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**Effectiveness of Different Post Harvest Treatments and Packaging Methods for  
Peeled Chestnuts**

By

Kuo-Chun Yen

A THESIS

Submitted to  
Michigan State University  
in partial fulfillment of the requirements  
for the degree of

MASTER OF SCIENCE

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## **ABSTRACT**

### **Effectiveness of Different Post Harvest Treatments and Packaging Methods for Peeled Chestnuts**

By

Kuo-Chun Yen

Almost all the American chestnut trees were destroyed in the early 20<sup>th</sup> century. Therefore, there is limited information on chestnuts grown in the US. The purpose of this study was to evaluate the baseline level of the microbial population and quality of chestnuts grown in the US and processed using the brulage chestnut peeler (Boema Company, Italy). The quality of stored, pretreated (hydrochloride and chlorine dioxide gas) and untreated fresh peeled chestnuts in different packaging material (Nylon, and PE) under vacuum and atmospheric conditions were evaluated. Chinese chestnuts (*Castanea sativa* Mill.) were harvested, peeled by the automatic peeling machine, treated with either sodium hypochlorite or chlorine dioxide, packaged in low density polyethylene (LDPE) and nylon/LDPE pouchs, and stored at either 4-5C and 10-12C for 4 weeks. The texture, moisture content, color and microbial population of the chestnuts were evaluated periodically in storage. Packaging methods and materials had no significant effect on extending the shelf life. Treatment with sodium hypochlorite or chlorine dioxide reduced the initial microbial population but only extended the shelf life to 14 days when stored at 4-5C. Low temperature storage provided some additional quality preservation. A trained sensory panel evaluated the appearance, texture, and off- flavor of the product. Sensory scores indicated that there was no significant different between treated and untreated samples before and after storage.

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## **1. INTRODUCTION**

Before the chestnut blight devastated the American chestnut population in the early 20<sup>th</sup> century, the chestnut was a major forest component in the eastern United States. Today, production of edible chestnuts is making a comeback. Michigan State University and Chestnuts Grower Inc. are working together to re-establish the chestnut industry in Michigan by producing added value pre-peeled chestnuts.

The minimum processed fruit and vegetable market has grown rapidly in the past 10 years. Retail sales in the US in 1994 were 5.8 billion (Hodge, 1995) and had reached 19 billion by 2003 (Greenleaf, 1999). Valued-added minimal processed chestnut products have great potential for the fresh produce industry.

However, quality parameters effecting browning, growth of microorganisms and texture are major problems for the minimal processed fruit and vegetable industry. Many methods have been reported as beneficial to maintain quality including chemical dips (Gorny et al., 1999; McHugh and Senesi, 2000; Dong et al., 2000), control atmosphere packaging ( Gorny et al., 1999; Lakakul et al., 1999), modified atmosphere packaging (Hotchkiss and Banco, 1992), high pressure processing (Boynton, 1999), and irradiation (Prakash, 2000) are reported to reduce the number of microorganisms on fruits and vegetables. However, there is no recent published research related to pre-peeled chestnuts. Microbial contamination can occur during transportation, storage, and processing. Cross-contamination usually occurs during cutting or shredding (Grag et al., 1990). Soil and water present on the surface of the produce can also support the growth of microorganisms, including pathogens such as *L. monocytogenes* (Al-Gahazali and Al-Azawi, 1990). The automatic chestnut peeling line is the first of its kind in the US and

there is no research related to quality of chestnuts produced by this peeling machine, nor the number of microorganisms.

**The objectives of this research are:**

1. Determine baseline levels of microorganisms for the chestnuts in the field prior to harvest, during storage at 4-5C and 10-12C, just prior to peeling and post peeling prior to freezing.
2. Determine microbial count at various points along the peeling line where the peeling process may introduce micro flora.
3. Determine the effect of different packaging materials and closure methods, and different storage temperatures on the shelf life of fresh peeled chestnuts.
4. Compare the efficacy of sodium hypochlorite and chlorine dioxide gas in inactivating yeast, mold and bacteria on chestnuts.

## **2. Literature Review**

### **History of Chestnuts**

Chestnuts (*Castanea*) have played an important role in the human history of Europe, Asia, and North American (Smith, 1953). In China, Chestnuts have been used for food and timber since ancient times (Payne et al., 1983). The American chestnut was one of the most important forest trees from Maine South to Florida, and from the Piedmont West to the Ohio valley (Fig 1). In 1904 a fungus was discovered which was attacking the trees in the Bronx Zoological Park in New York City (Merkel, 1906). It killed those chestnuts and spread quickly. Detailed accounts of the origin, progression, and consequences of the blight epidemic have been described in several reviews (Anagnostakis, 1987; MacDonald and Fulbright, 1991; Fulbright, 1999). By 1950 the most plentiful tree on over nine million acres was virtually extinct – almost every single one of them a victim of the blight (Anagnostakis, 1982).

Chestnut blight, or chestnut bark disease, is caused by an introduced fungus, *Cryphonectria parasitica* (Murrill) Barr, (formerly *Endothia parasitica* [Murrill] Anderson & Anderson). The fungus enters wounds, grows in and under the bark, and eventually kills the cambium all the way around the twig, branch, or trunk (Metcalf, 1912).

The Native American chestnut was almost entirely destroyed by the chestnut blight. Breeders have produced new cultivars of blight-resistant chestnuts by hybridizing *C.*

*dentata* with *C. mollissima* (Jaynes 1979) and backcrossing. Significant progress in controlling blight has been made over the last 20 years (Griffin 1986).

Michigan is outside of the natural range of the American Chestnut. But Michigan provides a unique opportunity for recovering populations and producing of the American chestnut because the blight-resistant gene spread naturally in Michigan (Fulbright et al., 1983). Michigan farmers are just beginning to plant and grow Asian and European hybrid varieties of chestnuts (Fulbright & Mandujano, 2000). For the North American market, these domestically grown, in-shell chestnuts are fresher than imported nuts, and with improved post harvest handling, increased production should be available for longer periods.

### **The chestnuts industry**

Current chestnut worldwide production is over 500,000 tons. China is the leading producer with 40%, followed by Korea with 15%, Italy, and Turkey, and Japan with 10% each. The US grows less than 1% (Olsen, 2000). The annual consumption of chestnuts in the US is less than 0.02 Kg/person (Burnett, 1987). This low consumption rate is not due to lack of interest in chestnuts, but to the availability of good chestnuts, and to the fact that consumers are not familiar with chestnuts products.

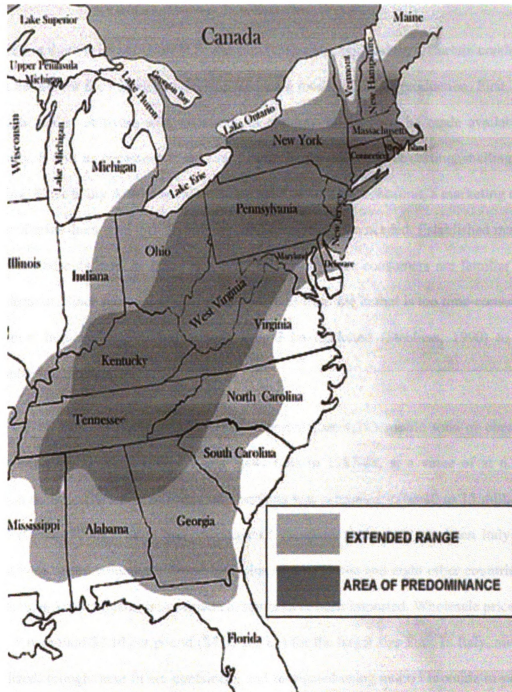


Fig 1. The American chestnut tree reigned over 200 million acres of eastern woodlands from Maine to Florida, and from the Piedmont plateau in the Carolinas west to the Ohio Valley, until succumbing to a lethal fungus infestation, known as the chestnut blight, during the first half of the 20th century. An estimated 4 billion American chestnuts, up to 1/4 of the hardwood tree population, grew within this range. (The American Chestnuts Foundation, [http://www.acf.org/Chestnut\\_history.htm](http://www.acf.org/Chestnut_history.htm))

At present there is a very small U.S. chestnut (*Castanea* spp.) industry. Certain conditions must be met for the industry to develop to even a modest level of production. First, trees of high quality cultivars with resistance to chestnut blight must be made available in quantity. It will be necessary to develop or improve mechanical harvesting, shelling, and peeling. Since many Americans alive today have never seen a chestnut, a marketing effort to familiarize them with this "exotic" nut and its uses will be needed. Established markets for exported nuts exist in Japan and other countries where consumers are familiar with the chestnut. Since removal of the "skin" (pellicle) from the kernel is too time-consuming for most homemakers, peeled kernels should be marketed (Stebbins, 1990) to take advantage of the demand.

The USDA Foreign Agricultural Service reported that 4,293 metric tons of chestnuts were imported in fiscal 1986-87, and 3,441 tons in 1987-88, at a value of at 6.5-6.7 million dollars. The value of imported chestnuts was estimated to be 10 to 15 million in 2000 (Olsen, 2000). The primary exporter of chestnuts to the U.S. has been Italy with about 84% of the volume, followed by China, Spain, Korea and eight other countries. In addition, unknown quantities of dried chestnuts have been imported. Wholesale prices are generally around \$2.10 per pound (\$4.62 per kg) for the larger size nuts. In Italy, nuts are sterilized, brought here in sea containers, and fumigated using methyl bromide in port as required by the USDA Animal and Plant Health Inspection Service (APHIS). Fresh nuts should be refrigerated (Stebbins, 1990).





U.S. producers could displace imports and expand the U.S. market for in-shell chestnuts, and a much larger tonnage could be marketed if a peeled product were made available (Miller 1988). It is unlikely that many homemakers will want to peel chestnuts as it is a time consuming task.

Currently, the majority of chestnuts marketed in the United States are imported from Italy and the wholesale value is \$20 million to \$40 million per year. Fresh Michigan-grown chestnuts are able to provide a better quality product for consumers than Italian imports. Michigan and the United States in general have great potential for increased plantings of chestnuts. With only a small increase in domestic consumption, the industry could be worth \$600 to \$800 million annually (Vossen, 2000).

### **Nutritional value of chestnuts**

Unlike most other tree nuts, chestnuts are low in protein and fat but high in carbohydrate (McCarthy and Meredith, 1988). Nutritional data on chestnuts grown outside of the US have been reported for Europe by Dudek and Elkin (1984) and Beaubatie (1979); for China by Harris et al. (1949); for Japan by Ha et al. (1982) and Rhee and Kim (1982). McCarthy and Meredith (1988) have reported in detail the nutritional data of the American (*Castanea dentate*) and Chinese chestnuts (*Castanea mollissima*) grown in the US and compared this data to that of imported Italian chestnuts (*Castanea sativa*) (table 1, 2 and 3). Nutritional data on chestnuts grown in Michigan also have been reported (Harte, 2003) (Table 4 and 5). Chestnuts are also a source of essential fatty acids (linoleic and linolenic acid) (Kunsch et al., 1999).

Table 1. Proximate composition of raw chestnut kernels (amount in 100g, edible portion)a.

| Chestnuts, raw                   |                   |      |                   |      |                   |      |
|----------------------------------|-------------------|------|-------------------|------|-------------------|------|
| Proximates                       | American          |      | Chinese           |      | European          |      |
|                                  | Mean (g)          | S.E. | Mean (g)          | S.E. | Mean (g)          | S.E. |
| Water <sup>b</sup>               | 43.70             |      | 43.95             |      | 54.88             |      |
| Protein (N*5.30)                 | 4.83 <sup>e</sup> | 0.06 | 4.20 <sup>f</sup> | 0.01 | 1.98 <sup>g</sup> | 0.00 |
| Fat <sup>c</sup>                 | 1.32 <sup>g</sup> | 0.04 | 1.11 <sup>f</sup> | 0.05 | 1.63 <sup>e</sup> | 0.03 |
| Carbohydrate, total <sup>d</sup> | 48.57             |      | 49.07             |      | 40.28             |      |
| Crude fiber                      | 1.91 <sup>e</sup> | 0.01 | 1.64 <sup>f</sup> | 0.04 | 1.36 <sup>g</sup> | 0.04 |
| Ash                              | 1.58 <sup>f</sup> | 0.02 | 1.67 <sup>e</sup> | 0.00 | 1.23 <sup>g</sup> | 0.00 |

a Duplicate determination

b One analysis

c our analyses for American chestnuts

d Calculated by difference

e-g Means with different superscripts differ (p<0.01)

(McCarthy and Meredith 1988)

Table 2. Mineral composition of raw chestnut kernels (amount in 100g, edible portion)a.

