that were untreated. Therefore, when mushrooms are vacuum hydrated, direct-contact steam should provide a more uniform, faster heating media than water.

In conventional canning of mushrooms, mushrooms are blanched before being packed in the cans, not so much for the purpose of denaturing the browning enzymes—because these enzymes would be denatured in the cooking process—but instead, to avoid in-can shrinkage. The result is more mushroom weight per volume of can. Canned mushrooms are sold on a drained weight basis.

Nutrient retention

Thermal resistance (z), activation energy (E_a) and thermal death time ($D_{121°C}$) of food constituents are listed in Table **3.1**. Vitamins, color, texture and flavor are up to 6 orders of magnitude more resistant to thermal destruction than spores and vegetative cells (Lund, 1974). Blanching processes are typically designed for enzyme inactivation. Since some thermally resistant enzymes have temperature dependencies similar to nutrients, process optimization is dependent on leaching and oxidative losses of nutrients. Based on these considerations, the optimum blanching operation may be high temperature-short time (HTST) in steam since both excessive leaching of water-soluble vitamins and oxidation would be minimized (Lund, 1974).

Table 3.1: Activation energy and thermal resistance of food constituents (Adapted from Lund, 1974)

Constituent	z (°C)	E_a (kcal/mole)	$D_{121°C}$ (min)
Vitamins	25-31	20-30	100-1000
Color, texture, flavor	25-44	10-30	5-500
Enzymes	7-56	12-100	1-10
Vegetative cells	4-7	100-120	0.002-0.02
Spores	7-12	53-83	0.1-5.0

Optimization of commercial sterilization processes is not straightforward. The primary concern when designing a sterilization process is destruction of pathogenic microorganisms. Often destruction of thermophilic microorganisms and heat resistant enzymes that may lead to economic spoilage or long-term quality degradation may actually dictate the scheduled process. At low temperatures the rate of destruction of enzymes is greater than that of microorganisms. At higher processing temperatures the rate of destruction of microorganisms increases faster than for enzymes. The temperature range where the destruction rate of enzymes equals that for microorganisms is 132°C to 143°C; above this range, inactivation of enzymes must be the basis of process design (Lund, 1974).

HTST processes for commercial sterilization will also result in maximum nutrient retention and quality factors in foods heated by convection since the product will heat rather uniformly. For foods heated by conduction, optimization of nutrient retention and quality attributes is more difficult. A HTST process is

optimum for a nutrient with a high z-value, but the optimum retention of a low z-value nutrient is obtained at a low temperature-long time process (Lund, 1974).

Objective

Compare yield and quality of continuous steam sterilized segmented-flow aseptically processed mushrooms with that of conventionally canned mushrooms. Segmented-flow aseptic processing under HTST processing conditions was expected to improve appearance, taste, texture, and processing yield compared to canned product.

Procedure

Mushrooms

The mushroom products studied in this project were small whole mushrooms (2.5 to 4.0 cm cap diameter) and sliced mushrooms (7 mm thickness, 2.5 to 4.0 cm cap diameter), species *Agaricus bisporus*. Raw mushrooms, supplied by the Mushroom Test Demonstration Facility (MTDF), Penn State University (University Park, PA), were of the same commercial quality typical for canned mushrooms.

Quality Tests

Quality tests were conducted on whole and sliced mushrooms using three independent mushroom crops for each on separate occasions. Approximately 5 kg of mushrooms with 2.5 to 4.0 cm nominal cap diameters were selected from the crop and washed in cold tap water. Washed mushrooms were randomly divided into two lots—one for processing aseptically and the other for canning conventionally—and stored for18 to 24 hr at 4°C.

A total of 96 mushrooms were used from each crop for processing—48 were canned, 48 were aseptically processed. Twelve additional mushrooms were selected randomly from the two lots to characterize the texture of raw mushrooms. Prior to processing, raw mushrooms were randomly selected and removed from cold storage for weight and color measurements 12 at time to minimize quality degradation, particularly color, due to the ambient environmental conditions of the laboratory. Sliced mushrooms were prepared on a mandolin by cutting to 7 mm thickness. Only slices cut from the center of the mushroom were used in quality studies; outer edges slices were discarded. Each mushroom piece, whole or sliced, was uniquely marked for identification purposes. Once weight and color measurements were made on raw mushrooms, the mushrooms were returned to cold storage and held at 4°C for approximately 0.5 to 1 hr until processing commenced.

Physical attributes of quality, yield, color, and texture, were measured using industry accepted tests.

Yield

Weight shrinkage was measured as yield weight. Raw mushrooms were weighed before vacuum hydration and thermal processing and the drained weight of the same mushrooms were measured 18 to 24 hr after processing. The net yield was calculated using Equation **3.1**.

$$\text{Yield}_{\text{net}} = \frac{\text{drained weight after processing}}{\text{fresh weight before processing}} \qquad \textbf{3.1}$$

Color

L (degree of whiteness) and ΔE (degree of color change with reference to a white calibration tile with characteristics: L = 97.00, a = -2.00, and b = 0.0) color values were measured with a Minolta Chromameter Model CR-200 (Konica Minolta, Mahwah, NJ). Color measurements were made at two locations on the cap of whole mushrooms and once per side of sliced mushrooms. Mushrooms were evaluated in their raw state before vacuum hydration and processing and again 18-24 hours after processing (Okereke et al., 1990). The net change in L value was evaluated using Equation **3.2** and the net change in ΔE was evaluated using Equation **3.3.**

$$\text{L}_{\text{net}} = \frac{\text{L value after processing}}{\text{L value before processing}} \qquad \textbf{3.2}$$

$$\Delta E_{net} = \frac{\Delta E \text{ after processing}}{\Delta E \text{ before processing}} \qquad \textbf{3.3}$$

Texture

Texture was evaluated by measuring the work per unit mass required to shear individual mushrooms. An Instron Model 4444 Universal Testing Machine (Instron, Norwood, MA) was equipped with a Kramer shear cell (Instron, Norwood, MA). Figure **3.3** shows a similar model Instron machine equipped with a Kramer shear cell. During shear tests, a single whole mushroom was placed on its side or a single sliced mushroom was laid flat on one cut side in the center of the shear cell; in both cases, stems were oriented perpendicular to the shear blades. Individual mushrooms were sheared at a rate of 22 cm/min and data were collected every 0.25 and 0.5 seconds, resulting in a data point every 1 and 2 mm for sliced and whole mushrooms, respectively.

Figure 3.3: Instron Universal Test Machine with a Kramer shear cell attachment (Instron, 2006)

At the start of each trial, twelve raw mushrooms were randomly selected from the two lots and sliced, if appropriate. Since texture analysis was a destructive test, individual whole or sliced raw mushrooms were sheared to characterize raw mushroom texture. Following weight and color measurements of processed mushrooms, individual mushrooms were sheared to characterize post-processing texture. Work was calculated using stepwise numerical integration of Equation 3.4, where F is force and y is displacement. The net change in work was evaluated using Equation 3.5. The net change in texture was evaluated using Equation 3.6.

$$Work = \int_0^y F \cdot dy \qquad 3.4$$

$$\text{Work}_{\text{net}} = \frac{Work\ for\ processed\ mushrooms}{Work\ for\ raw\ mushrooms} \quad \textbf{3.5}$$

$$\text{Texture}_{\text{net}} = \frac{Work_{\text{net}}}{\text{mass after processing}} \quad \textbf{3.6}$$

Processing

Mushrooms were vacuum hydrated following the procedure outlined by McCardle et al. (1974). Raw mushrooms were submerged in cold tap water inside a vacuum kettle. Mushrooms were held under a vacuum of 94 kPa (28in Hg) for five minutes. The vacuum was released and mushrooms were held an additional minimum 5 minutes under ambient conditions.

Aseptic Processing

Vacuum hydrated mushrooms were processed in the aseptic processing system according to the scheduled processes developed in Chapter 2:130°C for 11.05 minutes and 3.74 minutes for whole and sliced mushrooms, respectively. Heat treated mushrooms entered the cooling column filled with cool, approximately 15°C, sterile water. Mushrooms cooled during their descent through the cool sterile water circulating in the cooling column and were discharged directly into a sterile glove box. Inside the sterile glove box, cool,

aseptically processed mushrooms were loosely packed in 8 oz. glass jars. Sterile cool water was added to the jars of mushrooms until nearly full. Jars were sealed using lids and screw-on bands common to home canning. Sealed jars were removed from the sterile glove box and allowed to equilibrate to ambient room temperature.

Canning in a Still Retort

On the same day as aseptic processing, vacuum hydrated mushrooms from the same crop used in the aseptic processing studies were blanched in approximately 90°C water. After 5 minutes, blanched mushrooms were promptly removed from the hot water and 145 g were added to 8 oz. jars. Jars were topped with boiling water and tightly sealed. Sealed jars, with a minimum initial temperature of 65°C, were placed in a small pressure kettle and processed following the scheduled process developed in Chapter 2. Whole and sliced mushrooms were processed at 121°C for 21 and 18 minutes, respectively, then cooled with tap water. Cool jars of mushrooms were removed from the retort and allowed to equilibrate to ambient room temperature.

Data Analysis

The difference in yield, L value, ΔE peak force and work measurements for each crop of aseptically processed and conventionally canned mushrooms were compared using the ratio presented in Equation **3.7**.

$$\frac{\left(\overline{X}_{net\ aseptic} - \overline{X}_{net\ canned}\right)}{\overline{X}_{net\ canned}} \times 100\% \qquad \textbf{3.7}$$

Analysis of Variance (ANOVA) was used to determine the effects of aseptic (Method 1) or canned (Method 2) processing methods and mushroom crop on yield, L value, ΔE, and texture (work/unit-mass) quality factors. The ANOVA General Linear Model used in statistical analysis for quality experiments with multiple experimental units for each processing method in each crop of mushrooms is given in Equation **3.8** , where y_{ij} is the random variable representing the response for the j^{th} processing method observed in mushroom crop i, μ is the overall mean quality characteristic, α_i is the effect of the i^{th} crop (i = 1,2,3,4), β_j is the effect of the j^{th} processing method (j = 1,2), $(\alpha\beta)_{ij}$ represents the general non-additive effects of the general processing method x crop interaction for the k^{th} processing unit (k=1,2,…,u), and ε_{ijk} are the random experimental errors for the j^{th} processing method in the i^{th} crop for the k^{th} experimental unit.

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk} \qquad \textbf{3.8}$$

(Adapted from Kuehl, 2000)

To test the equality of the treatment (processing method) means, the null hypothesis, H_O: $\mu_1 = \mu_2$, i.e., aseptic and canned processing methods gave the same mean quality, was tested against the alternative hypothesis, H_A: $\mu_1 \neq \mu_2$.

Tukey's Least Significant Difference test was used to support the findings of the ANOVA tests.

Results and Discussion

Yield, L value, ΔE, and texture quality data are summarized in Table **3.2** and Table **3.3** for whole and sliced mushrooms, respectively. Raw data are given in Appendix B. Detailed results of ANOVA are presented in Appendix C.

Yield

Whole mushroom yield was 6.1% greater and sliced mushroom yield was 6.6% greater for aseptic versus canned processing methods (Table **3.2** and Table **3.3**, respectively). Of particular importance to yield from the perspective of thermal processing, were the significant impacts of a required separate blanching step, the relative lower process temperature (121°C) and longer process time for conventional canning. The improved yield under aseptic conditions reaffirm the findings of Anantheswaran et al. (1986) who found that the activation energy for mushroom yield was lower than for bacterial destruction and suggested "…a high temperature short time (HTST) sterilization for mushrooms…" would be

advantageous. Since mushrooms were canned in small jars, it is reasonable to conclude that improvement in yield may be even more significant when making comparisons to #10 cans of mushrooms that are processed for a much longer time.

Color

The positive difference in L value indicated that aseptically processed whole mushrooms were 3.1% whiter and sliced mushrooms were 4.7% whiter and than canned mushrooms (Table **3.2** and Table **3.3**, respectively). The negative difference in ΔE indicated that aseptically processed whole mushrooms experienced 6.0% less color difference and sliced mushrooms experienced 8.5% less color difference than canned mushrooms. Figure **3.4** and Figure **3.5** illustrate the visibly superior quality of aseptically processed whole mushrooms. Since, color degradation is enhanced by exposure to heat until denaturation of tyrosinase (Beelman, 2002), HTST conditions and the higher heat transfer of steam, minimize the impact of enzymatic browning.

Texture

Aseptically processed whole mushrooms required 3.9% less work per unit-mass and sliced mushrooms required 4.6% less work per unit-mass to shear than canned mushrooms (Table **3.2** and Table **3.3**, respectively). Figure **3.6** and

Figure 3.7 reveal that when similar sized whole and sliced mushrooms are compared, the texture of a typical aseptically processed mushroom more closely matched the texture of a typical raw mushroom versus a canned mushroom. Figure 3.8 illustrates the three stages of a texture test: compression, extrusion and shear. While these stages are not apparent in the texture plot, they are more apparent in force plots (Figure 3.9 and Figure 3.10), particularly the force plot of whole mushrooms (Figure 3.9). During the compression stage of a whole raw mushroom nearly constant force was exerted. Then, during the extrusion stage, force (negative) decreased sharply. Finally, at the shear stage, failure occurred at the point of peak force. Since less intercellular damaged had occurred in the aseptically processed mushroom, greater compression was exhibited versus the canned mushroom. Much less compression occurred in the sliced mushrooms since they were of uniform thickness and relatively fragile. The raw mushroom slice, which was very crisp, fractured with the least amount of effort and the aseptically processed slice, required more work than the raw sliced, but less than the canned slice. These observations indicate that the texture of aseptically processed whole and sliced mushrooms more closely matched the texture of raw mushrooms.

Table **3.2**: Whole mushroom quality data.

	Aseptic					Canned					Difference				
Crop	Yield	L	Δ E	Peak Force	Work (g^{-1})	Yield	L	Δ E	Peak Force	Work (g^{-1})	Yield	L	Δ E	Peak Force	Work (g^{-1})
1	0.70	0.75	2.39	0.59	0.37	0.64	0.72	2.57	0.56	0.38	9.8%	3.3%	-7.3%	4.8%	-1.5%
2	0.67	0.77	2.20	0.65	0.40	0.63	0.73	2.37	0.68	0.41	6.6%	5.2%	-7.1%	-3.1%	-5.6%
3	0.71	0.74	2.43	0.63	0.39	0.67	0.71	2.56	0.67	0.42	5.3%	3.3%	-5.0%	-6.7%	-4.2%
4	0.71	0.69	2.45	0.67	0.42	0.69	0.69	2.57	0.69	0.44	2.8%	0.5%	-4.8%	-3.4%	-4.2%
Mean	0.70	0.74	2.37	0.64	0.40	0.66	0.71	2.52	0.65	0.41	6.1%	3.1%	-6.0%	-2.1%	-3.9%
SD	0.02	0.03	0.11	0.03	0.02	0.03	0.02	0.10	0.06	0.03	2.9%[a]	1.9%[a]	1.3%[a]	4.9%	1.7%[a]

* Since quality data are calculated as a ratio of post-process/pre-process measurements, all data are unit-less with the exception of texture data that are the work ratio/post process mass (g).

a Since P-value < 0.05, there is sufficient evidence to support the claim that the means from the two processing methods differ. Peak force was not evaluated statistically.

Table **3.3**: Sliced mushroom quality data.

	Aseptic					Canned					Difference				
Crop	Yield	L	Δ E	Peak Force	Work (g^{-1})	Yield	L	Δ E	Peak Force	Work (g^{-1})	Yield	L	Δ E	Peak Force	Work (g^{-1})
1	0.69	0.80	1.72	0.77	0.57	0.64	0.77	1.84	0.79	0.57	7.8%	4.3%	-6.9%	-2.2%	0.0%
2	0.67	0.81	1.66	0.81	0.53	0.62	0.77	1.84	0.82	0.56	7.9%	5.4%	-9.8%	-0.7%	-5.4%
3	0.71	0.83	1.60	0.70	0.55	0.68	0.80	1.76	0.71	0.60	4.1%	4.3%	-8.8%	-1.4%	-8.3%
Mean	0.69	0.81	1.66	0.76	0.55	0.65	0.78	1.81	0.77	0.58	6.6%	4.7%	-8.5%	-1.5%	-4.6%
SD	0.02	0.01	0.06	0.06	0.02	0.03	0.02	0.05	0.06	0.02	2.2%[a]	0.7%[a]	1.5%[a]	0.8%	4.2%[b]

* Since quality data are calculated as a ratio of post-process/pre-process measurements, all data are unit-less with the exception of texture data that are the work ratio/post process mass (g).

a Since P-value < 0.05, there is sufficient evidence to support the claim that the means from the two processing methods differ. Peak force was not evaluated statistically.

b Since the P-value > 0.05 there is not sufficient evidence to support the claim that the means from the two processing methods differ at the 95% confidence level.

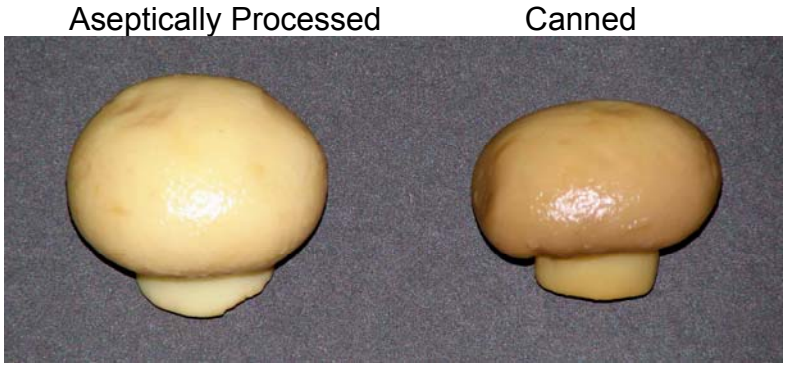

Figure **3.4**: Visibly superior quality of an aseptically processed whole mushroom.

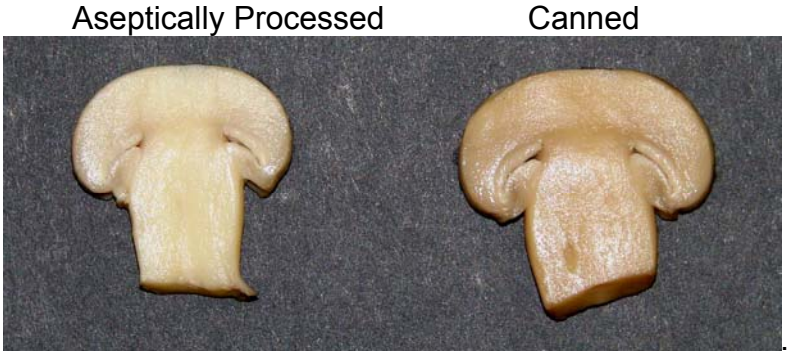

Figure **3.5**: Visibly superior quality of an aseptically processed sliced mushroom

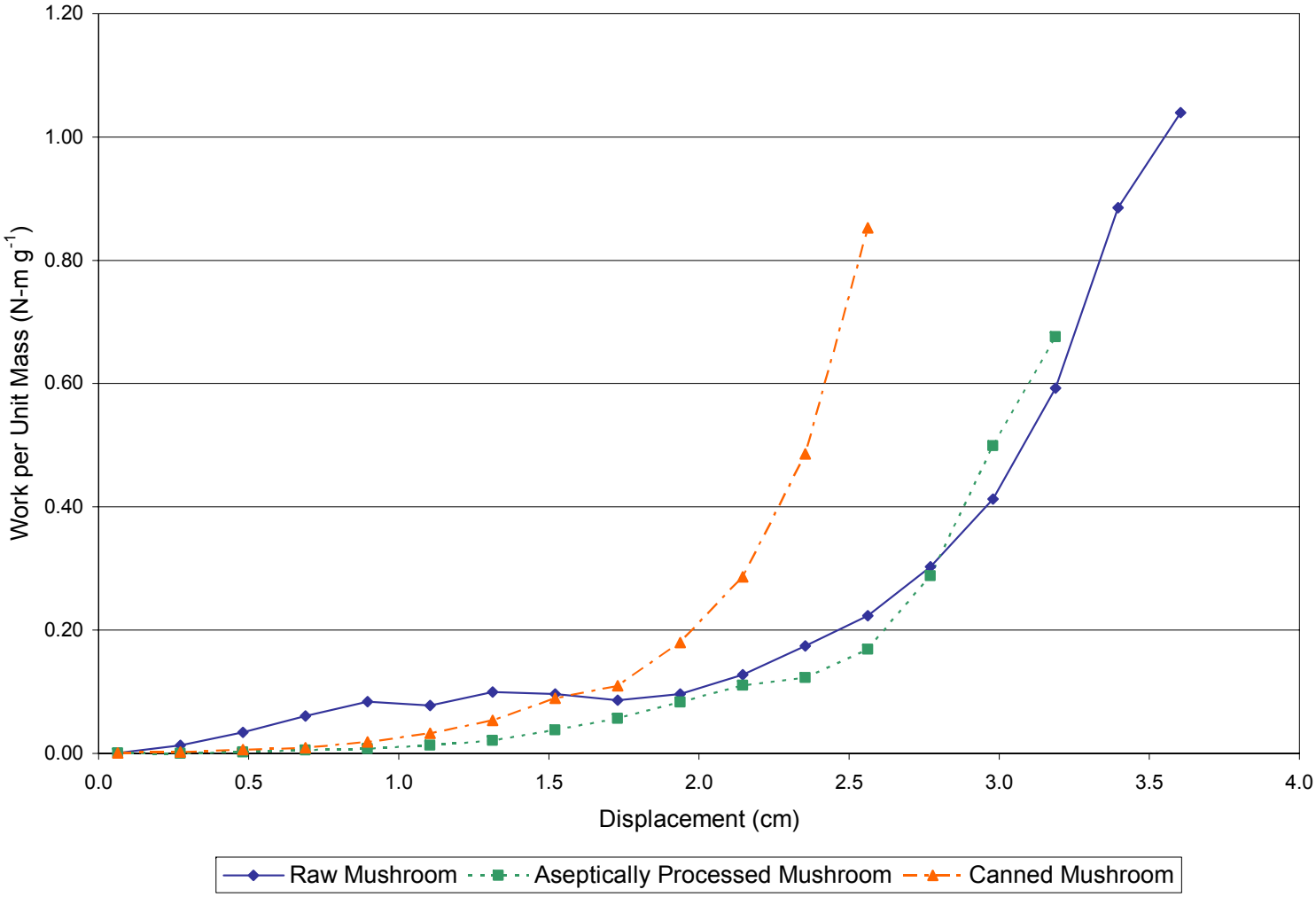

Figure **3.6**: Texture plots for similar sized individual raw, aseptically processed and canned whole mushrooms (raw weight ~21 g).

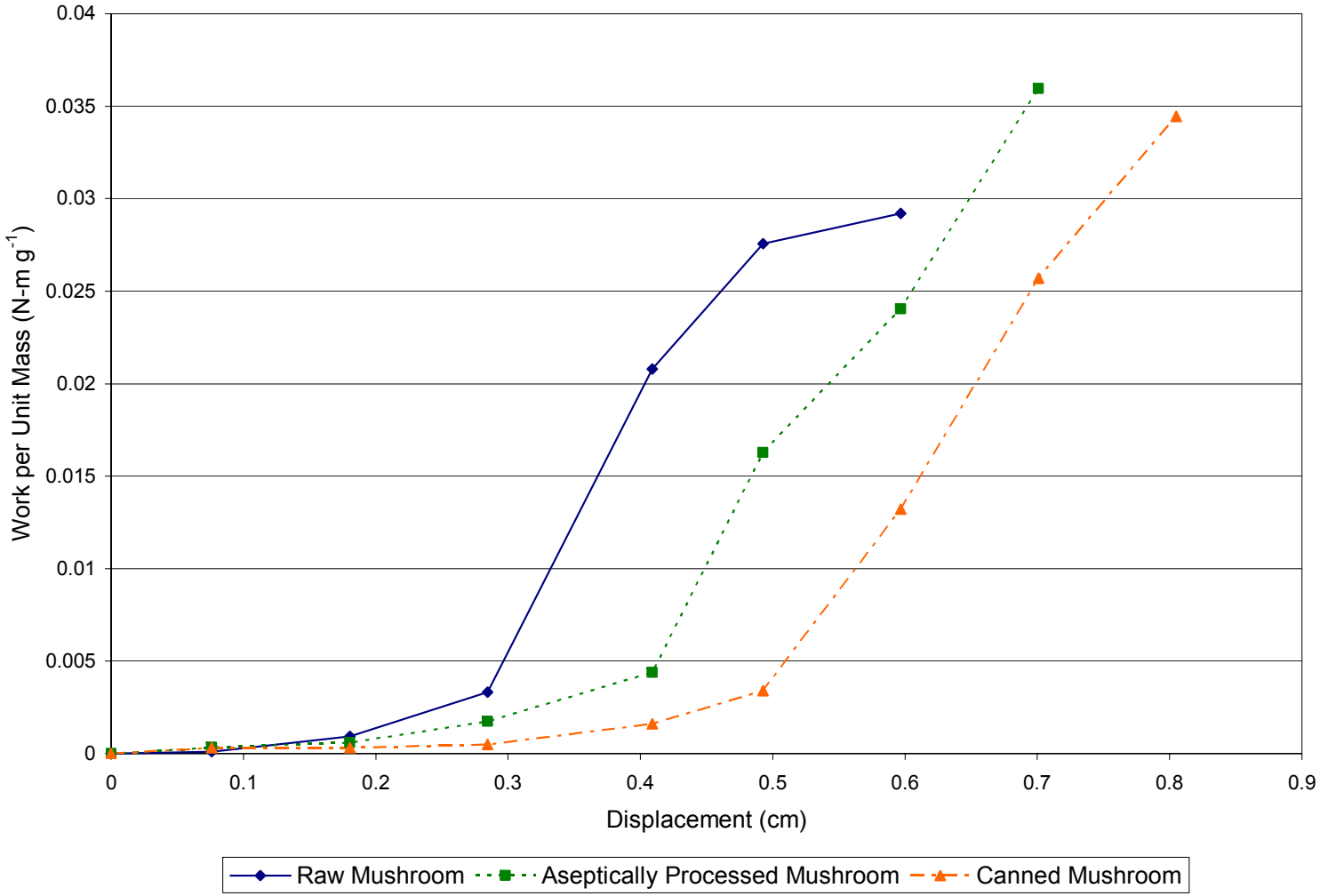

Figure **3.7**: Texture plots of similar sized individual raw, aseptically processed and canned mushroom slices (raw weight ~5.5g).

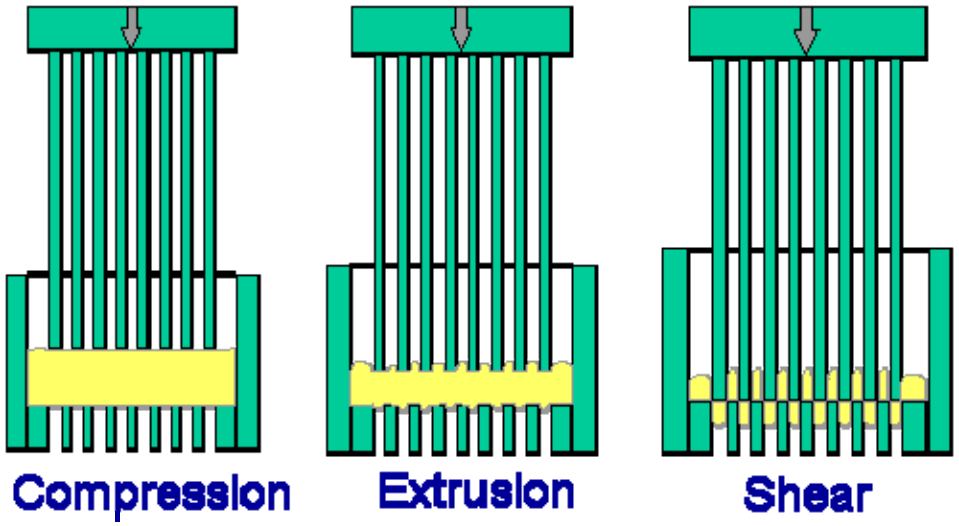

Figure 3.8: Three stages of texture analysis in a Kramer shear cell (Instron, 2006).

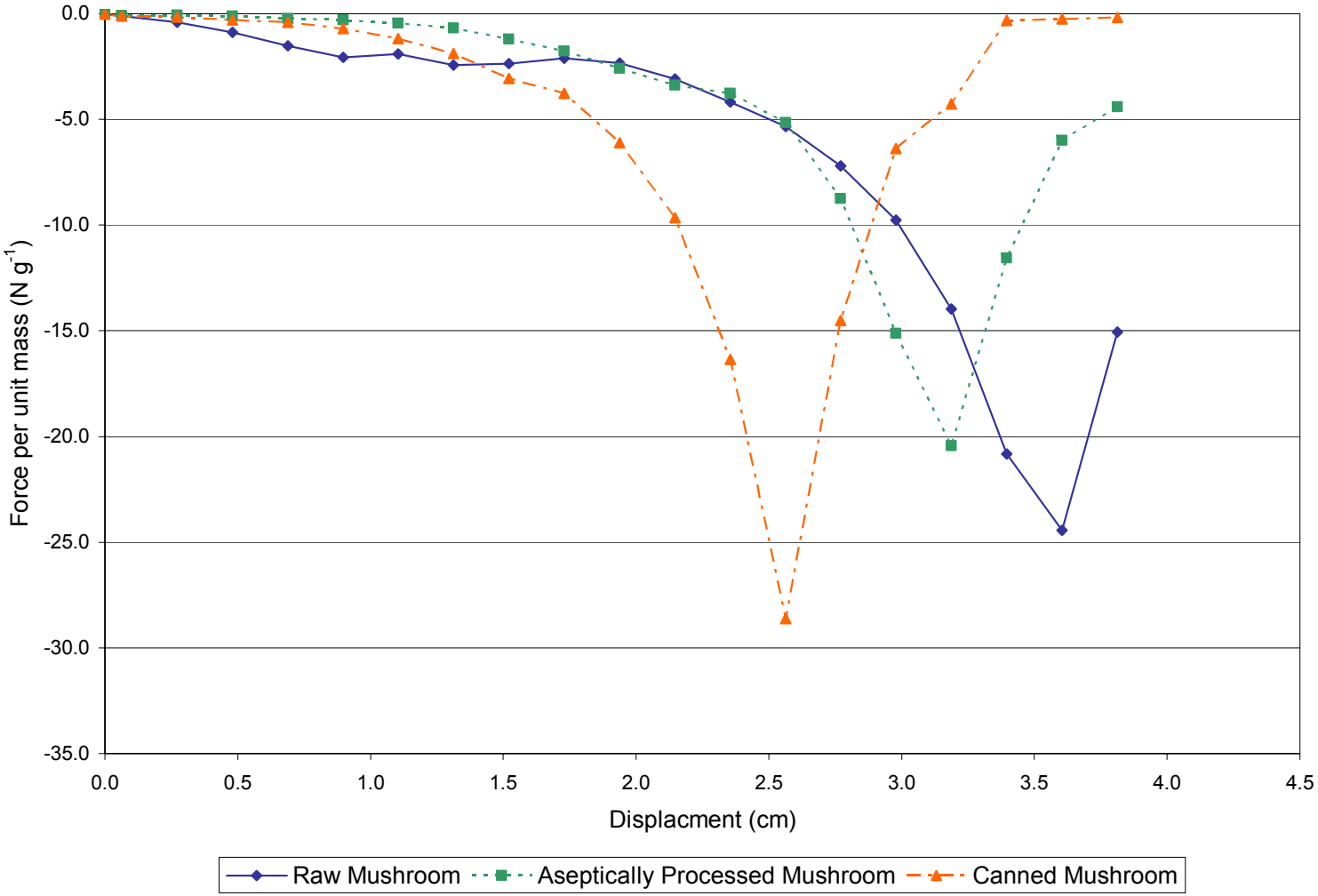

Figure **3.9**: Force plots for similar sized (raw weight ~21 g) individual raw, aseptically processed and canned whole mushrooms. Force is reported as negative since the shear head was under compression.

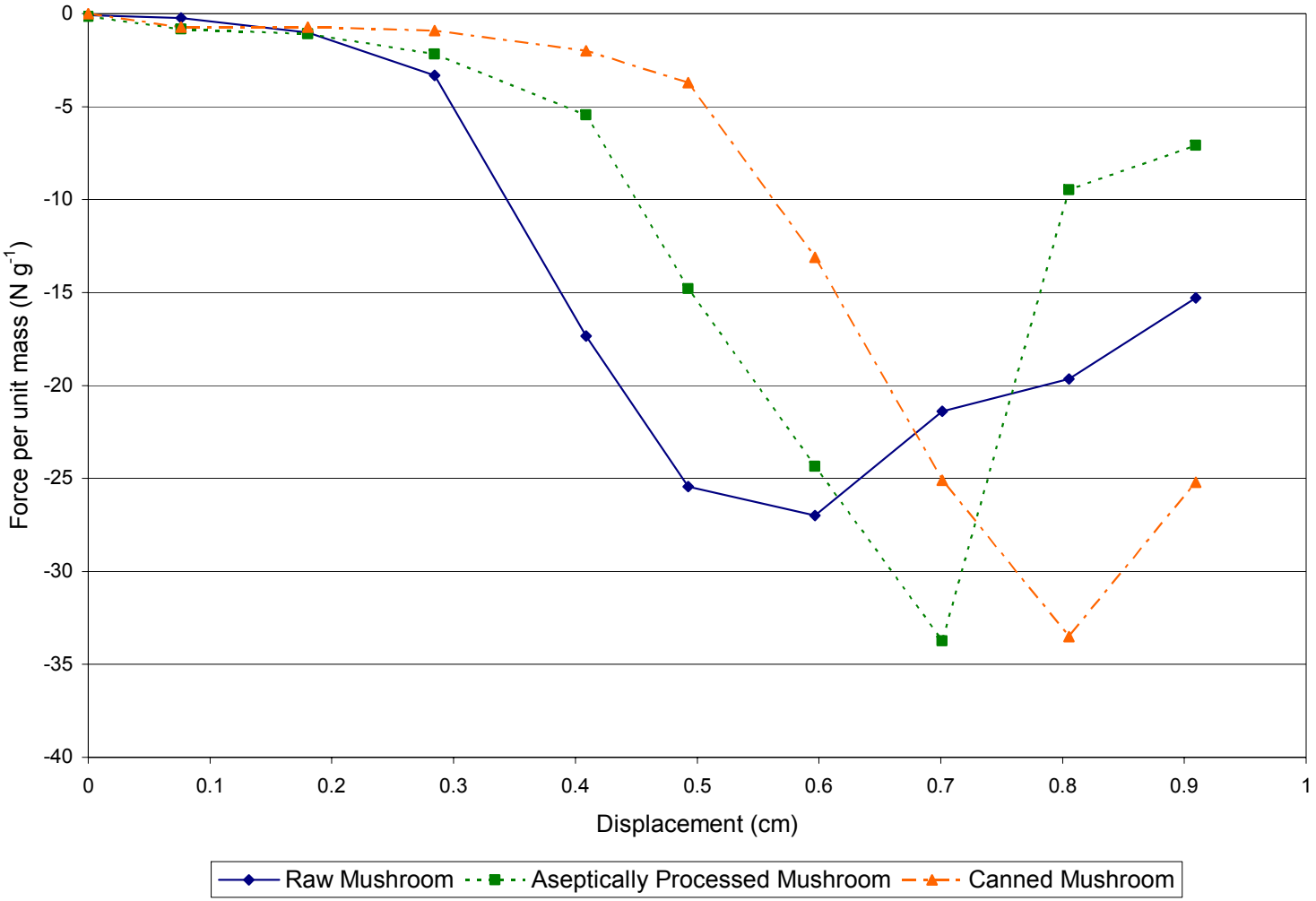

Figure **3.10**: Force plots for similar sized (raw weight ~5.5g) individual raw, aseptically processed and canned mushroom slices. Force is reported as negative since the shear head was under compression.

Conclusion

A continuous steam sterilization segmented-flow aseptic processing unit produced shelf-stable aseptically processed mushrooms of superior quality to conventionally canned mushrooms. When compared to conventionally canned mushrooms, aseptically processed yield (weight basis) increased 6.1% (SD=2.9%) and 6.6% (SD=2.2%), whiteness (L) improved 3.1% (SD=1.9%) and 4.7% (SD=0.7%), color difference (ΔE) improved 6.0% (SD=1.3%) and 8.5% (SD=1.5%), and texture improved 3.9% (SD=1.7%) and 4.6% (SD=4.2%), for whole and sliced mushrooms, respectively. The segmented-flow processing system utilized HTST processing conditions, eliminated a separate blanching step, eliminated the unnecessary packaging of water and promoted the use of bag-in-box and other versatile aseptic packaging methods.

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Chapter 4

Thermal Death Time and Inoculated Pack Studies with *Clostridium sporogenes* to Validate Steam Sterilization Segmented Flow Aseptic Processing of Mushrooms

Abstract

Thermal death time studies revealed close agreement between the $D_{121°C}$ for *Clostridium sporogenes* spores obtained in mushroom slurry (0.90 minutes) and whole mushrooms processed aseptically (0.87 minutes). Inoculated pack studies proved the development of a scheduled process based on temperature penetration data of the slowest heating particle lead to predictable destruction of *Clostridium sporogenes* spores. At an inoculation level of approximately 10^3 CFU/container, viable spores were eliminated after 9.3 minutes process time, indicating that process time could be likely reduced from more conservative processes.

Introduction

As indicated in Chapter 2, *Clostridium botulinum*, a toxin producing spore forming mesophilic microorganism, is a significant threat to public health in canned mushrooms. *Clostridium botulinum* types A and B, typically associated with soil, occur naturally on fresh mushrooms at an estimated incidence of 0.08 to 0.16 CFU per 100 g of mushrooms (Notermans et al., 1989). *Clostridium*

sporogenes PA3679 has physiological similarities to *Clostridium botulinum* and a higher resistance to heat. A typical 5D process for *Clostridium sporogenes* is more conservative than a traditional 12D process for *Clostridium botulinum.* "Therefore, the increased resistance of (*Clostridium sporogenes*) over that of *Clostridium botulinum* provides a built-in safety factor when using (*Clostridium sporogenes*) in inoculated pack studies (Okereke et al., 1988)." Since it is a nonpathogenic, putrefactive, and a voluminous gas producer, *Clostridium sporogenes* is a choice organism for determining the adequacy of thermal designs (Okereke et al., 1988). The F_o =7 minutes suggested by FPA (Deniston, 2003) for small button mushrooms packaged in brine in a U.S. standard 211 x 212 can, with diameter 6.8 cm (2-11/16 inches) and height 7.0 cm (2-12/16 inches), and processed in a still retort is based on a 5D process for *Clostridium sporogenes.* Larger can sizes, the use of agitated retorts, and the presence of thermophilic microorganisms will influence the F_o; the typical range of F_o is thus 7 to 11 minutes for canned mushrooms (Deniston, 2003).

Okereke et al. (1988) conducted thermal death studies on spore crops of *Clostridium sporogenes* PA3679 in Sorenson's phosphate buffer and mushroom slurry. Glass vials (2 mL capacity) were filled with 1.5 mL of a standard spore suspension of 10^6 CFU/mL. Flame sealed vials were placed in a 121°C oil bath. Vials were removed from the oil bath, cooled and plated on Eugon agar. Plates were incubated anaerobically at 35°C for 5 to 7 days. The log of the viable plate count was plotted against time and a best-fit, straight line drawn through the points. The decimal reduction time (D) was the negative reciprocal of the slope (-1/slope) of the line (Paustian, 2006). Okereke et al. (1990) reported D-values for *Clostridium sporogenes* of $D_{121\,^{\circ}C}$ = 1.31 to 1.80 minutes.

Okereke et al. (1988) also conducted an inoculated pack study on canned mushrooms. Mushrooms were inoculated through vacuum hydration in a standard 10^7 CFU/mL solution. Mushrooms were then blanched in a hot water bath. Inoculated mushrooms (25 g) were added to cans of uninoculated mushrooms (100g) and topped with hot water. Cans were sealed and retorted. Thermally process cans were incubated at 35°C for an extended period. Spoilage (swelled cans) was used to evaluate the effectiveness of the thermal processes.

Objectives

Determine the thermal death time of a suitable *Clostridium sporogenes* spore crop.

Conduct an inoculated pack study to validate the temperature penetration tests and scheduled process developed for whole mushrooms.

Procedure

A spore crop of *Clostridium sporogenes* was obtained from The National Food Laboratory, Inc. (The NFL, Dublin, CA) for use in these studies. The spore crop characteristics are presented in Table **4.1.** While the specific protocol used by The NFL for preparation and calibration [i.e., determination of heat resistance (D) and heat resistance (z)] are proprietary the general methods are presented in Appendix D.

Table **4.1**: *Clostridium sporogenes* spore crop characteristics (Willette, 2006)

Description	Value
Total Volume	5 mL
Concentration	$5x10^8$ CFU/mL
$D_{121°C}$	0.79 minutes
Z	10.4 °C

Determination of Heat Resistance in Sorenson Phosphate Buffer (SPB)

To verify the heat resistance of the spore crop obtained from The NFL and to provide a benchmark for the researcher's own methods, it was desirable to determine the $D_{121°C}$. The spore suspension was agitated using a vortexer. Using a sterile syringe, 0.05 mL of $5x10^8$ CFU/mL spore suspension was removed and added to 25 mL of SPB (Appendix E for SPB formulation) to yield 25 mL with concentration of $1x10^6$ CFU/mL. Diluted spore suspension (1.5 mL) was placed in 2 mL capacity glass ampoules. A thermocouple was inserted into one of these ampoules and sealed using epoxy; the remaining ampoules were flame-sealed. Then all of the ampoules were placed in an ice-water bath and allowed to equilibrate.