| Chestnuts, raw |                    |       |                    |       |                    |       |
|----------------|--------------------|-------|--------------------|-------|--------------------|-------|
| Minerals       | American           |       | Chinese            |       | European           |       |
|                | Mean (g)           | S.E.  | Mean (g)           | S.E.  | Mean (g)           | S.E.  |
| Calcium        | 24                 | 0     | 18                 | 2     | 23                 | 1     |
| Iron           | 1.52 <sup>b</sup>  | 0.11  | 1.41 <sup>b</sup>  | 0.02  | 0.71 <sup>c</sup>  | 0.01  |
| Magnesium      | 79 <sup>b</sup>    | 0     | 84 <sup>b</sup>    | 2     | 32 <sup>c</sup>    | 0     |
| Phosphorus     | 96 <sup>b</sup>    | 0     | 96 <sup>b</sup>    | 2     | 50 <sup>c</sup>    | 1     |
| Potassium      | 504                | 32    | 447                | 15    | 378                | 15    |
| Sodium         | 3                  | 0     | 3                  | 0     | 3                  | 0     |
| Zinc           | 1.16               | 0.21  | 0.87               | 0.09  | 0.44               | 0.01  |
| Copper         | 0.390 <sup>b</sup> | 0.004 | 0.363 <sup>c</sup> | 0.002 | 0.208 <sup>d</sup> | 0.003 |
| Manganese      | 2.164              | 0.296 | 1.601              | 0.403 | 0.563              | 0.019 |

a Duplicate determination

b-d Means with different superscripts differ (p<0.01)

(McCarthy and Meredith 1988)

**Table 3. Amino Acid composition of raw chestnut kernels (g of amino acid per g of nitrogen)**

| <b>Amino acids</b> | <b>American</b> | <b>Chinese</b> | <b>European</b> |
|--------------------|-----------------|----------------|-----------------|
| Tryptophan         | 0.059           | 0.062          | 0.059           |
| Threonine          | 0.209           | 0.228          | 0.195           |
| Isoleucine         | 0.212           | 0.205          | 0.209           |
| Leucine            | 0.329           | 0.341          | 0.306           |
| Lysine             | 0.295           | 0.292          | 0.318           |
| Methionine         | 0.098           | 0.128          | 0.125           |
| Cystine            | 0.118           | 0.139          | 0.168           |
| Phenylalanine      | 0.231           | 0.275          | 0.225           |
| Tyrosine           | 0.174           | 0.194          | 0.152           |
| Valine             | 0.285           | 0.282          | 0.289           |
| Arginine           | 0.418           | 0.557          | 0.388           |
| Histidine          | 0.135           | 0.160          | 0.148           |
| Alanine            | 0.271           | 0.254          | 0.369           |
| Aspartic acid      | 0.886           | 1.076          | 0.904           |
| Glutamic acid      | 0.681           | 0.681          | 0.684           |
| Glycine            | 0.249           | 0.232          | 0.286           |
| Proline            | 0.235           | 0.206          | 0.270           |
| Serine             | 0.228           | 0.231          | 0.260           |

**a Mean values**

**(Kunsch et al., 1999)**

**Table 4. Proximate composition of raw chestnut (Colossal) grown in Michigan**

| <u>Analysis</u>         | <u>per 100gm</u> |            |
|-------------------------|------------------|------------|
| Calories                | 170              |            |
| Calories from Total Fat | 9                |            |
| Total Fat               | 1.01             | gm         |
| Saturated Fat           | 0.2              | gm (18.8%) |
| *Polyunsaturated Fat    | 0.5              | gm (52.3%) |
| *Monounsaturated Fat    | 0.3              | gm (28.9%) |
| Cholesterol             | < 1              | mg         |
| Sodium                  | 19               | mg         |
| Total Carbohydrate      | 37.2             | gm         |
| Dietary Fiber           | 6.3              | gm         |
| Sugars                  | 4.6              | gm         |
| Protein                 | 2.99             | gm         |
| Vitamin A               | 12               | IU         |
| Vitamin A               | 1                | RE         |
| Vitamin C               | 1.7              | mg         |
| Calcium                 | 15               | mg         |
| Iron                    | 1.26             | mg         |

\* = Non-mandatory label declarations (voluntary)

gm = grams

mg = milligrams

IU = International Units

RE = Retinol Equivalents

< = Less than

**Table 5. Amino Acid composition of raw chestnut (Colossal) grown in Michigan**

| <u>Fatty Acid</u>           | <u>% of Total Fatty Acids</u> |
|-----------------------------|-------------------------------|
| Saturated Fatty Acids       | 18.75                         |
| C12:0 Lauric Acid           | 0.18                          |
| C14:0 Myristic Acid         | 0.36                          |
| C15:0 Pentadecylic Acid     | 0.18                          |
| C16:0 Palmitic Acid         | 14.89                         |
| C17:0 Margaric Acid         | 0.25                          |
| C18:0 Stearic Acid          | 1.02                          |
| C20:0 Arachidic Acid        | 0.51                          |
| C21:0 Heneicosanoic Acid    | 0.18                          |
| C22:0 Behenic Acid          | 0.67                          |
| C23:0 Tricosanoic Acid      | 0.07                          |
| C24:0 Lignoceric Acid       | 0.44                          |
| Monounsaturated Fatty Acids | 28.93                         |
| C16:1 Palmitoleic Acid      | 0.43                          |
| C18:1 Oleic Acid            | 27.61                         |
| C20:1 Gondoic Acid          | 0.89                          |
| Polyunsaturated Fatty Acids | 52.32                         |
| C18:2 Linoleic Acid         | 44.93                         |
| C18:3 Linolenic Acid        | 6.94                          |
| C20:2 Eicosadienoic Acid    | 0.17                          |
| C20:4 Arachidonic Acid      | 0.28                          |

### **Problems associated with chestnuts during the post harvest period**

As new disease-resistant varieties of chestnut trees mature, the domestic grower will be able to compete favorably with imported chestnuts for the fresh chestnut market. Also, peeled fresh chestnuts add convenience and thus have the potential to be very successful in several market categories. However, peeled fresh chestnuts have been found to have a limited shelf life because of color change and microbial growth during storage. Dooley et al. (1980) stored European chestnuts, in air. Fungal infection was 10-15% in the air stored nuts.

Chestnuts have higher water activity (aw) level, moisture content and starch and lower protein level than most other nuts (Beuchat, 1978). Consequently, they have the potential to support the growth of a broad spectrum of fungi and bacteria, which are responsible for significant losses during storage. Fungal colonization of the nuts may occur at any stage from flowering through harvest, storage, sorting or transport via damage of the outer shell. Early studies on fungal spoilage were published by Wright (1960) and Wells and Payne (1975a) for *Castanea mollissima*, and Lanza (1950) and Riccardo (1963) for *Castanea sativa*. These studies reported that *Penicillium* spp. were most frequently (40.7%) isolated from infected chestnuts. Next, in order of frequency of occurrence, were *Rhizopus*, *Alternaria* and *Aspergillus*; each comprising about 17% of the total mycoflora isolated. Washington et al. (1997) reported that the fungi most commonly isolated from rotted chestnuts in Australia comprised *Penicillium* spp., *Botrytis cinerea*, *Phomopsis castanea*, *Fusarium* spp., *Mucor* spp., *Truncatella* spp. and *Cytospora* spp. Overy et al. (2003), in a market survey of Canadian grocery stores, isolated three dominant mycotoxigenic fungal species; *Penicillium crustosum*, *Penicillium glabrum* and

*Penicillium discolor* at frequencies of 67.1%, 18.6% and 17.7%, respectively. In the same study, the presence of the mycotoxins penitrem A, chaetoglobosin A and C, emodin and ochratoxin A was confirmed in extracts prepared from nut tissue. Thus, chestnut spoilage by fungi not only affects the aesthetic value of the nut due to the presence of fungal mycelium, but infected nuts may contain mycotoxins if they are not properly handled during post harvest operations.

Contamination of the tissue also occurs while peeling the pellicle of the chestnut from the kernel. About 80% of the labor in processing is needed in the peeling process, which is mostly done by hand using a knife. This results in the high cost of chestnut confectioneries (Harris and Smith 1988).

### **Non-heat Sanitation methods**

#### **A. Irradiation**

The application of irradiation to food has been approved by FAO/WHO for more than 50 types of food (Mertens and Knorr, 1992). The technology uses ionizing radiation for inactivating microorganisms (Thayer, 1994). Cobalt-60, x-ray, Beta electrons, IR etc. are common sources of Irradiation processing. Sterilization of packaging materials for aseptic packaging by irradiation has been done for several years. The package materials that can be used with irradiated foods are formed in the US Code of Federal Regulation title 21.149.5. However, irradiation is not widely used because acceptance by the public is limited.



## B. Sanitization (Chlorine, chlorine dioxide)

Chlorine is widely used in the washing and sanitizing of fresh produce and is highly effective in reducing the bacteria and mold population on product surfaces (Beuchat, 1998). Chlorine sanitization usually can reduce bacteria populations by 1 to 2 log (Pirovani et al., 2000; Sapers et al., 1999; Beuchat et al., 1998). Typically, chlorine is applied at a concentration of 200ppm or less. Some commercial products contain sodium hypochlorite, buffer, and surfactant to provide a high concentration of hypochlorous acid (Tenzer, 1997; Wartanessian, 1997). Chlorine dioxide is also used as an anti-microbial agent and has been approved for use on raw products (21CFR173.25, 2000).

Table 6. Advantages and Disadvantages of chlorine and chlorine dioxide for washing fresh fruits and vegetables.

| Agent            | Used Level (ppm) | Advantages                                                                                                                                                                                                                                                    | Disadvantages                                                                                                                                                                         |
|------------------|------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Chlorine         | 50-200           | Easy to apply<br>Inexpensive<br>Effective against all microbial form<br>Not affected by hot water<br>Easy to monitor<br>FDA approved                                                                                                                          | Decomposed by organic matter<br>Reaction products may be hazardous<br>Corrosive to metals<br>Irritating to skin<br>Activity pH-dependent<br>Population reductions limited to 1-2 logs |
| Chlorine dioxide | 1-5              | More potent than chlorine<br>Activity not pH dependent<br>Fewer chlorinated reaction products formed than with Cl <sub>2</sub><br>Effective against biofilms<br>FDA approved<br>Residual antimicrobial action<br>Less corrosive than Cl <sub>2</sub> or Ozone | Must be generated on-site<br>Explosive at high concentration<br>Not permitted for cut fruits and vegetables<br>Population reductions limited to 1-2 logs                              |

(Novak et al, 2003)

### **C. High Pressure processing**

With increasing consumer demand for light-processed fresh-like food, several non-thermal food processing methods have been developed such as pulse electrical fields, irradiation, and high pressure processing (HPP). HPP is a novel food preservation method that can produce food products with improved shelf-life, nutritional quality, flavor, texture, reduced enzymatic activity, and decreased microbial risk (Cheftel, 1995). HPP at 100-800 MPa is commercialized in the USA, Europe and Japan for processing products such as fruit juices, guacamole, tomato-based salsa and fresh oysters. Processing of pre-packaged foods is typically done using a batch-type system in concert with flexible packaging. Flexible packaging films are made by single layer or multi-layers using coated or uncoated polymeric materials. Ideally, HPP should not affect packaging material properties, however, recent research has showed that changes occurred in the barrier properties of PET and EVOH films after HPP. This occurred because both PET and EVOH showed crystallinity changes when exposed to high pressure (Lambert et al. 2000; Caner et al, 2000).

In addition to mechanical considerations, HPP may affect sorption parameters (Matsui et al. 1992). This could affect the organoleptic and nutritional quality attributes of packaged products (Gavara et al. 1996). Sorption refers to the uptake of volatile components from a food product to plastic materials. Sorption, including flavor scalping, can occur in foods such as juice and fruit-flavor beverages. Sorption may cause the degradation of flavor quality of packaged food during storage or processing which causes a reduction in shelf-life (Fukamachi et al. 1996). Generally, polyolefins such as PE and PP absorb larger quantities of aroma compounds than the polar polymers such as PET. It is likely that

enzyme activity would be reduced and the microbial population of fresh peeled chestnuts would be lowered by HPP treatment.

#### **D. Modified Atmosphere Packaging**

A general definition of modified atmosphere packaging (MAP) is that the package atmosphere is different from the normal composition of air (21% Oxygen, 0.03% Carbon dioxide, 78.97% Nitrogen)(Mathlouthi, 1994). Using this definition, several packaging techniques can be included such as vacuum packaging, control atmosphere packaging and modified atmosphere packaging. Vacuum packaging is the most common method of modifying the internal packaging atmosphere. In vacuum packaging, the product is placed in a package of low oxygen permeability, the air is evacuated and the package sealed in the absence of any other gas. Typically, an atmosphere with elevated levels of carbon dioxide develops as the food product and contaminating microorganisms consume any residual oxygen and produce carbon dioxide (Devlieghere and Debevere, 2000). Control atmosphere packaging includes systems to maintain a certain atmosphere around the product. This technique tends to be limited to storage in the warehouse or in bulk packs. Modified atmosphere packaging is established by gas flushing or by allowing air-packed products to generate an internal atmosphere as a result of respiration.

Modified atmosphere packaging can extend the shelf life of food by inhibiting chemical, enzymatic and microbial spoilage. This allows preservation of the food without heat and chemical treatment. The gases used in most MAP applications include carbon dioxide, oxygen and nitrogen, and different products need different combinations of gases. Carbon dioxide is used primarily to inhibit the growth of microorganisms. Carbon dioxide

typically inhibits the growth of gram negative bacteria which usually grow rapidly and produce off flavors associated with spoilage of many foods (Devlieghere and Debevere, 2000). The amount of oxygen in the package depends on the product. Oxygen is used with gas packs of fresh meat to maintain desired color. The level of oxygen needs to be carefully determined, because if the level is too low, it may result in anaerobic respiration and produce off flavors. Nitrogen is an inert gas usually used as a headspace filler. Many types of food products are currently being packed by modified atmosphere packaging, and one has to be careful to select a suitable gas combination. Fresh peeled chestnuts can be packed under modified atmosphere in order to decrease the microbiological risk. Again, a suitable gas composition must be used.