The ampoules were heated in a 121°C oil bath. Once the thermocouple equipped ampoule had reached 121°C, one ampoule was removed to establish the come-up time. Ampoules were removed every 36 seconds (75% of published D-value) thereafter. Once removed from the oil bath, ampoules were immediately placed in an ice-water bath. Ampoules were cleaned with ethanol to remove residual oil from the bath. Samples were serially diluted in 0.1% peptone water (See Appendix E for peptone water formulation.), spread-plated on Eugon agar (See Appendix E for Eugon agar formulation.) and incubated at 35°C for 48 hours anaerobically in anaerobic jars with Gas Pak Plus hydrogen and carbon dioxide producing gas envelopes (BD, Franklin Lakes, NJ) maintaining anaerobic conditions. The log of the viable plate count was plotted against time and a best-

fit, straight line drawn through the points. The decimal reduction time (D) was the negative reciprocal of the slope (-1/slope) of the line (Paustian, 2006).

Determination of Heat Resistance in Mushroom Slurry

The heat resistance of the spore crop in mushroom slurry was determined using the following steps. Raw mushrooms, species *Agaricus bisporus*, supplied by the Mushroom Test Demonstration Facility (MTDF), Penn State University (University Park, PA), were washed in cold tap water and stored for 18 to 24 hours at 4°C. Mushrooms were vacuum hydrated following the procedure outlined by McArdle et al. (1974). Raw mushrooms (100g) were submerged in 300 mL of SPB in a one liter blender vessel inside a vacuum kettle. Mushrooms were held under a vacuum of 88 kPa (26 in Hg) for five minutes. The vacuum was released and mushrooms were held an additional minimum 5 minutes under ambient conditions. Vacuum hydrated mushrooms and remaining SPB were blended for 15 sec to produce slurry.

The same procedure for microbiological analysis used in the previous section was used here with the exception of ampoule preparation. In this case, 0.05 mL of $5x10^8$ CFU/mL spore suspension was removed and added to 8.33 mL of SPB to yield 8.38 mL with concentration of $3x10^6$ CFU/mL. Then the diluted spore suspension (0.5 mL) and 1 mL of mushroom slurry were placed in a 2 mL capacity glass ampoule to yield a final dilution of $1x10^6$ CFU/mL of mushroom slurry was required for thermal death studies of mushroom slurry.

Determination of Heat Resistance in Individual Whole Mushrooms Processed Aseptically

The heat resistance of the spore crop in mushroom slurry was determined using the following steps. Twenty raw mushrooms, supplied by the Mushroom Test Demonstration Facility (MTDF), Penn State University (University Park, PA), pre-selected to be nominal cap diameter of 4.0 cm, were washed in cold tap water and stored for 18 to 24 hours at 4°C. Individual mushrooms were uniquely marked for identification, and cap diameter and raw weight were measured.

Using a sterile syringe, 0.16 mL of $5x10^8$ CFU/mL spore suspension was added to 1.8 mL of SPB to yield 1.96 mL with concentration of $4.5x10^7$ CFU/mL. To inoculate the test mushrooms, a sterile syringe was inserted through the base of the stem and the tip extended to the geometric center (Figure **2.3**) of the mushroom cap before 0.1 mL of the diluted spore suspension was injected into each individual mushroom. Mushrooms were vacuum hydrated following the procedure outlined by McArdle et al. (1974). Raw mushrooms (100 g) were submerged in SPB in a small stainless steel pot inside a vacuum kettle. Mushrooms were held under a vacuum of 88 kPa (26 in Hg) for five minutes. The vacuum was released and mushrooms were held an additional minimum 5 minutes under ambient conditions.

Vacuum hydrated mushrooms were processed at 130°C in the segmented flow aseptic processing system described in Chapter 1. Mushrooms were processed based on mushroom heating observed during temperature penetration studies described in Chapter 2. Table **4.2** lists the target lethality and equivalent

process times for the fastest heating particle as determined using Equation **2.2** and Equation **2.3** in the temperature penetration study conducted in Chapter 2 (See Table **2.3** for summary data of the fastest and slowest heating mushrooms). Three randomly selected mushrooms were processed at each process time. Process times were not randomly ordered. Rather, tests were done in order according to process time, with the longest processing times first. This minimized the potential for cross-contamination between tests, particularly in the cooling water. Processed mushrooms were packaged individually in glass jars and capped with metal lids and screw-on rings inside the sterile glove box. Samples of the vacuum hydration water and cooling water after each treatment were also collected.

Table **4.2**: Target spore reduction and process time for mushrooms injected with *Clostridium sporogenes.*

Target Reduction (log)	Process Time (minutes)
0	Unheated
0.5	5.48
1	5.97
2	6.28
3	6.93
4	7.25

* Target reduction based on the fastest heating mushroom in temperature penetration studies conducted in Chapter 2.

Jars containing aseptically processed mushrooms were removed from the glove box. Individual mushrooms were transferred aseptically to 532 mL (11.5 cm x 23 cm) sterile Whirl-Pak bags (Nasco, Modesto, CA). The bag openings

were folded once and secured with the wire bag closure. The entire Whirl-Pak bag was then placed inside a resealable polyethylene bag. The double-bagged mushrooms were then pressed in large flat-jaw vise to expel the liquid and spores from the mushroom tissue. The jaw pressure was released, bag position adjusted to maximize jaw-to-mushroom contact, and pressure reapplied. This release and reapplication of pressure was repeated several times for each mushroom to maximize liquid expulsion. The expelled liquid and pressed mushroom tissue were carefully positioned to keep the liquid separate inside the Whirl-Pak bag. Freshly pressed mushroom liquid was serially diluted in peptone water. Pour-plate techniques were used to accommodate 1 mL of diluted suspension added to each Petri dish and covered with approximately 20 mL of Eugon agar and swirled to disperse the spores.

Inoculated vacuum hydration water and water sampled from the cooling column of the aseptic processor were filter (0.22 μm nominal retention) concentrated. The filters were placed in Petri dishes and covered with approximately 20 mL of Eugon agar and swirled to disperse the spores.

Plates were incubated at 35°C for 48 hours in anaerobic jars with AnaeroPack carbon dioxide producing gas packs (Mitsubishi Gas and Chemical, Inc., Tokyo, Japan) maintaining anaerobic conditions. The log of the viable plate count was plotted against time and a best-fit, straight line drawn through the points. The decimal reduction time (D) was the negative reciprocal of the slope (-1/slope) of the line (Paustian, 2006).

Effect of Aseptic Processing on the Spoilage of Mushroom Packs Inoculated with *Clostridium sporogenes* Spores

To further validate the effectiveness of the scheduled process developed in Chapter 2, it was desirable to conduct an inoculated pack study. The mushrooms, selected to have a nominal cap diameter in the range of 2.5 to 4.0 cm, were supplied by the Mushroom Test Demonstration Facility (MTDF), Penn State University (University Park, PA). Mushrooms were washed in cold tap water and stored for18 to 24 hours at 4°C.

Inoculated pack study procedures outlined by the National Canners Association (1968) suggest inoculating a minimum of 100 containers, each with 10^4-10^5 CFU/container. Preliminary tests indicated that 133 g of fresh mushrooms would take up 92 g of water during vacuum hydration and yield 100 g of processed mushrooms. Therefore, if mushrooms were vacuum hydrated in a standard spore suspension of 1.08×10^2 CFU/mL the researchers expected to yields to be approximately 10^4 CFU per container (100 g of mushrooms).

Using a sterile syringe, 0.1 mL of 5×10^8 CFU/mL spore suspension was removed and added to 4.9 mL of SPB to yield 5 mL with concentration of 1×10^7 CFU/mL. A sterile syringe was then used to transfer 2.05 mL of the diluted spore suspension to the vacuum chamber containing 19 L of distilled water to yield a diluted spore suspension with 1.08×10^2 CFU/mL.

Raw mushrooms (4.5 kg) were submerged in the diluted spore suspension. Mushrooms were vacuum hydrated following the procedure outlined by Okereke et al. (1988). Mushrooms were held under a vacuum of 88 kPa (26

in Hg) for 20 minutes; the vacuum was then released and mushrooms were held an additional minimum 7 minutes at atmospheric pressure. Vacuum was applied again for 10 minutes and released for 7 minutes. The prolonged vacuum and a second vacuum application were applied to maximize potential for spores to reach the mushroom center.

The goal of an inoculated pack study is to yield nearly 100% and 0% spoilage for at least two processes, and partial spoilage for at least one process (NCA, 1968). The results from the study of individual mushrooms (See previous section.) indicated that the thermal death rate of *Clostridium sporogenes* closely followed the least conservative temperature penetration data (Results discussed in detail later.). Since, mushroom size ranged in cap diameter from 2.5 to 4.0 cm in the inoculated pack test, it was thought that if anything, inoculated pack tests would result in greater spore lethality. Therefore, in Trial 1, vacuum hydrated inoculated mushrooms were processed in the range of 6.0 to 8.4 minutes at 130°C in the segmented flow aseptic processing system to achieve target reductions of 0.1 to 1.8 logs based on the slowest heating and therefore most conservative temperature penetration data collected in Chapter 2. The logic used was that the most conservative process would result in 0% spoilage at 10^4 CFU/container inoculation level with a 2 log reduction target process and would likely result in 100% spoilage at a 0.1 log reduction target. Table **4.3** lists the process times and target reductions for Trial 1.

Table 4.3: Target spore reduction and process time for mushrooms inoculated with *Clostridium sporogenes* based on the slowest (most conservative) temperature penetration data.

Trial	Process Time (minutes)	Target log reduction*
1	6.0	0.1
	6.9	0.3
	7.5	0.6
	8.0	1.4
	8.4	1.8
2	7.4	0.5
	8.5	2.0
	9.3	4.0
	10.1	7.0
	10.5	9.0

* Target log reduction based on the slowest heating mushroom in temperature penetration studies conducted in Chapter 2.

The results of Trial 1 indicated that spoilage occurred for every process applied in Trial 1 and more conservative target reductions were required (Results discussed in detail later.) Therefore, in Trial 2, mushrooms were processed in the range of 7.4 to 10.5 minutes at 130°C to achieve target reductions of 0.5 to 9 logs (Table 4.3).

In order to minimize the potential for spore cross-contamination, particularly in the cooling water, tests were completed for the longest process times first. Due to space, time and cost considerations, approximately 1 kg of randomly selected vacuum hydrated mushrooms were processed at each process time to yield approximately 12 containers per time of the inoculated pack

study. Cool processed mushrooms (100 g) were packaged in glass jars and topped with cool water from the aseptic processing system, capped with metal lids and screw-on rings inside the sterile glove box. Samples of vacuum hydration water and cooling water were also collected.

Following processing, jars of aseptically processed mushrooms were removed the glove box and incubated at 35°C for an extended time period. Jars were regularly evaluated for signs of spoilage. Jars that exhibited visible gas production in the form of gas bubbles, turbidity of the liquid or swelling were counted as spoiled and removed from the incubator, autoclaved and disposed.

Two samples (100 g) of raw vacuum hydrated inoculated mushrooms were evaluated to determine the initial challenge level per container (N_0). Raw vacuum hydrated mushrooms were transferred aseptically to a sterile Stomacher bag. Since vacuum hydrated samples contain substantial liquid, too much sample volume existed in the bags to press in a vise as before; therefore, the samples were homogenized with no additional liquid added in a Stomacher for 30 seconds at 250 rpm. The entire bag was then submerged in a 90°C water bath for 10 minutes to heat activate the spores. Mushroom liquid was serially diluted in peptone water. Pour-plate techniques were used to accommodate 1 mL of diluted suspension added to each Petri dish and covered with approximately 20 mL of Eugon agar. Poured plates were swirled to disperse the spores.

Inoculated vacuum hydration water was also heat treated in a 90°C water bath for 10 minutes to heat activate the spores. Heat treated vacuum hydration water and water sampled from the cooling column of the aseptic processor were

filter (0.22 µm) concentrated. The filters were placed in Petri dishes, covered with approximately 20 mL of Eugon agar and swirled to disperse the spores.

Plates were incubated at 35°C for 48 hours in anaerobic jars with AnaeroPack (Mitsubishi Gas and Chemical, Inc., Tokyo, Japan) carbon dioxide producing packets maintaining anaerobic conditions. After the incubation period, plates were enumerated.

After an extended incubation period of 45 days, spoilage was calculated as the percent of swelled jars. The most probable number (MPN) of spores capable of outgrowth was calculated with Equation **4.1**, n is the number of processed jars and q is the number of non-swollen/non-spoiled containers (Halverson and Ziegler,1933).

$$\mathrm{MPN} = \ln\left(\frac{\mathrm{n}}{\mathrm{q}}\right) \qquad \textbf{4.1}$$

The probability (P) of a single spore causing spoilage within the incubation period was calculated using Equation **4.2**.

$$P = \frac{MPN}{N_O} \qquad \textbf{4.2}$$

Results and Discussion

Determination of Heat Resistance of *Clostridium sporogenes* in SPB

The resistance of *Clostridium sporogenes* spores to heat was determined in SPB. Heat resistance was determined in 2 mL capacity ampules in a 121°C oil bath. Each ampoule contained 1.5 mL of $1x10^6$ CFU/mL SPB. Though the $D_{121°C}$ The NFL calibration for the *Clostridium sporogenes* spore crop was 0.79 minutes, the value obtained in this study was close; a $D_{121°C}$ = 0.67 minutes was calculated as the negative inverse of the slope of the fitted line shown in Figure **4.1**. The fitted line exhibited close fit (R^2 = 0.9686) with the data. Time zero, the come-up time at which ampoule temperature reached the oil bath temperature (121°C), occurred 2.80 minutes after the ampoules placed in the oil bath.

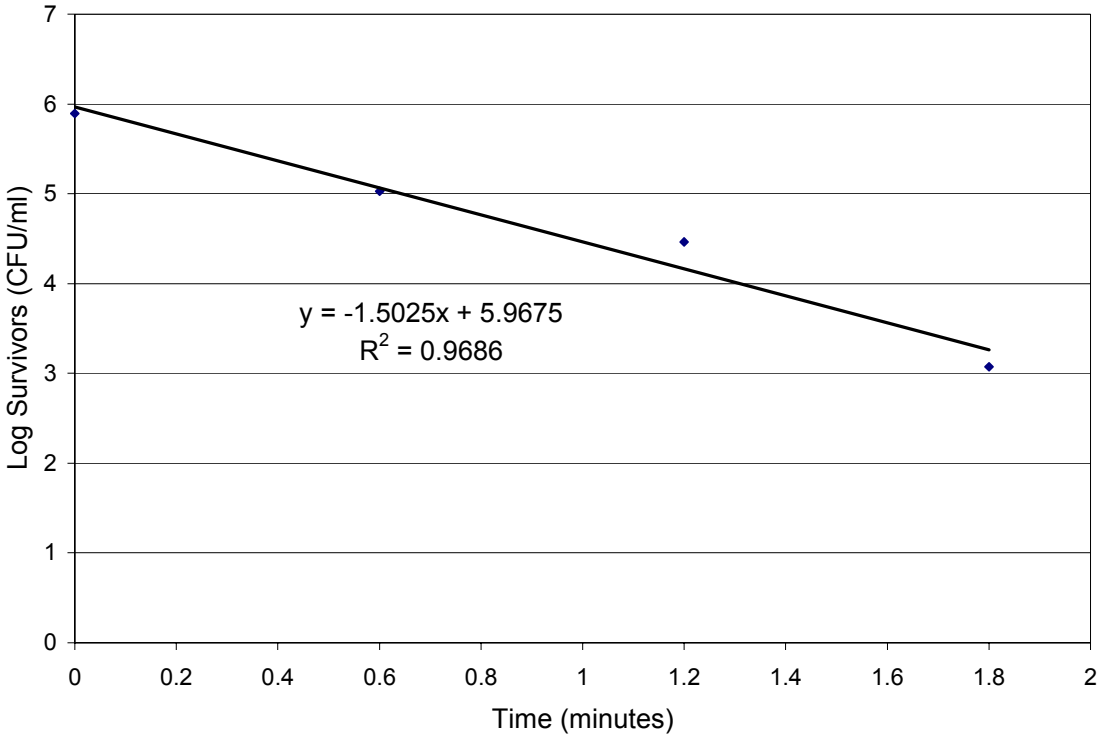


Figure **4.1**: Number of *Clostridium sporogenes* spores surviving heat treatments in SPB at 121°C. $D_{121°C}$ = 0.67 minutes.

Determination of Heat Resistance of *Clostridium sporogenes* in Mushroom Slurry

The resistance of *Clostridium sporogenes* spores to heat was determined in mushroom slurry. Heat resistance was determined in 2 mL capacity ampules in a 121°C oil bath. Each ampoule contained 0.5 mL of $3x10^6$ CFU/mL and 1 mL of mushroom slurry.

Though the come-up time for the mushroom slurry was 4.20 minutes, ampoules were removed beginning at 2.80 minutes to coincide with the come-up

time of the heat resistance test in SPB. As a result, a temperature lag resulted and a "shoulder" is exhibited in the data (see Figure 4.2). Because the "shoulder" data do not represent the reference temperature of 121°C, these points were removed from consideration. $D_{121°C}$ = 0.90 minutes was calculated as the negative inverse of the slope of the fitted line shown in Figure 4.2. The fitted line exhibited close fit (R^2 = 0.986) with the data.

In this study, the higher D value (0.90 minutes) obtained in mushroom slurry as compared to SPB (0.67 minutes) agreed with the previous findings of Okereke et al. (1988), who found that *Clostridium sporogenes* spores were more susceptible to thermal destruction in SPB than in mushroom slurry.

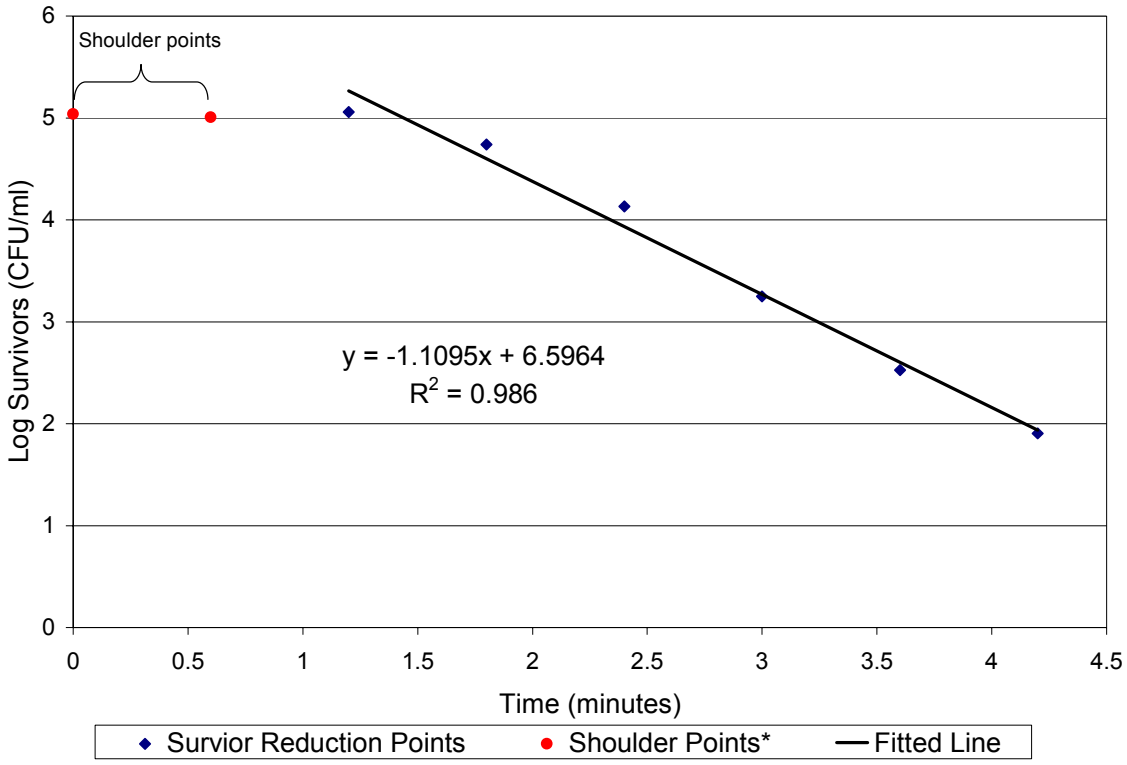


Figure 4.2: Number of *Clostridium sporogenes* spores surviving heat treatments in mushroom slurry at 121°C. $D_{121°C}$ = 0.90 minutes.

Determination of Heat Resistance of *Clostridium sporogenes* in Individual Whole Mushrooms Processed Aseptically

The resistance of *Clostridium sporogenes* spores to heat was determined in whole mushrooms processed aseptically. Eighteen 4.0 cm nominal cap diameter mushrooms (minimum: 3.92 cm, mean: 4.04 cm, maximum: 4.13 cm) were injected with 4.5×10^6 CFU per mushroom. Injected mushrooms were vacuum hydrated. Three vacuum hydrated mushrooms were processed at 130°C for each of the times listed in Table **4.2**. Processed mushrooms were pressed in a parallel faced vice and the liquid serially diluted and pour plated in Eugon Agar. Plates were incubated at 35°C for 48 hours. The results of this test are shown in Figure **4.3**. Since the heating rates of mushrooms varied, spores were eliminated in as few as 7.2 minutes of processing. However, when a line was fitted through the most conservative points (representing the single mushroom for each processing time that exhibited the least amount of kill off), it gave a $D_{121°C}$ = 0.87 minutes (R^2 = 0.986). Thus, given the variability of heating rates of mushrooms, the thermal death rate closely matched the value obtained in mushroom slurry under the more controlled conditions of an oil bath.

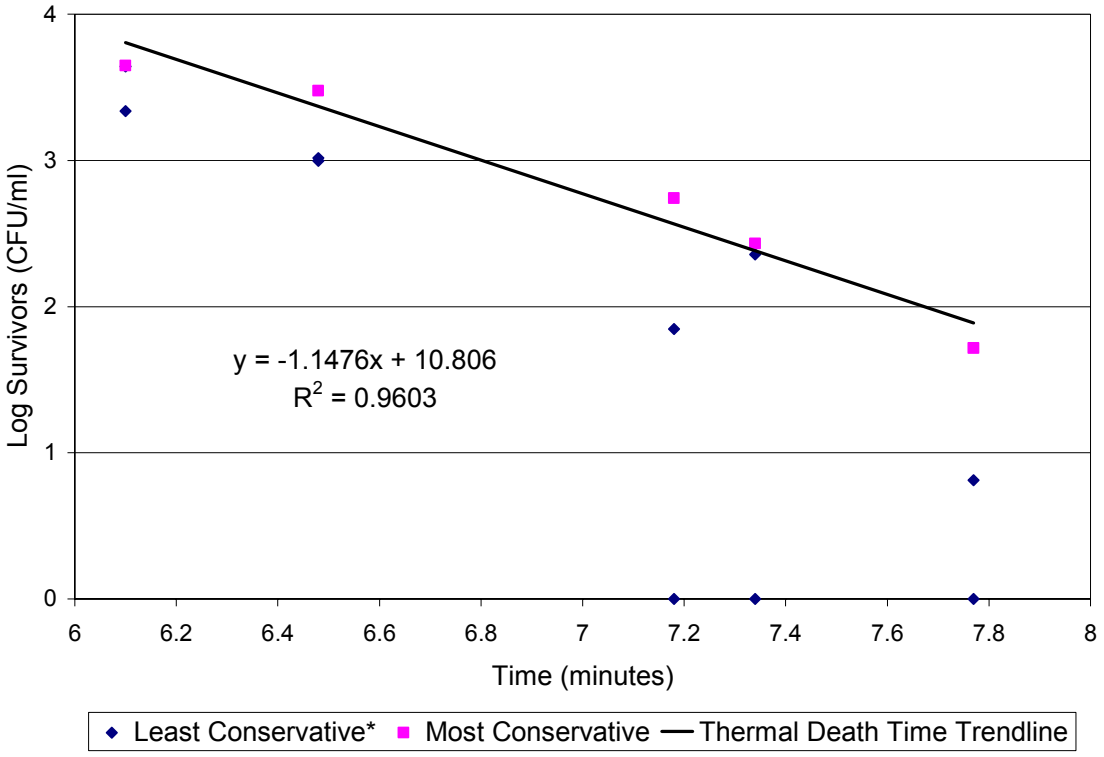


Figure **4.3**: Number of *Clostridium sporogenes* spores surviving heat treatments in mushrooms processed aseptically at 130°C. $D_{121°C}$ = 0.87 minutes.

Based on temperature penetration data collected in Chapter 2, approximately a one log reduction was expected after 6.1 minutes of processing. The spore counts of the 6.1 minute data points were $2.17x10^3$ to $4.45x10^3$ CFU/mL (Figure **4.3**); therefore, it can be concluded that the target level of inoculation of $4.5x10^4$ CFU/mL was achieved even though no viable spores were counted in the raw inoculated mushroom. Only 62 CFU/mL were counted in the vacuum hydration water, which indicates the number of spores that migrated out of the injected mushrooms and into the vacuum hydration water was relatively small; however, there was no heat applied to these samples either. Since no

heat was applied to the raw mushrooms or hydration water, it is thought that the spores were not activated and an accurate spore count was not achieved. One viable spore was counted in 500 mL of filter concentrated chill water analyzed from the sample taken following all the tests. Chill water was also not heat activated as any spores that made it into the chill water had to have survived heat treatment in the steam sterilization chamber. Since the shortest processing times had surviving spores, it was not surprising to find a spore in the chill water.

Effect of Aseptic Processing on the Spoilage of Mushroom Packs Inoculated with *Clostridium sporogenes* Spores

In Trial 1, the intent was to process mushrooms (2.5 to 4.0 cm cap diameter) using less conservative treatments. However, none of these treatments resulted in 0% spoilage (Discussed in detail later in this section.). Therefore, a second trial was conducted. In Trial 2, mushrooms were processed under the same conditions as before, but this time the process times were more conservative. The process time and target log reduction for both trials based on the slowest heating particle are presented in Table **4.2**.

Percent spoilage data for Trial 1 and Trial 2 are presented in Figure **4.4**. In Trial 1, some jars spoiled for all process times; however, in Trial 2, no heat treatment resulted in 100% spoilage. The processes were based on the largest mushrooms (4.0 cm cap diameter), and smaller mushrooms (2.5 cm < cap diameter < 4.0 cm) were also used in the study. Since the smaller mushrooms heated faster, variation in the effectiveness of the processes resulted. For

instance, at the shortest processing time (6.1 minutes), one jar did not spoil, which indicted that the mushrooms in that particular jar heated more rapidly because they had less than 4.0 cm cap diameters or other characteristics, such as a loose cap or open veil. However, overall, the actual thermal inactivation whole mushrooms inoculated with *Clostridium sporogenes* spores closely followed the most conservative, slowest heating particle temperature penetration data developed in Chapter 2.

While the intent was to achieve the NCA (1968) inoculated pack study recommendation to inoculate at 10^4 to 10^5 CFU/container, heat activated (90°C, 15 minutes) mushrooms pour plated on Eugon agar indicated that mushrooms were only inoculated with $1.38x10^2$ to $1.91x10^2$ *Clostridium sporogenes* spores per container using vacuum hydration. However, a 100 mL filter concentrated sample taken following vacuum hydration indicated that there were 20 CFU/mL of inoculation water. Since 92 mL of inoculation water was taken up during vacuum hydration by 100 g net of processed mushrooms, mushrooms should have contained approximately $1.9x10^3$ CFU/container. In Trial 2, 92% spoilage occurred at 7.4 minutes (target spore reduction = 0.5 log) and 8% spoilage at 8.3 minutes (target spore reduction = 2.0 log), indicating approximately a 0.5 log and 2 log reduction in viable spore count at these treatments, respectively (Figure **4.4** and Figure **4.5**). The fact that no spoilage occurred after a 9.3 minute process indicated that the initial spore population was greater than 10^2 CFU/container and less than 10^4 CFU/container. Though a discrepancy in the initial spore count per container was recognized, it could not be resolved from this data. However,

it appeared that the initial inoculation level was approximately 10^3 CFU/container. Thus, since no spoilage occurred in 13 jars at a target spore reduction of 4 log, at least a 4 log reduction in viable spore count was achieved (Figure **4.5**).

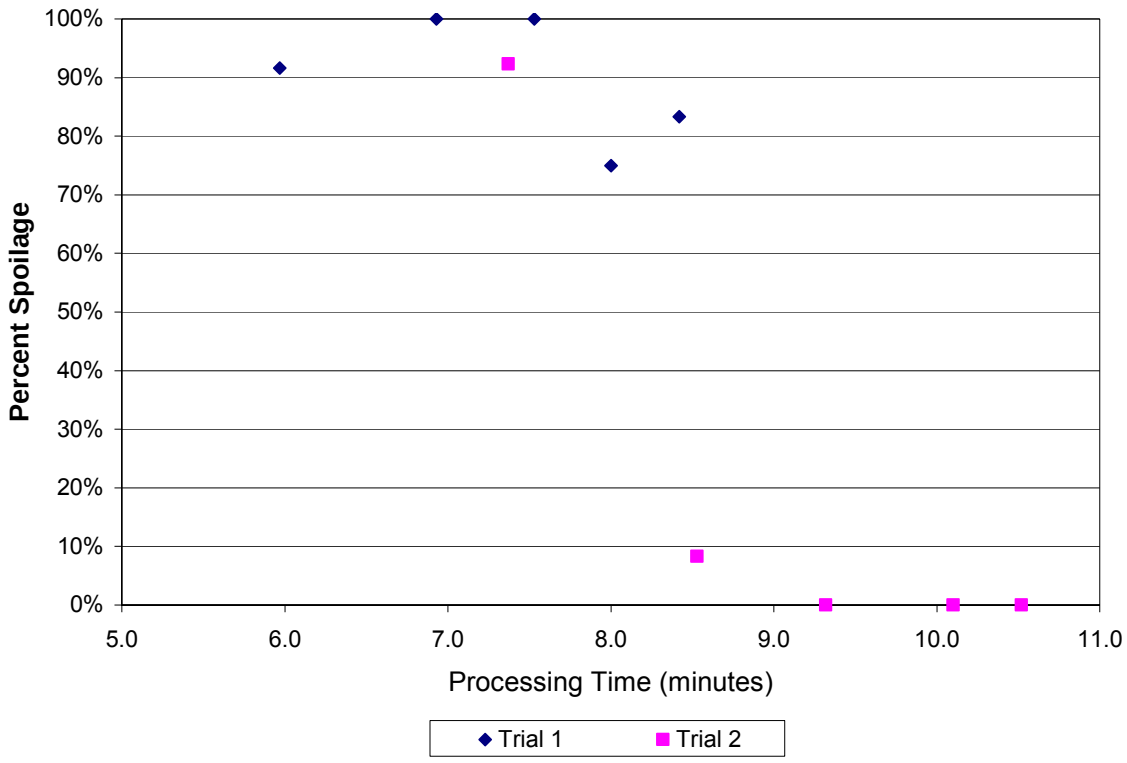
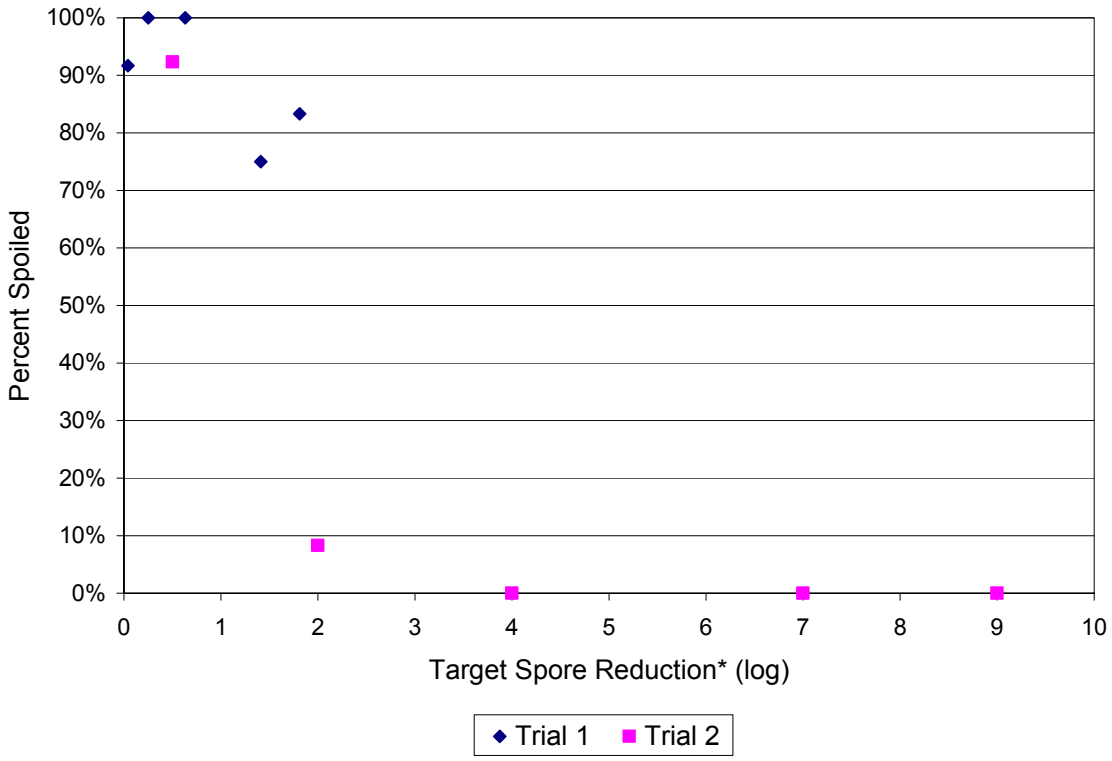


Figure **4.4**: Effect of processing time at 130°C on the spoilage of aseptically processed mushrooms inoculated with 10^3 *Clostridium sporogenes* spores per container.



* Target spore reduction based on the slowest heating mushroom in temperature penetration studies conducted in Chapter 2.

Figure **4.5**: Effect of heat treatments at 130°C on the spoilage of aseptically processed mushrooms inoculated with 10^3 *Clostridium sporogenes* spores per container.

The probability (P) of a single spore causing spoilage in aseptically processed mushrooms was calculated using Equation **4.1** and Equation **4.2**. Table **4.4** shows that at a target log 2 reduction, the probability of a non-sterile unit (PNSU) was 4.6×10^{-4}. In other words, the number of non-sterile units that would be expected per million cans would be 460—clearly unacceptable from a public safety standpoint. Even though the calculated probability was zero at target reductions of 4, 7, 9 log (i.e., no spoilage occurred), it cannot be concluded that processes delivered were adequate since the initial inoculation level was

only approximately 10^3 CFU/container; however, given the strong agreement between temperature penetration data and inoculated pack data illustrated in Figure **4.4** and Figure **4.5**, an adequate process can be accurately predicted and delivered based on temperature penetration data. In fact, an adequate process would likely be significantly shorter than the scheduled process (11.0 minutes) developed in Chapter 2. That process likely produced a 12.7 log reduction of *Clostridium sporogenes*; whereas, a minimum 5 log reduction is recommended by the NCA (1968). In other words, a 5D process could likely be obtained in 9.6 minutes rather than 11.0 minutes used in Chapter 3. As a result, one would expect even greater quality improvement than was attained during the quality studies conducted in Chapter 3.

Table **4.4**: Probability of a non-sterile unit (PNSU) of aseptically processed mushrooms inoculated with *Clostridium sporogenes* spores.

Trial	Actual Processing Time, F(131°C, z=10°C) (min)	Target Reduction (log)	P
1	6.0	0.1	1.8E-02
	6.9	0.3	1.0
	7.5	0.6	1.0
	8.0	1.4	1.0E-02
	8.4	1.8	1.3E-02
2	7.4	0.5	1.4E-02
	8.5	2.0	4.6E-04
	9.3	4.0	0.0
	10.1	7.0	0.0
	10.5	9.0	0.0

* Target reduction based on the slowest heating mushroom in temperature penetration studies conducted in Chapter 2.

Conclusion

Thermal death time studies revealed close agreement between the $D_{121°C}$ for *Clostridium sporogenes* spores obtained in mushroom slurry (0.90 minutes) and whole mushrooms processed aseptically (0.87 min). Inoculated pack studies proved the development of a scheduled process based on temperature penetration data of the slowest heating particle lead to predictable destruction of *Clostridium sporogenes* spores. At an inoculation level of approximately 10^3 CFU/container, viable spores were eliminated with 9.3 minutes process time at 130°C. Inoculated pack studies indicate that process time could be reduced by at least 1.0 minute from the more conservative scheduled process of 11.0 minutes proposed in Chapter 2 and utilized in quality studies in Chapter 3.

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Chapter 5

Changes in Mushroom Properties Following Vacuum Hydration and Aseptic Processing

Abstract

Dimensional, mass and volumetric changes in mushrooms that were vacuum hydrated and aseptically processed were studied. Following vacuum hydration, mass had increased 71.9% (SD=10.2%), volume 11.6% (SD=4.0%) and characteristic mushroom dimensions in the range of 2.9% (SD=1.5%) to 5.9% (SD=6%); however, on average, diameter increased 34.78% more than length. Following aseptic processing, mass had decreased 24.4% (SD=2.9%) and 55.9% (SD=3.1%), volume 51.8% (SD=1.9%) and 56.8% (SD=3.3%), and characteristic mushroom dimensions 4.7% (SD=5.8%) to 28.4% (SD=5.2%) and 9.0% (SD=3.8%) to 30.4% (SD=4.4%) for aseptically processed versus raw, and aseptically processed versus vacuum hydrated mushrooms, respectively. However, on average, length decreased 96.6% and 63.8% more than diameter. Vacuum hydration increased (dry basis) moisture content (dry basis) by 71.5% relative to raw mushrooms. Aseptic processing decreased moisture content (dry basis) by 27.4% and 57.7% relative to raw and vacuum hydrated mushrooms, respectively.

Mushrooms are anisotropic and non-homogenetic. Moisture content, density and volume of the mushroom body are ever changing as it shrinks. These factors make it difficult to accurately predict the temperature distribution of vacuum hydrated and unblanched mushrooms being heated by steam in an aseptic processor.

Introduction

Development and validation of a scheduled process is critical to ensuring public safety. The accuracy with which the heat distribution inside a mushroom is predicted influences greatly, the ability to design and validate an effective scheduled process. For this reason, it is desirable to predict the heat distribution in mushrooms during aseptic processing.

Mushrooms, like many foods, are irregularly shaped with anisotropic properties making it difficult to predict the heating characteristics. Sastry et al. (1985) determined experimentally the thermal conductivity (k), specific heat (C_p), convective heat transfer coefficient (h_c), density (ρ) and the convective mass transfer coefficient (h_m) (Table **5.1**), and then used these parameters in a three-dimensional finite element model to accurately predict the heat distribution of whole mushrooms canned in brine and processed in a still retort.

Table 5.1: Parameters used in a three-dimensional finite element model to accurately predict the heat distribution of whole mushrooms canned in brine and processed in a still retort (Sastry et al., 1985)

Parameter	Value
Thermal conductivity, k	0.4324 W m^{-1} °C^{-1}
Surface heat transfer coeeficient, h_c	500 W m^{-2} °C^{-1}
Specific heat, C_p	3.883 kJ kg^{-1} °C^{-1}
Density, ρ	980 kg m^{-3}
Mass transfer coefficient, h_m	1.006x10^{-5} m s^{-1}

However, Sastry et al. (1985) did not model the blanching step where heating is impacted by simultaneous shrinkage of the mushroom. Biekman et al. (1997) determined that mushroom shrinkage occurs in the temperature range of 45-85°C and leads to a 1.5-2 fold increase in the rate constant for the temperature increase at the mushroom center.

Objective

Determine the dimensional, volumetric and moisture changes in mushrooms following vacuum hydration and aseptic processing in a pressurized steam environment.

Procedure

The mushrooms, species *Agaricus bisporus,* studied in this project were whole mushrooms with approximately 4.0 cm cap diameter obtained from the Mushroom Test Demonstration Facility (MTDF), Penn State University (University Park, PA). Mushrooms were washed in cold tap water and stored for 18-24 hours at 4˚C. Three trials were conducted on separate occasions, each using approximately 100g raw weight of mushrooms to characterize raw moisture content, approximately 100 g raw weight to characterize vacuum hydration moisture content, approximately 100g raw weight to characterize processed moisture content and 12 mushrooms (approximately 4.0 cm cap diameter) to characterize dimensional and volumetric changes.

Mass, Volume, Density and Dimensional Changes

Volumetric and density changes were calculated using Equation **5.1** and Equation **5.2**, respectively. Mass of each individual mushroom was measured on a balance. Then apparent submerged weight was measured by suspending each mushroom, submerged in water, from the scale using a rigid, copper-wire frame and using a apparatus similar to that pictured in Figure **5.1**.

$$Volume = \frac{Mass - Submerged\ Weight}{Density\ of\ Water} \qquad \textbf{5.1}$$

$$Density = \frac{Mass}{Volume} \qquad 5.2$$

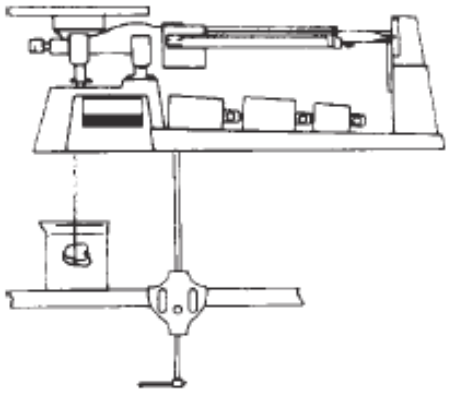

Figure 5.1: Apparatus for measuring the submerged mass of mushrooms in water. Though a beam balance is pictured here, a digital scale was used in actual measurements (Ohaus, 1998).

Dimensional measurements, made with Vernier calipers, were taken as illustrated in Figure 5.2.

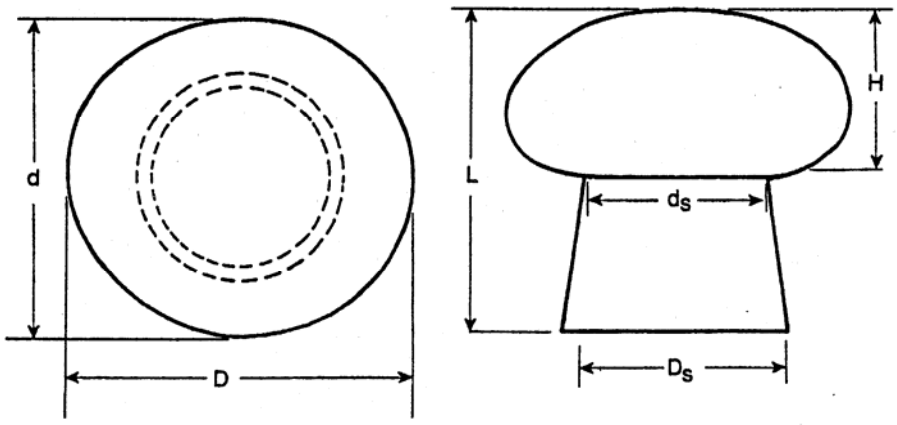

Figure 5.2: Dimensional measurements taken of mushrooms (adapted from Sastry et al., 1985).

Moisture content was measured by recording the initial raw mass, vacuum hydrated mass, post-process mass, and the mass after drying in an oven at 100°C for 24 hours. Three randomly sampled groups of mushrooms (approximately 100g raw weight each) were required due to the destructive nature of these tests.

Individually twelve mushrooms were selected at random from each lot, uniquely marked for identification, weighed and dimensional measurements taken. Since raw mushrooms float, they produced a negative (upward) force during measurements of submerged mass.

Samples were then vacuum hydrated following the procedure outlined by McArdle et al. (1974). Raw mushrooms were submerged in cold tap water inside a vacuum kettle. Mushrooms were held under a vacuum of 94 kPa (28 in Hg) for five minutes. The vacuum was released and mushrooms were held submerged an additional minimum 5 minutes under ambient conditions. Vacuum hydrated samples were removed individually from the vacuum chamber and drained in a sieve for 3 minutes and measurements repeated. Vacuum hydrated samples sink, so submerged mass was recorded as a positive (downward) force.

After measurements on vacuum hydrated mushrooms were made, the same mushrooms were processed aseptically in the steam sterilization segmented flow aseptic processing unit at 130°C for 11.05 minutes—the scheduled process developed in Chapter 2. Processed mushrooms were packed in jars, topped with cool water and sealed with a metal, screw-on lid. Jars were promptly removed from the sterile glove box. Processed mushrooms were again

drained and measurements of mass, submerged mass, and dimensions taken as outlined previously.

Volume and density were then calculated from the measured data.

Moisture Content

To characterize the moisture content mushrooms, raw mushrooms were selected at random for each trial and grouped (approximately 100 g per group). Each group of mushrooms was uniquely marked as raw (**R**), vacuum hydrated (**V**) or aseptically processed (**P**). The initial mass was taken by weighing each group in a single foil pan. The **R** group was then placed in a drying oven at 100°C for 24 hours.

Groups **V** and **P** were then vacuum hydrated per the procedure described earlier. Both groups were removed from the vacuum chamber and drained in a sieve for 3 minutes. Mushrooms were placed in a foil pan, weighed and vacuum hydrated mass recorded. Group **V** mushrooms were placed in the drying oven at 100°C for 24 hours.

Group **P** mushrooms were then aseptically processed per the procedure described earlier. Processed mushrooms were removed from the jar and drained in a sieve for 3 minutes. Mushrooms were weighed in a foil pan and placed in the drying oven at 100°C for 24 hours.

After 24 hours of drying, Groups **R**, **V** and **P** mushrooms were weighed. Samples were reweighed one hour later to ensure mass was unchanging.

Following these last measurements, dry basis moisture content was calculated.

Results and Discussion

Mass, Volume, Density and Dimensional Changes

Data for three trials used to characterize dimensional, mass and volumetric changes in mushrooms at the three stages of processing (raw, vacuum hydration, aseptic processing) are summarized in Table **5.2**. To illustrate the significance of these changes, percent change for vacuum hydrated versus raw, aseptically processed versus raw, and aseptically processed versus vacuum hydrated is illustrated in Figure **5.3**. When raw mushrooms were vacuum hydrated, cap height (H) and overall length (L) increased in the range of 2.9% (SD=1.5%) to 3.5% (SD=3.6%), while the most significant dimensional changes were related to diameter cap (D and d) and stem (Ds and ds), which increased in the range of 4.5% (SD=0.6%) to 5.9% (SD=5.6%); therefore, on average, diameter increased 34.8% more than length. Significant changes in mass and submerged apparent mass also occurred during vacuum hydration. Mass increased 71.9% (SD=10.2%), apparent mass decreased 105.4% (SD=1.5%), volume increased 11.6% (SD=4.0%), and density increased 54.0%

(SD=5.1%). This data provides evidence that during vacuum hydration, air was removed from the intercellular spaces of mushrooms when the vacuum was pulled. When the vacuum was released, a greater volume of water was pulled into the empty intercellular spaces causing the mushroom to swell.

When raw mushrooms were aseptically processed, cap diameter (D and d) and stem diameter (Ds and ds) decreased in the range of 4.7% (SD=5.8%) to 19.7% (SD=1.9%) and 9.0% (SD=3.8%) to 23.9% (SD=2.2%), while the most significant dimensional changes cap height (H) and overall length (L) decreased in the range of 25.0% (SD=2.9%) to 28.4% (SD=5.2%) and 27.5% (SD=3.5%) to 30.4% (SD=4.4%) for aseptically processed versus raw, and aseptically processed versus vacuum hydrated mushrooms, respectively; therefore, on average, length decreased in the range of 63.8% to 96.6% more than diameter. Mass decreased 24.4% (SD=2.9%) and 55.9% (SD=3.1%), apparent mass decreased 105.2% (SD=1.0%) and 4.3% (SD=17.9%), volume decreased 51.8% (SD=1.9%) and 56.8% (SD=3.3%), and density increased 57.0% (SD=5.5%) and 2.0% (SD=0.7%) for aseptically processed versus raw, and aseptically processed versus vacuum hydrated mushrooms, respectively. This data provides evidence that even though mushrooms increase in volume when vacuum hydrated, during aseptic processing liquid was expelled not only from intercellular spaces, but also from the internal compartments of cells as cellular destruction took place during exposure to steam heat resulting in net volumetric shrink.

Moisture Content

Data for three trials used to characterize changes in moisture content (dry and wet basis) of mushrooms at the three stages of processing (raw, vacuum hydration, aseptic processing) are summarized in Table **5.3**. On average, the dry basis moisture contents were 2038% (SD=238%), 3495% (SD=561%), and 1480% (SD=155%) for raw, vacuum hydrated, and aseptically processed mushrooms, respectively. Vacuum hydration increased moisture content by 71% (dry basis) relative to raw mushrooms. Aseptic processing decreased moisture content by 27% (dry basis) and 57% (dry basis) relative to raw and vacuum hydrated mushrooms, respectively.

Table 5.2: Mean dimensions, mass, apparent mass, volume and density of mushrooms taken at each stage of processing: raw, vacuum hydration and aseptic processing. Standard deviation is presented in parenthesis.

Processing Stage	D (mm)	d (mm)	L (mm)	H (mm)	Ds (mm)	ds (mm)	Mass (g)	Apparent Mass (g)	Volume (cm^3)	Density ($g\ cm^{-3}$)
Raw	41.1(0.4)	39.8(0.3)	39.1(2.6)	22.5(0.5)	17.2(3.5)	17.4(1.8)	18.1(1.5)	-9.3(0.3)	27.3(1.4)	0.7(0.0)
Vacuum Hydrated	42.9(0.2)	42.1(0.1)	40.5(3.7)	23.2(0.8)	18.1(3.3)	18.2(1.7)	30.9(0.8)	0.5(0.1)	30.5(0.7)	1.0(0.0)
Aseptically Processed	33.5(1.0)	32.0(1.0)	29.3(2.9)	16.1(1.2)	15.3(2.3)	16.5(1.1)	13.6(1.1)	0.5(0.1)	13.2(1.1)	1.0(0.0)

Table 5.3: Moisture content of mushrooms taken following each stage of processing: raw, vacuum hydration and aseptic processing.

	Moisture Content (dry basis)					Moisture Content (wet basis)				
Process Stage	Trial 1	Trial 2	Trial 3	Mean	SD	Trial 1	Trial 2	Trial 3	Mean	SD
Raw	1745%	2310%	2060%	2038%	283%	95%	96%	95%	95%	1%
Vacuum Hydrated	2859%	3920%	3706%	3495%	561%	97%	98%	97%	97%	0%
Aseptically Processed	1302%	1556%	1583%	1480%	155%	93%	94%	94%	94%	1%

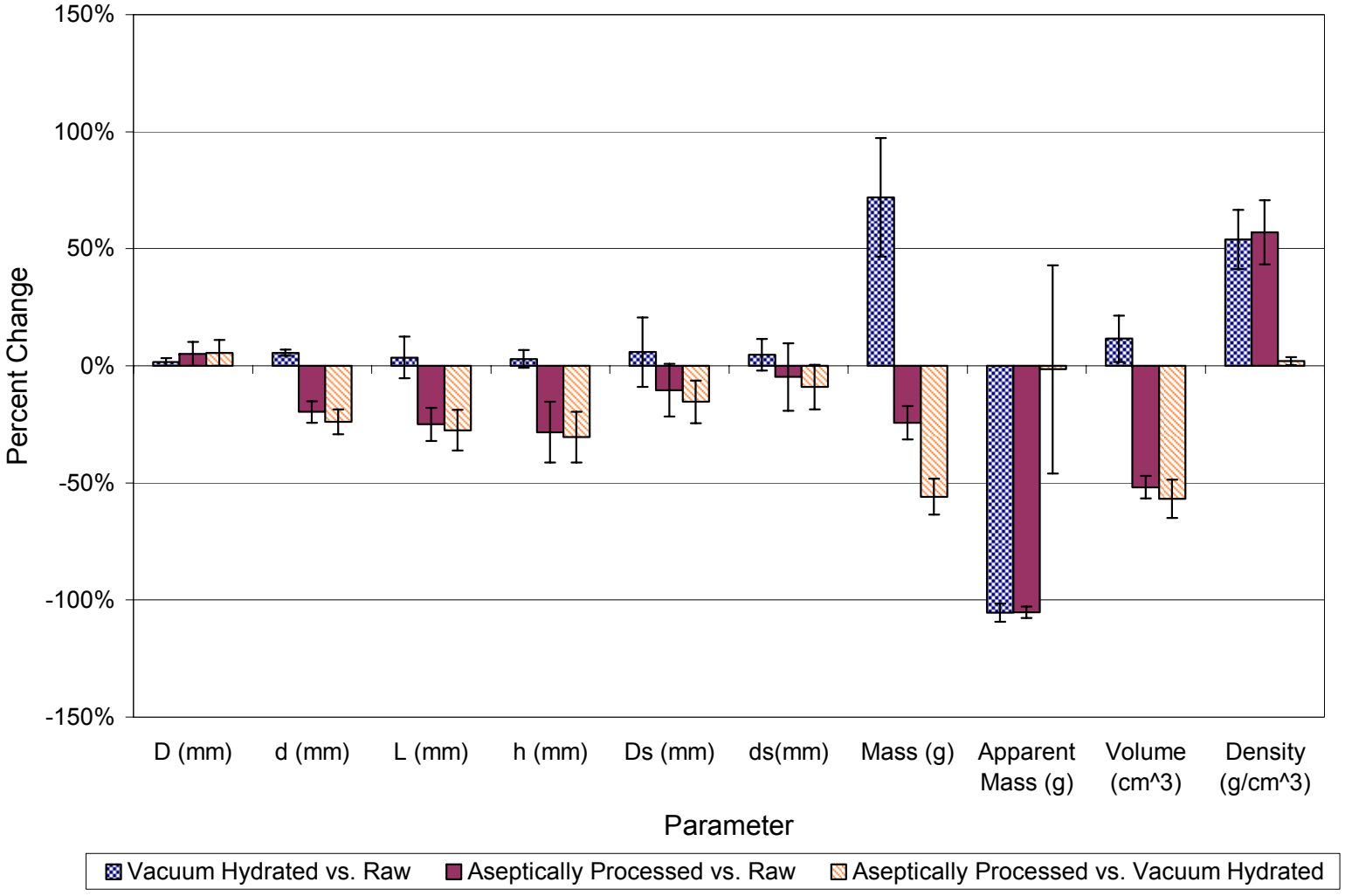

Figure **5.3**: Percent change of parameters with 95% confidence intervals.

Conclusion

Dimensional, mass and volumetric changes in mushrooms that were vacuum hydrated and aseptically processed were studied. Following vacuum hydration, mass had increased 71.9% (SD=10.2%), volume 11.6% (SD=4.0%) and characteristic mushroom dimensions in the range of 2.9% (SD=1.5%) to 5.9% (SD=6%); however, on average, diameter increased 34.78% more than length. Following aseptic processing, mass had decreased 24.4% (SD=2.9%) and 55.9% (SD=3.1%), volume 51.8% (SD=1.9%) and 56.8% (SD=3.3%), and characteristic mushroom dimensions 4.7% (SD=5.8%) to 28.4% (SD=5.2%) and 9.0% (SD=3.8%) to 30.4% (SD=4.4%) for aseptically processed versus raw, and aseptically processed versus vacuum hydrated mushrooms, respectively. However, on average, length decreased 96.6% and 63.8% more than diameter. Vacuum hydration increased (dry basis) moisture content (dry basis) by 71.5% relative to raw mushrooms. Aseptic processing decreased moisture content (dry basis) by 27.4% and 57.7% relative to raw and vacuum hydrated mushrooms, respectively.

Sastry et al. (1985) applied constants (Table **5.1**) when modeling the heat distribution of mushrooms canned in brine and processed in a still retort. The results obtained in this study indicate that in addition to being anisotropic, moisture content, density and volume of the mushroom body are ever changing, particularly during the shrinking stage. These ever-changing conditions would

make it very difficult to accurately predict the heat distribution of vacuum hydrated, unblanched mushrooms being heated by steam in an aseptic processor. Substantial experimentation would be required to characterize these parameters during dynamic change.

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Chapter 6

Future Research

The intent of this research was to explore the possibility of producing commercially sterile aseptically processed particle foods. To do this, a new processing system was designed and a pilot-scale processor constructed, heat distribution and heat penetration tests were conducted, a scheduled process was developed, quality of mushrooms processed aseptically was compared to canned mushrooms, a microbiological pack study was completed and properties of mushrooms evaluated. However, this study is just a stepping stone and a great deal of additional future research is required to produce a finished product that is safe for human consumption.

Due to limits of the resources available the pilot-scale processor, though was constructed predominantly of sanitary stainless steel components, contained some components (such as ball valves and threaded pipe used in the cooling loop) not generally considered suitable in a commercial aseptic processing system. There are other obvious components such as the conveyor which is fastened together using screws that could potentially come loose must also be redesigned to eliminate potential safety hazards. Replacement of such components with ones that can maintain an aseptic processing environment and eliminate potential hazards to the consumer would be necessary in any scale-up

of the system. In addition, as noted earlier, pumps could replace the double-valve inlet and outlet control system to increase throughput.

The temperature distribution study conducted in this research was enough to verify the uniformity of steam temperature in the sterilization chamber, but a typical heat distribution study of a commercial aseptic processing system would include testing all components (heat, holding, cooling, packaging) from start-up through sterilization and production operation of the system. Though temperature was monitored at key points (inlet water temperature, chamber temperature, cooling column inlet and outlet and glove box), a thorough monitoring and control system was not installed. Critical areas to consider monitoring would be the valves, filters, heat exchanger and overflow line that may be troublesome to sterilize or maintain sterility. Though not generally accepted in the food industry for temperature measurement, thermistor temperature sensors provided accurate temperature measurement (±0.05°C) with rapid response (0.6 seconds) over a wide temperature range (25°C to 126°C). Further research of applicability, reliability, substitution of sensors should be conducted on thermistor temperature sensors.

As mentioned earlier, temperature penetration studies were conducted over a limited range of rather favorable conditions (limited throughput, limited operational time). A commercial system would be expected to operate for many hours, even days processing many tonnes of material before being shutdown for full cleaning and re-sterilization. During this extended processing time, small pieces and fines will accumulate in the system. Accumulated fines may fill voids

between mushrooms and limit steam exposure to these areas. As a result, temperature distribution may be influenced during heating. This influence was not studied in this research and must be considered during development of a commercial process.

Arrangement, as a function of bed depth, was evaluated under limited conditions and found not be a significant (at the 95% confidence level) influence on temperature penetration in this research; however, arrangement might be a significant area of concern in a commercial system. When processing sliced mushrooms in particular, arrangement is critical, as mushrooms may stack in layers. Stacking of thin slices will increase the effective thickness of mushroom slices, leading to slower temperature increase. If significant effects on temperature penetration are caused by stacking in a commercial system, processing time for sliced mushrooms may increase significantly and perhaps even exceed the processing time required for whole mushrooms. This influence must be considered during development of a commercial process.

In this research, aseptic processing resulted in improved yield, color and texture as compared to mushrooms from the same lot canned conventionally on the same day in a laboratory retort. Ideally mushrooms from the same lot would have been processed aseptically, canned conventionally in the lab and canned commercially at a processor, all on the same day. This would have provided the best foundation for comparison of quality.

Yield and color measurements are well refined and relatively easy to characterize. This is not the case when evaluating texture. Ultimately, texture is

a matter of consumer preference. It is very difficult to relate mechanical shear to consumer preference; therefore, consumer preference testing would be a more meaningful evaluation of texture analysis, and should be considered in future research.

Due to time limitations, the processing conditions used in the quality studies were estimated prior to conducting microbiological challenge studies. The processing conditions that ensure public safety should be developed and validated before any final evaluation of quality. Additionally, quality was evaluated only under one set of processing conditions. It is likely that these conditions were not optimum. Therefore, quality evaluation should be completed at several valid time-temperature combinations so that the optimum conditions for product quality can be identified using kinetics.

The size of the spore crop, time and resources available to conduct microbiological control studies in this research was limited. Therefore, in some instances, replicate testing was not performed, which weakens the validity of these tests. However, the goal of this research was to provide enough information to illustrate that the concepts were valid, not to develop a saleable consumer product. To develop a commercial process for a consumer product that would ensure public safety would require a great deal more time, effort and resources than were available to this project. Replicate testing D-value tests should be conducted, higher inoculation levels during inoculated pack studies would improve the adequacy of the test, and more containers (i.e., replicates) should be used during inoculated pack studies.

Aseptic processing of mushrooms caused significant dimensional, density and moisture changes during heating. While this research illustrates that point, it does not provide a means for predicting those changes. Measurement of these characteristics at several time-temperature combinations would provide kinetic data that may enable prediction of these changes.

Appendix A

Statistical Analysis of Heat Penetration Data

Statistical Analysis of Heat Penetration Data of Whole Mushrooms

Analysis of Variance (ANOVA) was used to determine the effects of bed position and mushroom block on processing time during heat penetration studies of whole mushrooms (Table **A.1**).

Table **A.1**: ANOVA: response variable process time for heat penetration tests of whole mushrooms.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Mushroom Block	4	1.2887	0.3222	0.660	0.639
Bed Location	2	0.2748	0.1374	0.280	0.763
Error	8	3.9236	0.4905		
Total	14	5.4872			

Before testing the hypothesis it was necessary to check the model for outliers, equal variance, interaction and normality. Plots of the standardized residuals versus mushroom block (Figure **A.1**) and bed location (Figure **A.2**) revealed one moderate outlier for block 1, location 1 (Top). However, a standardized residual of 2.09 is not unusual for 15 observations; therefore, the observation was not removed from the data set. The residual plots also suggested equal variance between blocks.

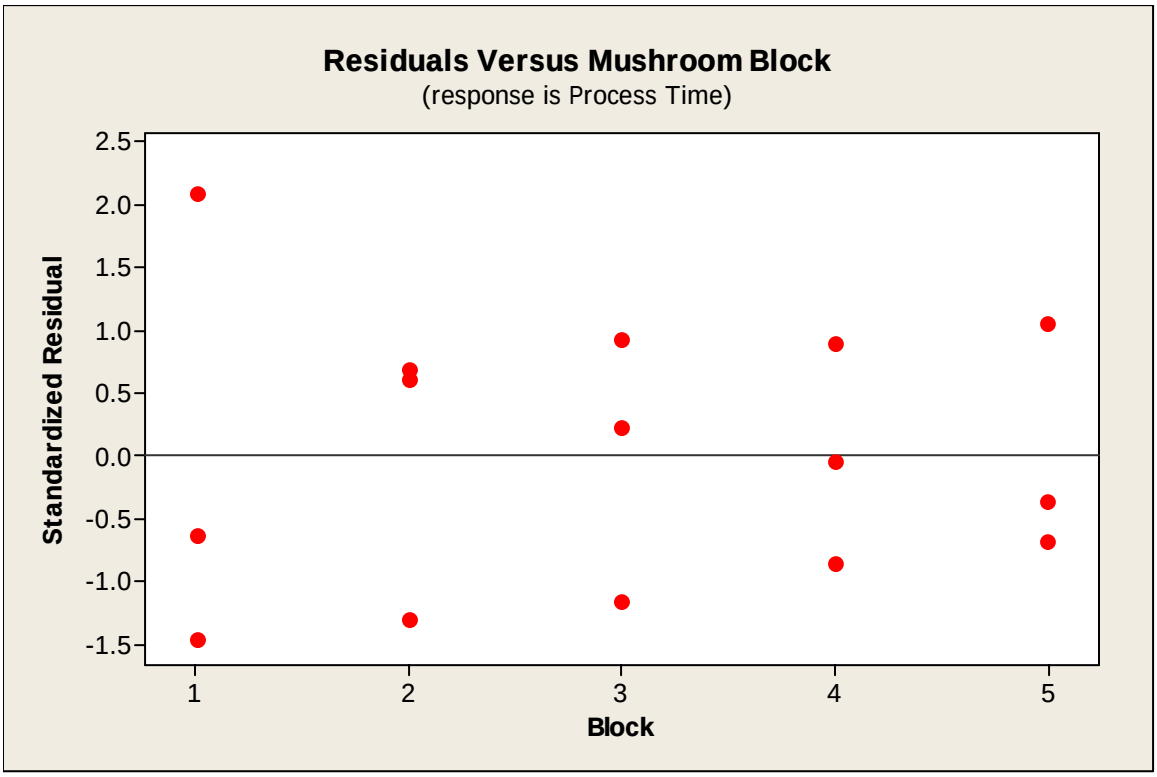

Figure **A.1**: Standardized residuals versus mushroom block for response variable process time of whole mushrooms.

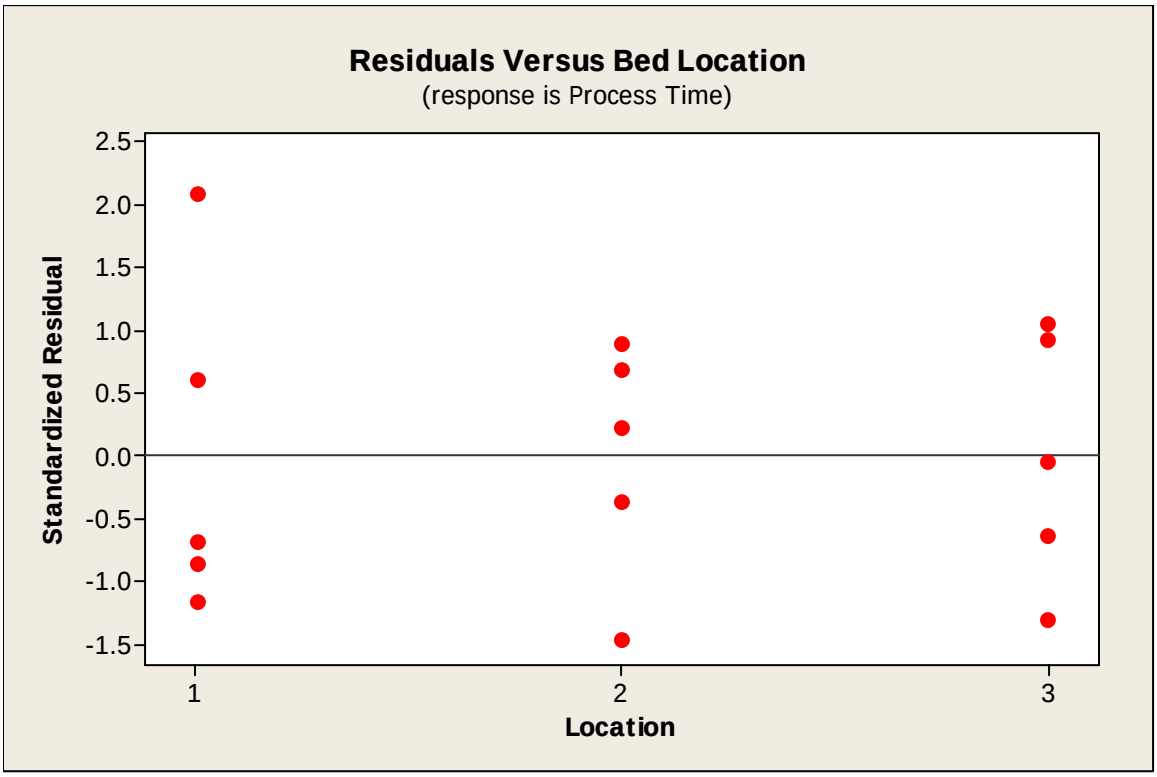

Figure **A.2**: Standardized residuals versus bed location for response variable process time of whole mushrooms.

The plot of standardized residuals versus fits (Figure **A.3**) shows no apparent patterns to suggest either interaction between block and location or a relationship between mean and variance.

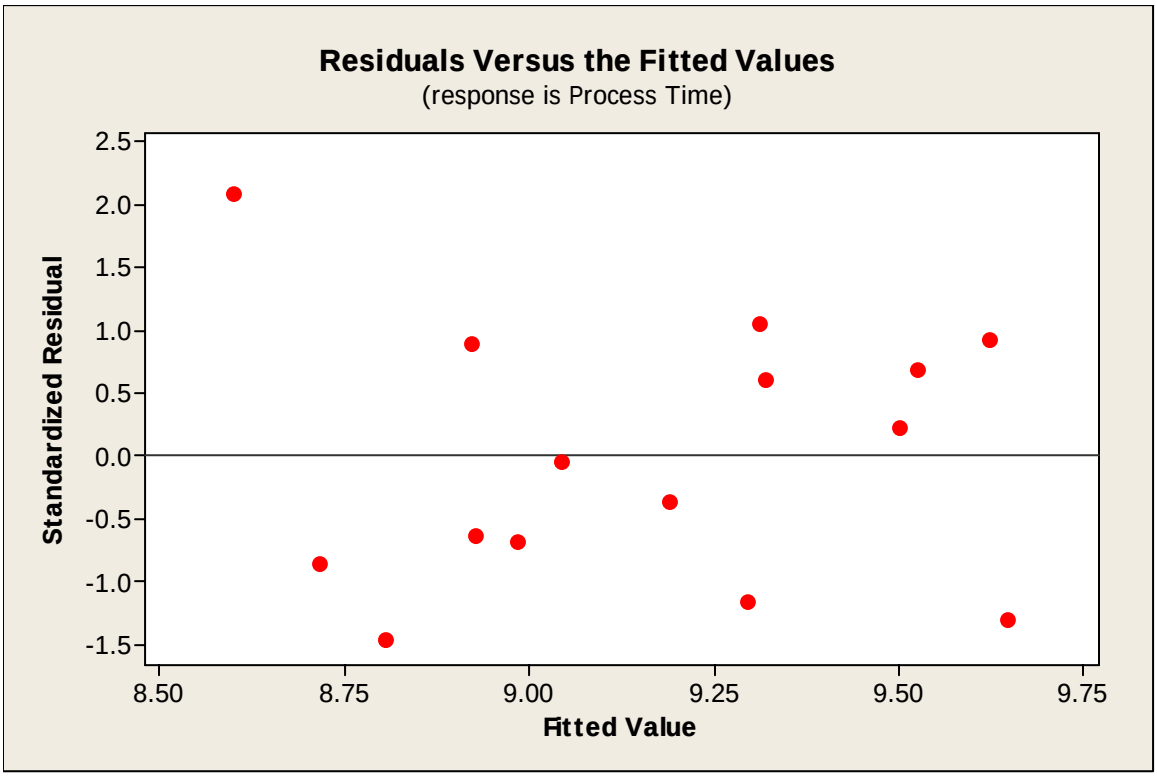

Figure **A.3**: Residuals versus the fitted values for response variable process time of whole mushrooms.

The normal probability plot (Figure **A.4**) seemed consistent with the normality of errors.

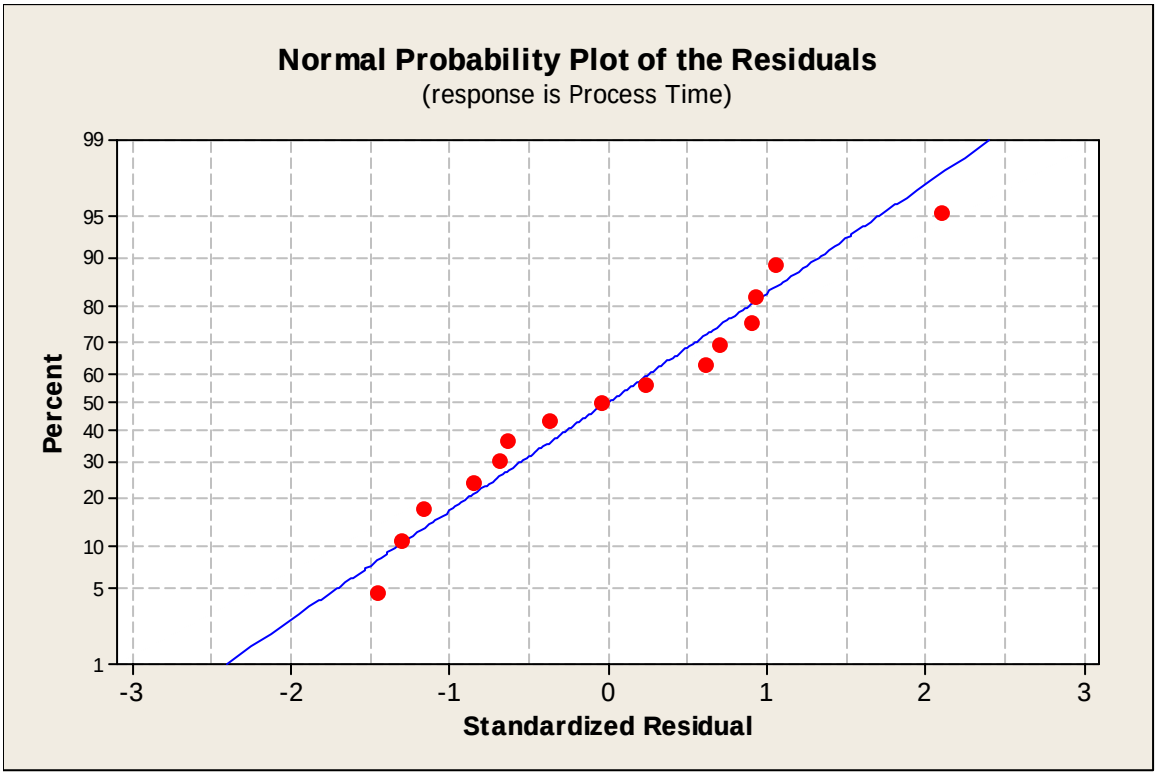

Figure **A.4**: Normality probability plot of the residuals for response variable process time of whole mushrooms.

Thus, it can be inferred that since the p-value = 0.763 > 0.05, the null hypothesis cannot be rejected, indicating all bed locations gave the same mean process time. This was further validated by employing a Tukey Least Significant Difference (LSD) test using 95% simultaneous confidence intervals (Figure **A.5**). Since zero was contained in each of the confidence intervals, the null hypothesis was not rejected.

```
Bed Location = 1  subtracted from:

Location   Lower  Center  Upper  ----+---------+---------+---------+--
2         -1.059  0.2060  1.471   (---------------*--------------)
3         -0.937  0.3280  1.593    (---------------*---------------)
                                 ----+---------+---------+---------+--
                                  -0.80      0.00      0.80      1.60
Bed Location = 2  subtracted from:

Location   Lower  Center  Upper  ----+---------+---------+---------+--
3         -1.143  0.1220  1.387  (---------------*--------------)
                                 ----+---------+---------+---------+--
                                  -0.80      0.00      0.80      1.60
```

Figure **A.5**: Tukey 95% simultaneous confidence intervals for all pairwise comparisons among levels of bed location for response variable process time of whole mushrooms.

Analysis of Variance (ANOVA) was used to determine the effects of bed position and mushroom block on cap diameter for heat penetration studies of whole mushrooms (Table **A.2**).

Table **A.2**: ANOVA: Response variable cap diameter for heat penetration tests of whole mushrooms.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Mushroom Block	4	9.00E-06	2.30E-06	1.39	0.320
Bed Location	2	4.00E-07	2.00E-07	0.13	0.883
Error	8	1.30E-05	1.60E-06		
Total	14	2.24E-05			

Before testing the hypothesis it was necessary to check the model for outliers, equal variance, interaction and normality. Plots of the standardized residuals versus mushroom block (Figure **A.6**) and bed location (Figure **A.7**) revealed one moderate outlier for block 3, location 3 (Top). However, a standardized residual of 2.55 is not unusual for 15 observations; therefore, the

observation was not removed from the data set. The residual plots also suggested equal variance between blocks.

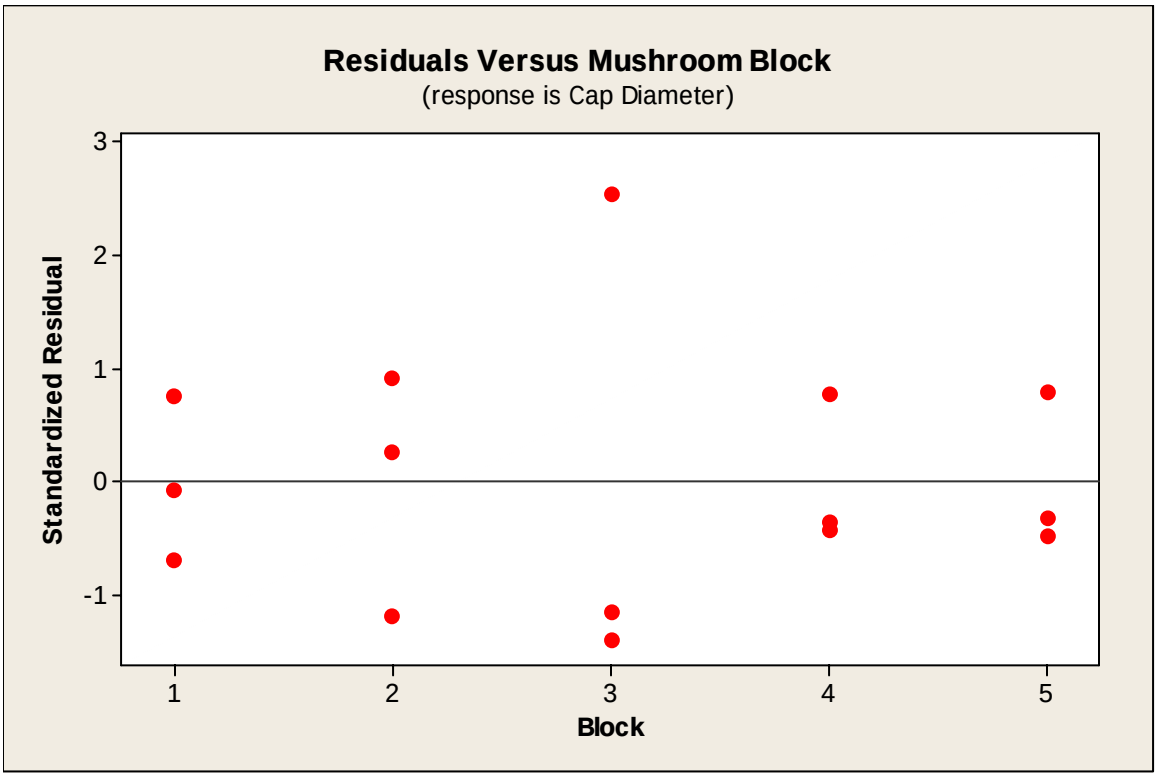

Figure **A.6**: Standardized residuals versus mushroom block for response variable cap diameter of whole mushrooms.

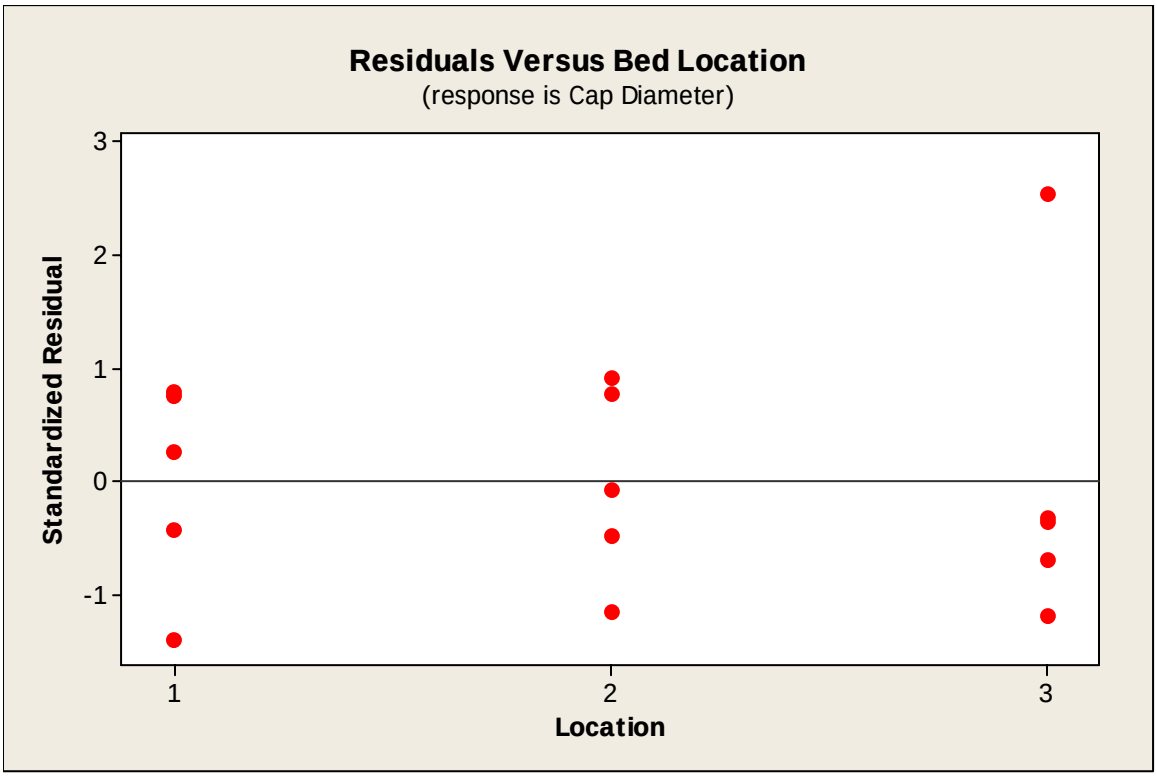

Figure **A.7**: Standardized residuals versus bed location for response variable cap diameter of whole mushrooms.

The plot of standardized residuals versus fits plot (**Figure A.8**) shows no apparent patterns to suggest either interaction between block and location or a relationship between mean and variance.

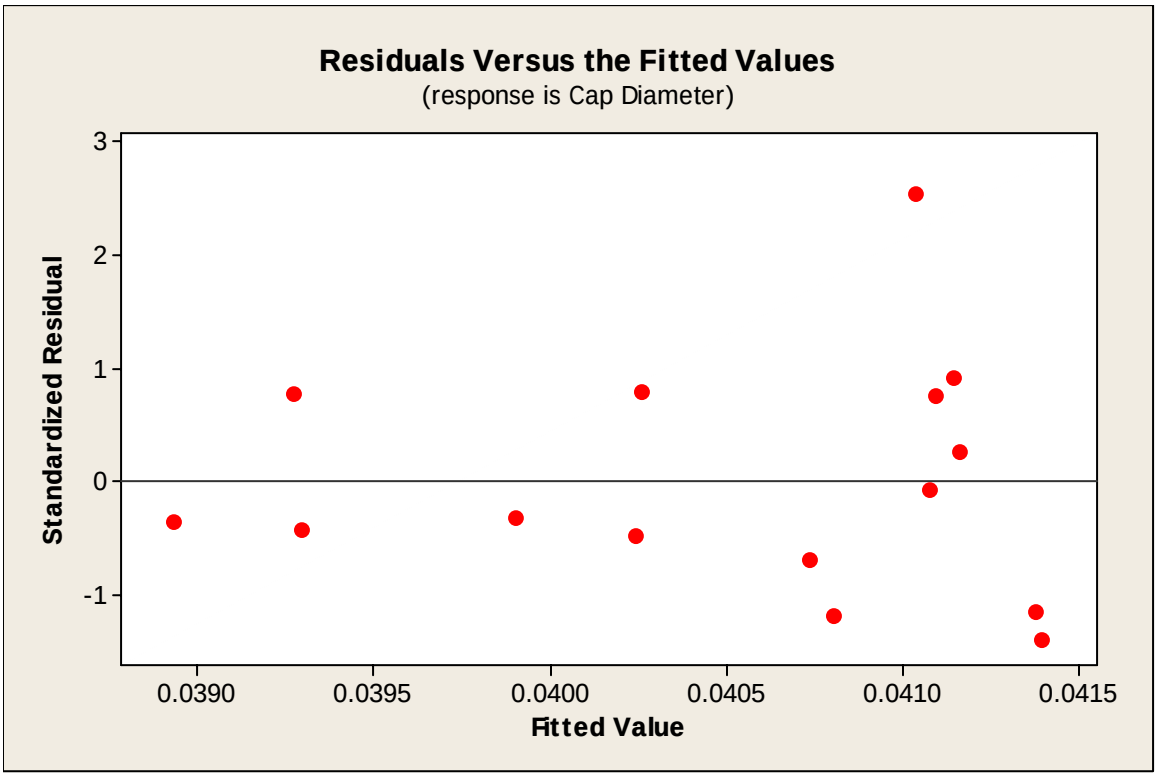

Figure **A.8**: Residuals versus the fitted values for response variable cap diameter of whole mushrooms.

The normal probability plot (Figure **A.9**) seemed consistent with the normality of errors.