Many researches have reported that the modified atmosphere packaging can inhibit the growth of microorganisms in order to extend product shelf life. Simon et al (2005) reported that modified atmosphere packaging with CO<sub>2</sub> levels of 2.5% and O<sub>2</sub> levels of 10-20 % had a beneficial effect on sliced mushrooms at 4C. Modified atmosphere packaging inhibited the growth of bacteria, improved slice appearance, and reduced the incidence of bacterial blotches. No microbial spoilage was observed in packages of blueberry stored under modified atmosphere with 6% O<sub>2</sub> and 4% CO<sub>2</sub> at 4C after 12 weeks (Day et al, 1990).

### **3. MATERIALS AND METHODS**

#### **Chestnuts**

Chinese chestnuts (*Castanea sativa* Mill.) were harvested from several local orchards in southwest Michigan (Benton Harbor) and northwest Michigan (Traverse City) in October 2003 and 2004. The chestnuts were washed, drained and transported to Michigan State University and stored at  $-2 \pm 2^{\circ}\text{C}$ . The chestnuts were then transported to the pilot plant in Jackson, MI for peeling and processing. The peeled chestnuts were then sent back to Michigan State University for further treatment and evaluation.

#### **Chestnuts Peeler – Project 1**

A new machine, called a Brulage chestnut peeler pilot plant (Boema) (Figure 2), was imported from Italy in 2001. The shelling equipment is the first of its kind in the United States. The chestnut peeler is made up of four parts: 1. a double burner which burns off the relatively thin chestnut shell and pellicle. 2. a thrasher which uses centripetal force to remove any remaining shell or pellicle. 3. a steamer that moistens and cleans the chestnuts. and 4. a brusher/washer which removes the remaining dirt, shell or pellicle. Chestnuts with the shell on are metered into the burner; conveyed through the oven within a screw auger cage to make the peel brittle; passed into a thrasher containing rubber-ended paddles moving against steel rods which break away the peel and all or part of the pellicle; conveyed to steamer which is a closed screw conveyor one-half filled with water and heated with steam to  $70\text{--}80^{\circ}\text{C}$  ( $\sim 158\text{--}176^{\circ}\text{F}$ ) to loosen any remaining shell or pellicle; then finally moved onto a brusher/washer which removes loose pellicle and shell with counter-rotating pairs of rollers, followed by cleaning rinse.

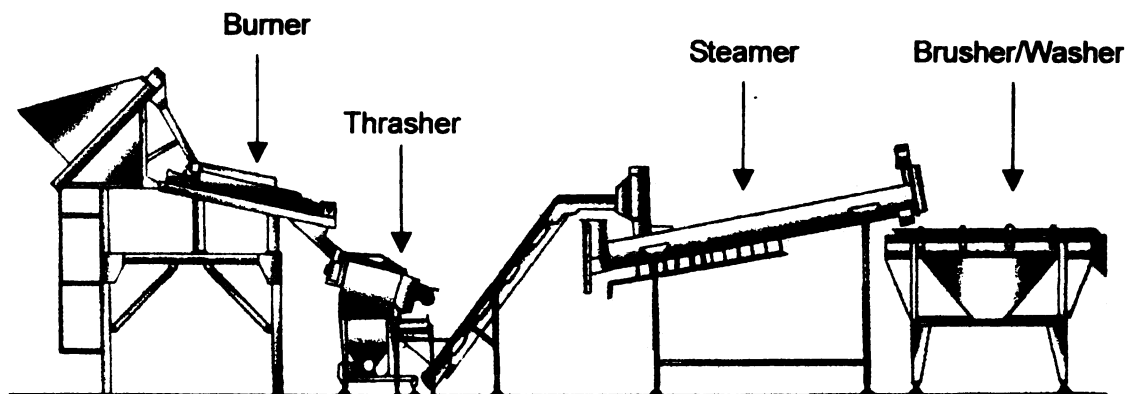


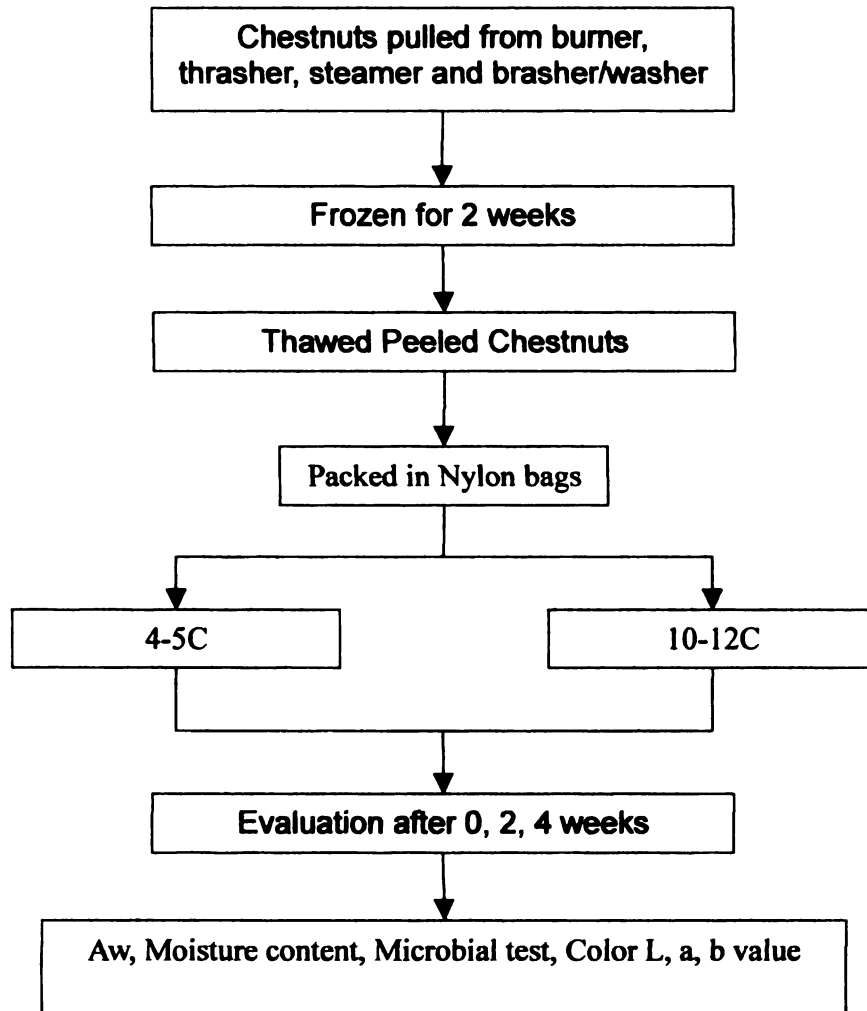
Figure 2. Schematic of integrated components of brulage chestnut peeling line

#### Chestnut samples used for microbial analysis

Chestnut (with the shell) microbial assays were performed at 0 time, 2 weeks, 2 months and 4 months storage. Chestnut shells were removed by hand in a laminar flow hood and a sanitized knife was used for removing the shells.

The baseline microbial populations of microorganisms for the four different parts of the peeler (burner, thrasher, steamer, and brusher/washer) were also determined. 15 lbs of chestnuts were collected from each part and divided into 3 sets. The first sets were used to determine the real time microbial populations. The second sets were packed in Nylon bags (225g), vacuum sealed and frozen at -17.6°C. After 2 weeks storage, the samples were pulled out and thawed at 25°C for 1 hour. The chestnut samples were then stored at 4-5°C and 10-12°C for 2 and 4 weeks and then assayed. The third set was packed in PE bags (225g) and stored at 4-5°C and 10-12°C for 2 and 4 weeks and then assayed.





**Fig 3. Flow diagram of Frozen and thawed peeled chestnut quality evaluation**

### **Packaging Materials**

Three different packaging materials, Nylon/Polyethylene (Koch Supplies Inc., Kansas City, MO), Polyethylene (VWR International, Batavia, IL) and Porous Polyethylene (12 0.2in diameter holes on the bag and covered by Tyvek®), were used in this study. For the Nylon/Polyethylene bags (12in x 8in, 3mil), vacuum and atmospheric packaging processes were employed. Vacuum packaging was accomplished using the MULTIVAC A 300/16 machine (MULTIVAC INC., Kansas City, MO) set at 800 mbar. For the



Polyethylene bags(12in x 8in, 2mil), punctured and non-punctured bags were used. Punctured PE bags were fabricated by placing 9 evenly distributed holes (about 0.5 cm diameter) on both sides of the pouch. These were then covered by Tyvek®. All bags, excepted the vacuum packed bags, were sealed using the SENCORP SC-12 impulse heat sealer (Sencorp System Inc., Hyannis, MA). Tyvek® is a bound polyethylene which allows free flow of gases such as oxygen.

#### Microbial assays

The total microbial count for each sample was determined using the APHA Standard Plate Count Method (Busta et al., 1984). 25g of chestnuts were taken and shaken together with 225ml of phosphate buffer (pH 7) containing 0.1% peptone water. A  $10^{-1}$  sample dilution was made by aseptically transferring 1 ml of the previous buffer solution into a tube containing 9 ml of sterile phosphate buffer solution and mixing well. Sample dilutions of  $10^{-2}$  to  $10^{-8}$  were used for microbial enumeration. A 0.1 ml sample was taken from each dilution to determine the microbial numbers for the different microorganisms (bacteria, yeast, mold, E. Coli), using standard pour plate techniques. All tests were done in duplicate. Media and incubating conditions for each type of microbial group were as follows:

Total bacteria count: Trypticase Soy Agar with 0.6% Yeast Extract (TSA-YE, Sigma, MA), incubated at 23C for 48-72 hours.

Yeast and Mold: Rose Bagel Agar (Sigma, MA), incubated at 23C for 5 days.

E. Coli: Petri film (3M, MN), incubated at 37C for 24-48 hours.

Colonies from each plate were counted and calculated as the average colony forming



units (CFU) per gram chestnuts.

The following general methods were used when determining the bacteria, yeast and mold population levels. A chestnut sample weighing ~25 grams was removed and soaked in 225 ml phosphate buffer and mixed using a pulsifier for 1 min. The yeast/mold and bacteria populations were determined by plating the buffer solution on rose bagel agar and trypticase soy agar containing 0.6% yeast extract (TSAYE), respectively and incubating at the appropriate temperature. All of the microbial assays were duplicated.

#### **Water Activity and Moisture Content**

The water activity of chestnuts was determined at 25C using an electronic dew-point water activity meter, Aqualab Series 3 model TE (Decagon Devices, Pullman, Washington, USA). For each determination five replicates were obtained and the average reported. Moisture content was measured by loss in weight after heating at 105 °C in the vacuum oven for 6 hours.

#### **Frozen and thawed peeled chestnut – Project 2**

Approximately 50 pounds of peeled chestnuts were stored at -20C in cooler and then thawed at 25C for 1 hour after 2 weeks. The thawed samples were packaged in 3mil nylon bags and the filled packages (225g) were vacuum-sealed and stored at 4-5C and 10-12C. The moisture content, color (Hunter Color Difference), water activity and microbial (bacteria, yeast and mold) counts of the chestnut samples were determined. Triplicate analyses were done for all of the tests.