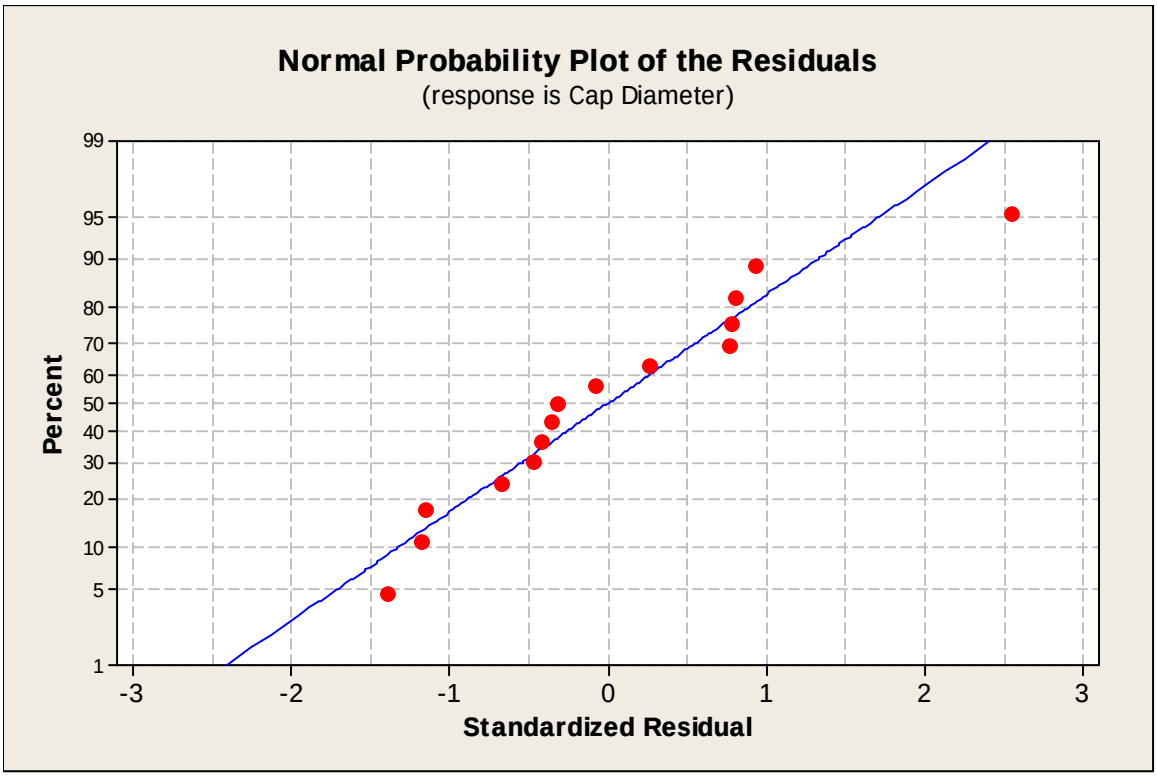

Figure **A.9**: Normality probability plot of the residuals for response variable cap diameter of whole mushrooms.

Thus, it can be inferred that since the p-value = 0.883 > 0.05, the null hypothesis cannot be rejected, indicating all bed locations gave the same mean cap diameter. This was further validated by employing a Tukey LSD test using 95% simultaneous confidence intervals (Figure **A.10**). Since zero was contained in each of the confidence intervals, the null hypothesis was not rejected.

```
Location = 1  subtracted from:

Location      Lower     Center     Upper  --------+---------+---------+--------
2         -0.002320  -0.000020  0.002280     (--------------*--------------)
3         -0.002660  -0.000360  0.001940  (---------------*--------------)
                                           --------+---------+---------+--------
                                                -0.0015    0.0000    0.0015
Location = 2  subtracted from:

Location      Lower     Center     Upper  --------+---------+---------+--------
3         -0.002640  -0.000340  0.001960  (---------------*--------------)
                                           --------+---------+---------+--------
                                                -0.0015    0.0000    0.0015
```

Figure **A.10**: Tukey 95% simultaneous confidence intervals for all pairwise comparisons among levels of bed location for response variable cap diameter of whole mushrooms.

Statistical Analysis of Heat Penetration Data of Sliced Mushrooms

Analysis of Variance (ANOVA) was used to determine the effects of bed position on processing time during heat penetration studies of sliced mushrooms (Table **A.3**). Since one data point was eliminated in testing because the temperature sensor did not stay in place, there were not enough degrees of freedom in the model to test the effect of blocking the data. Therefore, a single-factor General Linear Model was used in evaluating sliced mushroom heat penetration data.

Table **A.3**: ANOVA: Response variable process time for heat penetration tests of sliced mushrooms.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Bed Location	2	0.00579	0.00289	0.04	0.965
Error	11	0.88350	0.08032		
Total	13	0.88929			

Before testing the hypothesis it was necessary to check the model for outliers, equal variance, interaction and normality. Plots of the standardized residuals versus bed location (Figure **A.11**) suggested equal variance existed between bed locations for the response variable, process time.

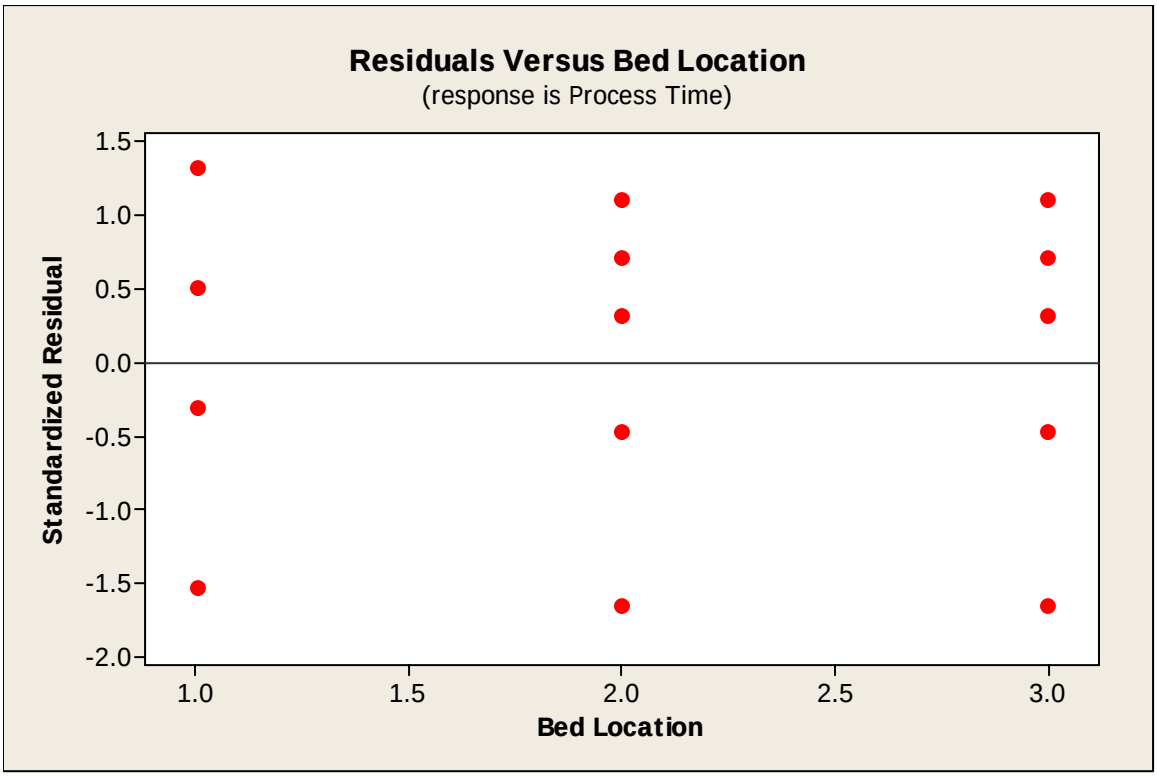

Figure **A.11**: Standardized residuals versus bed location for response variable process time of sliced mushrooms.

The plot of standardized residuals versus fits (Figure **A.12**) shows no apparent patterns to suggest interaction between location or a relationship between mean and variance.

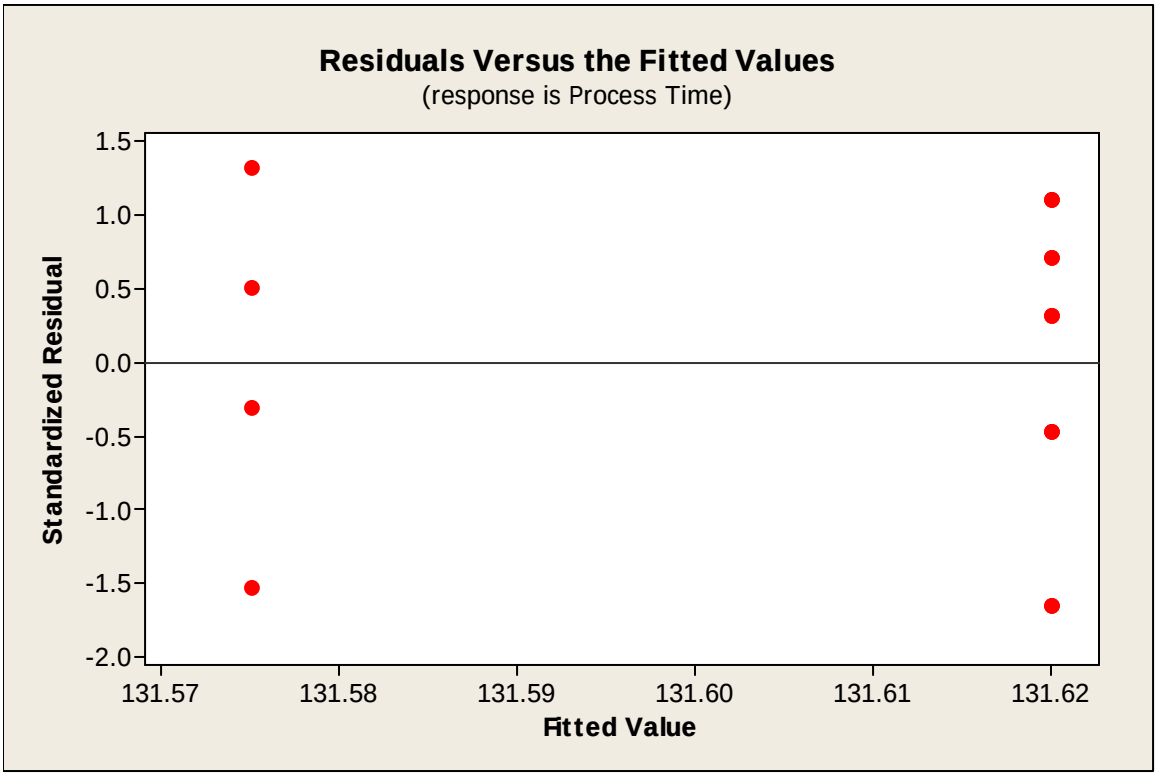

Figure **A.12**: Residuals versus the fitted values for response variable process time of sliced mushrooms.

The normal probability plot (Figure **A.13**) seemed consistent with the normality of errors.

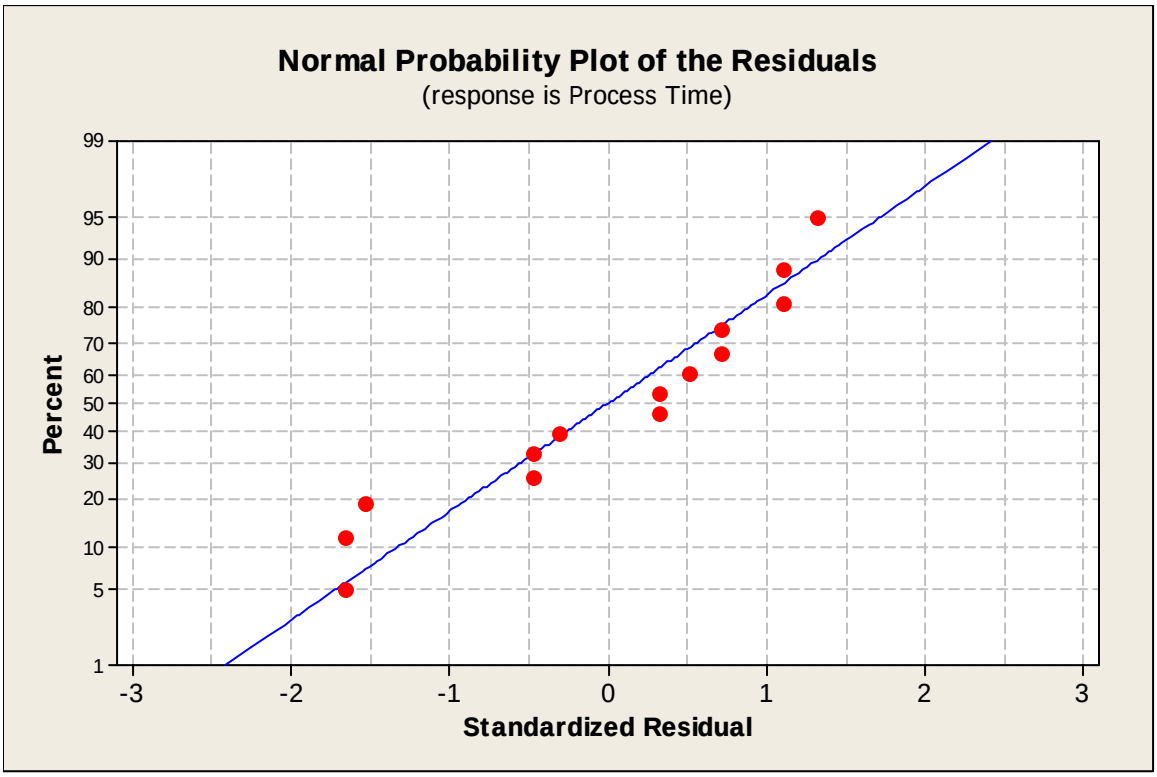

Figure **A.13**: Normality probability plot of the residuals for response variable process time of sliced mushrooms.

Thus, it can be inferred that since the p-value = 0.965 > 0.05, the null hypothesis cannot be rejected, indicating all bed locations gave the same mean process time. This was further validated by employing a Tukey LSD test using 95% simultaneous confidence intervals (Figure **A.14**). Since zero was contained in each of the confidence intervals, the null hypothesis was not rejected.

```
Bed Location = 1  subtracted from:

Location    Lower   Center   Upper  ------+---------+---------+---------+
2         -0.4685  0.04500  0.5585  (-----------------*----------------)
3         -0.4685  0.04500  0.5585  (-----------------*----------------)
                                    ------+---------+---------+---------+
                                       -0.30      0.00      0.30      0.60
Bed Location = 2  subtracted from:

Location    Lower     Center   Upper  ------+---------+---------+---------+
3         -0.4842  -0.000000  0.4842  (---------------*---------------)
                                    ------+---------+---------+---------+
                                       -0.30      0.00      0.30      0.60
```

Figure **A.14**: Tukey's 95% simultaneous confidence intervals for all pairwise comparisons among levels of bed location for response variable process time of sliced mushrooms.

Analysis of Variance (ANOVA) was used to determine the effects of bed position on slice thickness for heat penetration studies of sliced mushrooms (**Table A.4**).

Table **A.4**: ANOVA: Response variable slice thickness for heat penetration tests of sliced mushrooms.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Bed Location	2	0	0	0.17	0.848
Error	12	1.3E-06	1E-07		
Total	14	1.3E-06			

Before testing the hypothesis it was necessary to check the model for outliers, equal variance, interaction and normality. Plots of the standardized residuals versus bed location (Figure **A.15**) suggested equal variance existed between bed locations for the response variable, slice thickness.

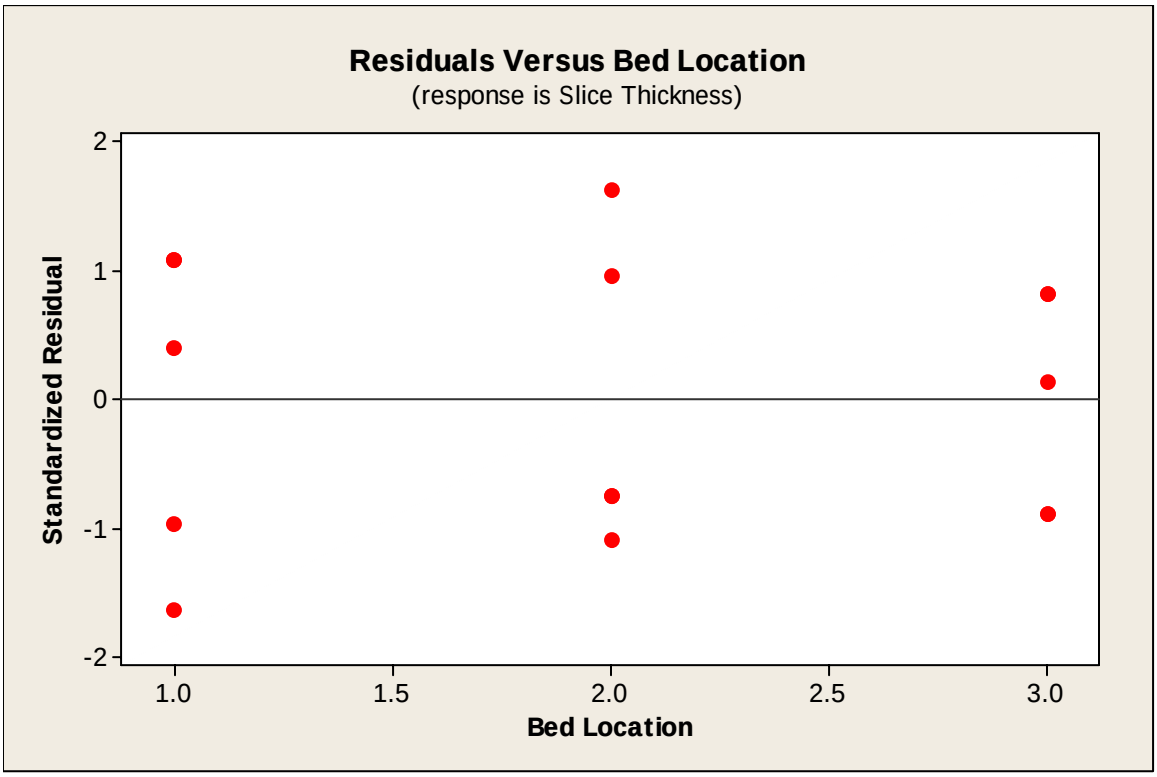

Figure **A.15**: Standardized residuals versus bed location for response variable slice thickness of sliced mushrooms.

The plot of standardized residuals versus fits plot (Figure **A.16**) shows no apparent patterns to suggest interaction between location or a relationship between mean and variance.

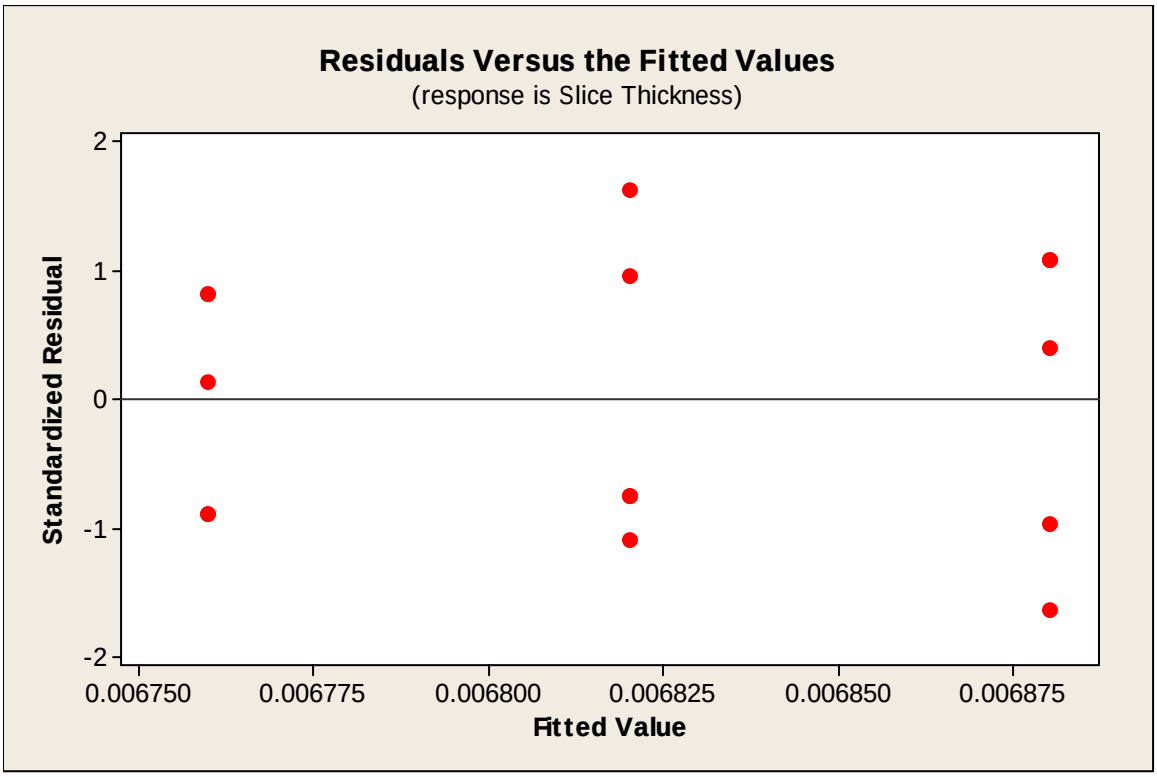

Figure **A.16**: Residuals versus the fitted values for response variable slice thickness of sliced mushrooms.

The normal probability plot (**Figure A.17**) seemed consistent with the normality of errors.

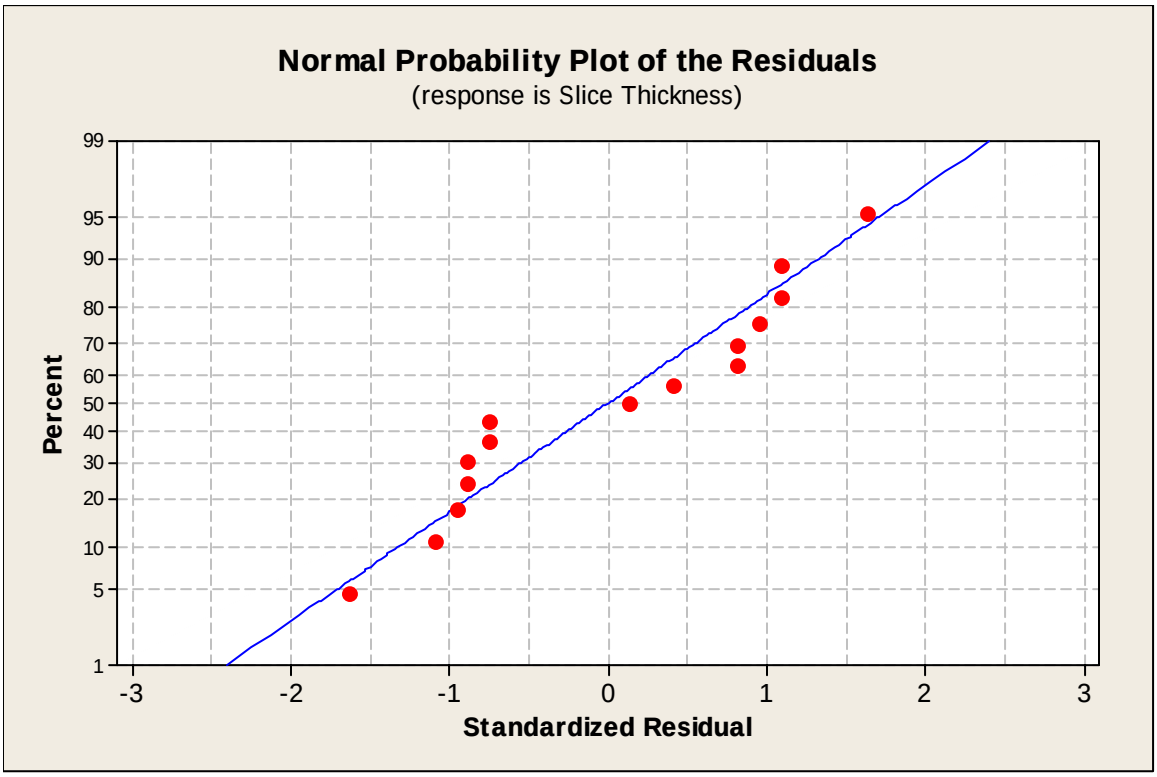

Figure **A.17**: Normality probability plot of the residuals for response variable slice thickness of sliced mushrooms.

Thus, it can be inferred that since the p-value = 0.848 > 0.05, the null hypothesis cannot be rejected, indicating all bed locations gave the same mean cap diameter. This was further validated by employing a Tukey LSD test using 95% simultaneous confidence intervals (Figure **A.18**). Since zero was contained in each of the confidence intervals, the null hypothesis was not rejected.

```
Bed Location = 1  subtracted from:

Location      Lower     Center     Upper  ---------+---------+---------+-------
2         -0.000612  -0.000060  0.000492    (--------------*--------------)
3         -0.000672  -0.000120  0.000432  (---------------*--------------)
                                           ---------+---------+---------+-------
                                                 -0.00035   0.00000   0.00035
Bed Location = 2  subtracted from:

Location      Lower     Center     Upper  ---------+---------+---------+-------
3         -0.000612  -0.000060  0.000492    (--------------*--------------)
                                           ---------+---------+---------+-------
                                                 -0.00035   0.00000   0.00035
```

Figure **A.18**: Tukey 95% simultaneous confidence intervals for all pairwise comparisons among levels of bed location for response variable slice thickness of sliced mushrooms.

Appendix B

Mushroom Quality Data

Sliced Mushroom Data

Table **B1**: Sliced mushroom quality raw data.

Identification Number	Process	Pre-process			Post-Process			Force (N)	Work (N-m)
		Mass (g)	L	DE	Mass (g)	L	DE		
06-17-05-13	Aseptic	5.41	92.55	19.40	3.56	72.52	33.44	-115.96	0.21
06-17-05-14	Aseptic	3.77	91.88	18.45	2.71	68.88	36.09	-111.38	0.18
06-17-05-16	Aseptic	4.16	90.09	19.91	2.75	74.41	32.21	-93.67	0.14
06-17-05-17	Aseptic	4.33	88.39	20.42	2.86	67.71	37.71	-114.58	0.22
06-17-05-18	Aseptic	3.67	87.69	20.71	2.69	69.09	36.78	-74.19	0.10
06-17-05-20	Aseptic	5.47	82.58	24.91	3.63	71.22	33.71	0.00	0.00
06-17-05-21	Aseptic	3.75	93.33	16.24	2.87	73.76	32.01	-144.07	0.20
06-17-05-22	Aseptic	3.64	91.11	18.46	2.66	72.26	33.43	-101.15	0.17
06-17-05-23	Aseptic	4.86	91.23	19.00	3.24	74.09	32.03	-105.64	0.23
06-17-05-24	Aseptic	4.47	88.27	20.21	2.74	72.11	33.54	-93.76	0.14
06-17-05-28	Aseptic	3.79	87.69	20.50	2.71	70.31	34.76	-123.39	0.24
06-17-05-30	Aseptic	4.84	90.00	19.37	3.28	70.14	35.56	-96.83	0.26
06-17-05-33	Aseptic	2.69	87.79	20.69	1.79	69.57	36.06	-46.21	0.11
06-17-05-34	Aseptic	3.24	88.85	20.56	2.36	70.18	35.82	-82.20	0.21
06-17-05-35	Aseptic	5.54	90.51	19.41	3.88	74.23	32.43	-148.61	0.21
06-17-05-37	Aseptic	3.81	91.93	19.46	2.65	74.35	33.29	-92.56	0.17
06-17-05-38	Aseptic	3.04	84.78	23.10	2.15	68.06	37.15	-65.47	0.10
06-17-05-40	Aseptic	4.16	89.87	19.60	2.83	71.12	34.37	-102.08	0.23
06-17-05-43	Aseptic	3.97	86.84	20.67	2.72	70.38	34.66	-90.65	0.18
06-17-05-44	Aseptic	3.73	89.01	21.24	2.50	74.73	31.94	-72.32	0.16
06-17-05-45	Aseptic	2.89	89.27	20.15	1.99	72.80	33.57	-52.35	0.07
06-17-05-46	Aseptic	6.20	92.53	17.56	4.24	75.06	31.20	-158.48	0.36
06-17-05-49	Aseptic	5.17	94.19	17.16	3.58	70.30	35.79	-108.71	0.24
06-17-05-53	Aseptic	4.64	91.32	19.73	3.36	72.72	34.90	-119.25	0.14
06-17-05-55	Aseptic	4.08	90.30	20.44	2.92	72.86	34.47	-77.53	0.18
06-17-05-56	Aseptic	3.99	91.24	20.00	2.71	74.45	32.79	-77.04	0.18
06-17-05-57	Aseptic	4.50	88.08	20.92	2.98	69.82	35.78	-133.17	0.22
06-17-05-58	Aseptic	3.72	91.06	19.83	2.56	74.63	33.31	-78.33	0.16
06-17-05-63	Aseptic	5.42	92.46	19.02	3.79	74.45	33.37	-145.00	0.23
06-17-05-64	Aseptic	5.00	89.02	19.97	3.47	72.41	33.23	-102.08	0.23
06-17-05-66	Aseptic	4.55	90.44	19.37	3.16	74.37	32.02	-113.20	0.18
06-17-05-67	Aseptic	4.10	89.61	19.72	2.74	71.11	34.49	-75.75	0.14
06-17-05-68	Aseptic	3.46	85.31	23.31	2.27	70.36	35.15	-74.55	0.17
06-17-05-70	Aseptic	4.72	89.58	20.53	3.36	72.47	33.77	-147.45	0.27
06-17-05-72	Aseptic	3.59	89.99	19.67	2.40	70.50	34.43	-99.64	0.15
08-11-05-42	Aseptic	6.43	83.42	25.42	4.51	73.30	33.25	-166.76	0.26
08-11-05-43	Aseptic	5.72	90.77	19.36	3.86	70.03	36.55	-134.33	0.28
08-11-05-44	Aseptic	5.05	89.07	21.04	3.56	69.58	37.72	-124.28	0.23
08-11-05-45	Aseptic	4.06	86.15	22.62	2.72	69.60	37.41	-83.53	0.12
08-11-05-47	Aseptic	5.08	87.00	22.55	3.34	72.05	34.82	-94.08	0.21
08-11-05-48	Aseptic	4.59	90.91	19.56	3.08	71.89	34.87	-105.37	0.16
08-11-05-49	Aseptic	5.09	89.34	20.54	3.39	72.40	34.10	-109.47	0.21
08-11-05-50	Aseptic	3.97	91.31	19.57	2.52	70.14	36.54	-72.95	0.12
08-11-05-52	Aseptic	5.06	89.96	19.81	3.49	70.48	36.54	-124.19	0.24
08-11-05-53	Aseptic	4.81	87.11	22.16	3.35	73.08	34.47	-106.00	0.19

08-11-05-54	Aseptic	4.62	87.56	22.68	3.05	73.64	32.88	-82.69	0.19
08-11-05-55	Aseptic	4.24	89.62	20.36	2.74	72.26	34.70	-97.63	0.17
08-11-05-56	Aseptic	4.79	86.70	22.89	3.16	71.33	35.72	-105.11	0.17
08-11-05-58	Aseptic	5.39	89.54	20.42	3.70	71.48	35.44	-121.25	0.23
08-11-05-59	Aseptic	4.93	89.20	21.43	3.14	70.94	36.39	-104.84	0.14
08-11-05-63	Aseptic	5.74	86.75	22.82	3.65	70.83	35.22	-123.21	0.30
08-11-05-65	Aseptic	4.68	85.57	23.15	3.15	72.66	34.97	-102.66	0.19
08-11-05-67	Aseptic	5.22	88.07	20.89	3.65	72.65	33.77	-117.78	0.20
08-11-05-69	Aseptic	6.65	92.36	18.56	4.46	72.36	34.81	-153.81	0.13
08-11-05-70	Aseptic	6.03	89.09	20.69	4.00	74.01	32.65	-138.29	0.26
08-25-05-01	Aseptic	4.25	84.68	24.46	3.21	70.26	37.23	-100.88	0.16
08-25-05-03	Aseptic	6.36	88.17	20.92	4.60	74.08	33.34	-186.64	0.30
08-25-05-04	Aseptic	6.05	86.82	22.29	4.40	70.05	37.11	-163.64	0.34
08-25-05-07	Aseptic	4.76	83.69	23.81	3.55	69.89	36.85	-115.16	0.28
08-25-05-08	Aseptic	3.85	89.03	20.88	2.61	72.43	36.63	-91.45	0.15
08-25-05-11	Aseptic	4.31	84.60	24.65	2.96	69.68	38.04	-95.72	0.19
08-25-05-13	Aseptic	4.34	85.95	21.99	3.02	70.93	36.40	-93.67	0.13
08-25-05-24	Aseptic	4.50	89.33	21.17	3.31	69.57	38.63	-95.37	0.19
08-25-05-26	Aseptic	6.54	87.02	21.79	4.62	72.94	35.50	-162.00	0.36
08-25-05-31	Aseptic	5.52	92.44	19.96	3.53	72.35	34.64	-119.07	0.25
08-25-05-37	Aseptic	5.55	87.40	22.14	3.87	72.97	35.70	-112.80	0.19
08-25-05-38	Aseptic	4.63	88.51	21.65	3.27	73.54	34.58	-110.44	0.19
08-25-05-39	Aseptic	4.47	88.54	21.28	3.06	74.34	34.74	-88.38	0.15
08-25-05-42	Aseptic	5.26	84.93	23.21	3.09	73.91	33.50	-102.13	0.20
08-25-05-43	Aseptic	3.99	85.01	23.89	2.77	72.70	34.54	-93.94	0.18
08-25-05-45	Aseptic	4.90	82.55	24.36	3.65	70.88	35.48	-121.30	0.19
08-25-05-49	Aseptic	4.63	86.03	22.54	3.11	72.63	35.07	-102.26	0.22
08-25-05-54	Aseptic	6.28	88.59	20.47	4.45	72.72	35.05	-172.40	0.39
08-25-05-55	Aseptic	4.77	83.97	24.21	3.55	71.18	36.14	-152.03	0.25
08-25-05-56	Aseptic	6.02	86.30	23.59	4.55	72.02	35.47	-141.80	0.25
08-25-05-58	Aseptic	4.52	88.22	21.04	3.16	74.61	33.68	-108.62	0.19
08-25-05-59	Aseptic	4.64	89.79	21.05	3.40	72.59	37.12	-101.95	0.17
06-17-05-100	Can	3.81	88.30	21.53	2.27	66.64	39.11	-80.51	0.13
06-17-05-101	Can	3.66	84.68	23.31	2.23	66.88	38.21	-82.47	0.19
06-17-05-102	Can	3.54	92.73	19.02	2.36	72.05	35.69	-82.15	0.20
06-17-05-103	Can	3.18	91.12	18.53	2.12	62.98	41.71	-80.24	0.13
06-17-05-104	Can	3.47	87.57	21.77	2.25	69.01	37.19	-60.23	0.08
06-17-05-105	Can	4.63	87.17	22.33	2.93	68.35	37.85	-103.73	0.18
06-17-05-106	Can	4.22	89.14	20.79	2.73	67.84	38.53	-91.90	0.20
06-17-05-107	Can	3.97	92.46	19.43	2.60	70.39	37.62	-90.61	0.13
06-17-05-108	Can	4.29	91.40	18.50	2.69	70.84	35.74	-90.52	0.16
06-17-05-109	Can	3.25	91.31	20.07	1.99	71.93	36.17	-69.12	0.13
06-17-05-110	Can	4.21	86.20	22.78	2.57	69.74	36.48	-87.63	0.15
06-17-05-111	Can	4.53	89.62	20.82	2.95	68.91	37.79	-95.05	0.14
06-17-05-112	Can	4.42	87.33	22.83	2.94	66.84	38.57	-112.18	0.16
06-17-05-113	Can	5.68	88.65	20.33	3.71	66.13	39.02	-140.56	0.31
06-17-05-114	Can	5.39	90.45	19.84	3.35	67.82	38.33	-107.82	0.16
06-17-05-115	Can	4.17	88.39	21.69	2.59	70.22	36.51	-96.66	0.19
06-17-05-116	Can	3.46	91.57	19.85	2.20	70.27	37.38	-81.89	0.16
06-17-05-117	Can	3.98	93.73	16.69	2.68	67.56	39.11	-91.54	0.18
06-17-05-118	Can	4.45	87.84	22.06	2.71	70.37	36.38	-87.94	0.19
06-17-05-119	Can	3.19	90.86	20.45	1.99	70.17	37.60	-64.63	0.12
06-17-05-120	Can	5.27	92.17	18.84	3.64	68.79	38.53	-150.12	0.26
06-17-05-73	Can	3.49	86.27	23.08	2.15	66.87	39.56	-78.77	0.13

06-17-05-74	Can	5.10	86.89	23.31	3.12	69.35	37.88	-118.49	0.19
06-17-05-75	Can	4.78	87.73	21.56	2.87	66.05	39.61	-115.20	0.19
06-17-05-76	Can	3.49	93.92	18.17	2.58	70.05	36.83	-88.74	0.18
06-17-05-77	Can	6.24	93.53	18.27	4.14	69.48	38.31	-179.48	0.33
06-17-05-78	Can	4.23	92.47	19.03	2.75	72.71	35.29	-107.95	0.16
06-17-05-79	Can	4.27	90.48	19.72	2.83	71.03	36.11	-92.74	0.19
06-17-05-80	Can	3.06	89.06	20.80	1.97	69.75	37.11	-57.78	0.13
06-17-05-81	Can	5.89	92.24	19.12	3.93	66.97	39.71	-141.54	0.24
06-17-05-82	Can	3.27	92.60	18.79	2.12	71.53	36.22	-70.37	0.15
06-17-05-83	Can	5.32	91.17	19.57	3.29	66.63	39.29	-108.89	0.20
06-17-05-84	Can	4.23	88.02	20.44	2.57	66.10	39.28	-89.72	0.17
06-17-05-85	Can	4.36	88.93	20.76	2.73	67.57	38.35	-112.80	0.22
06-17-05-86	Can	4.29	91.71	19.75	2.80	70.88	36.23	-92.34	0.13
06-17-05-87	Can	4.79	89.21	21.53	3.05	68.65	37.68	0.00	0.00
06-17-05-88	Can	5.29	89.83	20.47	3.33	71.29	35.48	-117.12	0.30
06-17-05-89	Can	3.90	90.99	20.20	2.51	68.00	38.70	-72.37	0.12
06-17-05-90	Can	3.95	87.48	20.71	2.60	67.60	38.07	-106.84	0.24
06-17-05-91	Can	4.96	89.51	20.35	3.06	72.12	35.08	-98.83	0.15
06-17-05-92	Can	4.35	86.98	22.92	2.80	69.42	37.22	-93.01	0.21
06-17-05-93	Can	4.08	86.84	22.39	2.51	69.81	36.45	-83.62	0.13
06-17-05-94	Can	4.44	85.96	23.01	2.70	68.65	37.56	-111.82	0.19
06-17-05-95	Can	4.81	88.40	21.48	2.95	70.09	36.64	-111.29	0.20
06-17-05-96	Can	6.14	92.28	18.79	4.19	70.19	36.69	-164.53	0.22
06-17-05-97	Can	3.93	90.59	20.16	2.61	65.50	40.87	-84.60	0.17
06-17-05-98	Can	4.35	92.86	17.72	2.72	68.88	37.12	-90.21	0.19
06-17-05-99	Can	4.36	87.41	22.42	2.71	71.44	35.25	-90.34	0.16
08-11-05-100	Can	5.36	89.73	20.82	3.36	69.62	38.27	-115.20	0.21
08-11-05-101	Can	4.75	84.19	24.89	2.91	66.50	40.17	-92.12	0.20
08-11-05-102	Can	4.03	88.72	21.61	2.55	68.90	38.02	-89.09	0.17
08-11-05-103	Can	5.87	88.02	21.50	3.88	70.05	38.79	-138.64	0.20
08-11-05-104	Can	6.52	88.07	21.57	4.05	69.53	37.63	-151.50	0.27
08-11-05-105	Can	4.40	83.28	24.79	2.69	65.12	41.06	-94.52	0.23
08-11-05-106	Can	4.37	84.18	25.33	2.77	66.14	40.45	-95.10	0.18
08-11-05-107	Can	3.42	91.15	20.58	2.20	69.40	38.22	-60.85	0.12
08-11-05-108	Can	4.74	84.93	24.50	2.80	67.23	40.33	-105.42	0.19
08-11-05-109	Can	3.76	91.14	19.39	2.45	67.36	39.35	-81.67	0.11
08-11-05-110	Can	4.96	89.20	20.34	3.06	69.62	38.32	-102.66	0.22
08-11-05-111	Can	5.69	87.12	21.75	3.54	67.68	38.85	-133.35	0.24
08-11-05-112	Can	4.09	91.40	19.62	2.46	71.30	36.79	-64.36	0.16
08-11-05-113	Can	4.98	86.76	22.39	3.08	65.95	40.46	-101.55	0.23
08-11-05-114	Can	6.06	91.37	20.19	3.97	69.39	39.14	-135.75	0.27
08-11-05-115	Can	6.01	92.66	17.92	3.85	69.95	38.72	-145.63	0.19
08-11-05-116	Can	6.65	88.34	21.85	4.19	67.20	39.90	-154.43	0.22
08-11-05-117	Can	5.12	87.83	22.12	3.19	71.70	36.27	-105.20	0.21
08-11-05-118	Can	3.56	88.33	21.05	2.12	68.39	38.73	-59.16	0.14
08-11-05-119	Can	4.73	89.03	21.31	2.91	65.84	40.77	-100.12	0.13
08-11-05-120	Can	4.59	86.41	22.64	2.64	70.02	37.44	-92.03	0.15
08-11-05-73	Can	5.58	91.33	19.10	3.54	70.23	37.81	-117.25	0.26
08-11-05-74	Can	5.60	88.79	21.22	3.61	66.25	40.61	-140.91	0.24
08-11-05-75	Can	5.93	87.78	20.64	3.60	62.68	42.87	-134.60	0.34
08-11-05-76	Can	5.50	88.11	20.74	3.28	67.07	39.67	-131.22	0.29
08-11-05-77	Can	5.93	83.27	24.88	3.87	64.12	41.76	-144.92	0.26
08-11-05-78	Can	5.06	89.83	20.76	3.08	68.79	39.46	-119.74	0.22
08-11-05-79	Can	4.45	90.93	19.86	2.53	67.11	39.73	-85.54	0.15
08-11-05-80	Can	6.39	90.34	20.08	4.00	67.47	40.72	-133.75	0.23