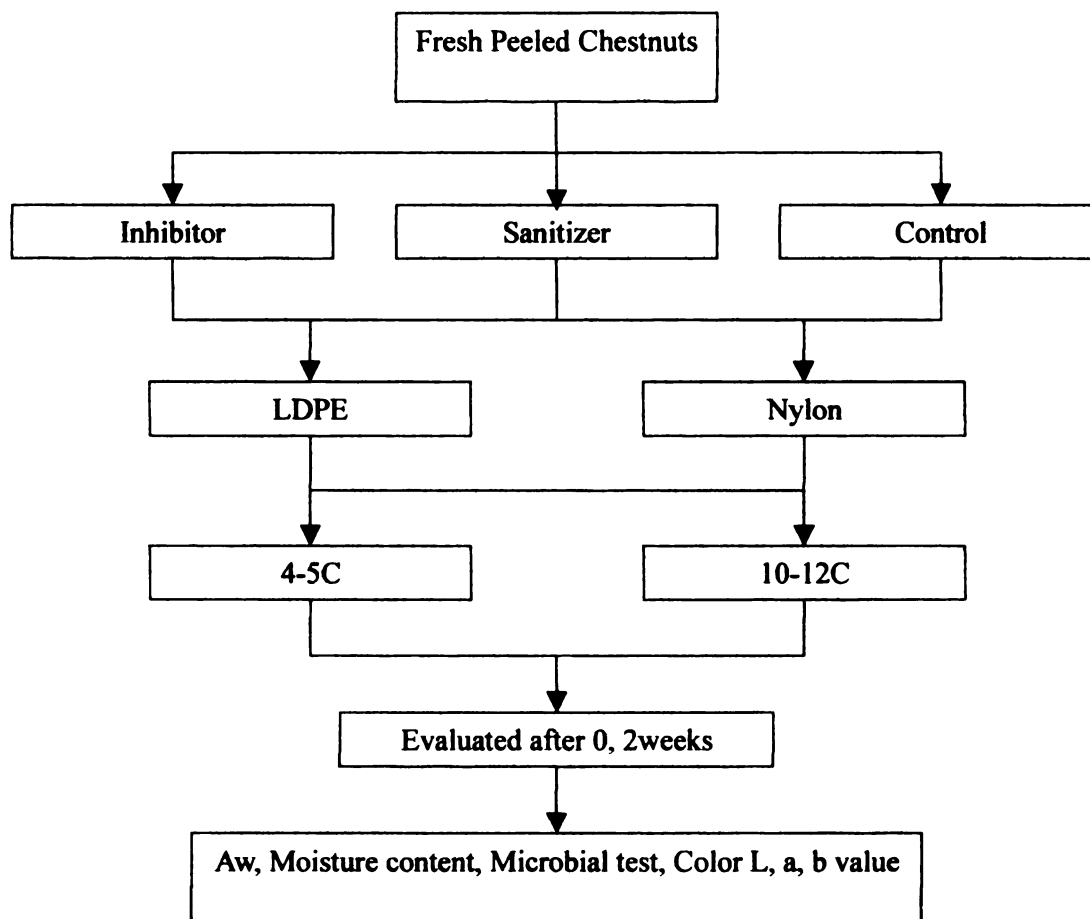


Fig 4. Flow diagram of Fresh, Peeled Chestnuts subjected to sanitizer treatments

### **Fresh, Peeled Chestnut treated with sanitizing agents – Project 3**

Approximately 100 pounds of peeled chestnuts which harvested and peeled in October, 2004 were used in this study. Two thirds of the chestnuts were dipped into a 0.1% solution of potassium sorbate or commercial chlorine sanitizer (80 ppm) and then drained for 5 minutes. All of the control and treated samples were packaged using three different bag materials, low-density polyethylene (LDPE) 3mils thick, nylon 3 mils thick and

micro-porous PE bags 3mils thick. All filled packages (225g) were weighed, heat-sealed to closure and stored at 4-5C or 10-12C for 14 days. Evaluation of chestnut moisture content, color (Hunter Color Difference, L, a, b values), water activity and microbial population (bacteria, yeast and mold) was made. Triplicate analyses were done for all tests.

#### **Fresh peeled chestnuts with alternate sanitizers – Project 4**

Fresh peeled chestnuts were immersed in distilled water containing sodium hypochlorite (100, 200ppm) (SC Johnson, US) then pulled out and drained, or boxed in plastic boxes with holes and exposed to chlorine dioxide gas using a novel Z-series 2000G solid release chlorine dioxide delivery system (ICA TriNova LLC, GA). Distilled water dipping of the chestnuts without sanitizer served as the control. After 5 and 10 minutes of hypochlorite solution immersion, or 12 hours chlorine dioxide exposure, chestnut samples were assayed for bacteria, yeast and mold populations. The remaining treated samples were vacuum packed in Nylon pouches and held at 4-5C or 10-12C for 2 and 4 weeks storage. Sensory evaluation and microbial count assays were then performed on the chestnut.

#### **Sensory evaluation**

A eight-member trained sensory panel evaluated the quality of the chestnuts. A nine-point scale was used for all attributes except texture. All sensory testing was conducted in the Sensory Laboratory at Michigan State University. Panelists were MSU faculty and

students who were selected on the basis of their ability to detect specific product attributes and their availability. Eight panelists participated in the training sessions before every sensory evaluation, at which the training samples varied in color (1 = dull chestnuts, 9 = fresh peeled chestnuts), flavor (1= water, 9 = strong chlorine flavor, 200ppm) and texture (1= cooked chestnuts, 9 = dried chestnuts) in order to give the panelists a range of attribute intensities.

### **Statistical Analysis**

Statistical analysis of all of the data was performed using an Analysis of Variance and Duncan multiple-range test using the statistical software program, SAS version 8.01. Statistical significance was defined as  $p < 0.05$ .

## 4. RESULTS & DISCUSSION

### Baseline levels of microorganisms

Chestnuts harvested at the end of September 2003 from several orchards in Benton Harbor, MI, were transported and stored at  $-2C \pm 2C$  at Michigan State University. The initial microbial populations of these fresh chestnuts were determined after removing the shells by hand under sterile conditions. Results showed high bacteria (total plate count) populations of  $\text{Log } 10^7$  CFU/g chestnut. After 2 months storage, the bacterial populations increased to  $\text{Log } 10^9$  CFU/g. After 4 months storage, the microbial populations decreased slightly to  $1.5 \times 10^8$  CFU/g (Table 4). Yeast, mold, and E. coli were not detected or populations were very low. These results indicate that the chestnuts naturally harbor large populations of bacteria prior to the peeling process. Also, because of the chestnut metabolism, the starch is converted to glucose which is easy to use by spoilage microorganisms even in low temperature storage (Kawano et al., 1984). Kawano et al. (1984) indicated that the shelf life of fresh chestnut (in the shell) is 3 months at  $1C$ , 2 months at  $5C$ , and 1 month at  $10C$  storage.

Table 7. Microbial populations of chestnuts stored at  $2^\circ C$  before peeling.<sup>1,2</sup>

| Amount CFU/g | 0 Day           | 2 weeks           | 2 months        | 4 months          |
|--------------|-----------------|-------------------|-----------------|-------------------|
| Bacteria     | $1 \times 10^7$ | $1 \times 10^7$   | $2 \times 10^9$ | $1.5 \times 10^8$ |
| Yeast        | -               | $1.5 \times 10^1$ | -               | $2 \times 10^2$   |
| Mold         | -               | $2.5 \times 10^1$ | -               | $4 \times 10^2$   |
| E. coli      | -               | -                 | -               | -                 |

<sup>1</sup> (–) indicates no colonies detected

<sup>2</sup> Results are the average of duplicate analyses

### **Contamination from the peeling line**

Chestnuts were removed from sections of the peeling line including the burner, the tangential thrasher, the steamer and the washer/brusher, packed in PE (3 mil) bags and stored at 4C and 10C. Initial bacterial counts from the different peeler positions were Log  $10^7$ , Log  $10^7$ , Log  $10^6$ , and Log  $10^8$  CFU/g (Table 5), respectively. After 2 weeks storage, samples taken from the burner, thrasher, and steamer had bacterial populations of approximately Log  $10^8$  CFU/g. The samples taken from the washer/brusher had bacteria populations  $> \text{Log } 10^{11}$  CFU/g (Table 6 and 7). The results showed that the steamer reduced log populations by one log, but after chestnuts passed through the washer/brusher, bacterial numbers again increased. This may have been due to brush contamination. Yeast and mold were not found or were less than  $10^2$  CFU/g, except for the yeast population of the chestnuts stored at 10C after 14days. This was probably due to contamination during sampling or microbial testing. The *E. coli* populations were less than 1 CFU/g before and after storage. The results showed that the initial populations of yeast, mold and *E. coli* were at very low levels.

Frozen chestnut samples from the 4 different parts of the peeling line were packed in a commercial barrier material (LDPE/Nylon, 3mil) and tested immediately after thawing and again after 2 and 4 weeks storage at 4 and  $10^0\text{C}$ . The initial bacteria counts were approximately  $10^5$  CFU/g (Table 8). After 2 weeks storage at 4 and  $10^0\text{C}$ , the bacteria populations had increased to approximately  $1 \times 10^7$  and  $1 \times 10^8$  CFU/g, respectively, with populations of  $> 1 \times 10^{12}$  CFU/g on samples taken from the washer/brusher (Table 9 and 10). Yeast, mold and *E. coli* were less than  $10^2$  CFU/g which indicates that the initial yeast, mold, and *E. coli* counts were low. The results also indicate that most of the



contamination was from the washer/brusher. Chestnuts packed in both packaging materials had results about the same, and thus neither material had any significant effect on controlling growth of microorganism.

**Table 8. Microbial populations on chestnuts packed in PE bags from different locations within the chestnut peeler (day 0).<sup>1,2</sup>**

| Amount CFU/g | Burner          | Thrasher        | Steamer         | Brusher/Washer  |
|--------------|-----------------|-----------------|-----------------|-----------------|
| Bacteria     | $2 \times 10^7$ | $2 \times 10^7$ | $1 \times 10^6$ | $3 \times 10^8$ |
| Yeast        | -               | -               | -               | -               |
| Mold         | -               | -               | -               | $4 \times 10^2$ |
| E. Coli      | -               | -               | -               | -               |

<sup>1</sup> ( - ) indicates no colonies detected

<sup>2</sup> Results are the average of duplicate analyses

**Table 9. Microbial populations on chestnut from different locations within the chestnut peeler, packed in PE bags and stored at 4C for 14days.<sup>1,2</sup>**

| Amount CFU/g | Burner          | Thrasher | Steamer         | Brusher/Washer |
|--------------|-----------------|----------|-----------------|----------------|
| Bacteria     | $2 \times 10^8$ | -        | $2 \times 10^7$ | $> 10^{12}$    |
| Yeast        | -               | -        | -               | -              |
| Mold         | -               | -        | -               | -              |
| E. Coli      | -               | -        | -               | -              |

<sup>1</sup> ( - ) indicates no colonies detected

<sup>2</sup> Results are the average of duplicate analyses

**Table 10. Microbial populations on chestnuts from different locations within the chestnut peeler and packed in PE and stored at 10C for 14days. <sup>1,2</sup>**

| Amount CFU/g | Burner          | Thrasher        | Steamer         | Brusher/Washer |
|--------------|-----------------|-----------------|-----------------|----------------|
| Bacteria     | $6 \times 10^8$ | $4 \times 10^7$ | $3 \times 10^8$ | $>10^{12}$     |
| Yeast        | -               | $3 \times 10^7$ | -               | $>10^{12}$     |
| Mold         | -               | -               | -               | -              |
| E. Coli      | -               | -               | -               | -              |

<sup>1</sup> ( - ) indicates no colonies detected

<sup>2</sup> Results are the average of duplicate analyses

**Table 11. Microbial populations on chestnuts from different locations within the chestnut peeler and packed in Nylon bags (day 0). <sup>1,2</sup>**

| Amount CFU/g | Burner          | Thrasher        | Steamer           | Brusher/Washer    |
|--------------|-----------------|-----------------|-------------------|-------------------|
| Bacteria     | $1 \times 10^5$ | $1 \times 10^5$ | $1.5 \times 10^4$ | $3.5 \times 10^5$ |
| Yeast        | -               | -               | -                 | $2 \times 10^1$   |
| Mold         | -               | -               | -                 | $1 \times 10^2$   |
| E. Coli      | -               | -               | -                 | -                 |

<sup>1</sup> ( - ) indicates no colonies detected

<sup>2</sup> Results are the average of duplicate analyses

**Table 12. Microbial populations on chestnuts from different locations within the chestnut peeler and packed in Nylon bags and stored at 4C for after 14 days. <sup>1,2</sup>**

| Amount CFU/g    | Burner            | Thrasher        | Steamer           | Brusher/Washer |
|-----------------|-------------------|-----------------|-------------------|----------------|
| <b>Bacteria</b> | $1.4 \times 10^7$ | $1 \times 10^7$ | $1.2 \times 10^7$ | $>10^{12}$     |
| <b>Yeast</b>    | -                 | -               | -                 | -              |
| <b>Mold</b>     | -                 | -               | -                 | -              |
| <b>E. Coli</b>  | -                 | -               | -                 | -              |

<sup>1</sup> ( - ) indicates no colonies detected

<sup>2</sup> Results are the average of duplicate analyses

**Table 13. Microbial populations on chestnuts from different locations within the chestnut peeler and packed in Nylon bags and stored at 10C for 14 days. <sup>1,2</sup>**

| Amount CFU/g    | Burner            | Thrasher        | Steamer           | Brusher/Washer |
|-----------------|-------------------|-----------------|-------------------|----------------|
| <b>Bacteria</b> | $2.5 \times 10^8$ | $3 \times 10^8$ | $4.5 \times 10^8$ | $>10^{12}$     |
| <b>Yeast</b>    | -                 | -               | -                 | -              |
| <b>Mold</b>     | -                 | -               | -                 | -              |
| <b>E. Coli</b>  | -                 | -               | -                 | -              |

<sup>1</sup> ( - ) indicates no colonies detected

<sup>2</sup> Results are the average of duplicate analyses

### Quality of frozen and thawed peeled chestnuts stored at 4 and 10C

The moisture content and water activity of frozen and thawed peeled chestnuts stored at 4 and 10 °C are shown in Figures 5 and 6. The storage period and the storage temperature had no significant effect on moisture content and water activity. The highest moisture value was determined on day 0 (packing day) and decreased slightly during the storage period at both 4 and 10 °C. The water activity of the chestnuts did not vary significantly between 0 and 28 days at 4 and 10 °C.