08-11-05-81	Can	5.84	87.17	21.71	3.69	67.70	39.00	-130.33	0.20
08-11-05-82	Can	5.92	93.54	17.44	3.68	67.70	39.24	-130.77	0.27
08-11-05-83	Can	5.41	93.40	18.65	3.63	71.19	37.74	-123.48	0.18
08-11-05-84	Can	5.56	86.14	22.31	3.51	67.64	38.70	-125.48	0.23
08-11-05-85	Can	4.95	86.94	22.07	3.04	68.28	38.76	-96.34	0.16
08-11-05-86	Can	4.47	90.02	20.56	2.78	66.86	39.93	-90.07	0.13
08-11-05-87	Can	4.71	84.26	24.56	2.87	66.36	39.52	-96.43	0.15
08-11-05-88	Can	5.29	83.70	24.53	3.17	67.33	39.36	-95.81	0.22
08-11-05-89	Can	4.47	92.66	19.09	2.88	70.50	38.26	-89.89	0.20
08-11-05-90	Can	5.44	86.91	23.18	3.19	66.88	39.77	-105.91	0.19
08-11-05-91	Can	4.61	88.64	21.19	2.74	68.67	37.92	-108.98	0.19
08-11-05-92	Can	5.82	87.89	21.39	3.75	66.96	39.02	-147.18	0.30
08-11-05-93	Can	4.58	90.97	19.31	2.77	66.38	39.94	-98.75	0.16
08-11-05-94	Can	6.57	86.44	22.59	3.92	69.41	38.29	-166.84	0.27
08-11-05-95	Can	5.03	87.94	21.75	3.21	68.05	39.05	-105.95	0.16
08-11-05-96	Can	5.69	86.32	22.63	3.50	65.94	40.62	-126.32	0.24
08-11-05-97	Can	3.89	88.23	20.64	2.49	69.41	38.01	-74.82	0.09
08-11-05-98	Can	5.15	87.56	21.21	3.10	66.90	39.40	-94.30	0.12
08-11-05-99	Can	5.47	87.74	21.43	3.34	67.65	39.38	-122.28	0.28
08-25-05-100	Can	4.89	90.59	21.10	2.84	72.73	37.60	-91.90	0.18
08-25-05-102	Can	3.95	87.75	22.17	2.54	69.95	39.27	-94.34	0.18
08-25-05-103	Can	5.00	81.50	26.54	3.40	69.47	38.75	-127.48	0.22
08-25-05-104	Can	6.38	87.74	21.07	4.30	70.76	37.76	-153.14	0.26
08-25-05-105	Can	4.30	85.18	24.41	3.74	69.94	39.03	-120.32	0.23
08-25-05-106	Can	4.25	89.19	20.90	2.81	67.61	41.16	-93.90	0.12
08-25-05-107	Can	4.62	85.38	23.58	2.80	69.52	39.75	-98.30	0.16
08-25-05-108	Can	4.97	87.32	22.74	3.33	69.45	39.25	-110.13	0.26
08-25-05-61	Can	4.97	87.33	21.20	3.26	68.71	39.57	-119.34	0.18
08-25-05-62	Can	5.36	84.94	23.04	3.48	68.56	39.37	-140.07	0.27
08-25-05-63	Can	5.57	88.05	21.60	3.79	68.13	40.22	-134.60	0.34
08-25-05-64	Can	5.37	85.15	22.84	3.63	69.15	38.61	-130.64	0.34
08-25-05-65	Can	4.98	84.60	24.62	3.54	66.97	40.86	-124.32	0.31
08-25-05-66	Can	4.54	89.26	21.22	2.97	68.77	40.31	-97.99	0.16
08-25-05-67	Can	4.41	90.59	20.09	3.06	68.91	40.21	-83.76	0.13
08-25-05-69	Can	5.29	84.04	24.60	3.53	69.99	39.36	-92.74	0.24
08-25-05-70	Can	5.84	82.46	26.45	4.03	69.23	39.25	-146.43	0.32
08-25-05-71	Can	4.01	90.80	19.84	2.68	69.44	38.46	-82.02	0.20
08-25-05-72	Can	6.13	87.36	22.19	4.14	70.51	38.54	-134.06	0.31
08-25-05-73	Can	3.60	90.77	20.02	2.37	68.91	39.77	-93.72	0.20
08-25-05-74	Can	7.13	88.91	21.11	4.98	69.35	39.41	-187.04	0.32
08-25-05-76	Can	5.40	83.41	25.09	3.69	67.74	39.56	-169.56	0.28
08-25-05-77	Can	4.77	86.31	22.65	3.15	66.49	40.85	-109.64	0.19
08-25-05-78	Can	5.55	82.31	24.89	3.82	65.39	41.99	-127.97	0.25
08-25-05-79	Can	5.06	85.60	23.04	3.48	69.11	39.21	-117.38	0.19
08-25-05-80	Can	4.67	88.60	21.87	3.04	68.49	39.18	-118.94	0.22
08-25-05-81	Can	4.86	86.08	22.53	3.25	69.31	39.09	-125.57	0.26
08-25-05-82	Can	4.54	85.60	23.64	3.15	70.20	39.09	-94.70	0.13
08-25-05-83	Can	4.99	89.24	20.41	3.39	68.17	40.87	-111.20	0.32
08-25-05-84	Can	5.87	87.71	20.90	3.94	68.78	40.23	-148.30	0.24
08-25-05-85	Can	6.17	88.62	20.78	4.24	69.30	39.22	-166.31	0.27
08-25-05-86	Can	4.73	86.56	21.56	3.29	68.04	40.20	-113.91	0.22
08-25-05-87	Can	6.53	89.36	21.72	4.53	70.47	39.08	-147.72	0.34
08-25-05-88	Can	4.44	83.46	25.99	2.89	68.36	40.29	-100.17	0.20
08-25-05-89	Can	4.68	84.04	25.03	3.00	70.84	37.68	-91.85	0.12
08-25-05-90	Can	5.82	87.08	21.41	4.07	68.65	39.95	-146.65	0.23

08-25-05-91	Can	5.24	82.07	24.92	3.61	66.36	40.39	-117.61	0.24
08-25-05-92	Can	4.83	87.29	21.73	3.10	67.98	40.56	-114.76	0.20
08-25-05-93	Can	5.24	88.76	21.29	3.84	67.22	41.09	-159.82	0.29
08-25-05-94	Can	4.68	88.07	21.59	3.28	69.62	39.28	-105.37	0.26
08-25-05-95	Can	4.26	89.24	21.75	2.83	67.33	41.35	-85.62	0.11
08-25-05-96	Can	5.13	84.30	23.07	3.83	69.29	39.25	-177.96	0.32
08-25-05-97	Can	5.37	84.29	24.99	3.64	67.03	40.11	-123.57	0.30
08-25-05-98	Can	5.60	88.59	22.32	3.67	72.26	38.16	-119.92	0.26
08-25-05-99	Can	4.45	85.77	24.47	2.94	71.11	38.18	-110.93	0.23
06-17-05-01	RAW	5.65						-172.98	0.47
06-17-05-02	RAW	3.40						-109.06	0.17
06-17-05-03	RAW	3.25						-94.39	0.21
06-17-05-04	RAW	3.44						-105.46	0.29
06-17-05-05	RAW	5.86						-175.96	0.55
06-17-05-06	RAW	4.85						-164.80	0.50
06-17-05-07	RAW	3.60						-96.70	0.21
06-17-05-08	RAW	2.86						-94.88	0.27
06-17-05-09	RAW	4.43						-159.33	0.44
06-17-05-10	RAW	3.34						-99.59	0.36
06-17-05-11	RAW	3.04						-95.28	0.25
06-17-05-12	RAW	4.24						-131.30	0.45
08-11-05-01	RAW	4.64						-119.21	0.23
08-11-05-02	RAW	6.14						-168.53	0.31
08-11-05-03	RAW	6.13						-156.39	0.49
08-11-05-04	RAW	4.28						-98.83	0.25
08-11-05-05	RAW	3.96						-100.97	0.20
08-11-05-06	RAW	5.82						-143.63	0.52
08-11-05-07	RAW	4.28						0.00	0.00
08-11-05-08	RAW	5.28						-132.73	0.39
08-11-05-09	RAW	5.99						-181.79	0.26
08-11-05-10	RAW	4.47						-118.05	0.23
08-11-05-11	RAW	4.12						-102.66	0.35
08-11-05-12	RAW	6.28						-178.45	0.64
08-25-05-01	RAW	4.91						-161.82	0.31
08-25-05-02	RAW	5.98						-193.35	0.35
08-25-05-03	RAW	4.81						-155.55	0.36
08-25-05-04	RAW	5.71						-165.78	0.33
08-25-05-05	RAW	6.19						-208.92	0.33
08-25-05-06	RAW	5.49						-148.47	0.34
08-25-05-07	RAW	4.87						-152.21	0.30
08-25-05-08	RAW	5.40						-155.32	0.27
08-25-05-09	RAW	6.02						-179.03	0.43
08-25-05-10	RAW	4.33						-133.26	0.29
08-25-05-11	RAW	6.22						-217.15	0.48
08-25-05-12	RAW	5.66						-183.04	0.34

Whole Mushroom Data

Table **B.2**: Sliced mushroom quality raw data.

Identification Number	Process	Pre-process			Post-Process			Force (N)	Work (N-m)
		Mass (g)	L	DE	Mass (g)	L	DE		
04-06-05-13	Aseptic	10.52	96.48	17.10	7.42	73.42	37.80	-90.16	0.45
04-06-05-14	Aseptic	15.05	96.00	15.18	9.67	72.56	36.47	-145.67	0.66
04-06-05-15	Aseptic	16.58	97.27	16.25	11.80	74.35	36.42	-195.53	0.80
04-06-05-16	Aseptic	13.52	97.44	15.24	9.50	71.59	37.83	-184.64	0.86
04-06-05-17	Aseptic	14.62	97.82	15.05	10.21	74.89	35.36	-140.91	0.94
04-06-05-18	Aseptic	12.27	96.49	15.47	8.07	72.50	38.05	-95.05	0.81
04-06-05-19	Aseptic	11.27	97.79	15.29	7.98	73.66	37.13	-164.89	0.77
04-06-05-20	Aseptic	13.33	95.92	16.33	8.86	74.35	35.79	-111.87	0.97
04-06-05-21	Aseptic	14.85	96.96	15.24	9.98	72.87	36.49	-194.20	0.82
04-06-05-22	Aseptic	14.15	95.81	18.26	10.62	70.19	39.12	-108.93	0.49
04-06-05-23	Aseptic	10.98	97.39	14.83	7.50	71.53	36.70	-139.76	0.70
04-06-05-24	Aseptic	20.75	96.97	15.33	13.94	73.54	36.57	-289.52	1.22
04-06-05-37	Aseptic	10.65	97.28	15.39	7.40	66.51	40.81	-126.59	0.52
04-06-05-38	Aseptic	11.67	97.29	15.06	8.08	74.24	36.28	-157.28	0.69
04-06-05-39	Aseptic	19.81	95.86	17.38	14.58	72.77	37.03	-184.77	0.83
04-06-05-40	Aseptic	14.51	97.51	15.24	9.69	75.34	36.54	-152.34	1.05
04-06-05-41	Aseptic	18.48	97.23	15.88	13.69	72.65	37.42	-284.09	1.36
04-06-05-42	Aseptic	11.66	96.97	16.67	8.47	72.57	38.02	-181.97	0.78
04-06-05-43	Aseptic	17.33	96.50	17.40	12.49	71.47	38.02	-175.30	0.74
04-06-05-44	Aseptic	10.36	95.92	16.29	6.83	69.45	38.87	-143.63	0.63
04-06-05-45	Aseptic	20.22	95.29	17.91	14.67	72.10	38.03	-365.63	1.66
04-06-05-46	Aseptic	15.17	97.51	16.24	11.26	71.61	37.35	-187.31	0.81
04-06-05-47	Aseptic	10.42	96.54	16.42	7.33	72.77	37.23	-124.10	0.77
04-06-05-48	Aseptic	11.80	97.44	14.81	7.74	71.41	38.64	-157.28	0.70
04-06-05-61	Aseptic	24.77	95.45	17.20	16.60	66.49	40.59	-206.92	1.02
04-06-05-62	Aseptic	13.56	96.82	15.77	9.68	71.56	37.46	-167.07	0.98
04-06-05-63	Aseptic	10.75	97.46	16.30	8.12	73.22	35.80	-161.64	0.74
04-06-05-64	Aseptic	12.10	96.59	16.61	8.20	74.02	37.30	-152.26	0.88
04-06-05-65	Aseptic	16.13	96.91	15.85	11.08	68.10	40.04	-139.27	0.64
04-06-05-66	Aseptic	15.98	97.03	15.03	10.83	68.43	39.41	-212.84	0.95
04-06-05-67	Aseptic	20.00	97.31	14.62	12.71	72.32	36.03	-234.90	0.97
04-06-05-68	Aseptic	12.16	97.36	14.85	8.07	74.56	36.00	-127.70	0.84
04-06-05-69	Aseptic	11.83	98.04	16.34	9.01	72.62	37.85	-121.12	0.55
04-06-05-70	Aseptic	15.99	96.49	15.91	10.87	71.93	37.25	-251.80	1.16
04-06-05-71	Aseptic	10.85	98.08	16.20	8.42	74.80	37.67	-186.86	0.75
04-06-05-72	Aseptic	18.07	97.39	17.23	12.98	73.29	36.68	-275.42	1.36
04-06-05-85	Aseptic	19.51	97.80	15.46	13.70	73.24	36.68	-281.51	1.54
04-06-05-86	Aseptic	10.08	96.26	16.72	7.44	74.89	35.71	-126.10	0.72
04-06-05-87	Aseptic	19.60	95.23	18.84	13.90	72.54	37.59	-282.00	1.42
04-06-05-88	Aseptic	10.39	97.47	16.79	7.46	72.84	37.93	-97.77	0.48
04-06-05-89	Aseptic	22.34	96.16	16.71	16.10	73.78	35.68	-309.22	1.43
04-06-05-90	Aseptic	11.09	94.04	17.74	7.58	71.87	37.93	-151.81	0.74
04-06-05-91	Aseptic	11.09	97.09	16.39	8.49	73.86	37.11	-104.79	0.78
04-06-05-92	Aseptic	14.88	97.54	16.62	11.60	71.90	38.50	-228.89	0.94
04-06-05-93	Aseptic	11.22	96.72	15.31	7.88	72.59	36.95	-102.79	0.43
04-06-05-94	Aseptic	16.30	97.47	15.58	11.73	74.62	35.28	-201.54	1.44
04-06-05-95	Aseptic	17.43	95.45	17.35	12.28	73.47	37.28	-148.07	0.71
04-06-05-96	Aseptic	17.94	96.64	16.98	12.51	72.22	37.30	-263.54	1.30
05-05-05-13	Aseptic	10.71	96.65	16.96	7.44	74.86	35.01	-143.94	0.61

05-05-05-14	Aseptic	12.12	96.74	15.86	8.38	75.67	34.62	-140.33	0.86
05-05-05-15	Aseptic	10.49	94.17	17.87	7.03	72.28	35.75	-152.79	0.70
05-05-05-16	Aseptic	14.3	94.19	17.00	9.37	73.88	35.49	-168.62	0.89
05-05-05-17	Aseptic	11.31	96.77	16.48	7.71	74.68	35.16	-175.96	0.77
05-05-05-18	Aseptic	15.21	94.99	17.48	10.06	72.76	36.32	-291.83	1.33
05-05-05-19	Aseptic	12.39	96.49	17.35	9.07	72.62	39.89	-185.62	0.77
05-05-05-21	Aseptic	9.91	96.33	15.08	6.60	74.23	34.76	-162.49	0.58
05-05-05-22	Aseptic	14.13	95.71	16.29	9.15	74.32	36.90	-155.99	0.99
05-05-05-23	Aseptic	14.8	96.75	16.45	9.84	74.82	35.88	-186.19	0.84
05-05-05-24	Aseptic	17.29	94.75	18.38	11.90	74.25	35.15	-212.44	1.05
05-05-05-25	Aseptic	8.84	97.30	15.05	6.03	75.11	35.96	-143.94	0.73
05-05-05-26	Aseptic	12.29	95.66	16.44	8.42	72.64	36.29	-179.39	0.75
05-05-05-27	Aseptic	9.56	94.45	17.34	6.23	72.58	37.23	-150.61	0.64
05-05-05-28	Aseptic	21.88	94.88	16.58	14.30	71.95	37.84	-230.23	1.15
05-05-05-29	Aseptic	16.81	96.56	15.62	11.07	74.54	35.31	-321.55	1.25
05-05-05-30	Aseptic	15.88	96.35	15.99	10.48	73.33	35.74	-173.87	0.85
05-05-05-31	Aseptic	22.14	94.85	16.05	14.32	72.94	37.75	-260.88	1.17
05-05-05-32	Aseptic	13.78	96.67	15.68	9.31	72.69	36.72	-210.75	0.97
05-05-05-33	Aseptic	17.39	96.40	15.37	11.38	73.23	36.07	-205.28	1.32
05-05-05-34	Aseptic	10.84	96.70	15.58	7.11	73.35	34.84	-139.98	0.58
05-05-05-35	Aseptic	13.28	96.52	16.27	9.02	73.41	36.85	-189.04	1.00
05-05-05-36	Aseptic	8.44	95.88	17.21	5.46	72.86	35.69	-106.62	0.44
05-05-05-37	Aseptic	18.18	97.25	15.58	12.04	75.20	34.27	-188.37	0.93
05-05-05-38	Aseptic	12.45	97.30	14.17	8.15	73.67	34.99	-181.61	0.72
05-05-05-39	Aseptic	14.22	96.55	15.73	9.46	74.80	34.05	-169.56	0.89
05-05-05-40	Aseptic	11.51	95.23	16.61	7.19	75.27	34.91	-139.80	0.67
05-05-05-41	Aseptic	12.08	98.20	15.77	8.41	74.85	35.33	-144.74	0.56
05-05-05-42	Aseptic	9.14	97.38	17.00	6.91	72.70	37.69	-132.28	0.65
05-05-05-43	Aseptic	10.74	96.55	18.01	7.60	73.90	36.78	-159.15	0.85
05-05-05-44	Aseptic	12.87	95.14	15.64	8.86	70.66	37.12	-228.89	1.19
05-05-05-45	Aseptic	17.65	94.98	16.91	11.31	72.18	37.17	-244.73	1.11
05-05-05-46	Aseptic	13.18	96.51	15.45	8.43	75.96	34.73	-177.79	0.36
05-05-05-47	Aseptic	16.16	95.68	15.19	10.09	74.52	34.78	-199.00	0.92
05-05-05-48	Aseptic	16.63	97.16	17.20	11.54	75.35	34.67	-207.50	1.22
05-05-05-49	Aseptic	9.14	96.71	14.74	5.77	73.06	35.78	-86.34	0.52
05-05-05-50	Aseptic	12.57	97.65	16.10	8.12	74.56	35.20	-161.42	0.81
05-05-05-51	Aseptic	22.56	96.86	16.05	15.32	77.34	32.57	-347.97	1.89
05-05-05-52	Aseptic	13.98	95.20	16.33	9.27	73.92	36.26	-168.62	0.90
05-05-05-53	Aseptic	12.63	96.42	16.17	8.23	72.45	35.94	-154.52	0.91
05-05-05-54	Aseptic	9.92	95.32	17.01	6.36	72.69	36.54	-138.42	0.55
05-05-05-55	Aseptic	17.29	97.01	16.28	11.24	74.60	35.49	-205.85	1.11
05-05-05-56	Aseptic	10.08	94.73	17.46	6.69	73.48	37.14	-137.98	0.72
05-05-05-57	Aseptic	10.81	95.85	15.41	6.73	73.33	35.56	-138.42	0.77
05-05-05-58	Aseptic	16.08	97.57	16.13	10.99	76.74	33.38	-220.09	1.05
05-05-05-59	Aseptic	10.89	97.63	15.88	7.26	76.88	33.13	-127.97	0.80
05-05-05-60	Aseptic	15.24	96.39	15.64	10.05	74.07	35.63	-185.13	1.10
05-11-05-13	Aseptic	9.44	95.74	15.98	6.74	71.15	38.69	-138.47	0.64
05-11-05-14	Aseptic	20.65	95.61	17.06	15.40	71.43	39.09	-303.31	1.62
05-11-05-15	Aseptic	14.40	96.87	15.43	10.89	70.90	38.92	-213.99	1.07
05-11-05-16	Aseptic	13.04	94.68	16.28	9.05	71.62	38.59	-218.49	0.91
05-11-05-17	Aseptic	15.70	95.22	18.56	11.66	70.03	40.88	-249.31	1.05
05-11-05-18	Aseptic	9.68	96.66	16.26	6.59	71.99	39.13	-118.63	0.48
05-11-05-19	Aseptic	17.29	96.33	16.66	12.44	71.89	38.76	-231.65	1.17
05-11-05-20	Aseptic	9.58	91.92	18.46	6.94	67.51	40.50	-231.65	1.17
05-11-05-21	Aseptic	15.13	94.19	18.64	10.78	70.09	40.93	-220.67	1.09

05-11-05-22	Aseptic	12.05	96.98	16.19	8.86	71.04	39.03	-183.61	0.88
05-11-05-23	Aseptic	19.11	96.98	14.30	14.03	70.66	39.16	-300.24	1.53
05-11-05-24	Aseptic	12.76	96.61	14.37	8.88	71.28	38.62	-156.79	0.73
05-11-05-25	Aseptic	10.02	96.48	15.51	6.85	69.82	39.53	-114.67	0.73
05-11-05-26	Aseptic	17.95	94.76	15.35	13.49	68.87	39.54	-288.10	1.54
05-11-05-27	Aseptic	15.48	96.26	16.42	11.02	72.94	39.40	-202.92	1.06
05-11-05-28	Aseptic	22.88	96.48	16.83	17.66	70.65	39.29	-398.59	2.11
05-11-05-29	Aseptic	14.90	95.48	15.66	10.22	70.61	38.07	-204.03	0.98
05-11-05-30	Aseptic	14.88	97.03	15.27	10.84	71.47	38.78	-234.50	1.46
05-11-05-31	Aseptic	14.55	95.65	16.54	10.16	71.36	38.94	-230.50	1.34
05-11-05-32	Aseptic	16.42	96.66	15.31	11.90	70.77	38.50	-246.64	1.21
05-11-05-33	Aseptic	14.03	96.91	14.98	10.06	71.99	38.82	-206.03	1.03
05-11-05-34	Aseptic	9.83	96.48	17.34	7.21	68.37	40.54	-123.97	0.57
05-11-05-35	Aseptic	11.37	95.68	16.90	8.02	70.03	39.44	-196.20	0.85
05-11-05-36	Aseptic	13.69	95.07	17.33	10.53	71.07	38.65	-226.89	1.26
05-11-05-37	Aseptic	14.16	94.72	15.99	9.70	71.22	38.86	-213.77	0.91
05-11-05-38	Aseptic	13.95	95.91	17.10	10.24	67.94	40.41	-201.85	0.92
05-11-05-39	Aseptic	22.54	96.53	17.44	16.44	69.13	40.91	-332.18	2.07
05-11-05-40	Aseptic	11.02	91.66	17.56	8.09	67.55	40.57	-159.10	0.80
05-11-05-41	Aseptic	15.65	95.53	16.99	11.11	71.27	38.97	-282.18	1.47
05-11-05-42	Aseptic	9.11	93.71	16.88	6.24	68.84	39.39	-224.98	1.09
05-11-05-43	Aseptic	16.35	94.66	16.38	10.76	69.14	39.38	-214.17	1.19
05-11-05-44	Aseptic	9.80	97.00	14.77	4.58	71.51	40.05	-131.75	0.74
05-11-05-45	Aseptic	16.36	96.81	15.47	11.35	71.93	38.47	-224.00	1.01
05-11-05-46	Aseptic	19.34	95.92	16.32	13.56	71.11	39.00	-299.31	1.77
05-11-05-47	Aseptic	17.25	97.27	14.55	11.79	71.39	38.86	-126.23	0.55
05-11-05-48	Aseptic	12.71	96.07	15.90	9.22	69.76	38.92	-193.71	0.73
05-11-05-49	Aseptic	10.57	96.69	15.28	7.02	72.06	38.85	-130.86	0.60
05-11-05-50	Aseptic	21.24	97.10	15.31	14.80	72.28	40.17	-254.56	1.29
05-11-05-51	Aseptic	19.47	96.19	15.93	13.73	71.07	39.46	-267.37	1.26
05-11-05-52	Aseptic	11.08	95.58	15.41	7.48	70.37	39.85	-143.80	0.67
05-11-05-53	Aseptic	21.84	97.21	15.17	15.38	72.02	39.46	-291.66	1.92
05-11-05-54	Aseptic	19.08	96.20	15.09	13.48	70.69	38.81	-291.34	1.41
05-11-05-55	Aseptic	14.24	95.04	15.59	9.90	69.28	39.37	-186.10	0.87
05-11-05-56	Aseptic	14.05	93.90	18.34	10.13	70.07	40.13	-212.26	0.94
05-11-05-57	Aseptic	11.00	96.57	16.08	7.84	71.26	38.83	-163.82	0.87
05-11-05-58	Aseptic	23.78	94.43	17.58	16.61	70.71	40.08	-355.13	1.87
05-11-05-59	Aseptic	14.66	96.40	15.43	10.81	70.95	38.46	-259.54	1.34
05-11-05-60	Aseptic	9.32	94.64	16.73	6.80	72.37	37.74	-136.55	0.72
08-04-05-13	Aseptic	18.69	91.34	20.33	13.37	68.13	41.71	-290.19	1.30
08-04-05-15	Aseptic	14.28	96.71	16.36	10.27	65.38	43.27	-197.36	1.26
08-04-05-16	Aseptic	8.73	95.55	18.45	6.24	67.56	42.07	-94.30	0.43
08-04-05-17	Aseptic	17.86	95.57	17.50	12.83	68.96	40.35	-282.85	1.44
08-04-05-18	Aseptic	12.37	95.81	16.25	9.38	69.00	41.09	-184.46	1.01
08-04-05-19	Aseptic	12.41	94.70	17.36	8.69	68.09	42.29	-170.22	0.98
08-04-05-20	Aseptic	15.78	94.90	18.06	11.63	64.26	43.87	-170.22	0.98
08-04-05-21	Aseptic	10.45	94.41	18.79	8.62	63.14	43.57	-187.71	0.68
08-04-05-22	Aseptic	13.02	92.20	18.01	9.00	63.54	43.68	-237.57	0.96
08-04-05-23	Aseptic	9.25	95.33	14.87	6.76	68.47	41.27	-157.01	0.61
08-04-05-24	Aseptic	17.96	95.93	14.81	13.39	69.44	40.06	-335.69	1.38
08-04-05-25	Aseptic	12.82	95.54	16.00	9.09	66.46	42.17	-221.60	0.87
08-04-05-26	Aseptic	22.60	94.80	16.02	16.37	68.03	41.48	-364.91	1.62
08-04-05-27	Aseptic	9.48	90.80	19.16	6.94	61.30	44.94	-192.46	0.73
08-04-05-28	Aseptic	13.10	94.09	16.15	9.47	69.31	41.29	-237.97	0.99
08-04-05-29	Aseptic	17.03	93.54	16.86	12.17	67.03	41.51	-267.37	1.20
08-04-05-30	Aseptic	12.54	94.77	15.99	9.00	67.49	41.87	-234.05	0.97

08-04-05-31	Aseptic	20.71	94.08	16.20	14.93	68.16	41.63	-346.99	1.87
08-04-05-32	Aseptic	14.43	95.41	15.84	10.18	69.95	41.15	-210.52	1.28
08-04-05-33	Aseptic	13.73	96.18	15.30	11.34	68.70	41.84	-219.82	1.07
08-04-05-34	Aseptic	11.64	94.18	17.07	7.62	63.33	44.14	-162.93	0.76
08-04-05-35	Aseptic	12.35	93.65	16.69	8.91	62.99	43.91	-198.11	1.11
08-04-05-36	Aseptic	12.88	93.95	17.52	9.67	64.32	44.09	-222.58	0.84
08-04-05-37	Aseptic	15.72	90.22	19.67	11.06	62.99	44.16	-305.89	1.36
08-04-05-38	Aseptic	12.25	93.07	18.89	8.19	64.64	43.33	-222.58	0.87
08-04-05-39	Aseptic	12.85	92.92	18.89	9.08	67.07	42.43	-212.93	0.82
08-04-05-40	Aseptic	21.49	96.79	17.04	15.92	71.64	40.64	-325.37	1.78
08-04-05-41	Aseptic	13.37	91.65	19.19	9.35	66.42	42.70	-234.23	1.15
08-04-05-42	Aseptic	18.02	96.15	16.42	12.67	70.04	41.66	-236.23	1.43
08-04-05-43	Aseptic	14.79	93.68	16.32	10.68	68.98	40.57	-198.11	0.97
08-04-05-44	Aseptic	14.61	94.71	15.89	10.41	67.47	41.20	-257.54	1.02
08-04-05-45	Aseptic	10.61	97.36	15.85	7.69	69.85	41.01	-154.66	0.71
08-04-05-46	Aseptic	22.22	94.91	17.19	16.33	68.24	42.13	-332.53	1.43
08-04-05-47	Aseptic	14.52	95.14	17.67	10.84	65.30	43.30	-266.35	1.12
08-04-05-48	Aseptic	16.37	94.24	16.86	11.74	68.08	42.04	-257.45	1.24
08-04-05-49	Aseptic	14.52	91.16	19.90	10.20	66.58	42.24	-195.93	0.90
08-04-05-50	Aseptic	19.38	95.15	15.24	14.07	65.11	42.27	-381.86	1.54
08-04-05-51	Aseptic	14.08	96.28	17.04	10.68	66.45	42.95	-251.31	1.16
08-04-05-52	Aseptic	17.32	95.04	16.39	12.67	62.95	44.25	-347.26	1.59
08-04-05-53	Aseptic	16.59	95.64	15.58	12.30	69.36	41.39	-254.03	1.15
08-04-05-54	Aseptic	13.69	94.45	18.25	9.82	68.32	41.93	-225.42	0.99
08-04-05-55	Aseptic	10.44	96.32	16.83	7.14	65.48	43.37	-131.93	0.66
08-04-05-56	Aseptic	12.73	94.40	16.97	9.12	64.71	43.13	-256.87	1.01
08-04-05-57	Aseptic	13.01	93.93	17.64	10.36	63.32	43.08	-213.01	1.21
08-04-05-58	Aseptic	13.34	95.94	16.18	9.21	63.16	44.28	-206.83	1.13
08-04-05-59	Aseptic	16.49	95.75	14.94	11.95	68.80	41.35	-295.84	1.47
08-04-05-60	Aseptic	10.61	95.00	16.05	7.62	65.99	43.28	-210.26	0.96
04-06-05-100	Can	12.23	97.51	14.91	7.48	71.22	38.88	-188.02	0.80
04-06-05-101	Can	12.57	96.27	14.66	7.35	70.69	39.44	-170.71	0.87
04-06-05-102	Can	14.79	97.84	15.09	9.32	69.22	39.93	-146.92	1.13
04-06-05-103	Can	10.69	97.51	16.64	7.17	71.24	38.94	-97.63	0.46
04-06-05-104	Can	14.16	95.55	15.87	8.33	73.21	38.97	-188.86	0.95
04-06-05-105	Can	11.06	96.19	15.36	6.54	74.35	37.06	-148.83	0.77
04-06-05-106	Can	12.77	95.50	18.27	7.78	72.24	38.23	-106.35	0.55
04-06-05-107	Can	18.63	97.52	16.34	12.34	70.82	38.81	-206.16	1.43
04-06-05-108	Can	19.61	97.70	14.64	11.92	70.04	38.89	-218.66	1.40
04-06-05-25	Can	12.37	97.18	14.99	8.16	69.81	40.62	-140.91	0.62
04-06-05-26	Can	14.35	97.15	17.22	9.92	68.46	41.70	-168.62	0.87
04-06-05-27	Can	16.90	97.97	15.02	10.55	70.25	40.15	-135.49	0.69
04-06-05-28	Can	15.54	96.77	15.80	9.98	69.18	40.54	-146.16	1.25
04-06-05-29	Can	12.72	97.01	15.10	7.79	70.61	39.46	-184.95	0.84
04-06-05-30	Can	17.22	96.98	16.02	11.49	65.90	45.48	-152.12	0.77
04-06-05-31	Can	17.34	95.20	17.06	11.46	68.96	40.95	-267.01	1.08
04-06-05-32	Can	11.77	96.60	14.85	7.34	70.51	38.53	-88.25	0.80
04-06-05-33	Can	17.24	93.16	18.40	11.17	64.81	44.08	-232.23	1.19
04-06-05-34	Can	14.64	97.88	15.50	9.91	68.84	40.92	-155.37	0.70
04-06-05-35	Can	10.68	95.38	17.86	6.97	68.23	41.84	-196.29	0.83
04-06-05-36	Can	24.38	95.98	15.47	14.90	69.59	41.41	-249.31	1.91
04-06-05-49	Can	13.03	97.16	14.81	8.40	69.18	40.96	-256.87	1.07
04-06-05-50	Can	10.64	97.17	15.90	6.89	70.61	40.01	-124.77	0.58
04-06-05-51	Can	13.53	93.31	16.49	8.78	68.15	41.13	-105.06	1.07
04-06-05-52	Can	9.28	98.08	15.51	6.21	73.69	37.00	-104.22	0.46
04-06-05-53	Can	14.67	97.66	15.47	9.95	68.38	40.59	-215.11	0.96

04-06-05-54	Can	16.00	94.66	18.52	9.77	67.53	41.98	-154.39	1.16
04-06-05-55	Can	17.45	97.23	17.18	11.44	68.92	40.84	-259.18	1.22
04-06-05-56	Can	12.04	95.67	16.78	7.55	70.64	39.88	-100.08	0.52
04-06-05-57	Can	20.20	98.21	15.16	12.77	70.37	39.37	-151.37	0.81
04-06-05-58	Can	14.86	97.93	17.42	10.63	71.05	39.86	-279.42	1.21
04-06-05-59	Can	19.69	97.35	14.89	11.97	70.61	40.11	-186.95	1.24
04-06-05-60	Can	10.32	98.27	14.69	6.78	67.75	41.44	-76.19	0.38
04-06-05-73	Can	15.68	97.82	14.28	9.61	71.84	38.77	-165.91	0.75
04-06-05-74	Can	22.01	97.98	14.57	14.54	71.23	38.50	-296.24	1.23
04-06-05-75	Can	18.10	97.80	15.18	11.69	72.60	37.46	-204.03	0.63
04-06-05-76	Can	13.10	97.94	16.09	9.15	68.42	41.57	-206.16	0.84
04-06-05-77	Can	8.23	97.27	14.84	5.12	72.52	38.43	-65.34	0.11
04-06-05-78	Can	9.79	96.98	16.06	6.40	68.66	40.53	-76.46	0.38
04-06-05-79	Can	12.07	96.27	15.35	7.84	68.34	40.21	-206.83	1.09
04-06-05-80	Can	17.61	95.23	18.31	11.25	72.69	37.91	-221.24	1.38
04-06-05-81	Can	15.59	97.08	16.40	10.14	70.19	39.88	-149.41	1.05
04-06-05-82	Can	16.00	96.26	15.93	10.36	70.35	39.08	-275.51	1.18
04-06-05-83	Can	15.49	97.71	16.03	10.09	70.98	38.90	-119.78	1.08
04-06-05-84	Can	12.13	95.16	18.47	7.88	69.96	38.85	-173.38	0.94
04-06-05-97	Can	16.34	97.04	16.31	9.66	71.82	38.84	-204.12	0.84
04-06-05-98	Can	10.60	95.38	17.67	7.12	68.20	40.24	-109.78	0.52
05-05-05-100	Can	9.22	96.14	16.12	5.83	69.16	38.67	-151.85	0.72
05-05-05-101	Can	14.82	96.01	16.30	9.16	71.62	37.58	-203.76	0.92
05-05-05-102	Can	16.36	96.80	15.83	10.17	71.01	38.37	-218.35	0.95
05-05-05-103	Can	9.94	96.91	15.90	5.92	72.87	37.24	-144.07	0.70
05-05-05-104	Can	12.52	95.82	16.25	7.76	70.54	37.98	-167.16	0.91
05-05-05-105	Can	12.19	96.23	15.54	7.95	68.71	39.00	-224.67	0.94
05-05-05-106	Can	11.41	96.98	15.96	7.28	71.20	38.26	-207.01	0.85
05-05-05-107	Can	16.7	96.25	15.84	10.11	73.94	36.54	-231.38	1.35
05-05-05-108	Can	14.02	95.70	16.11	8.60	70.96	38.25	-191.13	1.14
05-05-05-61	Can	14.68	96.61	16.80	9.68	72.92	36.30	-259.85	1.04
05-05-05-62	Can	15.73	97.26	15.74	10.00	71.05	38.28	-248.06	1.27
05-05-05-63	Can	10.73	97.15	15.03	6.79	72.04	36.92	-161.60	0.81
05-05-05-64	Can	9.47	93.76	17.06	5.75	68.54	40.00	-126.59	0.75
05-05-05-65	Can	18.36	97.08	15.61	11.42	70.99	38.21	-241.66	1.18
05-05-05-66	Can	9.95	94.94	19.34	7.12	70.16	38.51	-161.51	0.69
05-05-05-67	Can	14.55	96.08	15.83	8.59	70.03	38.28	-190.77	1.00
05-05-05-68	Can	12.09	96.53	15.93	7.68	70.08	38.78	-204.12	0.90
05-05-05-69	Can	14.51	95.74	16.93	9.38	64.26	42.18	-263.77	1.33
05-05-05-70	Can	11.56	96.15	16.12	6.93	70.87	38.43	-153.46	0.66
05-05-05-71	Can	10.71	94.37	18.24	6.49	68.21	40.38	-157.41	0.69
05-05-05-72	Can	14.77	96.17	15.71	9.14	69.14	38.68	-201.36	1.22
05-05-05-73	Can	14.63	96.79	16.15	9.20	69.84	38.28	-215.99	0.90
05-05-05-74	Can	15.9	94.50	17.12	9.78	68.70	39.04	-209.28	0.99
05-05-05-75	Can	11.01	97.95	15.05	6.71	72.39	36.82	-140.65	0.76
05-05-05-76	Can	13.05	94.78	17.50	7.78	69.83	39.65	-206.16	1.08
05-05-05-77	Can	10.58	97.21	15.31	6.59	69.62	38.43	-146.21	0.60
05-05-05-78	Can	16.71	96.84	15.22	10.11	69.64	38.29	-214.26	1.02
05-05-05-79	Can	10.29	96.54	16.07	6.51	71.52	37.11	-132.06	0.61
05-05-05-80	Can	10.14	95.89	16.46	6.36	69.46	39.91	-123.39	0.63
05-05-05-81	Can	15.8	97.08	16.07	9.46	70.87	37.55	-196.87	0.99
05-05-05-82	Can	11.12	96.34	15.94	7.00	70.70	37.97	-151.63	0.70
05-05-05-83	Can	12.41	96.47	15.93	7.68	71.85	37.53	-198.51	1.00
05-05-05-84	Can	9.28	96.58	17.11	5.87	68.38	39.91	-163.55	0.69
05-05-05-85	Can	15.91	97.05	15.46	10.01	70.76	38.14	-166.22	0.66
05-05-05-86	Can	10.31	94.79	17.32	6.16	68.03	39.98	-133.88	0.56