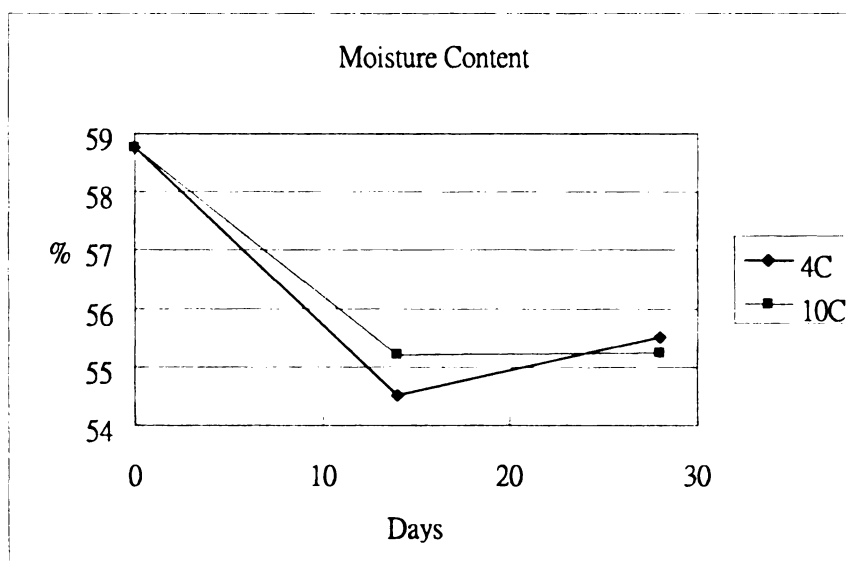
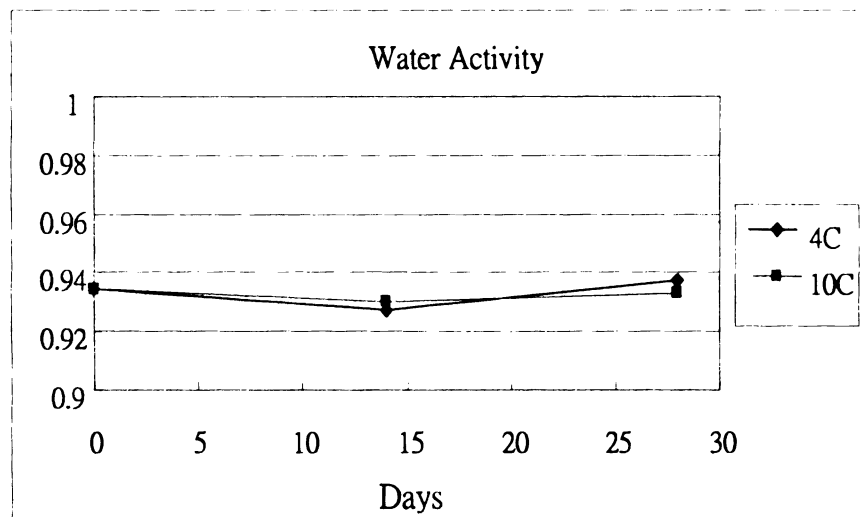


Figure 5. The moisture content of frozen and thawed chestnuts during storage



**Figure 6. The water activity of frozen and thawed chestnuts during storage**

Analysis of the color values showed that there was a significant difference in the “L” (white) values ( $P < 0.05$ ) for the outside and inside of the frozen and thawed peeled chestnuts after 28 days storage at 4-5 and 10-12C. The “L” (white) values for the outside surface of the chestnuts were higher than the inside values (Figure 7 and 8). There was no significant difference between the outside and inside of the chestnuts during storage at 10-12C. However, the chestnuts became darker after 28-days storage at 4-5C. The darkening of chestnuts likely occurred because of PPO activity even in low temperature storage (Xu, 2005).

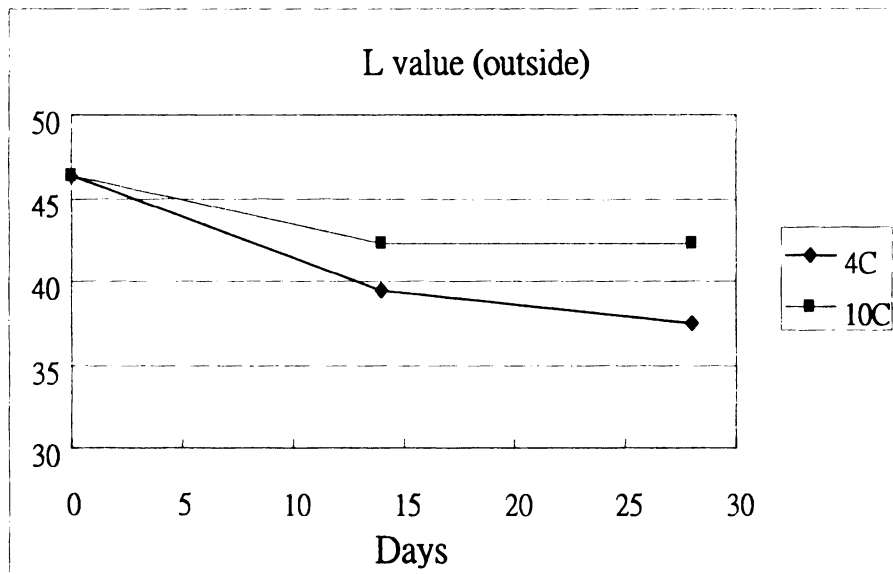


Figure 7. The L-value (outer layer) of chestnuts during storage

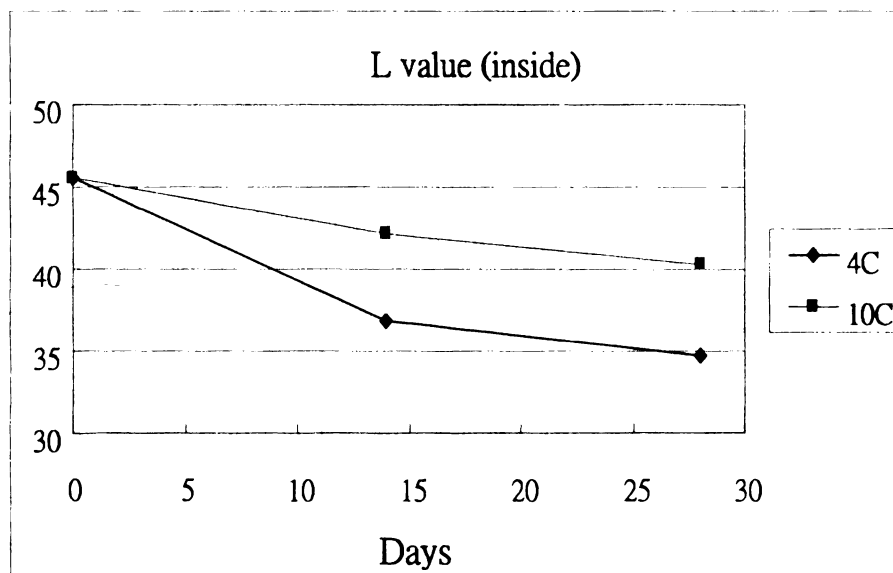


Figure 8. The L-value in the interior of the chestnuts during storage

The “a” (red) value for the interior of the chestnuts was higher than that for the outside, (Figure 8 and 9) at day 0. During storage, the a-value decreased on the inside of the chestnuts, but the a-value increased on the outside of the chestnuts. The “a” (red) values of the inside and outside of the frozen and thawed peeled chestnuts did not significantly differ after 28 days storage at 4-5 and 10-12C.

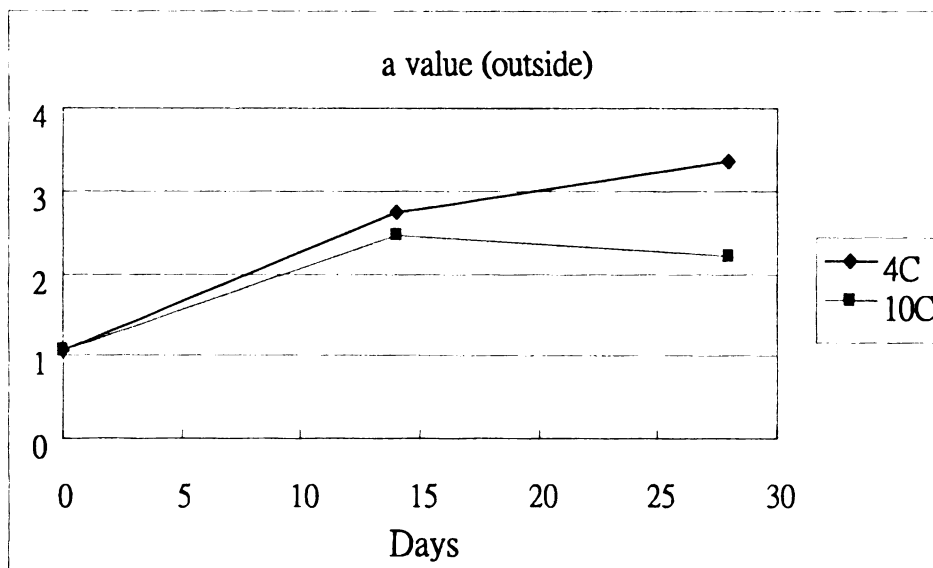
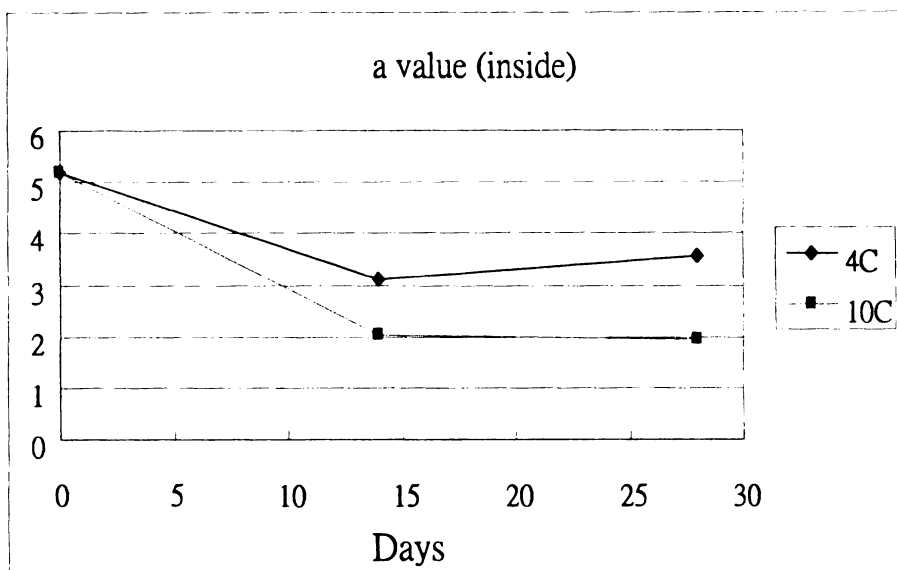


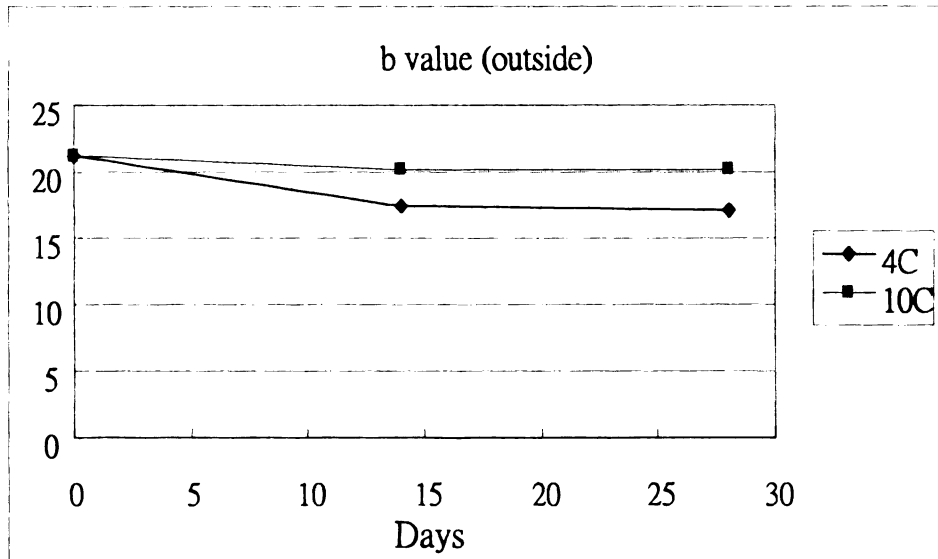
Figure 9. The a-value of the chestnuts exterior layer during storage



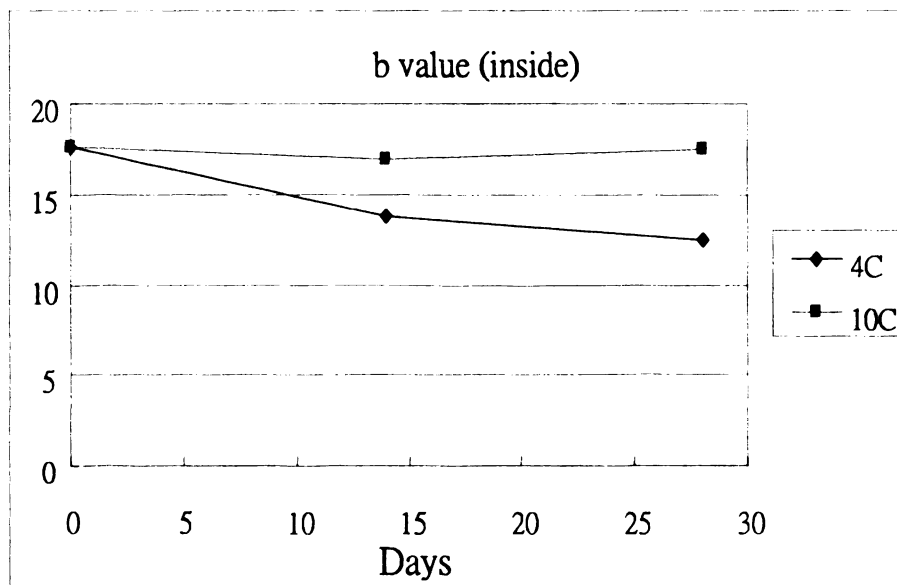
**Figure 10. The a-value on the interior of chestnuts during storage**

The “b” (yellow) values for the outside and the inside of the frozen and thawed peeled chestnuts were significantly different (Figure 11 and 12). However, there were no significant differences in b values between the outside of the chestnuts stored at 4-5 and 10-12C. There were, however, significant differences in b values in the interior of the chestnuts. The b values of chestnuts stored at 4-5C decreased during storage (Figure 12), but the b values of chestnuts stored at 10-12C were essentially the same.