05-05-05-87	Can	16.29	96.80	16.27	9.84	70.61	37.96	-231.25	1.35
05-05-05-88	Can	13.11	94.94	15.60	8.02	69.49	38.39	-254.20	1.16
05-05-05-89	Can	14.7	96.61	16.29	9.55	70.79	39.80	-146.21	1.02
05-05-05-90	Can	14.51	96.42	17.04	9.09	70.87	39.56	-196.69	0.91
05-05-05-91	Can	8.71	96.58	15.81	5.25	69.77	38.29	-117.47	0.59
05-05-05-92	Can	11.62	96.46	15.76	7.11	70.76	37.95	-181.48	0.78
05-05-05-93	Can	11.08	95.14	17.25	7.00	71.70	37.35	-149.68	0.80
05-05-05-94	Can	10.72	96.52	16.36	6.76	71.67	36.87	-166.22	0.66
05-05-05-95	Can	12.68	96.44	15.70	7.78	69.24	38.97	-202.43	1.20
05-05-05-96	Can	19.74	95.57	16.50	12.20	69.24	40.28	-261.54	1.20
05-05-05-97	Can	8.53	97.50	16.84	6.31	70.75	38.39	-173.96	0.83
05-05-05-98	Can	16.3	94.39	16.78	10.07	69.44	39.07	-214.17	0.99
05-05-05-99	Can	12.66	94.06	17.00	8.15	67.49	40.17	-214.17	0.99
05-11-05-100	Can	12.18	95.10	15.93	8.10	67.39	42.29	-197.36	1.11
05-11-05-101	Can	17.74	95.95	16.44	12.20	67.37	42.04	-264.21	1.51
05-11-05-102	Can	12.03	96.18	18.98	8.20	68.16	41.81	-194.87	0.97
05-11-05-103	Can	16.58	97.04	14.89	11.10	69.83	41.67	-223.07	1.23
05-11-05-104	Can	13.56	96.01	17.53	8.70	68.55	41.21	-193.53	1.26
05-11-05-105	Can	15.16	96.57	16.08	10.50	68.66	43.39	-224.76	1.13
05-11-05-106	Can	10.44	96.25	17.08	7.00	69.27	40.91	-154.48	0.73
05-11-05-107	Can	13.11	95.27	16.19	9.70	69.27	40.65	-264.03	1.12
05-11-05-108	Can	9.53	96.51	15.61	6.20	69.73	41.11	-134.82	0.63
05-11-05-61	Can	15.62	96.55	16.32	9.44	69.84	41.87	-217.15	1.32
05-11-05-62	Can	19.88	93.43	17.10	13.50	66.37	42.50	-344.01	1.52
05-11-05-63	Can	19.56	95.88	16.98	13.20	66.31	42.25	-469.71	2.36
05-11-05-64	Can	10.13	96.27	18.86	7.30	66.45	44.00	-145.49	0.56
05-11-05-65	Can	14.47	95.31	16.60	9.60	66.60	43.59	-182.63	0.88
05-11-05-66	Can	18.12	97.03	16.72	12.40	67.61	43.86	-315.32	1.50
05-11-05-67	Can	14.02	95.88	16.83	9.30	66.72	43.09	-218.66	1.08
05-11-05-68	Can	22.70	95.10	17.40	15.00	67.88	43.49	-393.56	1.72
05-11-05-69	Can	18.65	94.84	16.26	12.60	67.67	41.93	-285.16	1.29
05-11-05-70	Can	15.08	94.96	16.66	9.70	67.09	42.97	-232.05	1.31
05-11-05-71	Can	16.62	94.88	16.05	11.30	67.08	42.59	-261.72	1.46
05-11-05-72	Can	16.65	95.35	16.99	10.80	66.58	43.31	-245.22	1.18
05-11-05-73	Can	12.32	95.53	16.23	8.20	67.90	42.48	-190.95	0.97
05-11-05-74	Can	23.93	95.82	15.96	16.50	68.97	41.43	-460.37	2.11
05-11-05-75	Can	15.37	95.65	16.68	10.30	68.84	41.69	-207.10	0.99
05-11-05-76	Can	19.94	95.03	16.70	13.20	68.20	41.66	-290.19	1.62
05-11-05-77	Can	13.62	94.79	18.89	9.00	68.83	41.45	-242.15	1.08
05-11-05-78	Can	10.40	95.78	16.19	6.80	68.54	41.38	-181.03	0.78
05-11-05-79	Can	14.29	96.25	15.65	9.70	67.93	41.60	-192.38	0.91
05-11-05-80	Can	14.65	96.73	15.06	9.90	69.65	41.60	-206.25	1.04
05-11-05-81	Can	13.13	96.40	17.23	8.70	70.32	40.26	-197.27	1.27
05-11-05-82	Can	16.51	96.62	15.67	10.70	69.93	41.92	-269.59	1.35
05-11-05-83	Can	16.12	96.46	16.24	10.60	69.74	40.96	-223.73	1.13
05-11-05-84	Can	14.47	94.82	16.77	9.90	68.79	42.44	-236.90	1.48
05-11-05-85	Can	15.19	96.54	15.70	9.90	69.42	40.88	-201.18	0.84
05-11-05-86	Can	21.17	94.93	16.73	13.80	69.60	41.06	-331.02	1.62
05-11-05-87	Can	9.45	94.50	17.02	6.40	68.89	41.04	-155.55	0.74
05-11-05-88	Can	20.61	95.66	15.63	13.80	68.14	41.76	-309.40	1.69
05-11-05-89	Can	19.72	95.09	17.00	13.10	67.77	42.40	-287.52	1.55
05-11-05-90	Can	8.94	95.13	16.29	7.00	68.46	43.40	-148.43	0.92
05-11-05-91	Can	12.43	93.32	16.68	8.50	69.09	40.84	-211.59	0.78
05-11-05-92	Can	10.46	96.05	16.00	7.00	68.31	41.86	-132.46	0.73
05-11-05-93	Can	14.26	96.30	15.21	9.80	68.70	42.53	-181.48	1.11
05-11-05-94	Can	13.76	95.84	16.13	9.30	69.53	41.35	-173.07	0.94

05-11-05-95	Can	18.30	96.70	15.08	12.10	66.14	43.13	-278.27	1.34
05-11-05-96	Can	11.94	94.92	17.57	7.70	69.31	40.90	-195.45	0.87
05-11-05-97	Can	18.18	96.92	15.38	12.50	66.94	42.09	-339.83	1.25
05-11-05-98	Can	9.72	97.18	14.79	6.70	68.68	42.59	-143.23	0.84
05-11-05-99	Can	18.24	94.30	16.04	12.60	68.82	41.58	-143.23	0.84
08-04-05-100	Can	12.72	94.01	16.15	8.80	66.07	43.13	-241.13	1.22
08-04-05-101	Can	14.59	95.61	16.93	9.50	65.64	44.20	-282.18	1.29
08-04-05-102	Can	16.97	94.49	17.19	11.73	66.26	43.16	-272.71	1.22
08-04-05-103	Can	15.00	92.78	18.20	10.12	66.37	43.20	-196.60	0.95
08-04-05-104	Can	14.77	91.61	19.34	10.90	64.49	44.35	-270.62	1.37
08-04-05-105	Can	18.88	94.34	17.76	12.48	60.71	46.09	-344.19	1.46
08-04-05-106	Can	13.20	93.02	18.57	9.15	65.72	43.42	-250.56	1.16
08-04-05-107	Can	10.14	95.91	16.67	6.55	67.44	43.37	-135.09	0.81
08-04-05-108	Can	13.14	94.44	17.43	9.29	65.10	44.14	-218.09	0.91
08-04-05-61	Can	21.51	94.94	15.75	14.65	67.48	42.65	-408.37	1.84
08-04-05-62	Can	14.00	94.61	15.50	9.64	60.24	45.96	0.00	0.00
08-04-05-63	Can	11.11	92.95	18.69	8.29	64.54	44.27	-240.73	1.09
08-04-05-64	Can	23.29	96.78	16.66	16.03	66.96	43.96	-375.41	2.23
08-04-05-65	Can	22.22	96.48	17.29	16.29	66.45	44.47	-481.27	2.13
08-04-05-66	Can	16.00	95.75	16.85	10.87	65.62	44.76	-253.22	1.30
08-04-05-67	Can	13.43	95.05	16.88	8.82	68.33	42.07	-185.70	0.91
08-04-05-68	Can	9.72	92.00	16.65	6.89	62.85	45.06	-159.86	0.68
08-04-05-69	Can	11.85	93.62	18.85	8.10	66.08	44.24	-132.24	0.74
08-04-05-70	Can	17.97	94.20	16.52	12.73	67.94	41.10	-321.68	1.74
08-04-05-71	Can	12.97	93.91	17.13	8.05	59.81	47.23	-185.21	0.86
08-04-05-72	Can	11.34	95.61	16.80	7.38	65.74	43.18	-216.57	0.85
08-04-05-73	Can	12.60	96.21	16.20	8.74	62.99	45.36	-170.89	1.04
08-04-05-74	Can	17.31	93.82	18.53	11.60	66.10	43.88	-361.58	1.75
08-04-05-75	Can	21.47	92.84	17.72	14.35	65.09	44.41	-410.51	1.73
08-04-05-76	Can	11.32	94.68	17.36	7.21	65.90	43.93	-204.83	0.72
08-04-05-77	Can	19.86	95.39	18.02	14.69	60.00	48.57	-398.36	1.76
08-04-05-78	Can	10.44	97.61	17.88	8.48	69.87	43.08	-163.78	0.78
08-04-05-79	Can	18.06	95.05	16.33	12.38	64.86	44.20	-309.89	1.50
08-04-05-80	Can	10.17	92.38	18.26	7.10	66.23	43.41	-188.28	0.90
08-04-05-81	Can	11.72	93.57	17.01	7.97	64.94	44.33	-230.50	1.00
08-04-05-82	Can	12.50	93.79	19.12	8.90	65.90	44.51	-215.91	0.96
08-04-05-83	Can	13.65	97.12	15.24	9.50	63.61	44.66	-212.08	1.23
08-04-05-84	Can	20.03	94.40	17.14	14.05	65.99	43.30	-273.20	1.04
08-04-05-87	Can	14.16	95.82	16.67	9.41	67.78	43.10	-199.09	1.10
08-04-05-88	Can	13.74	94.63	15.51	9.82	58.00	47.83	-208.43	0.90
08-04-05-89	Can	13.27	95.13	17.68	8.87	64.52	44.60	-251.05	1.15
08-04-05-90	Can	21.13	95.69	16.71	14.51	64.28	44.17	-408.15	2.01
08-04-05-91	Can	14.46	92.05	18.18	9.92	65.52	43.73	-226.00	1.28
08-04-05-92	Can	14.94	94.00	17.25	10.93	60.18	46.19	-303.22	1.50
08-04-05-93	Can	17.52	92.30	18.46	12.36	63.29	43.98	-303.31	1.69
08-04-05-94	Can	15.13	94.47	17.37	9.94	63.98	44.55	-264.03	1.06
08-04-05-95	Can	13.27	92.88	17.51	8.89	64.06	44.00	-281.42	1.11
08-04-05-96	Can	16.43	94.71	17.02	10.88	66.47	43.01	-278.36	1.48
08-04-05-97	Can	17.51	96.29	16.75	11.99	67.69	42.91	-264.30	1.51
08-04-05-98	Can	9.44	95.56	17.07	6.30	65.76	43.73	-127.88	0.50
08-04-05-99	Can	12.17	92.65	18.24	8.46	63.50	44.58	-127.88	0.50
04-06-05-01	RAW	18.61						-429.41	3.57
04-06-05-02	RAW	9.37						-133.17	1.24
04-06-05-03	RAW	14.18						-325.19	2.09
04-06-05-04	RAW	9.90						-199.45	1.26
04-06-05-05	RAW	16.64						-353.22	2.69

04-06-05-06	RAW	13.63	-315.41	2.19
04-06-05-07	RAW	17.18	-305.89	2.99
04-06-05-08	RAW	18.03	-361.31	2.78
04-06-05-09	RAW	19.39	-378.30	3.50
04-06-05-10	RAW	15.51	-339.78	2.25
04-06-05-11	RAW	16.72	-330.62	2.64
04-06-05-12	RAW	11.80	-183.21	1.80
05-05-05-01	RAW	10.68	-230.14	2.14
05-05-05-02	RAW	12.4	-258.96	1.93
05-05-05-03	RAW	13.5	-288.99	2.84
05-05-05-04	RAW	14.55	-308.25	2.82
05-05-05-05	RAW	9.83	-192.29	2.02
05-05-05-06	RAW	18.31	-447.91	3.13
05-05-05-07	RAW	9.16	-165.82	1.15
05-05-05-08	RAW	9.57	-210.92	1.95
05-05-05-09	RAW	13.75	-267.46	1.93
05-05-05-10	RAW	11	-243.31	1.48
05-05-05-11	RAW	10.48	-291.66	1.68
05-05-05-12	RAW	20.11	-424.87	3.41
05-11-05-01	RAW	20.52	-500.40	3.94
05-11-05-02	RAW	11.12	-262.79	2.06
05-11-05-03	RAW	14.45	-341.52	3.52
05-11-05-04	RAW	10.81	-254.20	1.80
05-11-05-05	RAW	12.50	-287.43	2.46
05-11-05-06	RAW	21.78	-552.89	4.61
05-11-05-07	RAW	16.90	-480.38	3.48
05-11-05-08	RAW	10.72	-256.47	1.93
05-11-05-09	RAW	20.83	-457.25	3.92
05-11-05-10	RAW	11.06	-257.36	2.04
05-11-05-11	RAW	11.53	-289.25	2.36
05-11-05-12	RAW	10.90	-245.40	1.91
08-04-05-01	RAW	18.02	-423.89	2.86
08-04-05-02	RAW	11.12	-204.61	1.30
08-04-05-03	RAW	12.74	-257.36	1.97
08-04-05-04	RAW	18.28	-400.28	3.00
08-04-05-05	RAW	20.47	-500.84	3.96
08-04-05-06	RAW	18.31	-435.37	3.59
08-04-05-07	RAW	15.23	-246.46	2.03
08-04-05-08	RAW	13.34	-359.89	2.22
08-04-05-09	RAW	11.31	-251.80	2.04
08-04-05-10	RAW	16.52	-380.66	2.60
08-04-05-11	RAW	15.27	-351.53	2.49
08-04-05-12	RAW	16.13	-371.14	2.61

Appendix C

Statistical Analysis of Quality Data

Yield

Whole Mushrooms

Analysis of Variance (ANOVA) was used to determine the effects of mushroom crop, processing method and crop and processing method interaction during yield studies of whole mushrooms processed aseptically and canned (Table C.1).

Table C.1: ANOVA: Response variable yield for aseptically processed and canned whole mushrooms.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Crop	3	0.1904	0.0629	59.81	0.000
Processing Method	1	0.1785	0.1780	169.21	0.000
Crop*Processing Method	3	0.0118	0.0039	3.75	0.011
Error	369	0.3882	0.0011		
Total	376	0.7689			

Before testing the hypothesis it was necessary to check the model for outliers, equal variance, interaction and normality. Plots of the standardized residuals versus mushroom crop (Figure **C.1**) and processing method (Figure **C.2**) revealed one moderate outlier for crop 3, processing method 1 (Aseptic). However, given the natural variation inherent in mushrooms, a standardized residual of 7.42 is not unusual for 377 observations. The residual plots also suggested equal variance between crops.

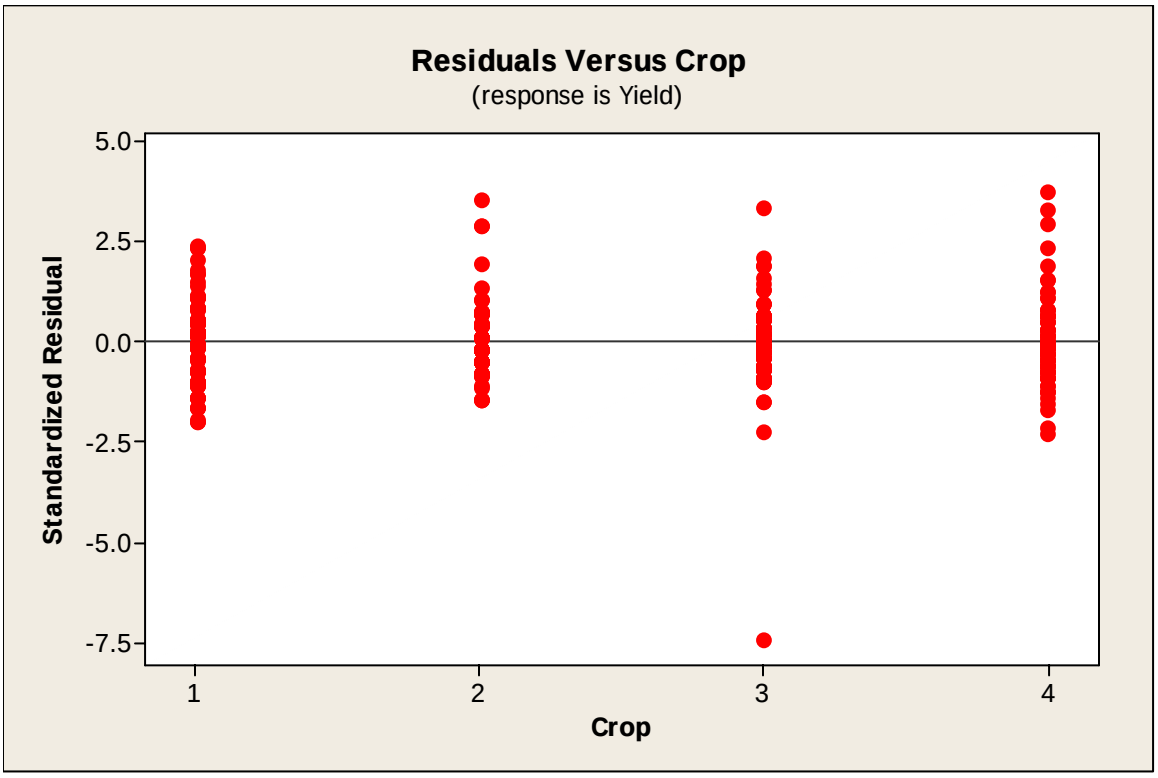

Figure **C.1**: Standardized residuals versus mushroom crop for response variable yield of whole mushrooms.

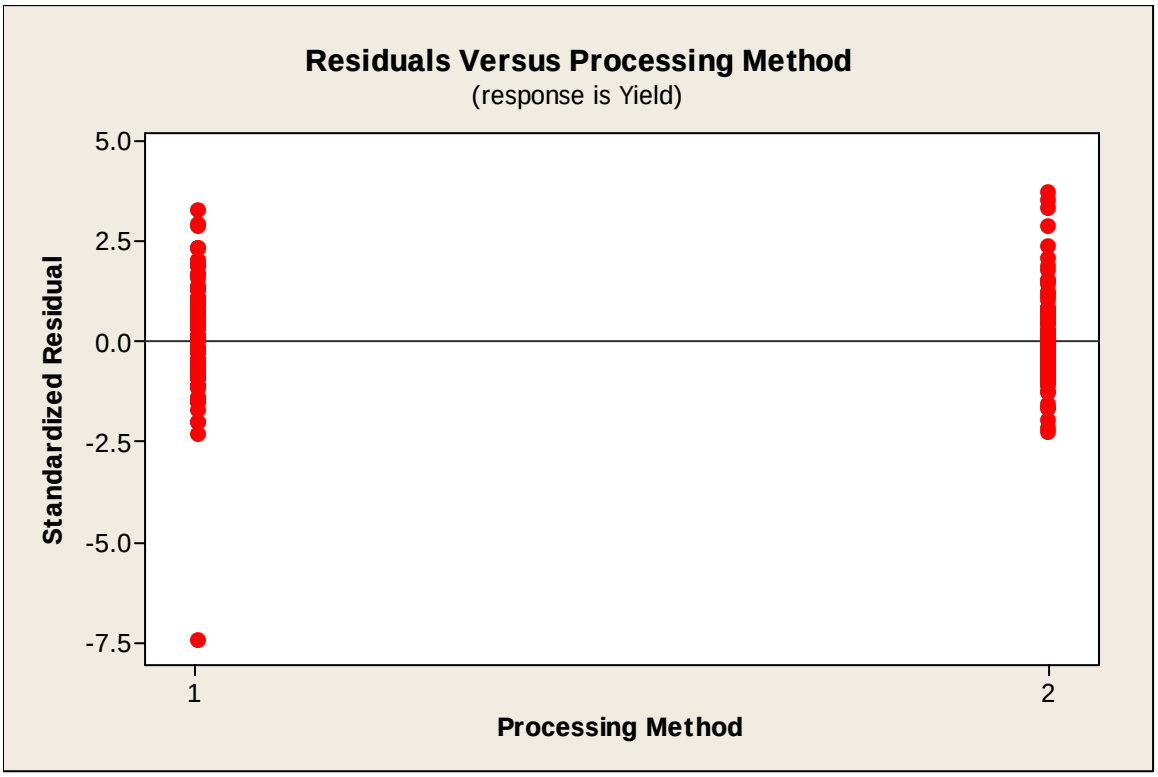

Figure C.2: Standardized residuals versus mushroom crop for response variable yield of whole mushrooms.

The plot of standardized residuals versus fits (Figure C.3) shows no apparent patterns to suggest either interaction between crop and processing method or a relationship between mean and variance.

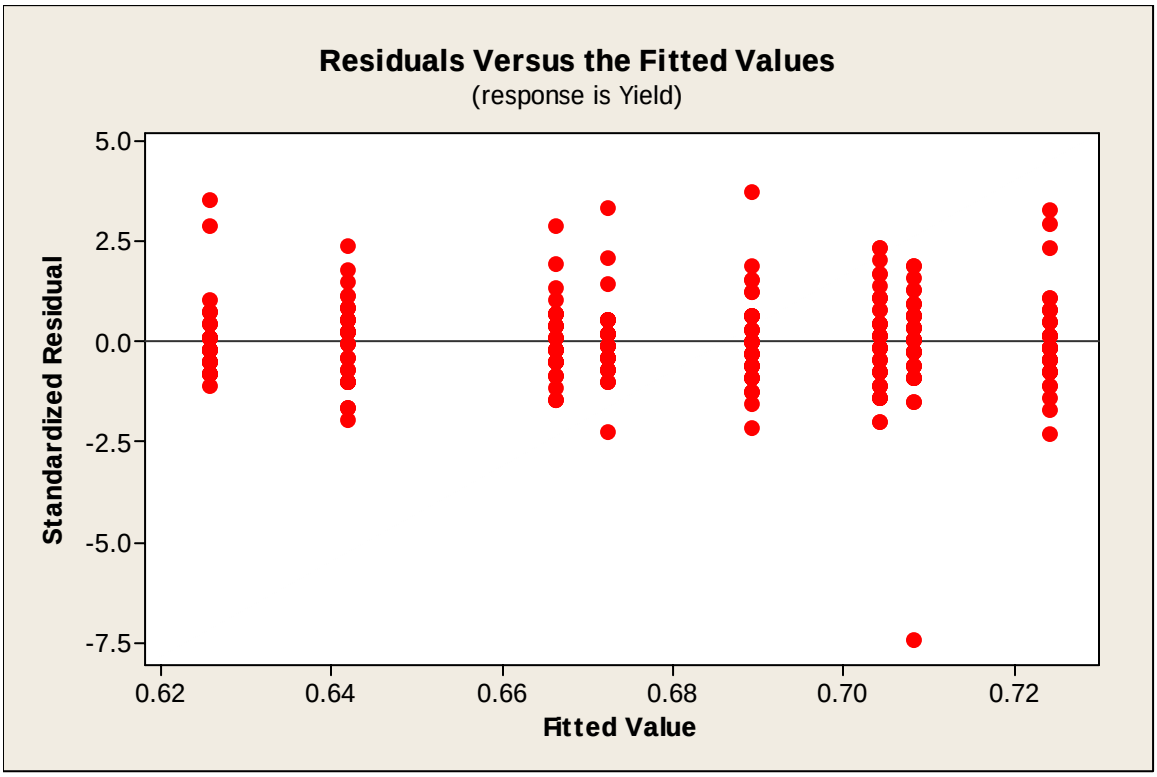

Figure **C.3**: Residuals versus the fitted values for response variable yield of whole mushrooms.

The normal probability plot (Figure **C.4**) seemed consistent with the normality of errors.

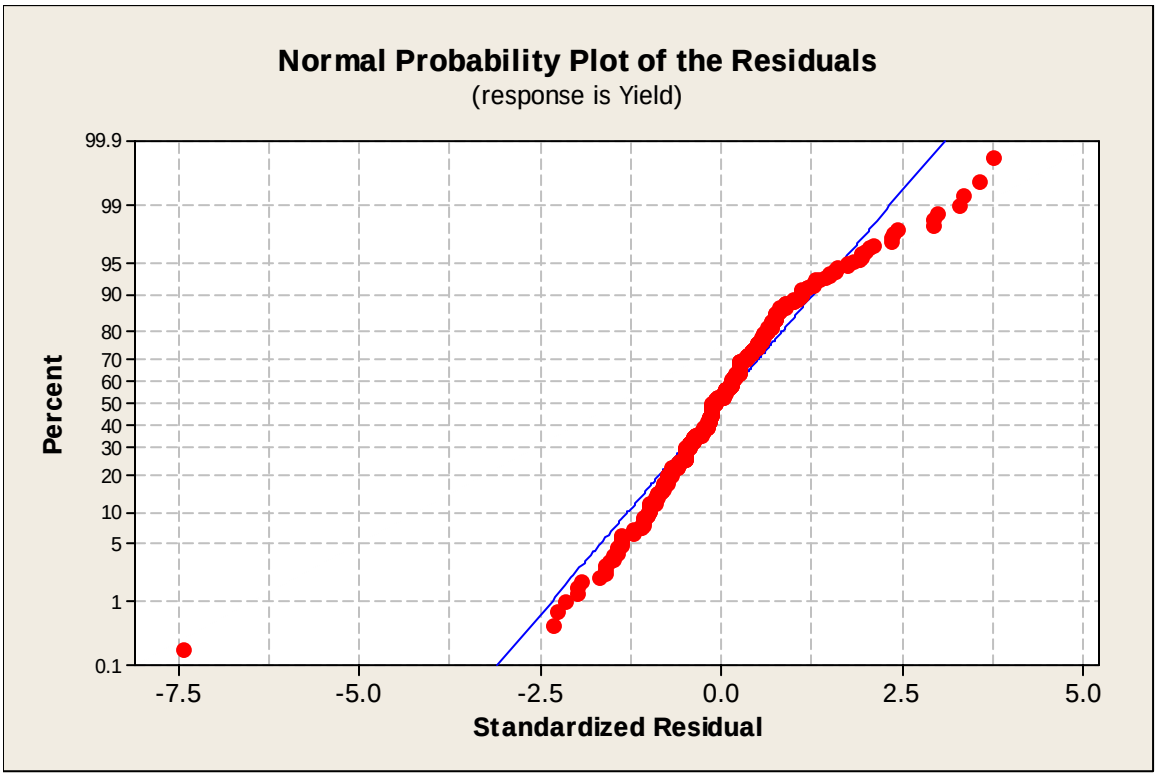

Figure **C.4**: Normality probability plot of the residuals for response variable yield of whole mushrooms.

Thus, it can be inferred that since the p-value = 0.000 < 0.05, the null hypothesis was rejected, indicating aseptic and canned processing methods did not have the same mean yield. This was further validated by employing a Tukey Least Significant Difference (LSD) test using 95% simultaneous confidence intervals (Figure **C.5**). Since zero was not contained in the confidence interval, the null hypothesis was rejected. In addition, the p-value =0.011 < 0.05, indicated that significant interaction between mushroom crop and processing method existed.

```
Processing Method = 1  subtracted from:

Processing
Method         Lower    Center     Upper  ---+---------+---------+---------+---
2           -0.05004  -0.04347  -0.03690  (---*---)
                                           ---+---------+---------+---------+---
                                           -0.045    -0.030    -0.015     0.000
```

Figure **C.5**: Tukey 95% simultaneous confidence interval for all pairwise comparisons among levels of processing method for response variable yield of whole mushrooms.

Sliced Mushrooms

Analysis of Variance (ANOVA) was used to determine the effects of mushroom crop, processing method and crop and processing method interaction during yield studies of sliced mushrooms processed aseptically and canned (Table **C.2**).

Table **C.2**: ANOVA: Response variable yield for aseptically processed and canned sliced mushrooms.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Crop	2	0.0765	0.0387	39.90	0.000
Processing Method	1	0.1093	0.0997	102.71	0.000
Crop*Processing Method	2	0.0047	0.0024	2.43	0.091
Error	230	0.2232	0.0010		
Total	235	0.4137			

Before testing the hypothesis it was necessary to check the model for outliers, equal variance, interaction and normality. Plots of the standardized residuals versus mushroom crop (Figure **C.6**) and processing method (Figure **C.7**) revealed one moderate outlier for crop 3, processing method 2

(Canned). However, given the natural variation inherent in mushrooms, a standardized residual of 6.25 is not unusual for 286 observations. The residual plots also suggested equal variance between crops.

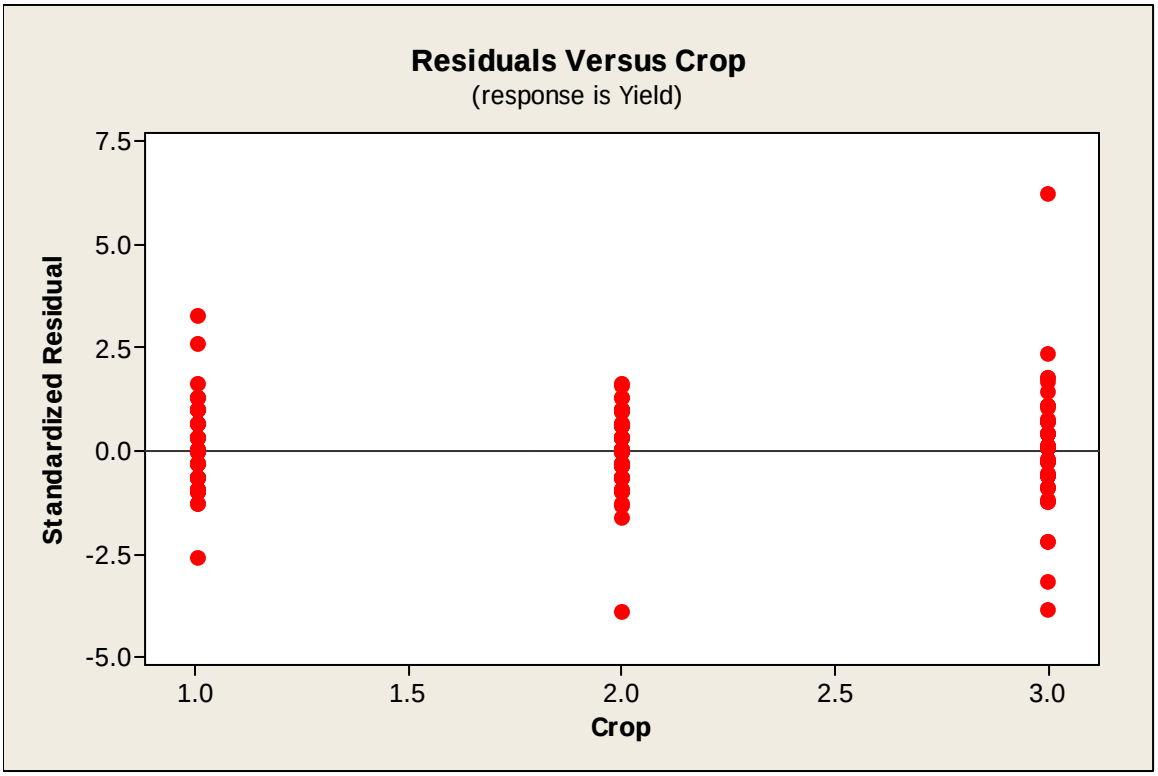

Figure C.6: Standardized residuals versus mushroom crop for response variable yield of sliced mushrooms.

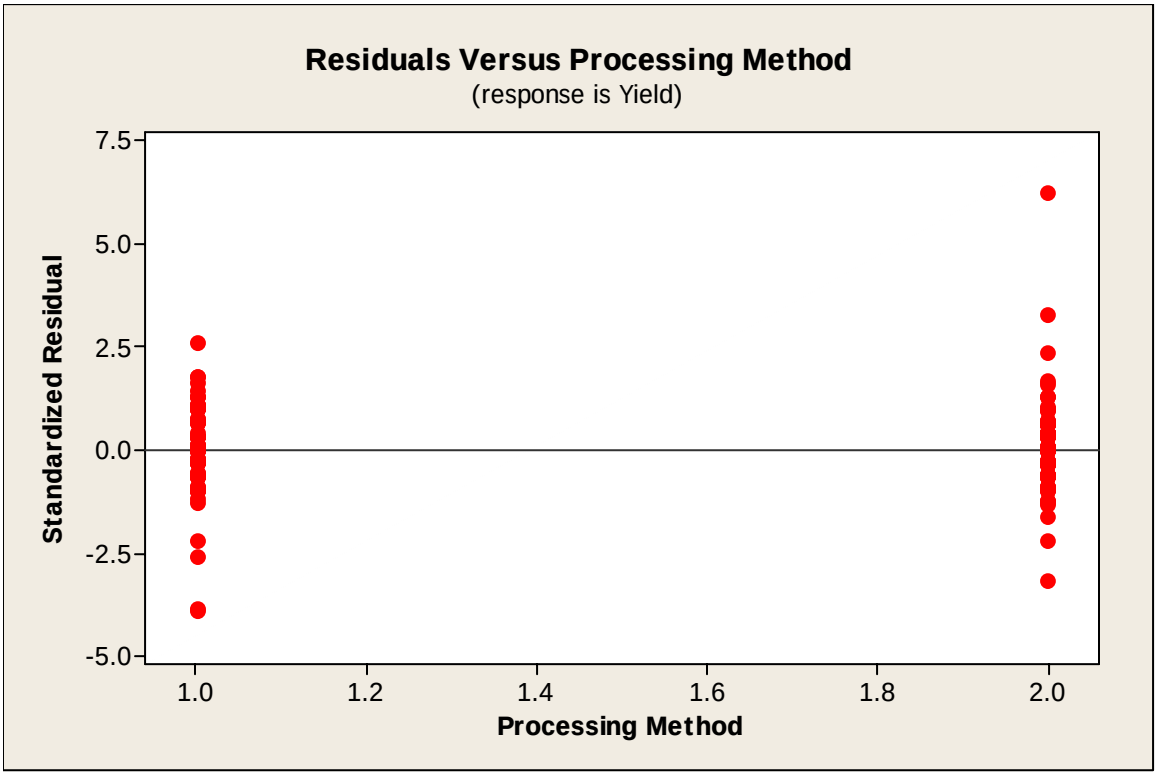

Figure C.7: Standardized residuals versus processing method for response variable yield of whole mushrooms.

The plot of standardized residuals versus fits (Figure C.8) shows no apparent patterns to suggest either interaction between crop and processing method or a relationship between mean and variance.

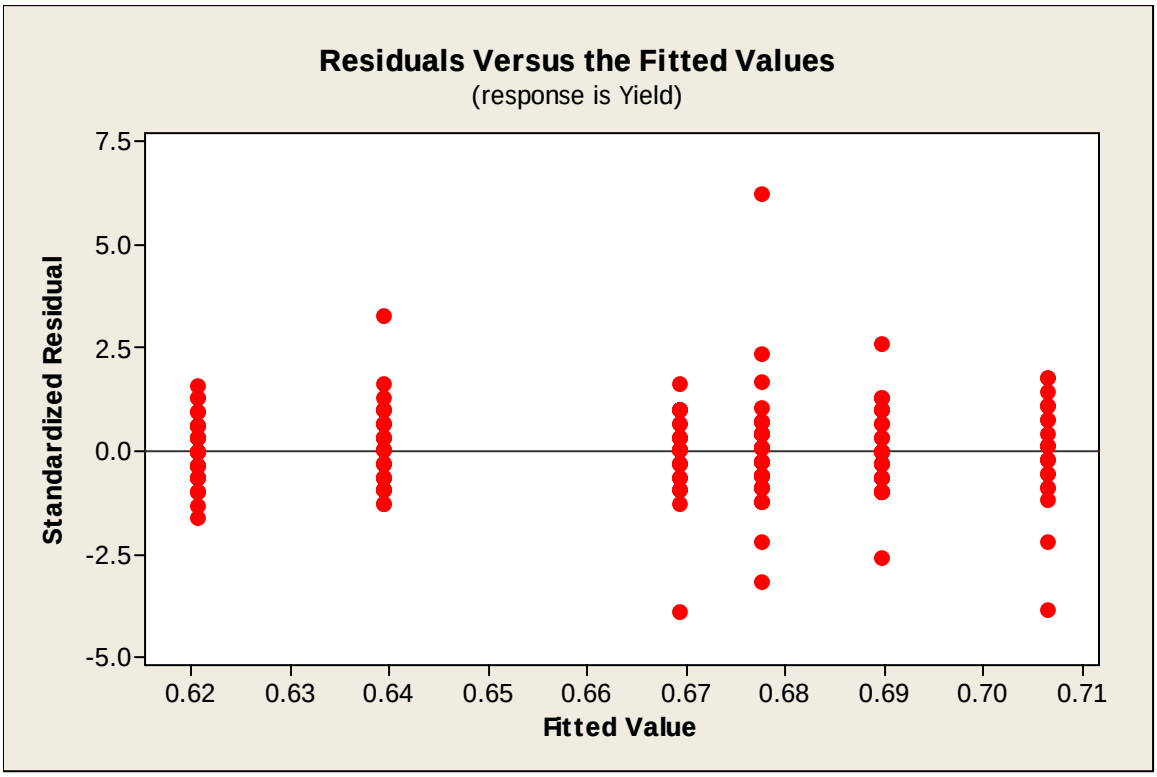

Figure C.8: Residuals versus the fitted values for response variable yield of sliced mushrooms.

The normal probability plot (Figure C.9) exhibited some tailing from straight line. Since, transformation of the data did not significantly improve tailing and other than tailing, the data seemed consistent with the normality of errors, no transformations were made.

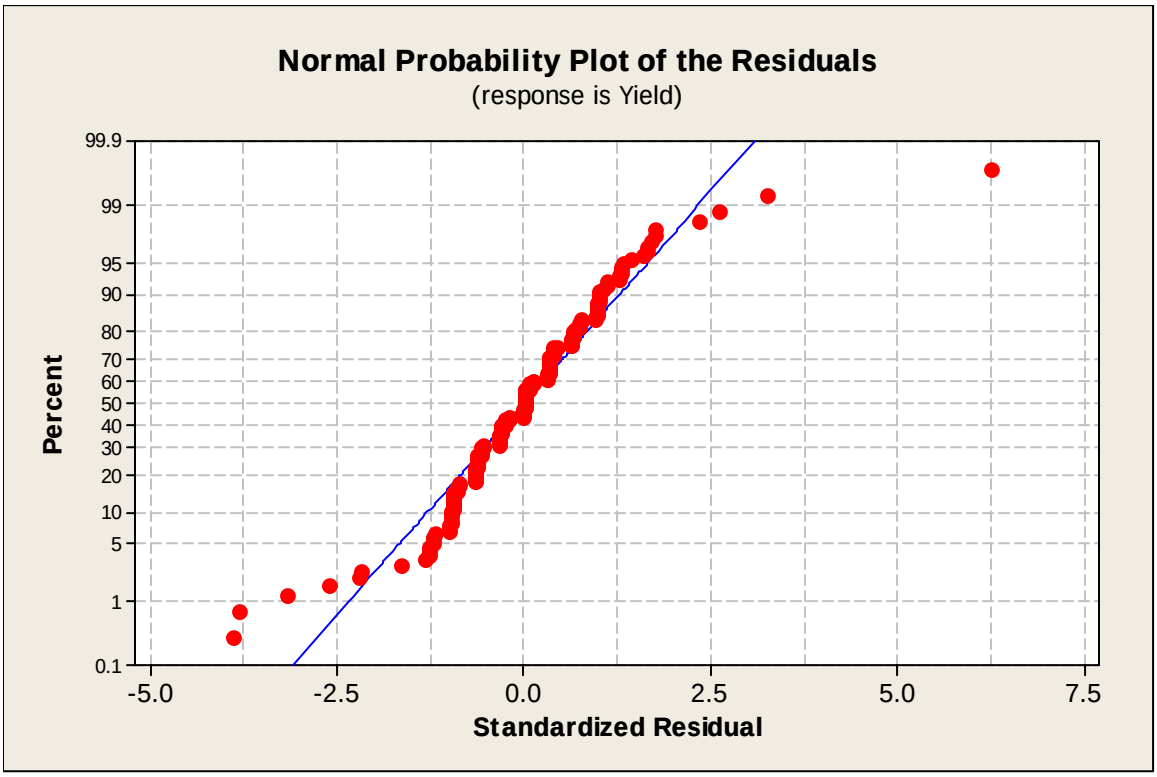

Figure **C.9**: Normality probability plot of the residuals for response variable yield of sliced mushrooms.

Thus, it can be inferred that since the p-value = 0.000 < 0.05, the null hypothesis was rejected, indicating aseptic and canned processing methods did not have the same mean yield. This was further validated by employing a Tukey Least Significant Difference (LSD) test using 95% simultaneous confidence intervals (Figure **C.10**). Since zero was not contained in the confidence interval, the null hypothesis was rejected. In addition, the p-value =0.091 > 0.05, indicated that no significant interaction between mushroom crop and processing method existed.

```
Processing Method = 1  subtracted from:

Processing
Method         Lower    Center     Upper  ----+---------+---------+---------+--
2           -0.05094  -0.04265  -0.03436  (-----*----)
                                           ----+---------+---------+---------+--
                                            -0.045    -0.030    -0.015     0.000
```

Figure **C.10**: Tukey 95% simultaneous confidence interval for all pairwise comparisons among levels of processing method for response variable yield of sliced mushrooms.

L value

Whole Mushrooms

Analysis of Variance (ANOVA) was used to determine the effects of mushroom crop, processing method, and crop and processing method interaction during L value studies of whole mushrooms processed aseptically and canned (Table **C.3**).

Table **C.3**: ANOVA: Response variable L value for aseptically processed and canned whole mushrooms.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Crop	3	0.1402	0.0470	141.16	0.000
Processing Method	1	0.0621	0.0619	186.11	0.000
Crop*Processing Method	3	0.0057	0.0019	5.72	0.001
Error	369	0.1228	0.0003		
Total	376	0.3309			

Before testing the hypothesis it was necessary to check the model for outliers, equal variance, interaction and normality. Plots of the standardized residuals versus mushroom crop (Figure C.11) and processing method (Figure C.12) revealed several observations with large standardized residuals. However, given the natural variation inherent in mushrooms, the residual plots suggested equal variance between crops.

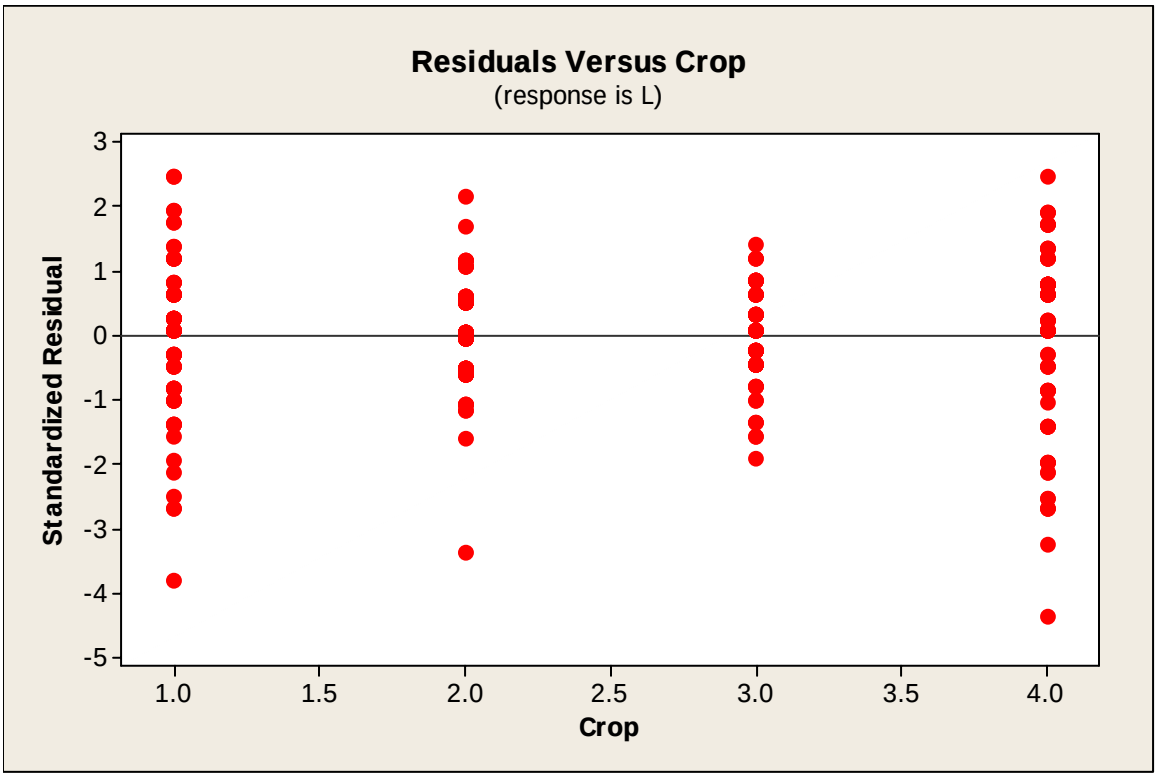

Figure C.11: Standardized residuals versus mushroom crop for response variable L value of whole mushrooms.