**Figure 11. The b-values of the exterior surface of the chestnuts during storage**



**Figure 12. The b-values of the interior of the chestnuts during storage**

### **Microbial populations of the chestnuts**

Mold did not grow on the samples probably because of the vacuum packaging and lower storage temperature. Bacteria and yeast numbers increased very quickly in the packaged products stored at 10-12C (Table 11 and 12). The data show that the lower storage temperature (4 – 5C) inhibited the growth of microorganisms. There were significant differences between chestnuts stored at the different temperatures after 14-days storage. Bacteria counts increased to  $10^{12}$  and  $10^6$  at 10-12C and 4-5C storage, respectively. Based on microbial numbers, the results also indicate that the shelf life of the chestnuts is about 2 weeks when stored at 4-5C and less than 2 weeks at 10-12C storage.

**Table 14. The bacteria count (TPC) of chestnuts during storage<sup>1</sup>**

|          | 0 days            | 14 days              | 28 days              |
|----------|-------------------|----------------------|----------------------|
| 4C – 5C  | $1.2 \times 10^4$ | $5.4 \times 10^6$    | $3.8 \times 10^{11}$ |
| 10 – 12C | $1.2 \times 10^4$ | $7.5 \times 10^{12}$ | $3.7 \times 10^9$    |

<sup>1</sup> Results are the average of duplicate analyses

**Table 15. The yeast count on chestnuts during storage<sup>1</sup>**

|          | 0 days            | 14 days              | 28 days              |
|----------|-------------------|----------------------|----------------------|
| 4C – 5C  | $4.5 \times 10^3$ | $6.5 \times 10^6$    | $2.4 \times 10^{11}$ |
| 10 – 12C | $4.5 \times 10^3$ | $2.5 \times 10^{12}$ | $5.4 \times 10^9$    |

<sup>1</sup> Results are the average of duplicate analyses

### **Fresh, Peeled Chestnut Packaging**

Chestnuts harvested and peeled in October, 2004 were used in this study. Two thirds of the chestnuts were dipped into a 0.1% solution of potassium sorbate or commercial chlorine sanitizer (80 ppm). All of the control and treated samples were packaged using three different bag materials, low-density polyethylene (LDPE) 3mils thick, nylon 3 mils thick and micro-porous PE bags 3mils thick. The moisture content and water activity did not change significantly during storage. The different treatments, packaging materials or storage temperatures did not have any significant effect on chestnut moisture content and water activity after 14 days storage (table 13 and 14). The moisture content and the water activity of the fresh peeled chestnuts were about the same as for frozen, thawed peeled chestnuts. Therefore, the moisture content and water activity did not change significantly during frozen storage.

**Table 16. The moisture content of fresh peeled chestnuts after 14 days storage<sup>1</sup>**

| Sample          | Moisture Content %(10-12C) | Moisture Content %(4-5C) |
|-----------------|----------------------------|--------------------------|
| Initial (day 0) | 59.14%                     | 59.14%                   |
| i-pe            | 59.68%                     | 58.87%                   |
| i-ppe           | 58.13%                     | 60.78%                   |
| i-ny            | 58.42%                     | 57.81%                   |
| s-pe            | 57.01%                     | 55.09%                   |
| s-ppe           | 55.57%                     | 58.17%                   |
| s-ny            | 57.07%                     | 59.72%                   |
| c-pe            | 55.80%                     | 59.55%                   |
| c-ppe           | 56.24%                     | 59.22%                   |
| c-ny            | 58.87%                     | 59.50%                   |

i = Inhibitor, s = Sanitizer, c = Control, pe = PE bag, ppe = Porous PE bag, ny= Nylon bag

<sup>1</sup>Results are the average of duplicate analyses

**Table 17. The water activity of fresh peeled chestnuts after 14 days storage <sup>1</sup>**

| <b>Sample</b>          | <b>Water activity(10-12C)</b> | <b>Water activity(4-5C)</b> |
|------------------------|-------------------------------|-----------------------------|
| <b>Initial (day 0)</b> | <b>0.935</b>                  | <b>0.935</b>                |
| <b>i-pe</b>            | <b>0.883</b>                  | <b>0.878</b>                |
| <b>i-ppe</b>           | <b>0.878</b>                  | <b>0.884</b>                |
| <b>i-ny</b>            | <b>0.890</b>                  | <b>0.919</b>                |
| <b>s-pe</b>            | <b>0.923</b>                  | <b>0.943</b>                |
| <b>s-ppe</b>           | <b>0.879</b>                  | <b>0.900</b>                |
| <b>s-ny</b>            | <b>0.894</b>                  | <b>0.877</b>                |
| <b>c-pe</b>            | <b>0.886</b>                  | <b>0.909</b>                |
| <b>c-ppe</b>           | <b>0.885</b>                  | <b>0.909</b>                |
| <b>c-ny</b>            | <b>0.889</b>                  | <b>0.902</b>                |

**i = Inhibitor**

**s = Sanitizer**

**c = Control**

**pe = PE bag**

**ppe = Porous PE bag**

**ny= Nylon bag**

<sup>1</sup> **Results are the average of duplicate analyses**

No significant change was observed in the L-value and b-value of the chestnut samples. The a-value increased during storage. This shows that the chestnuts were found to darken during storage. Polyphenol oxidase is responsible for the enzymatic browning reaction, which is triggered during the handling, storage and processing of fruit and vegetables (Macheix et al., 1990). It is also known that free phenolics in contact with PPO, cause substantial browning in plant materials. However, the phenolic content in chestnuts was not significant different between fresh and 6 months stored chestnuts at 4C (Xu, 2005).

Table 18. The L-value of fresh peeled chestnuts after 14 days chestnut storage <sup>1</sup>

| Sample          | L-value (10-12C) | L-value (4-5C) |
|-----------------|------------------|----------------|
| Initial (day 0) | 46.45            | 46.45          |
| i-pe            | 42               | 41.55          |
| i-ppe           | 41.65            | 39.9           |
| i-ny            | 43               | 43.6           |
| s-pe            | 42.75            | 41.35          |
| s-ppe           | 42               | 41.75          |
| s-ny            | 41.85            | 42.35          |
| c-pe            | 42.5             | 43.3           |
| c-ppe           | 42               | 41.95          |
| c-ny            | 42.5             | 42.5           |

i = Inhibitor

s = Sanitizer

c = Control

pe = PE bag

ppe = Porous PE bag

ny= Nylon bag

<sup>1</sup> Results are the average of duplicate analyses

**Table 19. The a-value of fresh peeled chestnuts after 14 days storage<sup>1</sup>**

| Sample          | a-value (10-12C) | a-value(4-5C) |
|-----------------|------------------|---------------|
| Initial (day 0) | 1                | 1.55          |
| i-pe            | 3.15             | 2.9           |
| i-ppe           | 3.9              | 3.25          |
| i-ny            | 2.45             | 2.55          |
| s-pe            | 2.65             | 2.85          |
| s-ppe           | 3.5              | 3.8           |
| s-ny            | 2.45             | 2.9           |
| c-pe            | 1.6              | 1.8           |
| c-ppe           | 3.85             | 3.15          |
| c-ny            | 2.45             | 3.1           |

i = Inhibitor

s = Sanitizer

c = Control

pe = PE bag

ppe = Porous PE bag

ny= Nylon bag

<sup>1</sup> Results are the average of duplicate analyses

**Table 20. The b-value of fresh peeled chestnuts after 14 days storage<sup>1</sup>**

| Sample          | b-value (10-12C) | b-value (4-5C) |
|-----------------|------------------|----------------|
| Initial (day 0) | 21.15            | 17.7           |
| i-pe            | 18.3             | 16.1           |
| i-ppe           | 17.25            | 16.05          |
| i-ny            | 19.6             | 16.75          |
| s-pe            | 18.55            | 16.75          |
| s-ppe           | 18.85            | 16.55          |
| s-ny            | 19.5             | 16.6           |
| c-pe            | 19.9             | 17.5           |
| c-ppe           | 18.65            | 17.25          |
| c-ny            | 18.65            | 16.6           |

i = Inhibitor

s = Sanitizer

c = Control

pe = PE bag

ppe = Porous PE bag

ny= Nylon bag

<sup>1</sup> Results are the average of duplicate analyses

The different packaging materials (PE/Nylon), sanitizer treatments, and storage temperature did not have a substantial effect on the growth of bacteria and yeast. This was probably because the initial loading of the chestnuts was so high; it overshadowed any possible benefit from the packaging system. The mold, however, only grew in the micro-porous PE bags stored at 10-12C probably because this bag allowed free flow of oxygen. Storage at 4-5C mostly inhibited the growth of the mold on chestnuts stored under these conditions.

**Table 21. The total plate count of fresh chestnuts stored at 10-12C after 14 days storage<sup>1</sup>**

| Sample | Initial (CFU/g)  | After storage        |
|--------|------------------|----------------------|
| i-pe   | $>2 \times 10^6$ | $4.0 \times 10^9$    |
| i-ppe  | $>2 \times 10^6$ | $>10^{11}$           |
| i-ny   | $>2 \times 10^6$ | $6.0 \times 10^9$    |
| s-pe   | $>2 \times 10^6$ | $7.5 \times 10^9$    |
| s-ppe  | $>2 \times 10^6$ | $>10^{11}$           |
| s-ny   | $>2 \times 10^6$ | $6.0 \times 10^9$    |
| c-pe   | $>2 \times 10^6$ | $7.5 \times 10^9$    |
| c-ppe  | $>2 \times 10^6$ | $>10^{11}$           |
| c-ny   | $>2 \times 10^6$ | $1.0 \times 10^{10}$ |

i = Inhibitor

s = Sanitizer

c = Control

pe = PE bag

ppe = Porous PE bag

ny= Nylon bag

<sup>1</sup> Results are the average of duplicate analyses

Table 22. The total plate count of fresh chestnuts stored at 4-5C after 14 days storage<sup>1</sup>

| Sample | Initial (CFU/g)    | After storage        |
|--------|--------------------|----------------------|
| i-pe   | $>2 \times 10^6$   | $2.2 \times 10^{10}$ |
| i-ppe  | $>2 \times 10^6$   | $7.4 \times 10^{10}$ |
| i-ny   | $>2 \times 10^6$   | $1.1 \times 10^{10}$ |
| s-pe   | $1.18 \times 10^5$ | $6.0 \times 10^9$    |
| s-ppe  | $1.18 \times 10^5$ | $6.2 \times 10^{10}$ |
| s-ny   | $1.45 \times 10^5$ | $1.5 \times 10^{10}$ |
| c-pe   | $1.45 \times 10^5$ | $3.1 \times 10^{10}$ |
| c-ppe  | $1.45 \times 10^5$ | $1.7 \times 10^{10}$ |
| c-ny   | $1.45 \times 10^5$ | $1.5 \times 10^{10}$ |

i = Inhibitor

s = Sanitizer

c = Control

pe = PE bag

ppe = Porous PE bag

ny= Nylon bag

<sup>1</sup> Results are the average of duplicate analyses

Table 23. The total yeast counts on fresh chestnuts at 10-12C after 14 days storage<sup>1</sup>

| Sample | Initial (CFU/g)    | After storage     |
|--------|--------------------|-------------------|
| i-pe   | $1.25 \times 10^5$ | $2.0 \times 10^9$ |
| i-ppe  | $2.0 \times 10^9$  | $>10^{11}$        |
| i-ny   | $8.5 \times 10^4$  | $7.0 \times 10^9$ |
| s-pe   | $>10^{11}$         | $4 \times 10^9$   |
| s-ppe  | $4.5 \times 10^4$  | $>10^{11}$        |
| s-ny   | $7.0 \times 10^9$  | $7.0 \times 10^9$ |
| c-pe   | $5.6 \times 10^4$  | $7.0 \times 10^9$ |
| c-ppe  | $4 \times 10^9$    | $>10^{11}$        |
| c-ny   | $7.2 \times 10^4$  | $5.5 \times 10^9$ |

i = Inhibitor

s = Sanitizer

c = Control

pe = PE bag

ppe = Porous PE bag

ny= Nylon bag

<sup>1</sup> Results are the average of duplicate analyses



**Table 24. The total yeast counts on fresh chestnuts stored at 4-5C after 14 days storage<sup>1</sup>**

| Sample | Initial (CFU/g)      | After storage        |
|--------|----------------------|----------------------|
| i-pe   | $1 \times 10^5$      | $2.9 \times 10^{10}$ |
| i-ppe  | $2.9 \times 10^{10}$ | $5.4 \times 10^{10}$ |
| i-ny   | $7.9 \times 10^4$    | $1.3 \times 10^{10}$ |
| s-pe   | $5.4 \times 10^{10}$ | $1.5 \times 10^{10}$ |
| s-ppe  | $4.5 \times 10^4$    | $7.5 \times 10^{10}$ |
| s-ny   | $1.3 \times 10^{10}$ | $1.0 \times 10^{10}$ |
| c-pe   | $5.2 \times 10^4$    | $3.4 \times 10^{10}$ |
| c-ppe  | $1.5 \times 10^{10}$ | $1.6 \times 10^{10}$ |
| c-ny   | $4.35 \times 10^5$   | $1.7 \times 10^{10}$ |

i = Inhibitor

s = Sanitizer

c = Control

pe = PE bag

ppe = Porous PE bag

ny= Nylon bag

<sup>1</sup> Results are the average of duplicate analyses

**Table 25. The total mold counts on fresh chestnuts stored at 10-12C after 14 days storage<sup>1</sup>**

| Sample | Initial (CFU/g)   | After storage |
|--------|-------------------|---------------|
| i-pe   | <1                | <1            |
| i-ppe  | $4.5 \times 10^3$ | $>10^{11}$    |
| i-ny   | $1 \times 10^3$   | <1            |
| s-pe   | <1                | <1            |
| s-ppe  | $1.5 \times 10^2$ | $>10^{11}$    |
| s-ny   | <1                | <1            |
| c-pe   | <1                | <1            |
| c-ppe  | <1                | $>10^{11}$    |
| c-ny   | <1                | <1            |

i = Inhibitor

s = Sanitizer

c = Control

pe = PE bag

ppe = Porous PE bag

ny= Nylon bag

<sup>1</sup> Results are the average of duplicate analyses

Table 26. The total mold counts on fresh chestnuts stored at 4-5°C after 14 days storage<sup>1</sup>

| Sample | Initial (CFU/g)   | After storage |
|--------|-------------------|---------------|
| i-pe   | $2.5 \times 10^5$ | <1            |
| i-ppe  | <1                | <1            |
| i-ny   | $1.5 \times 10^3$ | <1            |
| s-pe   | <1                | <1            |
| s-ppe  | <1                | <1            |
| s-ny   | <1                | <1            |
| c-pe   | $2 \times 10^3$   | <1            |
| c-ppe  | <1                | <1            |
| c-ny   | $2 \times 10^3$   | <1            |

i = Inhibitor

s = Sanitizer

c = Control

pe = PE bag

ppe = Porous PE bag

ny = Nylon bag

<sup>1</sup> Results are the average of duplicate analyses

### Effectiveness of different sanitizer

Hypochlorite solutions were prepared using sodium hypochlorite (5% active chlorine, SC Johnson, USA) and distilled water. Chlorine solutions of 100 and 200 ppm were prepared by appropriate dilutions. The 100 and 200 ppm chlorine solution reduced the bacterial populations by 5 logs. The results showed that the sodium hypochlorite was more successful with chestnuts than fresh apples because the sodium hypochlorite only reduced 1-2 logs on fresh apples (Beuchat et al., 1998). The bacterial populations on chestnuts treated with 200 ppm chlorine solution went up to  $\text{Log } 10^7$  and  $\text{Log } 10^{11}$  CFU/g after 2 weeks storage at 4°C and 10°C, respectively. The chestnuts treated with 100 ppm chlorine had population levels of  $\text{Log } 10^8$  and  $> \text{Log } 10^{11}$  CFU/g after 2 weeks storage at 4°C and

10°C, respectively. The results showed that hypochlorite effectively reduced the number of bacteria on the chestnuts after peeling, but then a more rapid increase in numbers (Table 24 and 25) occurred. Thus, the fresh peeled chestnuts had a shelf life of about 2 weeks when stored at 4°C and less than 2 weeks at 10°C.

The bacterial counts on chestnuts previously treated with 200 ppm chlorine solution increased to  $10^7$  and  $10^{11}$  CFU/g after 2 weeks storage at 4 and 10°C, respectively. The 100 ppm chlorine treated samples had population levels of  $10^8$  and  $>10^{11}$  CFU/g after 2 weeks storage at 4 and 10°C, respectively. Although hypochlorite reduced bacterial populations on the peeled chestnuts, these chestnuts only had a shelf-life of 2 weeks when stored at 4°C.

The initial microbial populations on peeled chestnuts treated with  $\text{ClO}_2$  gas and vacuum packed were reduced about 2 logs after 12 hrs exposure. After 2 weeks storage at 4 and 10°C, the microbial counts increased to  $10^9$ , which is higher than the results from hypochlorite treatment.

No significant difference in sensory results (day 4) were found for any of the chestnut samples in appearance/color (1 = worst, 9 = best), off-flavor (1 = no off flavor, 9 = strong off flavor), texture (1 = very soft, 9 = very hard), and overall quality (1 = worst, 9 = best). After 15 days storage, the results showed no significant difference between any of the samples. Therefore, the chlorine concentration and treatment time did not affect the appearance/color and texture, nor the overall sensory qualities.

**Table 27. Microbial populations on fresh chestnuts treated with different concentrations of hypochlorite (day 0, 4°C).<sup>1,2</sup>**

| Amount CFU/g | Control             | 100ppm, 5min      | 100ppm, 10min       | 200ppm, 5 min       | 200ppm, 10min       | CIO2                |
|--------------|---------------------|-------------------|---------------------|---------------------|---------------------|---------------------|
| Bacteria     | 1.5x10 <sup>7</sup> | 3x10 <sup>3</sup> | 4.9x10 <sup>3</sup> | 6.7x10 <sup>6</sup> | 5.8x10 <sup>3</sup> | 8.4x10 <sup>5</sup> |
| Yeast        | -                   | -                 | -                   | -                   | -                   | 3.5x10 <sup>2</sup> |
| Mold         | -                   | -                 | -                   | -                   | -                   | -                   |
| E. coli      | -                   | -                 | -                   | -                   | -                   | -                   |

<sup>1</sup> (–) indicates no colonies detected

<sup>2</sup> Results are the average of duplicate analyses

**Table 28. Microbial populations on fresh chestnut samples treat with different concentrations of hypochlorite and stored at 4C and assayed after 14 days.<sup>1,2</sup>**

| Amount CFU/g | Control           | 100ppm, 5min         | 100ppm, 10min        | 200ppm, 5 min       | 200ppm, 10min        | CIO2                |
|--------------|-------------------|----------------------|----------------------|---------------------|----------------------|---------------------|
| Bacteria     | >10 <sup>11</sup> | 1.14x10 <sup>8</sup> | 1.45x10 <sup>8</sup> | 2.4x10 <sup>7</sup> | 1.55x10 <sup>7</sup> | 2.9x10 <sup>9</sup> |
| Yeast        | -                 | -                    | -                    | -                   | -                    | 7.5x10 <sup>2</sup> |
| Mold         | -                 | -                    | -                    | -                   | -                    | -                   |
| E. coli      | -                 | -                    | -                    | -                   | -                    | -                   |

<sup>1</sup> (–) indicates no colonies detected

<sup>2</sup> Results are the average of duplicate analyses

**Table 29. Microbial populations on fresh chestnut samples treat with different concentrations of hypochlorite and stored at 4C and assayed after 21 days.<sup>1,2</sup>**

| Amount CFU/g | Control    | 100ppm, 5min      | 100ppm, 10min     | 200ppm, 5 min        | 200ppm, 10min      | CIO2            |
|--------------|------------|-------------------|-------------------|----------------------|--------------------|-----------------|
| Bacteria     | $>10^{11}$ | $3.2 \times 10^9$ | $6.2 \times 10^9$ | $7.2 \times 10^{10}$ | $8 \times 10^{10}$ | $9 \times 10^9$ |
| Yeast        | -          | -                 | -                 | -                    | -                  | -               |
| Mold         | -          | -                 | -                 | -                    | -                  | -               |
| E. coli      | -          | -                 | -                 | -                    | -                  | -               |

<sup>1</sup> ( - ) indicates no colonies detected

<sup>2</sup> Results are the average of duplicate analyses

**Table 30. The sensory results of chestnut samples after 4 days storage at 4C**

| Attribute         | 100ppm, 10min | 100ppm, 5min | 200ppm, 10 min | 200ppm, 5min | CIO2  | control |
|-------------------|---------------|--------------|----------------|--------------|-------|---------|
| Sliminess         | 3.38a         | 2.00ab       | 2.00ab         | 2.63ab       | 1.63b | 1.25b   |
| Appearance /color | 6.35a         | 6.20a        | 5.76a          | 5.40a        | 5.51a | 5.85a   |
| Off flavor        | 2.19a         | 3.48a        | 3.56a          | 3.24a        | 3.16a | 3.28a   |
| Texture           | 5.65a         | 5.51a        | 5.24a          | 5.24a        | 5.23a | 5.38a   |
| Overall           | 7.03a         | 6.44a        | 6.43a          | 6.01a        | 6.13a | 5.80a   |

a, b means within the same row with different superscript letters are different ( $p < 0.05$ ).

**Table 31. The sensory results of chestnut samples after 15 days storage at 4C**

| <b>Attribute</b>             | <b>100ppm,1<br/>0min</b> | <b>100 ppm,<br/>5min</b> | <b>200 ppm,<br/>10 min</b> | <b>200 ppm,<br/>5min</b> | <b>ClO2</b> | <b>control</b> |
|------------------------------|--------------------------|--------------------------|----------------------------|--------------------------|-------------|----------------|
| <b>Sliminess</b>             | 3.71a                    | 3.86a                    | 3.57a                      | 3.29a                    | 3.86a       | 2.57a          |
| <b>Appearance<br/>/color</b> | 6.01a                    | 6.07a                    | 5.09a                      | 4.36a                    | 5.41a       | 4.64a          |
| <b>Off flavor</b>            | 3.60a                    | 3.83a                    | 3.56a                      | 4.01a                    | 4.43a       | 3.39a          |
| <b>Texture</b>               | 5.83a                    | 4.84a                    | 5.41a                      | 5.06a                    | 4.70a       | 4.61a          |
| <b>Overall</b>               | 5.51a                    | 5.83a                    | 5.01a                      | 5.14a                    | 4.41a       | 5.46a          |

**a, b means within the same row with different superscript letters are different ( $p < 0.05$ ).**

## **Conclusion**

These results indicate that chestnuts naturally harbor large populations of bacteria prior to the peeling process. Also, because of chestnut metabolism, the starch will be converted to glucose which is easy to use by spoilage microorganism even at low storage temperature. The steamer reduced microbial populations by one log but after chestnuts passed through the washer/brusher, bacterial numbers were found to have increased. This may have been due to brush contamination. Chestnuts packed in both packaging materials had similar results and neither material had any significant effect on inhibiting the growth of microorganism. Mold did not grow on the chestnut samples probably because of vacuum packaging and lower storage temperature. Bacteria and yeast numbers increased very quickly in the packaged products stored at 10-12C. Therefore, low storage temperature can inhibit microbial growth. The shelf life of the chestnuts packed by different packaging materials and stored at 4-5C or 10-12C was less than 14days. The different sanitizer treatments did not significantly inhibit microbial growth. None of the treatments, packaging materials or storage temperatures had any significant effect on chestnut moisture content and water activity after 14 days storage. To extend the shelf life, MAP or CAP can probably be used and further research is needed to find suitable gas compositions. Other sanitation methods might be used such as ozone treatment or pasteurization methods such as high pressure processing.