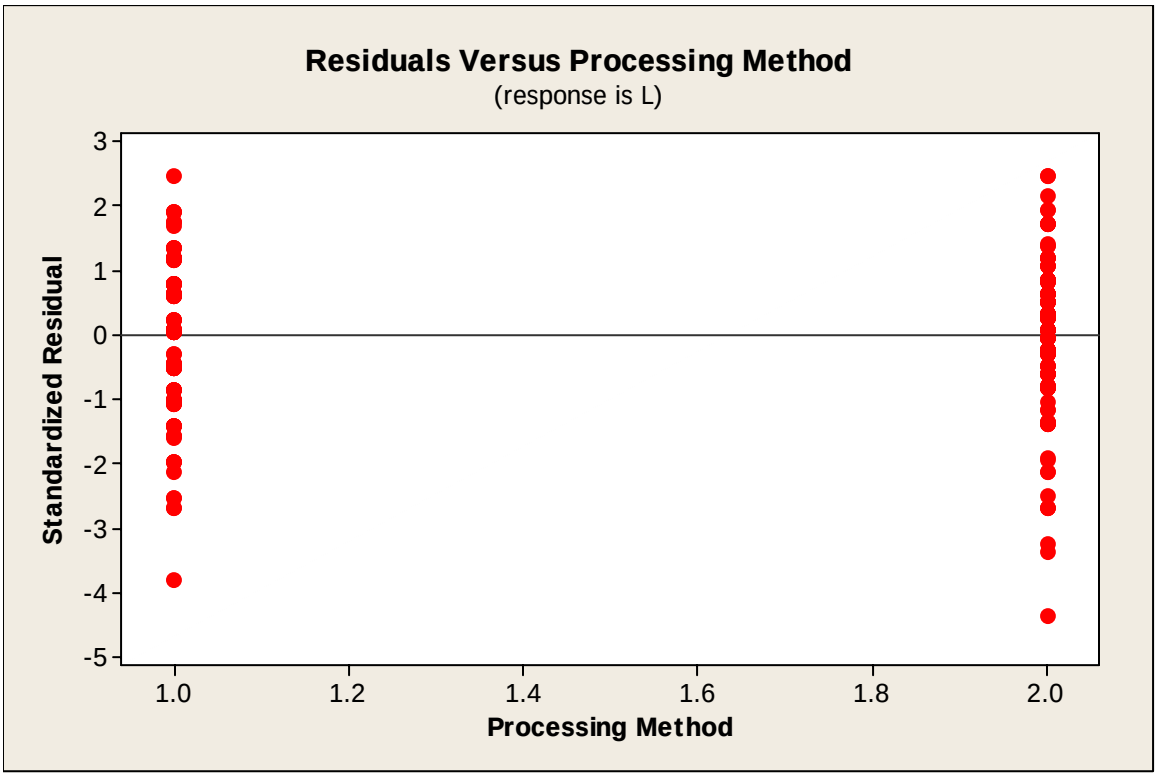

Figure C.12: Standardized residuals versus mushroom crop for response variable L value of whole mushrooms.

The plot of standardized residuals versus fits (Figure C.13) shows no apparent patterns to suggest either interaction between crop and processing method or a relationship between mean and variance.

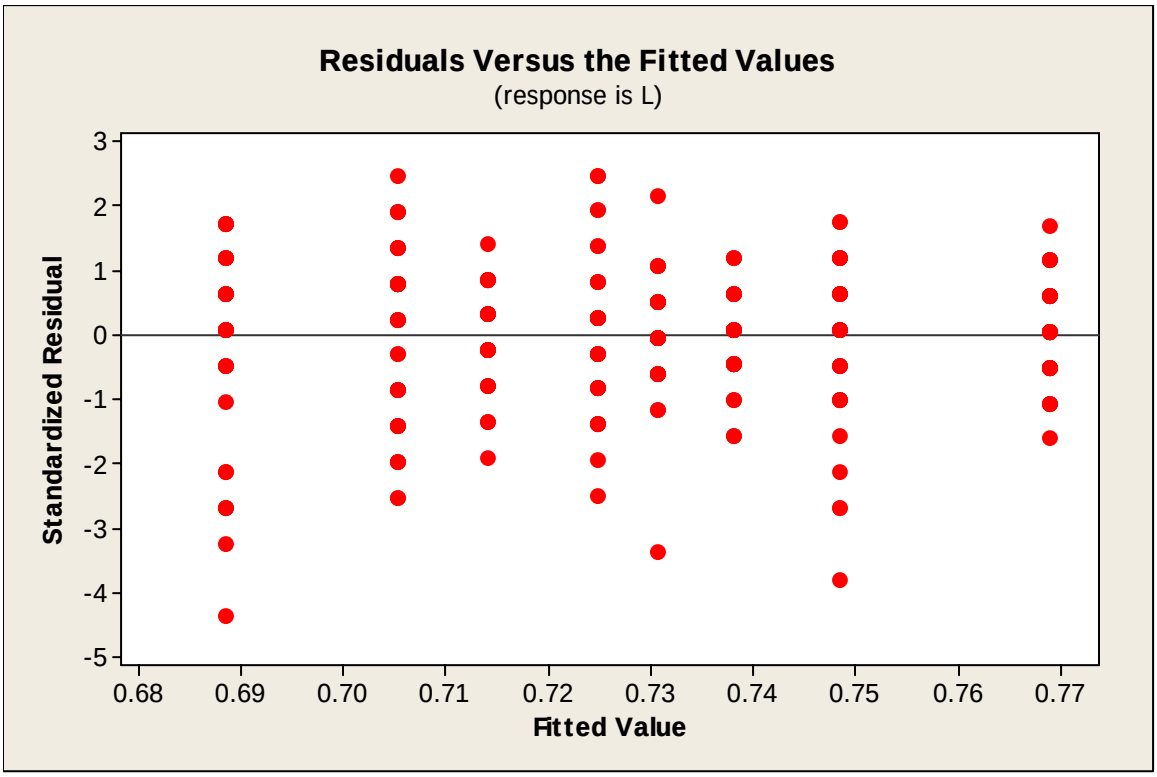

Figure **C.13**: Residuals versus the fitted values for response variable L value of whole mushrooms.

The normal probability plot (Figure **C.14**) exhibited slight tailing, but seemed consistent with the normality of errors.

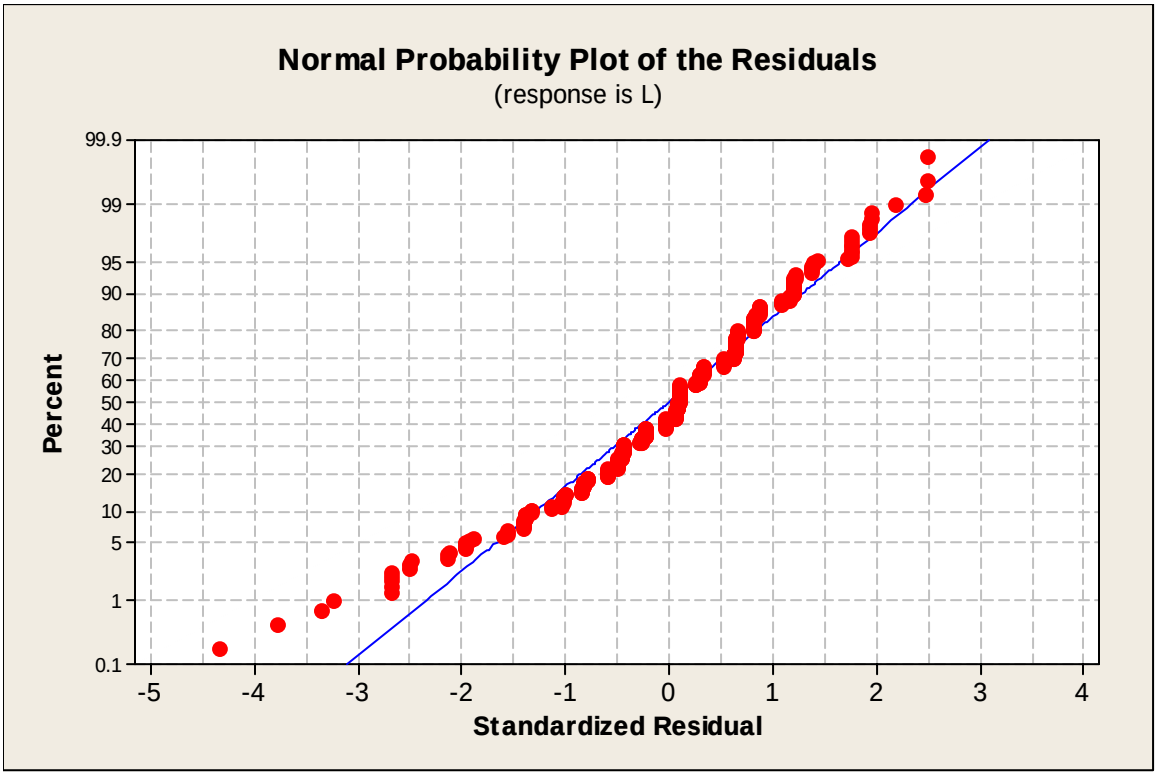

Figure **C.14**: Normality probability plot of the residuals for response variable L value of whole mushrooms.

Thus, it can be inferred that since the p-value = 0.000 < 0.05, the null hypothesis was rejected, indicating aseptic and canned processing methods did not have the same mean L value. This was further validated by employing a Tukey Least Significant Difference (LSD) test using 95% simultaneous confidence intervals (Figure **C.15**). Since zero was not contained in the confidence interval, the null hypothesis was rejected. In addition, the p-value = 0.001 < 0.05, indicated that significant interaction between mushroom crop and processing method existed.

```
Processing Method = 1  subtracted from:

Processing
Method         Lower    Center     Upper  -------+---------+---------+---------
2           -0.02934  -0.02564  -0.02195  (----*----)
                                           -------+---------+---------+---------
                                               -0.0240   -0.0160   -0.0080
```

Figure **C.15**: Tukey 95% simultaneous confidence interval for all pairwise comparisons among levels of processing method for response variable L value of whole mushrooms.

Sliced Mushrooms

Analysis of Variance (ANOVA) was used to determine the effects of mushroom crop, processing method and crop and processing method interaction during L value studies of sliced mushrooms processed aseptically and canned (Table **C.4**).

Table **C.4**: ANOVA: Response variable L value for aseptically processed and canned sliced mushrooms.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Crop	2	0.0283	0.0138	22.43	0.000
Processing Method	1	0.0635	0.0636	103.54	0.000
Crop*Processing Method	2	0.0007	0.0004	0.59	0.556
Error	212	0.1301	0.0006		
Total	217	0.2227			

Before testing the hypothesis it was necessary to check the model for outliers, equal variance, interaction and normality. Plots of the standardized residuals versus mushroom crop (Figure **C.16**) and processing method

(Figure C.17) revealed several observations with large standardized residuals. However, given the natural variation inherent in mushrooms, the residual plots suggested equal variance between crops.

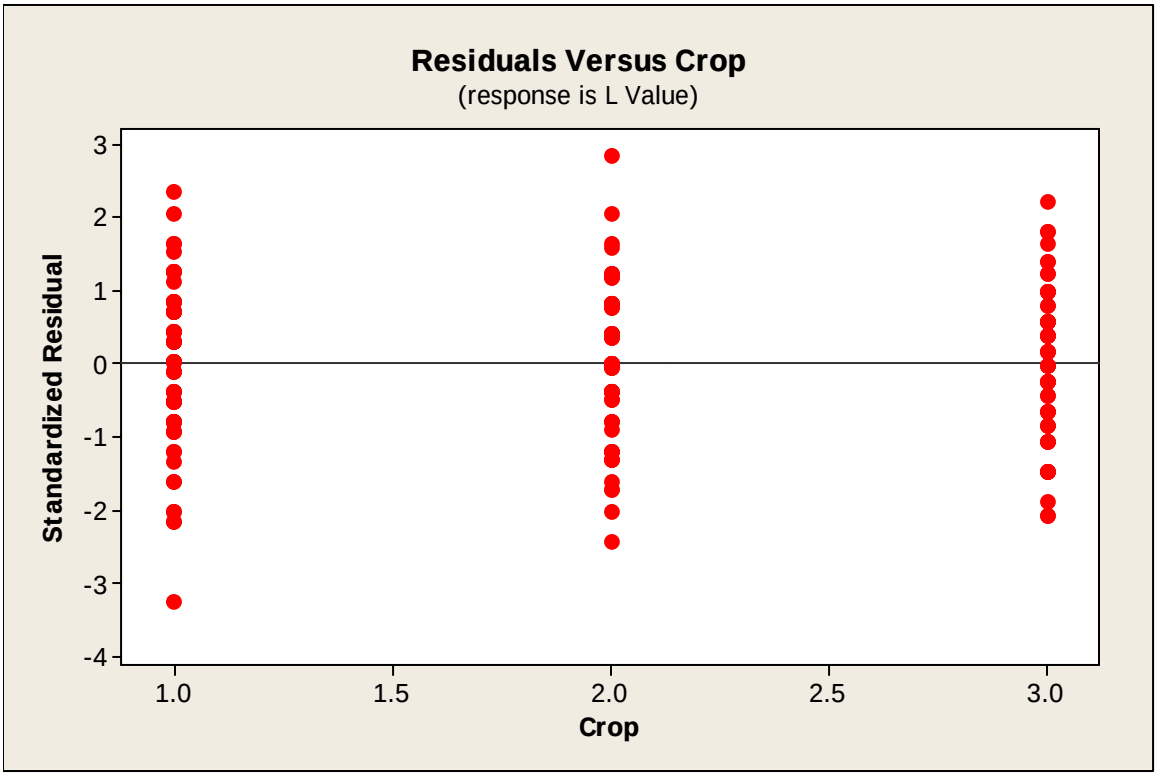

Figure C.16: Standardized residuals versus mushroom crop for response variable L value of sliced mushrooms.

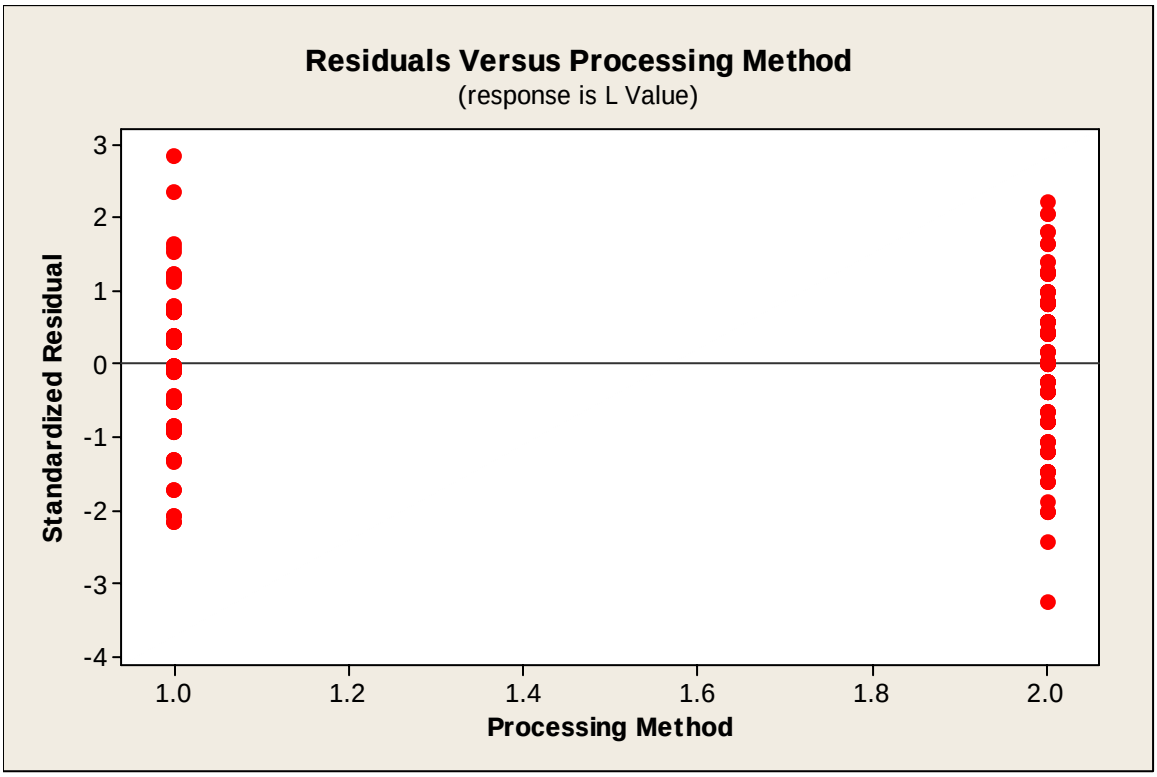

Figure **C.17**: Standardized residuals versus mushroom crop for response variable L value of whole mushrooms.

The plot of standardized residuals versus fits (Figure **C.18**) shows no apparent patterns to suggest either interaction between crop and processing method or a relationship between mean and variance.

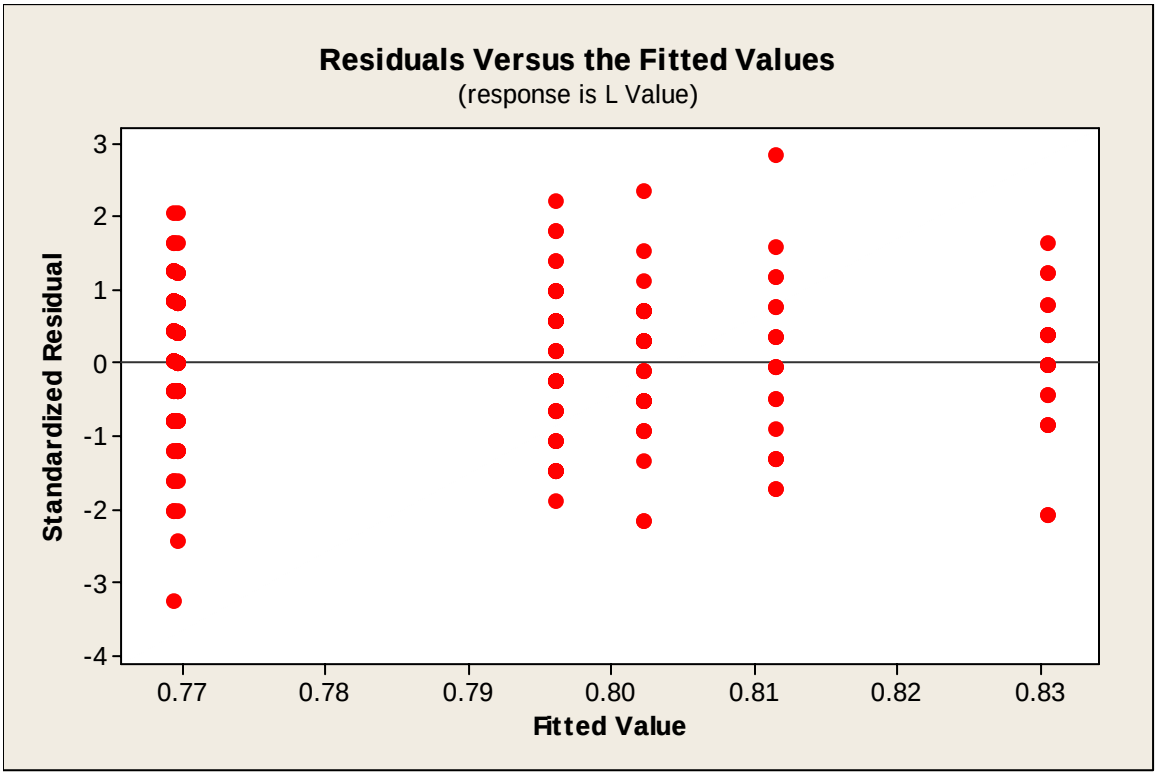

Figure **C.18**: Residuals versus the fitted values for response variable L value of sliced mushrooms.

The normal probability plot (Figure **C.19**) seemed consistent with the normality of errors.

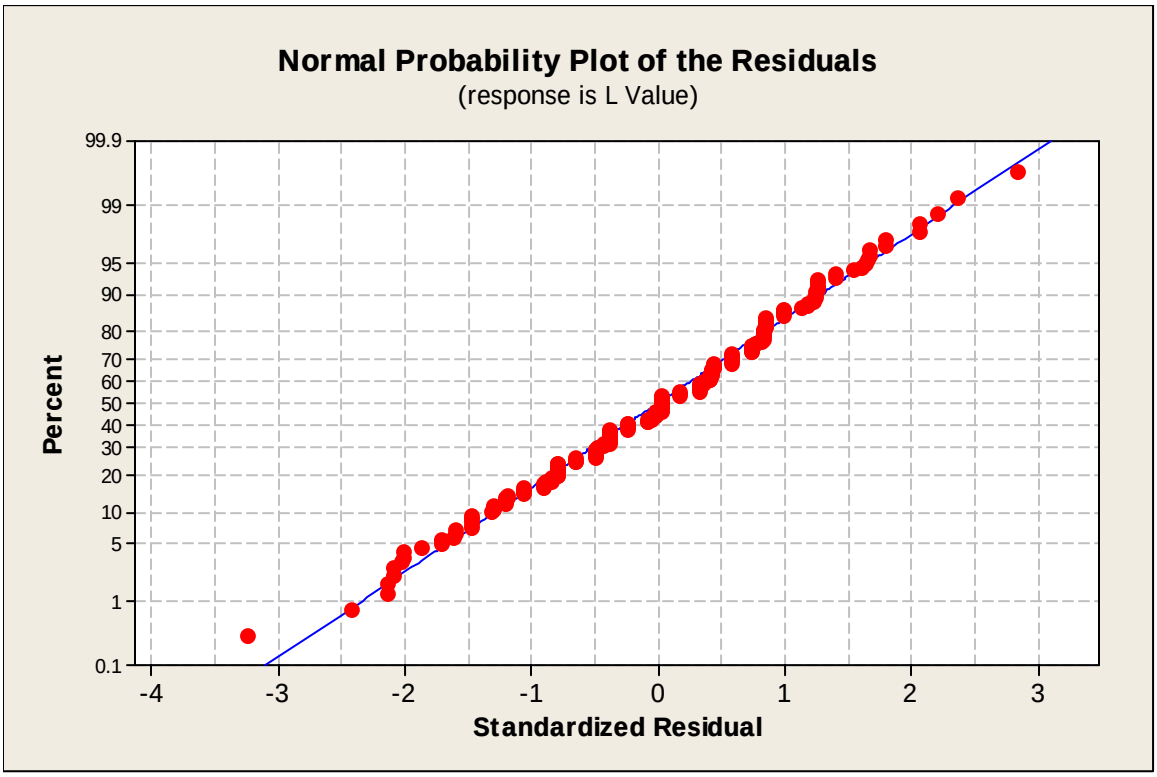

Figure **C.19**: Normality probability plot of the residuals for response variable L value of sliced mushrooms.

Thus, it can be inferred that since the p-value = 0.000 < 0.05, the null hypothesis was rejected, indicating aseptic and canned processing methods did not have the same mean L value. This was further validated by employing a Tukey Least Significant Difference (LSD) test using 95% simultaneous confidence intervals (Figure **C.20**). Since zero was not contained in the confidence interval, the null hypothesis was rejected. In addition, the p-value = 0.556 > 0.05, indicated that no significant interaction between mushroom crop and processing method existed.

```
Processing Method = 1  subtracted from:

Processing
Method         Lower    Center     Upper
2           -0.04348  -0.03643  -0.02937

Processing
Method      ------+---------+---------+---------+
2           (-----*-----)
            ------+---------+---------+---------+
               -0.036    -0.024    -0.012    -0.000
```

Figure **C.20**: Tukey 95% simultaneous confidence interval for all pairwise comparisons among levels of processing method for response variable L value of sliced mushrooms.

ΔE

Whole Mushrooms

Analysis of Variance (ANOVA) was used to determine the effects of mushroom crop, processing method, and crop and processing method interaction during ΔE studies of whole mushrooms processed aseptically and canned (Table **C.5**).

Table **C.5**: ANOVA: Response variable ΔE for aseptically processed and canned whole mushrooms.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Crop	3	3.3549	1.1206	50.31	0.000
Processing Method	1	1.7114	1.6974	76.20	0.000
Crop*Processing Method	3	0.2310	0.0770	3.46	0.017
Error	369	8.2196	0.0223		
Total	376	13.5170			

Before testing the hypothesis it was necessary to check the model for outliers, equal variance, interaction and normality. Plots of the standardized residuals versus mushroom crop (Figure C.21) and processing method (Figure C.22) revealed several observations with large standardized residuals. However, given the natural variation inherent in mushrooms, the residual plots suggested equal variance between crops.

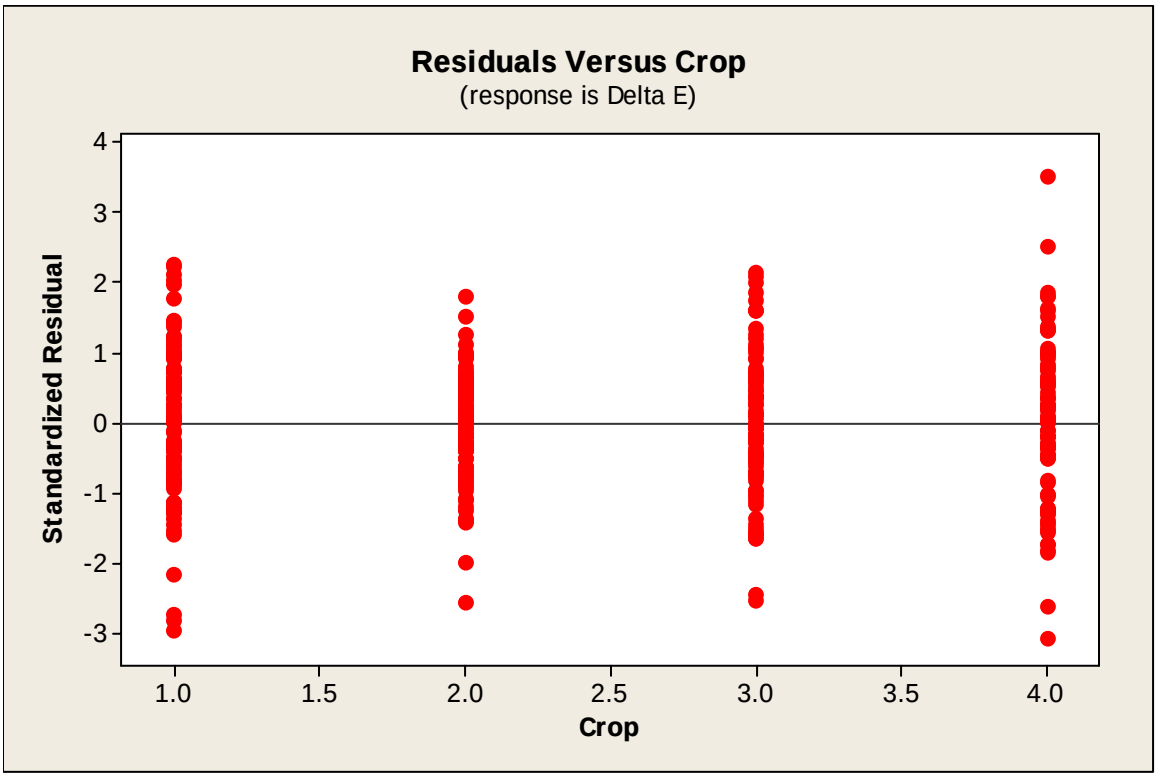

Figure C.21: Standardized residuals versus mushroom crop for response variable ΔE of whole mushrooms.

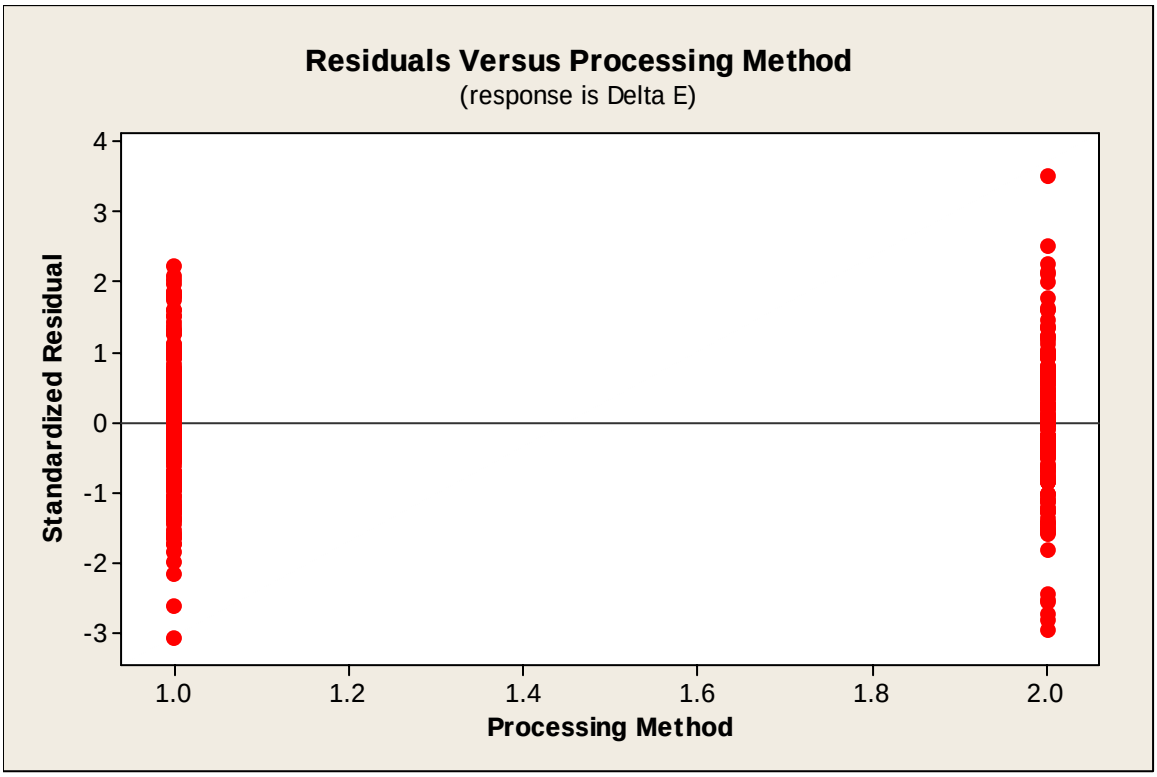

Figure C.22: Standardized residuals versus mushroom crop for response variable ΔE of whole mushrooms.

The plot of standardized residuals versus fits (Figure C.23) shows no apparent patterns to suggest either interaction between crop and processing method or a relationship between mean and variance.

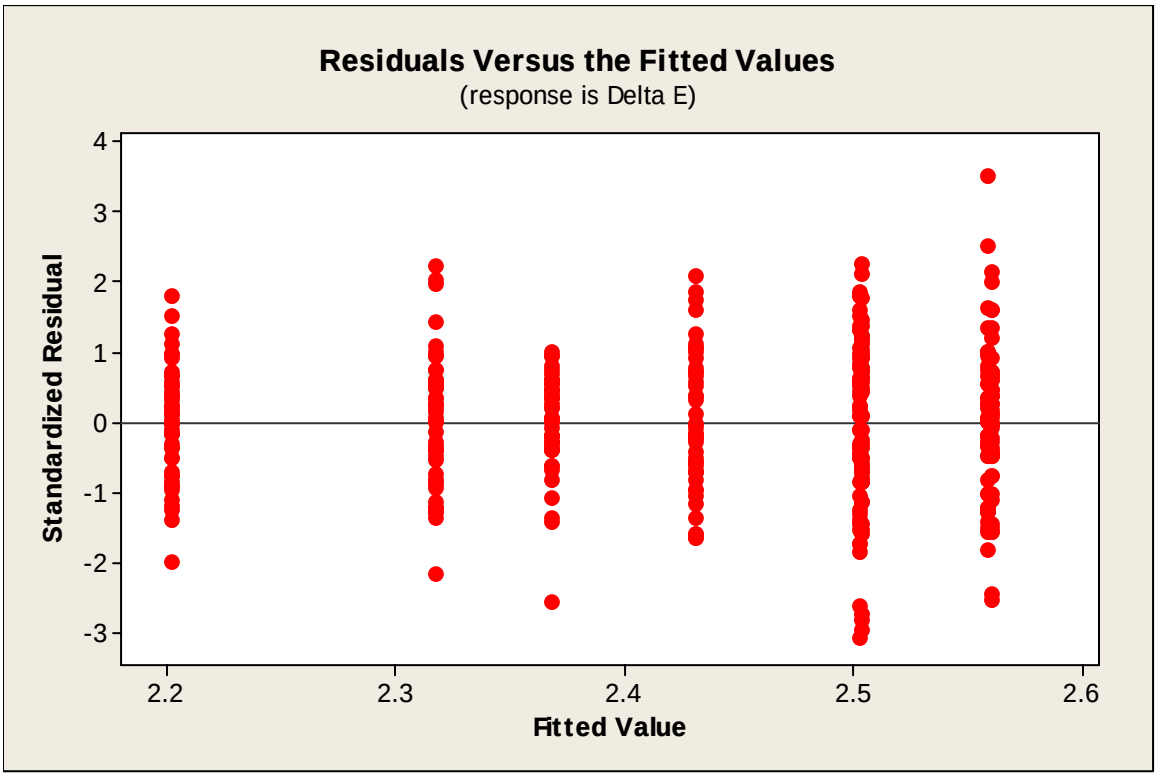

Figure **C.23**: Residuals versus the fitted values for response variable ΔE of whole mushrooms.

The normal probability plot (Figure **C.14**) exhibited slight tailing, but seemed consistent with the normality of errors.

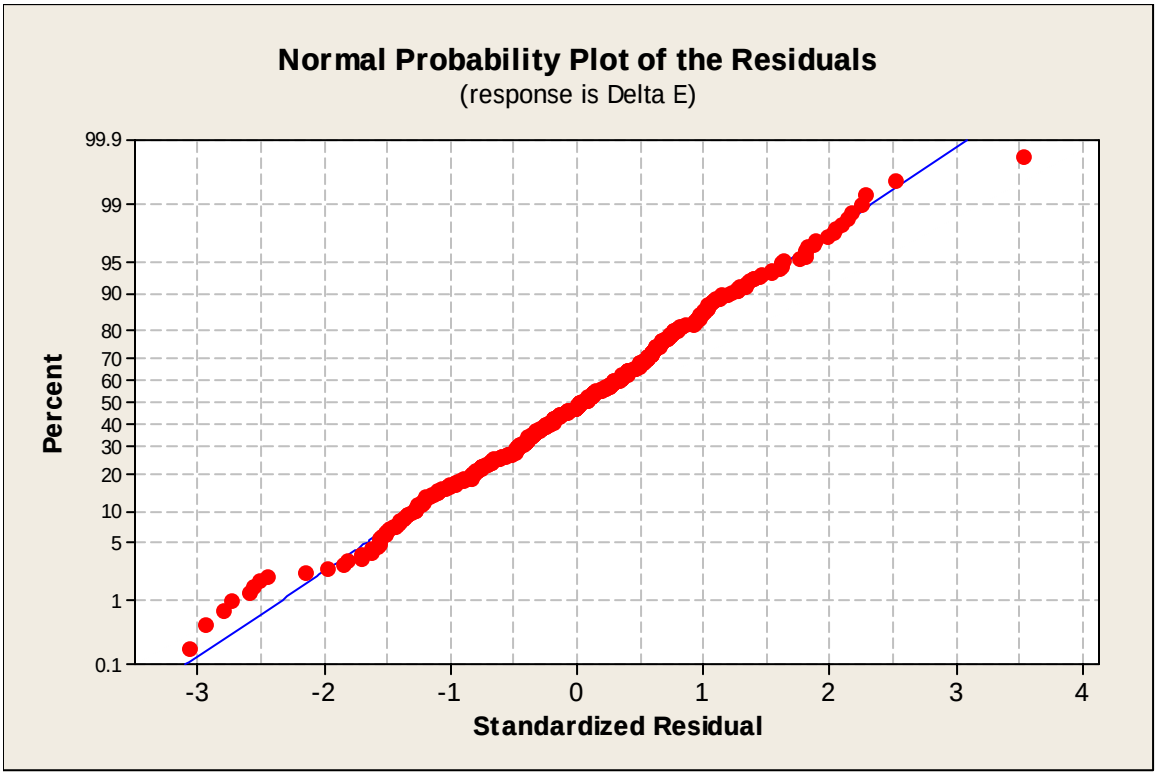

Figure **C.24**: Normality probability plot of the residuals for response variable ΔE of whole mushrooms.

Thus, it can be inferred that since the p-value = 0.000 < 0.05, the null hypothesis was rejected, indicating aseptic and canned processing methods did not have the same mean ΔE. This was further validated by employing a Tukey Least Significant Difference (LSD) test using 95% simultaneous confidence intervals (Figure **C.25**). Since zero was not contained in the confidence interval, the null hypothesis was rejected. In addition, the p-value = 0.017 < 0.05, indicated that significant interaction between mushroom crop and processing method existed.

```
Processing Method = 1  subtracted from:

Processing
Method       Lower  Center   Upper  --------+---------+---------+--------
2           0.1040  0.1342  0.1645  (--------------*--------------)
                                    --------+---------+---------+--------
                                          0.120     0.140     0.160
```

Figure **C.25**: Tukey 95% simultaneous confidence interval for all pairwise comparisons among levels of processing method for response variable ΔE of whole mushrooms.

Sliced Mushrooms

Analysis of Variance (ANOVA) was used to determine the effects of mushroom crop, processing method and crop and processing method interaction during ΔE studies of sliced mushrooms processed aseptically and canned (Table **C.6**).

Table **C.6**: ANOVA: Response variable ΔE for aseptically processed and canned sliced mushrooms.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Crop	2	0.3032	0.1703	7.73	0.001
Processing Method	1	1.1259	1.1483	52.09	0.000
Crop*Processing Method	2	0.0254	0.0127	0.58	0.563
Error	212	4.6735	0.0220		
Total	217	6.1280			

Before testing the hypothesis it was necessary to check the model for outliers, equal variance, interaction and normality. Plots of the standardized residuals versus mushroom crop (Figure **C.26**) and processing method (

Figure C.27) revealed several observations with large standardized residuals. However, given the natural variation inherent in mushrooms, the residual plots suggested equal variance between crops.

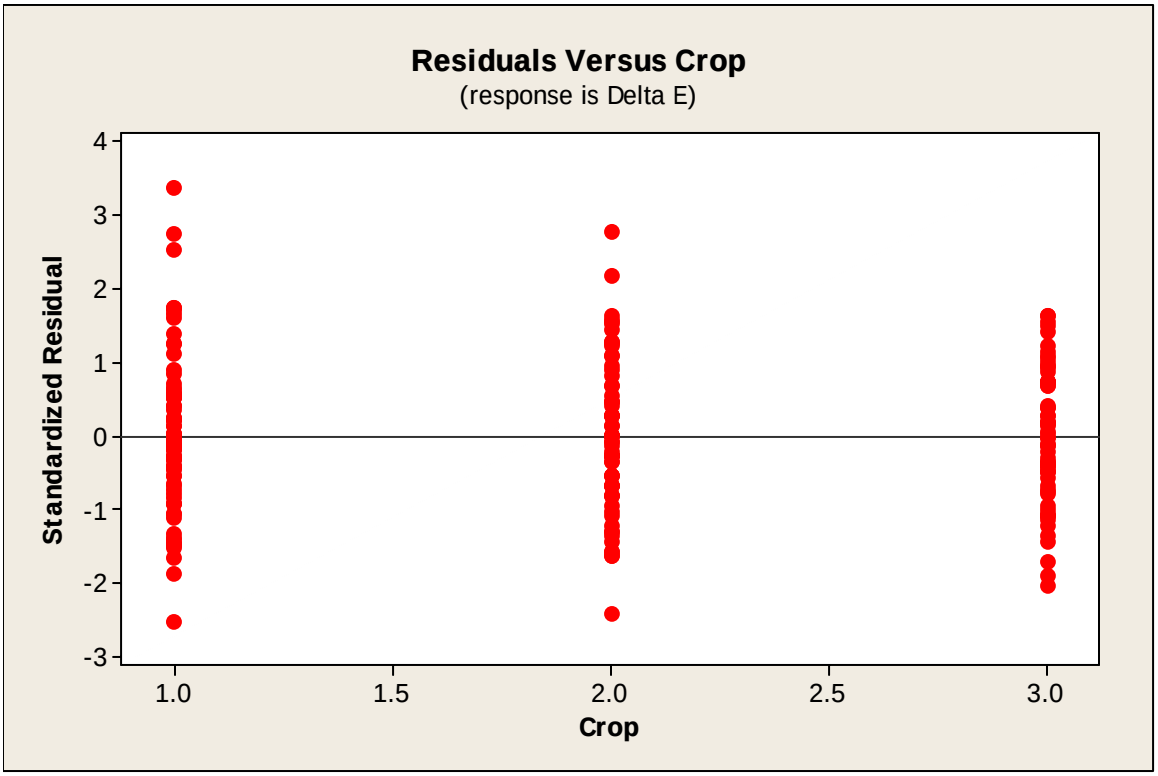

Figure C.26: Standardized residuals versus mushroom crop for response variable ΔE of sliced mushrooms.

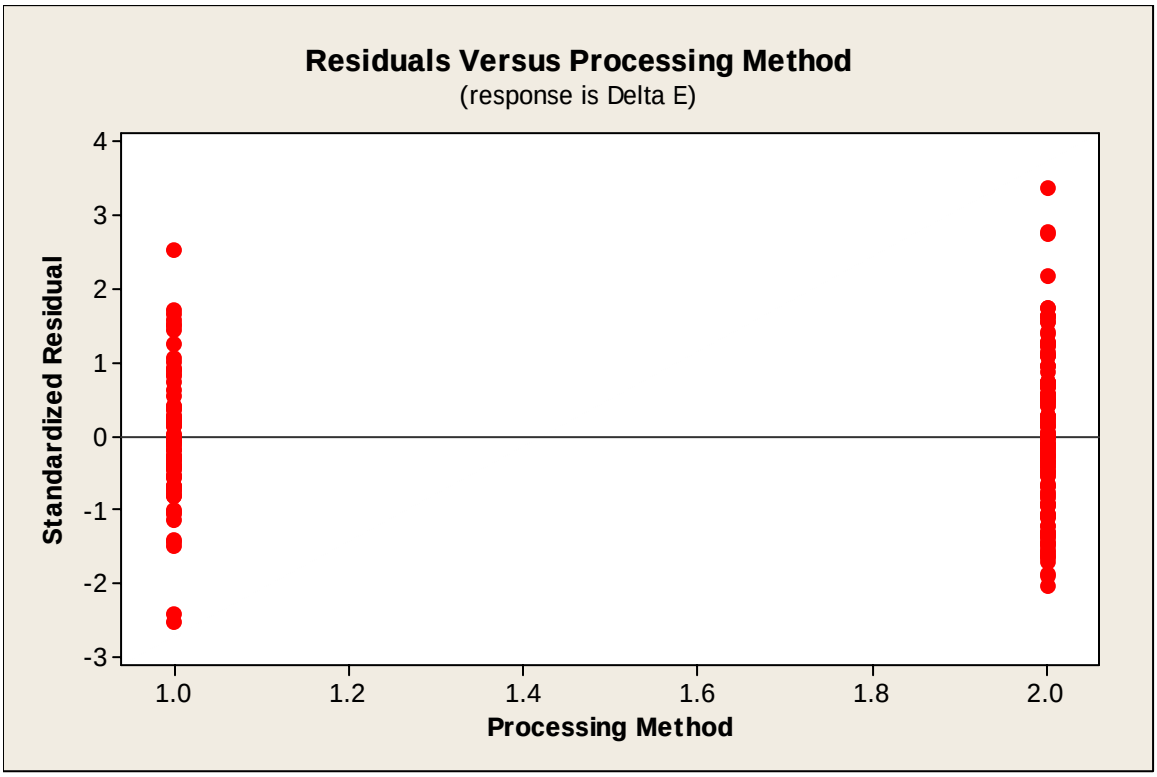

Figure C.27: Standardized residuals versus mushroom crop for response variable ΔE of whole mushrooms.

The plot of standardized residuals versus fits (Figure C.28) shows no apparent patterns to suggest either interaction between crop and processing method or a relationship between mean and variance.

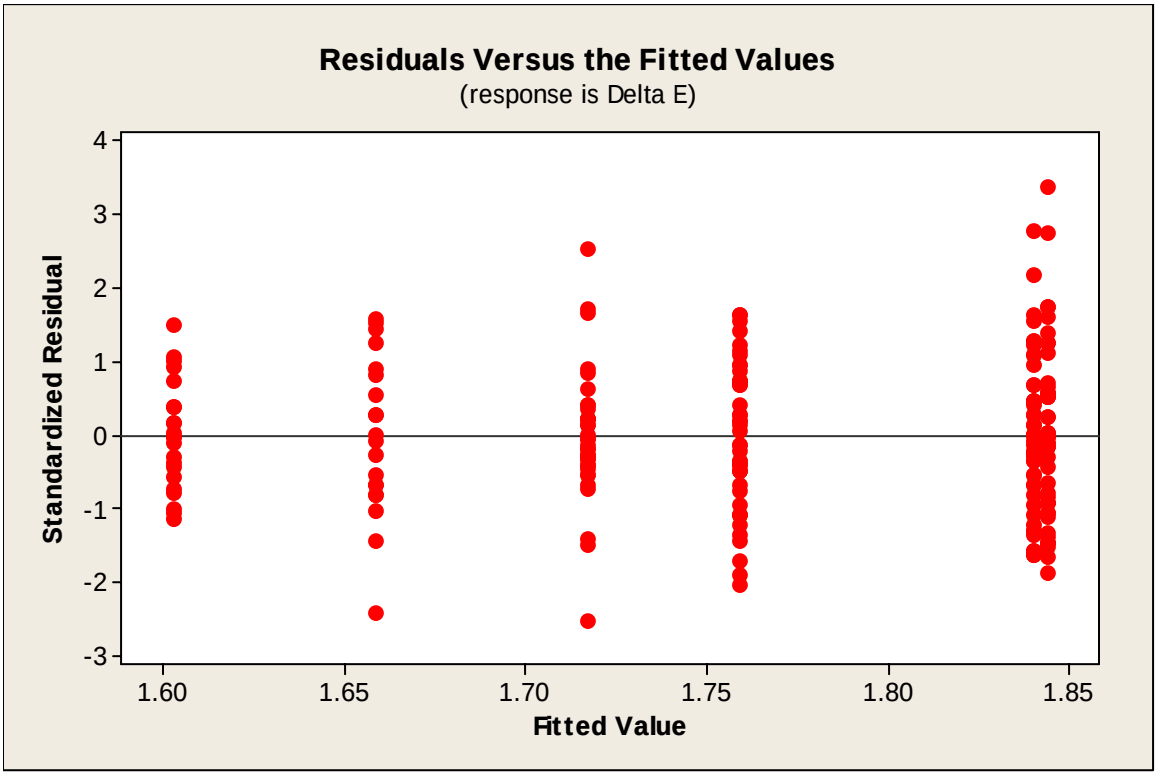

Figure **C.28**: Residuals versus the fitted values for response variable ΔE of sliced mushrooms.

The normal probability plot (Figure **C.29**) seemed consistent with the normality of errors.

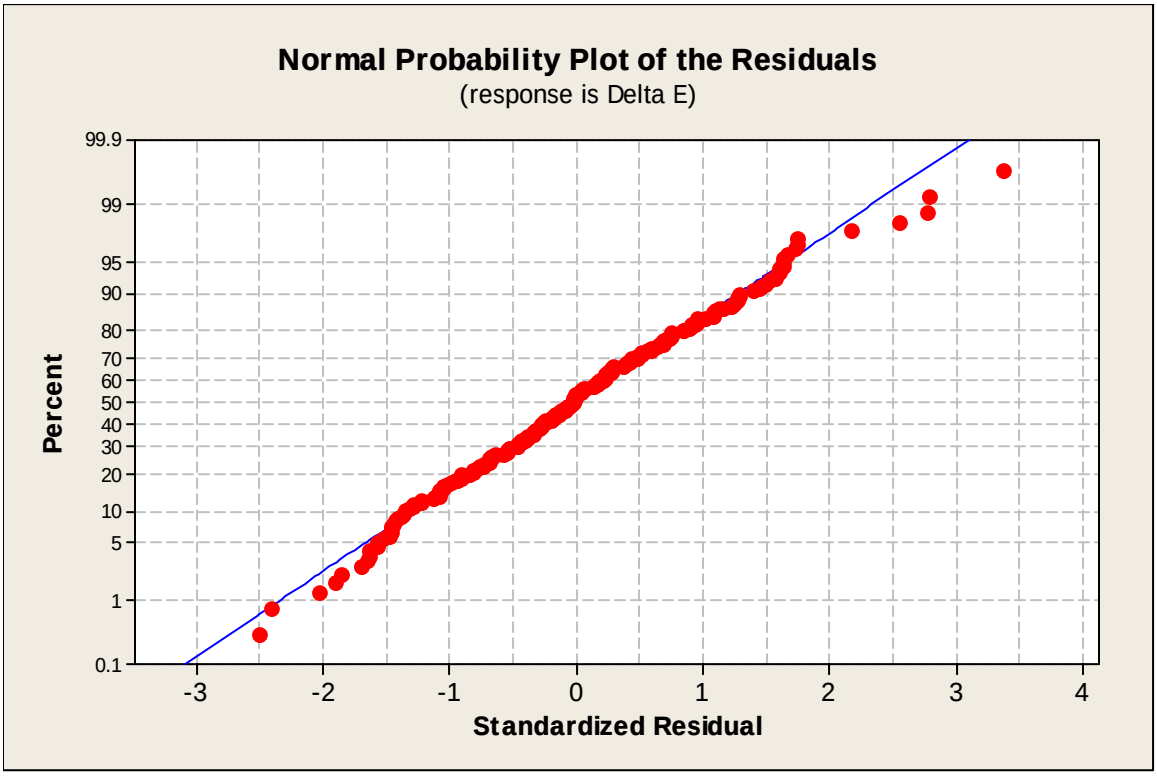

Figure **C.29**: Normality probability plot of the residuals for response variable ΔE of sliced mushrooms.

Thus, it can be inferred that since the p-value = 0.000 < 0.05, the null hypothesis was rejected, indicating aseptic and canned processing methods did not have the same mean ΔE. This was further validated by employing a Tukey Least Significant Difference (LSD) test using 95% simultaneous confidence intervals (Figure **C.30**). Since zero was not contained in the confidence interval, the null hypothesis was rejected. In addition, the p-value = 0.563 > 0.05, indicated that no significant interaction between mushroom crop and processing method existed.

```
Processing Method = 1  subtracted from:

Processing
Method       Lower  Center   Upper  -----+---------+---------+---------+-
2           0.1126  0.1548  0.1971  (----------------*----------------)
                                    -----+---------+---------+---------+-
                                       0.125     0.150     0.175     0.200
```

Figure C.30: Tukey 95% simultaneous confidence interval for all pairwise comparisons among levels of processing method for response variable ΔE of sliced mushrooms.

Texture

Whole Mushrooms

Analysis of Variance (ANOVA) was used to determine the effects of mushroom crop, processing method, and crop and processing method interaction during texture studies of whole mushrooms processed aseptically and canned (Table C.7).

Table C.7: ANOVA: Response variable work per unit mass for aseptically processed and canned whole mushrooms.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Crop	3	1.8211	0.6070	20.01	0.000
Processing Method	1	0.9524	0.9527	31.41	0.000
Crop*Processing Method	3	0.0538	0.0179	0.59	0.621
Error	369	11.2208	0.0303		
Total	376	14.0481			

Before testing the hypothesis it was necessary to check the model for outliers, equal variance, interaction and normality. Plots of the standardized residuals versus mushroom crop (Figure C.31) and processing method (Figure C.32) suggested equal variance between crops and processing methods.

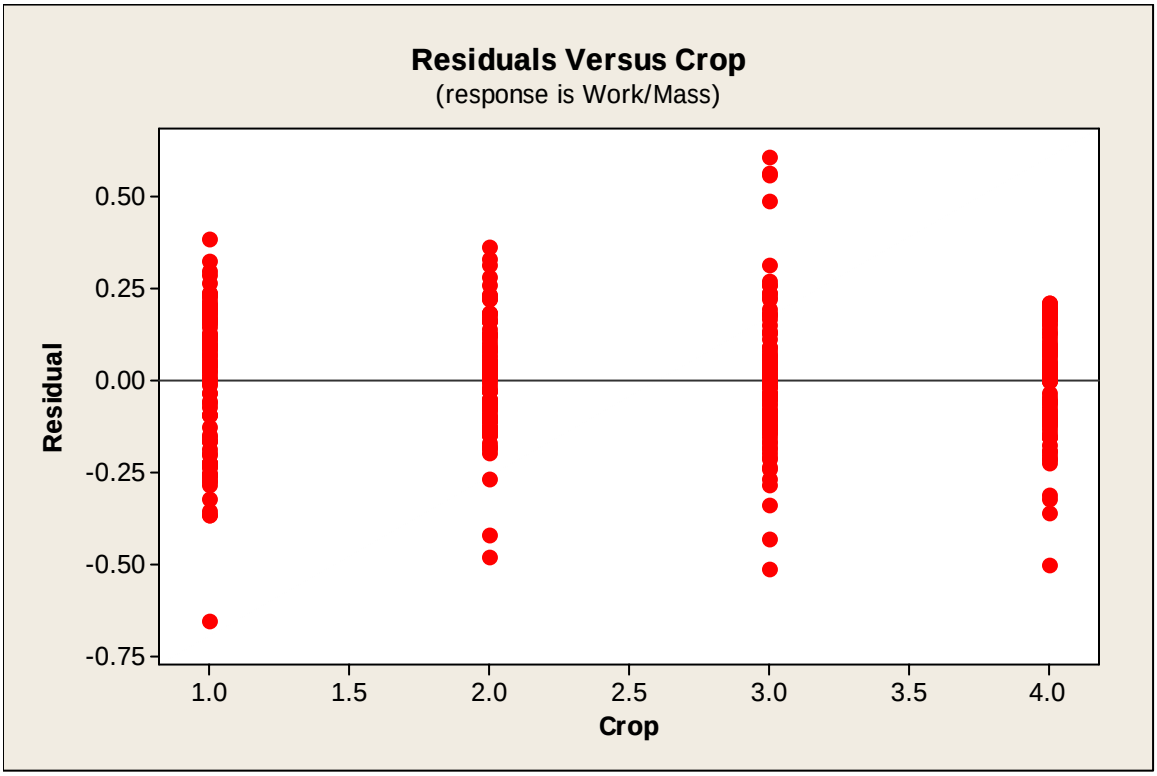

Figure C.31: Standardized residuals versus mushroom crop for response variable work per unit mass of whole mushrooms.

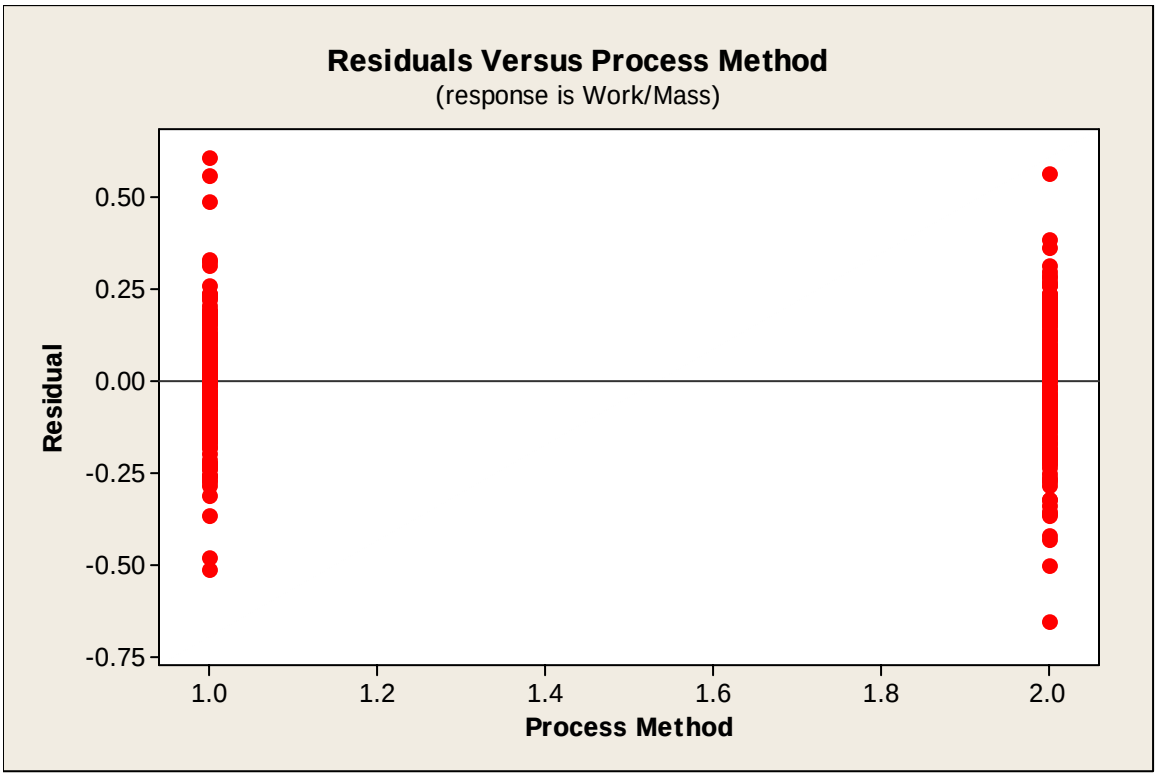

Figure **C.32**: Standardized residuals versus mushroom crop for response variable work per unit mass of whole mushrooms.

The plot of standardized residuals versus fits (Figure **C.33**) shows no apparent patterns to suggest either interaction between crop and processing method or a relationship between mean and variance.

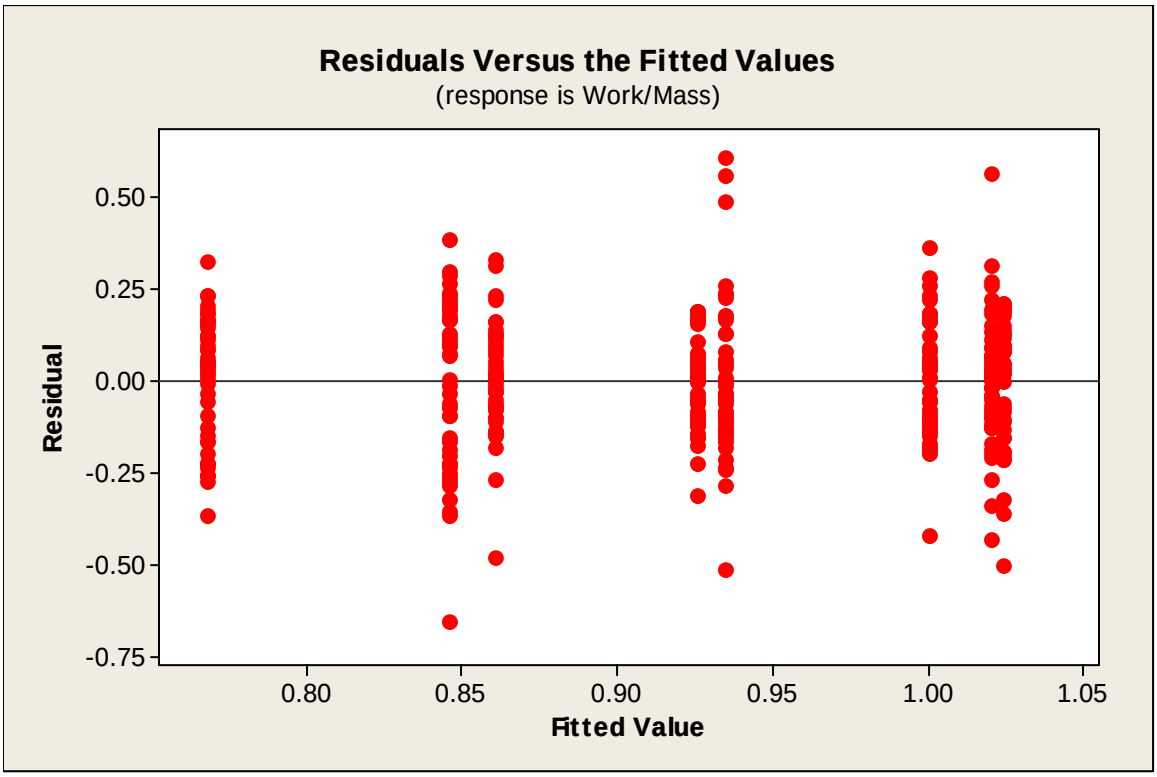

Figure **C.33**: Residuals versus the fitted values for response variable work per unit mass of whole mushrooms.

The normal probability plot (Figure **C.34**) exhibited slight tailing, but seemed consistent with the normality of errors.

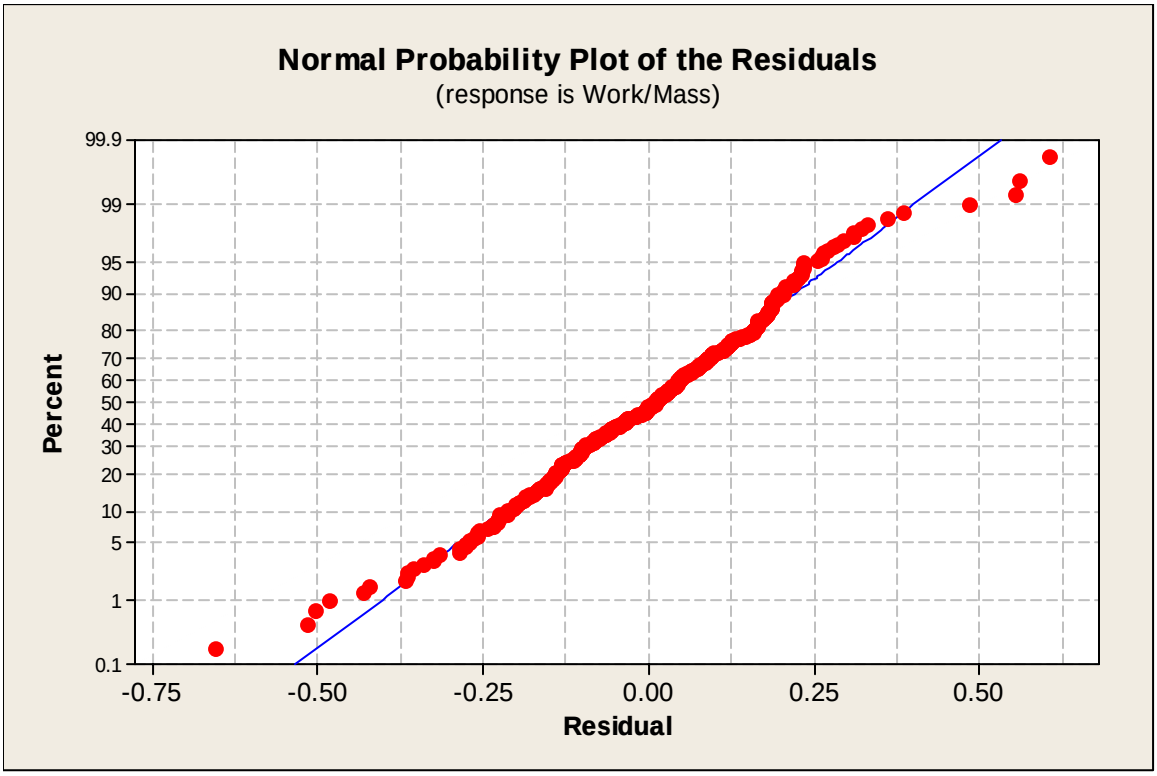

Figure **C.34**: Normality probability plot of the residuals for response variable work per unit mass of whole mushrooms.

Thus, it can be inferred that since the p-value = 0.000 < 0.05, the null hypothesis was rejected, indicating aseptic and canned processing methods did not have the same mean ΔE. This was further validated by employing a Tukey Least Significant Difference (LSD) test using 95% simultaneous confidence intervals (Figure **C.35**). Since zero was not contained in the confidence interval, the null hypothesis was rejected. In addition, the p-value = 0.621 > 0.05, indicated that no significant interaction between mushroom crop and processing method existed.

```
Process Method = 1  subtracted from:

Process
Method     Lower  Center   Upper  -------+---------+---------+---------
2        0.06519  0.1004  0.1357  (----------------*----------------)
                                  -------+---------+---------+---------
                                       0.080     0.100     0.120
```

Figure **C.35**: Tukey 95% simultaneous confidence interval for all pairwise comparisons among levels of processing method for response variable work per unit mass of whole mushrooms.

Sliced Mushrooms

Analysis of Variance (ANOVA) was used to determine the effects of mushroom crop, processing method and crop and processing method interaction during work per unit mass studies of sliced mushrooms processed aseptically and canned (Table **C.8**).

Table **C.8**: ANOVA: Response variable work per unit mass for aseptically processed and canned sliced mushrooms.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Crop	2	0.0580	0.0211	1.72	0.182
Processing Method	1	0.0384	0.0438	3.55	0.061
Crop*Processing Method	2	0.0203	0.0101	0.82	0.441
Error	227	2.7970	0.0123		
Total	232	2.9137			

Before testing the hypothesis it was necessary to check the model for outliers, equal variance, interaction and normality. Plots of the standardized residuals versus mushroom crop (Figure **C.36**) and processing method

(Figure C.37) revealed several observations with large standardized residuals. However, given the natural variation inherent in mushrooms, the residual plots suggested equal variance between crops.

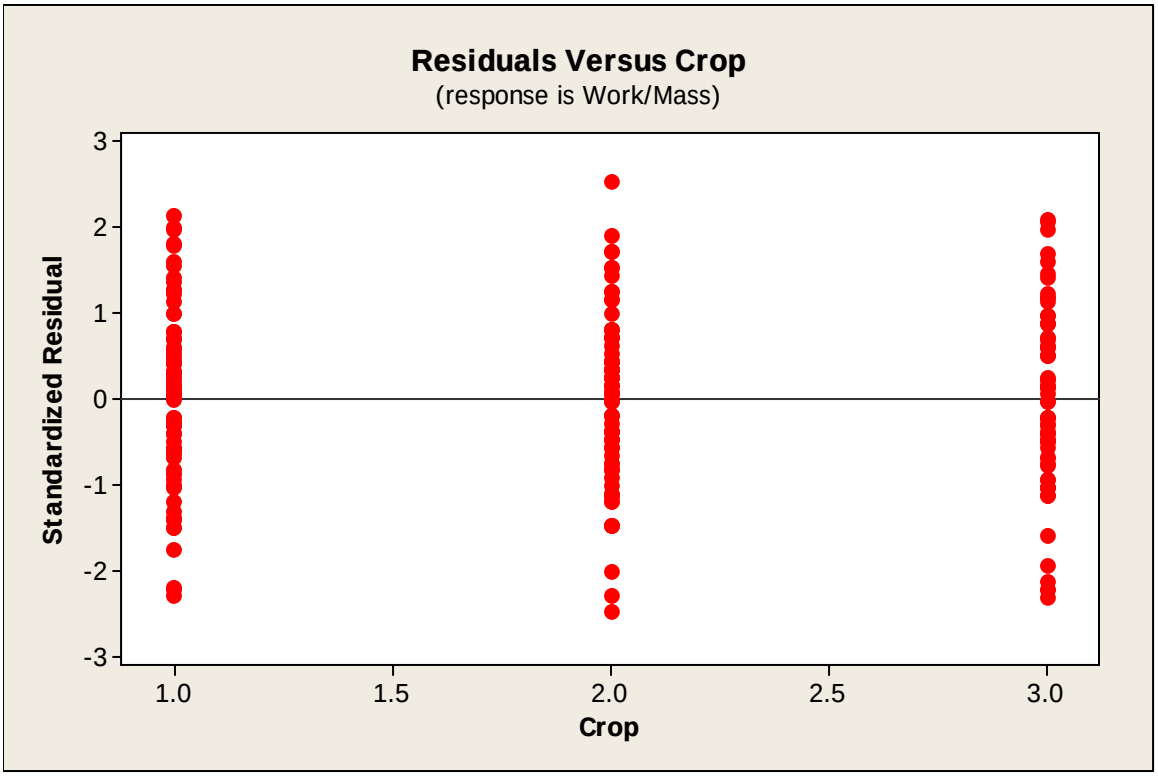

Figure C.36: Standardized residuals versus mushroom crop for response variable work per unit mass of sliced mushrooms.

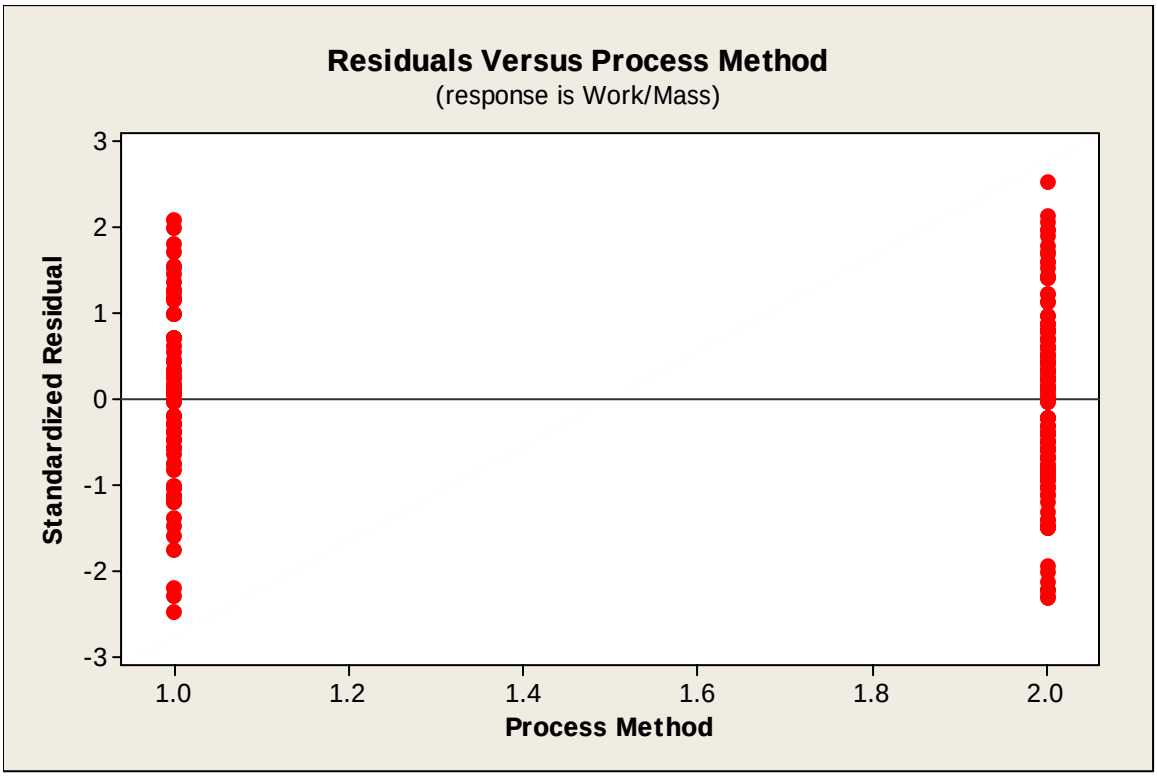

Figure **C.37**: Standardized residuals versus mushroom crop for response variable work per unit mass of whole mushrooms.

The plot of standardized residuals versus fits (Figure **C.38**) shows no apparent patterns to suggest either interaction between crop and processing method or a relationship between mean and variance.

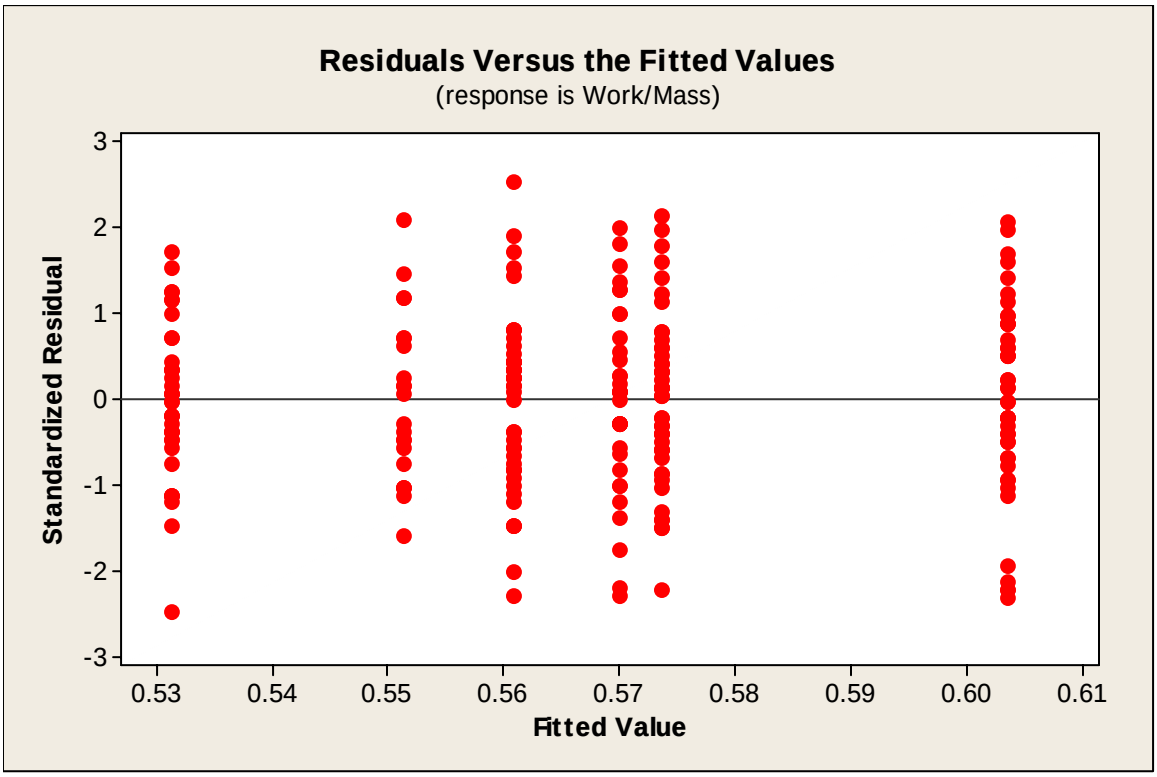

Figure **C.38**: Residuals versus the fitted values for response variable work per unit mass of sliced mushrooms.

The normal probability plot (Figure **C.39**) seemed consistent with the normality of errors.

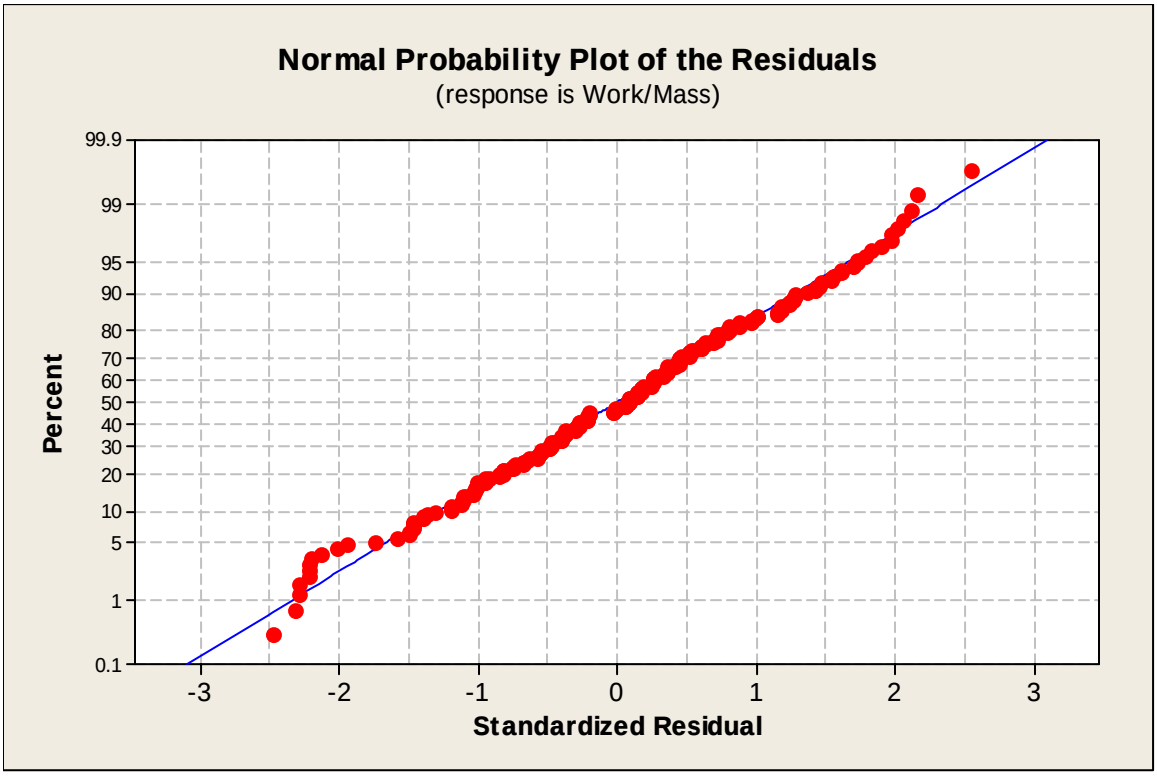

Figure **C.39**: Normality probability plot of the residuals for response variable work per unit mass of sliced mushrooms.

Since the p-value = 0.061 > 0.05, the null hypothesis was rejected, indicating aseptic and canned processing methods did have the same mean work per unit mass. This was further validated by employing a Tukey Least Significant Difference (LSD) test using 95% simultaneous confidence intervals (Figure **C.40**). Since zero was contained in the confidence interval, the null hypothesis was not rejected. In addition, the p-value = 0.441 > 0.05, indicated that no significant interaction between mushroom crop and processing method existed.

```
Process Method = 1  subtracted from:

Process
Method       Lower   Center    Upper   -+---------+---------+---------+-----
2        -0.001295  0.02843  0.05816   (--------------*--------------)
                                        -+---------+---------+---------+-----
                                      0.000     0.020     0.040     0.060
```

Figure **C.40**: Tukey 95% simultaneous confidence interval for all pairwise comparisons among levels of processing method for response variable work per unit mass of sliced mushrooms.

Appendix D

The National Food Lab, Inc. General Methods for Preparation and Calibration of *Clostridium Sporogenes* Spore Crop

Preparation of *Clostridium Sporogenes* Spore Crop

"The medium…used was a commercially available liver broth with liver particles made in-house. We incubated anaerobically at 30°C and checked weekly for spore growth. When the spores were determined to be of highest density and quality they were harvested. The crop was then washed, re-suspended in sterile (deionized) water and plated on TPGYE+thioglycollate agar for counting (Willette, 2006)."

Calibration of Spore Crop

"We use a wet steam thermoresistometer for calibration. The spore suspension was spot inoculated and allowed to dry on aluminum foil carriers. Times and temperatures were determined to establish the $D_{121°C}$ and z-value of the crop. After steam exposure the inoculated carriers were recovered in tubes of TPGYE+thio broth and covered with a layer of vaspar to create an anaerobic environment in the tube. The tubes were incubated for 30 days at 30°C (Willette, 2006)."

References

Willette, Julie. 2006. Personal communication. The National Food Lab, Inc. Dublin, CA. 19 April 2006.

Appendix E

Formulations for Sorenson Phosphate Buffer, Peptone Water and Eugon Agar

Sorenson Phosphate Buffer

Sorenson phosphate buffer (SPB) was prepared by adding

- Na_2HPO_4 (dibasic sodium phosphate) 5.676 g/l
- KH_2PO_4 (monobasic potassium phosphate) 3.631 g/l

to distilled water in an Erlenmeyer flask. The buffer solution was stirred until the phosphates had dissolved completely. Sorenson phosphate buffer was dispensed into 500 ml media bottles and autoclaved at 121°C for 25 minutes and cooled before use.

Peptone Water

Peptone water (1%) was prepared by adding

- Peptone 1 g/l

to reverse-osmosis water in an Erlenmeyer flask. The solution was stirred until the peptone had dissolved completely. Peptone water was dispensed into 500 ml media bottles and autoclaved at 121°C for 25 minutes and cooled before use.

Eugon Agar

Approximate formula for Difco Eugon agar [Becton, Dickenson Company (BD), Sparks, MD]:

- Tryptose 15.0 g/l
- Soytone 5.0 g/l
- Dextrose 5.5 g/l
- L-Cystine 0.7 g/l
- Sodium Chloride 4.0 g/l
- Sodium Sulfite 0.2 g/l
- Agar 15.0 g/l

"Directions: Suspend 45.4 g of the powder in 1 L of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to dissolve the powder. Autoclave at 121°C for 15 min [Becton, Dickenson Company (BD), Sparks, MD]."

VITA

EDUCATION

Ph.D. Food Engineering, Department of Agricultural and Biological Engineering, Penn State University, 2006
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B.S. Agricultural Engineering, Department of Agricultural Engineering, Minor Environmental Engineering, Penn State University, 1995

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Agricultural Engineer, Food and Drug Administration, Center for Food Safety and Applied Nutrition, 2006-present.
Production Supervisor, Land O' Lakes/AFP advanced food products, LLC. 2000-2002
Operations Manager, Walnut Acres Organic Farms, Inc., 1998-2000.

PUBLICATIONS

Anderson, N. M. and P. N. Walker. 2005. Continuous Steam Sterilization Segmented-Flow Aseptic Processing of Particulate Foods. Presented at the Int'l. ASAE Conference, Tampa, FL, July 17-20.

Anderson, N. M. and P. N. Walker. 2003a. Measuring Fat Content of Ground Beef Stream Using On-line Visible/NIR Spectroscopy. Transactions of the ASAE 46(1): 117-124.

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AWARDS AND HONORS

ASAE Paper Awards Honorable Mention, American Society of Agricultural Engineers, 2004 [for refereed paper Anderson and Walker (2003b) listed above.]
Alpha Epsilon, Honor Society of Agricultural, Food and Biological Engineering, 1997
Gamma Sigma Delta, Honor Society of Agriculture, 1997

PROFESSIONAL STATUS/TRAINING

Microbiology and Engineering of Sterilization Processes. Certificate awarded 2006.
Better Process Control School, Penn State University. Certificate awarded 1998.
Hazard Analysis Critical Control Point (HACCP) Training, TechniCal Inc. Certificate awarded 1998
Sanitation Short Course, Penn State University. Certificate awarded 1998
E.I.T., Bureau of Professional and Occupational Affairs, Pennsylvania Department of State. Awarded 1997.

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