## REFERENCES

- Anagnostakis, S.L. 1982. Biological control of chestnut blight. *Science* 215: 466-471.
- Anagnostakis, S.L. 1987. Chestnut blight: the classical problem of an introduced pathogen. *Mycologia*. 79:23-27.
- Al-Ghazali, M.R., and Al-Azawi, K., 1990. *Listeria monocytogenes* contamination of crops grown on soil treated with sewage sludge cake. *J. Appl. Bacteriol.* 69:642-647.
- Beaubatie, L. 1979. Etude des lipids, en particulier des acides gras, du fruit de *Castanea sativa* (Miller). *Bull. Soc. Pharm. Bordeaux*. 118:109-114.
- Beuchat, L.R. 1978. Relationship of water activity to moisture content in tree nuts. *Journal of Food Science*, 43, 754-5.
- Beuchat, L.R. 1998. Surface decontamination of fruits and vegetables eaten raw: a review. Food Safety Unit, World Health Organization. WHO/FSF/FOS/98.2.
- Beuchat, L.R. 1998. Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomato, lettuce. *J. Food Prot.* 61:1305-1311.
- Boynton, B.B. 1999. Quality and stability of pre cut mangos and carambolas subjected to high pressure processing, M.S. thesis, University of Florida, Gainesville.
- Burnett, M. 1987. The potential for a chestnut industry in the United State. Pp 2-4. In: Chestnuts and creating a commercial chestnut industry: Proc. Second Pacific Northwest Chestnut Cong. M.S. Burnett and R.D. Wallace (eds.) Chestnut Growers Exchange, P.O. Box 12632, Portland, OR 97212 USA.
- Busta, F.F., Peterson, E.H., Adams, D.M. and Johnson, M.G. 1984 Colony count methods in: Compendium of methods for the microbiological examination of foods. American Public Health Assoc. (APHA) Washington DC., p.63-83.
- Caner, C., Hernandez, R.J., and Parcall, M.A. 2000. Effect of high pressure processing on the permeance of selected high-barrier laminated films. *Packg. Technol. Sci.* 13: 183-195.
- Cheftel, J.C. 1995. Review: high pressure, microbial inactivation and food preservation. *Food Science Technol. Int.* 1: 75-90
- Dong, X., Worlstad, R.E., and Sugar, D., 2000. Extend shelf life of fresh cut pears. *J. Food Sci.* 65: 181-186.
- Dudek, J.A. and Elkins, E.R. 1984. Determination of the nutrition content of selected candies, nuts, condiments, beverages and vegetables. National Food Precursors Association Research Fundation. Washington, DC.



Day, N.B., Skura, B.J., and Powrie, W.D. 1990. Modified atmosphere packaging of blueberries: microbiological changes. *Canadian Institute of food Science and Technology Journal*. 23(1): 59-65.

Fukamachi, M., matsui, T., Hwang, T.W., Shimoda, M., and Osajima, Y. 1996. Sorption behavior of flavor compounds into packaging films from ethanol solution. *J. Agri. Food Chem.* 44: 2810-2813.

Fulbright, D.W., Weidlich, W.H., Haufler, K.Z., Thomas, C.S., and Paul, C.P. 1983. Chestnut blight and recovering American chestnut trees in Michigan. *Canadian Journal of Botany*. 61: 3164-3171.

Fulbright, D.W. 1999. Chestnut blight and hypovirulence, pp.57-79, in *Plant-microbe interaction*, vol. 4, edit by G. Stacey and N.T. Keen. APS Press, St. Paul, Minnesoda.  
MacDonald, W.L. and Fulbright, D.W. 1991. Biological control of chestnut blight: use and limitation of transmissible hypovirulence. *Plant Dis.* 75: 656-661.

Garg, N., Churey, J.J., and Splittstoesser, D.F. 1990. Effect of processing conditions on micro flora of the fresh cut vegetables. *J. Food Prot.* 53:701-703.

Gavara, R., Hernadez, R.J. and Giacin, J. 1996. Methods to determine partition coefficient of organic compounds in water/polystyrene systems. *J. food Sci.* 61: 947-952.

Gorny, J.R., Hess-Pierce, B., and Kader A.A., 1999. Quality changes in fresh cut peach and nectarin slices as affected by cultivar, storage atmosphere and chemical treatments. *J. Food Sci.*, 64: 429-432.

Greenleaf, C., 1999. Making the cut. *Amer. Veg. Grower*. March: 10-11.

Griffin, G.J. 1986. Chestnut blight and its control. *Hort. Rev.* 8:291-336.  
Dooley, L.B., R.L. Brohier, and C.R. Little. 1980. CA and air storage of chestnuts. *Hort. Res. Inst. Burwood Hwy, Knoxfield, Victoria, Australia*

Ha, B.S., Bae, M.S., Jeong, T.M., Sung, N.J., and Son, Y.O. 1982. Studies on constituent variation during storage after freeze-drying of chestnuts. *Korean J. Food Sci. Technol.* 14(2):97-105.

Harris, H. and D. Smith. 1988. Method and apparatus for thermal blast peeling, skinning, or shelling of food products. *Proc. 2nd PNW Chestnut Congress*. P.O. Box 12632, Portland, OR 97212

Harris, R.S., Wang, F.K.C., Wu, Y.H., Tsao, C.H.S., and Loe, L.Y.S. 1949. The composition of Chinese food. *J. Amer. Diet. Assoc.* 25:28-38.

Hodge, K., 1995. Fresh-Cut and the "perfect meal". *Fresh Cut*, 3(8): 12.

- Hotchkiss, J.H. and Banco, M.J. 1992. Influence of new packaging technologies on the growth of microorganism in produce. *J. Food Prot.* 55: 815-820.
- Jaynes, R.A. 1979. Chestnuts. p. 111-127. In: *Nut tree culture in North America*. The Northern Nut Growers Assoc. Inc.
- Kawano, S., Hayakawa, A., Iwamoto, M., Onta, H., and Sugawara, W. 1984. Precooling and Cold storage of chestnuts. *J. Japan. Soc. Hort. Sci.* 53(2): 194-201
- Kunsch, U., Scharer, H., Conedera, M., Sassella, A., and Jermini., M. 1999. Quality assessment of chestnut fruits. *Acta Hort.* 494: 119-125.
- Lakakul, R., Beaudry, R.M., and Hernandez, R.J. 1999. Modeling respiration of apple slices in modified atmosphere packages. *J. Food Sci.* 64: 105-110.
- Lambert, Y. Demazeau G, and Largeteau, A. 2000. Packaging for high pressure treatments in the food industry. *Packag. Technol. Sci.* 13: 63-71.
- Lanza, F. (1950). Sulla conservazione delle castagne destinate all' esportazione. *Annali Della Sperimentazione Agraria*, 4, 321-8.
- Macheix, J.J., Fleuriet, A., Billot, J., 1990. *Fruit Phenolics*. CRC Press, Florida, pp. 251–255.
- Matsui, T., nagashima, K., Fukamachi, M., Shimoda, M., and Osajima, J. 1992. *J. Agric. Food Chem.* 40: 1902-1905.
- McCarthy, M.A and Meredith, F.I. 1988. Nutrient data on the chestnuts consumed in the United State. *Economic Botany*. 42(1): 29-36.
- McHugh, T.H., and Senesi, E. 2000. Apple wraps: a novel method to improve the quality and extend the shelf life of fresh cut apples. *J. Food Sci.* 65: 480-485.
- Merkel, H.W. 1906. A deadly fungus on the American chestnut. *New York Zoo. Soc. Annu. Rep. for 1905.* 10: 97-103.
- Mertens, B. and Knorr, D., 1992. Developments of nonthermal processes for food preservation. *Food Technology*. 46(5): 124-126.
- Metcalf, H. 1912. The chestnut bark disease. p. 363-372 IN: "Yearbook of the Department of Agriculture for 1912," Washington, D.C.
- Miller, G. 1988. Considerations for commercial chestnut production in Eastern U.S.A. *Proc. 2nd PNW Chestnut Congress*. Chestnut Growers Exchange P.O. Box 12632, Portland, OR 97212.

- Olsen, J.L. 2000. Chestnut Production in the Northwestern United State. HortTechnology. 10(2): 296-297.
- Payne, J.A., Jaynes, R.A., Kays, S.J. 1983. Chestnut production in the United State: Practice, Problems, and Possible solutions. Econ. Bot. 37: 187-200.
- Pirovani, M.E. 2000. Survival of Samonella hadar after washing disinfection of minimally processed spinach. Lett. Appl. Microbiol. 31: 143-148.
- Prakash, A. 2000. Effect of low dose gamma irradiation on the shelf life and quality characteristics of cut romaine lettuce packaged under modified atmosphere. J. Food Sci. 65: 549-553.
- Rhee, C.O. and Kim, Z.U. 1982. Analysis of the lipid components in chestnut (*Castanea crenata*). Part 1. Composition of lipid fraction of inner and outer part of chest. J. Korean Agric. Chem. Soc. 25(4): 239-247.
- Rhodehamel, E.J., Reddy, N.R. and Pierson, M.D. 1992. Botulism: the causative agent and its control in foods. Food Control, 3:125-143.
- Riccardo, S. (1963). Secondo contributo sperimentale per lo studio delle alterazioni interne delle castagne. *Ricerche, Osservazioni e Divulgazioni Fitopatologiche, per la Campania ed il Mezzogiorno (Portici)*, 5, 1-14.
- Sapers, G.M., Miller R.L., and Mattrazzo, A.M. 1999. Effectiveness of sanitizing agents in inactivating *Escherichia coli* in Golden Delicious apples. J. Food Sci. 64: 734-737.
- Simon, A., Gonzalez-Fandos, E., and Tobar, V. 2005. The sensory and microbiological quality of fresh sliced mushroom (*Agaricus bisporus* L.) packaged in modified atmospheres. International J. of Food Sci and Technol. 40(9): 943-952.
- Smith J.R. 1953. Tree Crops, a permanent agriculture. Devin. Adair Co. NY.
- Stebbins, R.L. 1990. Requirements for a United States chestnut industry. p. 324-327. In: J. Janick and J.E. Simon (eds.), *Advances in new crops*. Timber Press, Portland, OR.
- Thayer, D.W. 1994. Wholesomeness of irradiated food. Food Technol. 48(5):132-134.
- Washington, W. S., Allen, A. D. and Dooley, L. B. (1997). Preliminary studies on *Phomopsis castanea* and other organisms associated with healthy and rotted chestnut fruit in storage. *Australasian Plant Pathology*, 26, 37-43.
- Wells, J. M. and Payne, J. A. (1975a). Toxigenic *Aspergillus* and *Penicillium* isolates from weevil-damaged chestnuts. *Applied Microbiology*, 30, 536-40.
- Wright, W. R. (1960). Storage decays of domestically grown chestnuts. *Plant Disease Report*, 44, 820-5.

**Xu, J. 2005. The effect of low-temperature storage on the activity of polyphenol oxidase in *Castanea henryi* chestnuts. Postharvest Biology and Technology 38, pp. 91–98.**

**Appendix A**

Name \_\_\_\_\_

Date \_\_\_\_\_

Please look at the chestnuts and answer the questions:

1. Please evaluate the **appearance/color** of the chestnuts on the following scale.

+-----+  
1 9  
dull, or mottled, atypical appearance typical appearance

2. Is there any presence of mold or decay? \_\_\_\_ Yes \_\_\_\_ NO

If yes, please rate the amount on the following scale and describe;

+-----+  
1 9  
large amount mold/decay on surface no mold/decay on surface  
(>50%)

3. Overall appearance quality?

+-----+  
1 9  
Very poor Very high

Please taste the samples:

4. Please rate the texture of the samples:

+-----+  
1 9  
very soft or chewy typical texture very hard or tough

5. Do you detect any off flavor in the samples?

+-----+  
1 9  
No off flavor Strong off flavor

**6. Please rate the Overall quality after tasting the chestnuts:**

+-----+  
1 9  
Very poor Very high

**Comments:**

**THANK YOU FOR YOUR PARTICIPATION**

## **Appendix B**

### **Do you eat chestnuts or want to try them? Participate in a quality evaluation of chestnuts!**

We are looking for panelists who consume and/or are familiar with chestnuts to participate in sensory evaluations of chestnut whole and peeled quality. Approximately 4-8 evaluations will be held over a 4-month period, beginning in October 2004. Each evaluation will take about 20 minutes of your time. For research purposes, we ask that you participate for all scheduled evaluations when possible. We will make every effort to accommodate your schedule.

**We will give you a food treats for participating each time.**

**102 TROUT FOOD SCIENCE**

**& HUMAN NUTRITION**

**(N. W. corner of Wilson Rd. and Farm Lane)**

**For any questions contact Janice Harte in the Department of Food Science and Human Nutrition Building, [harteja@msu.edu](mailto:harteja@msu.edu)**

**Or 355-8474, ext. 105**

## Appendix C

### Consent Form

#### **Microbial populations on peeled chestnuts and their inhibition**

Dear Participant:

Several Michigan State University researchers are investigating the quality of chestnuts. We would like you to take about 20 minutes (including the time you spend reading this letter) to help us samples of fresh and frozen chestnuts. We are asking for volunteers, over the age of 18, who eat or cook with chestnuts.

We are asking that panelists participate in an evaluation of chestnuts that will be conducted at intervals over a 4-month period starting October, 2004. Training will consist of approximately 1 session of 30-60 minutes. After training, evaluations will be scheduled up to eight times. You will be consulted prior to scheduling the tests in order to accommodate your schedule and availability. It is important for this research that we have the same panelists participate for each evaluation when ever possible. However, if you cannot attend any evaluation or continue as a panelist, please inform the researchers when contacted. Your signing this consent form will indicate your agreeing to participate when possible. You will be given a coupon or food treat worth less than \$5 for your participation and completion of the questionnaires.

*If you have a known food allergy to chestnuts or other tree nuts please do not participate in this study.*

If you believe there is a potential of an **allergic reaction upon sniffing and tasting**, notify the on-site sensory evaluation coordinator and/or principle investigator immediately. You will be released from participating in this study. Please note if you are injured as a result of your participation in this research project, Michigan State University will assist you in obtaining emergency care, if necessary, for you research related injuries. If you have insurance for medical care, your insurance carrier will be billed in the ordinary manner. As with any medical insurance, any costs that are not covered or in excess of whatever are paid by your insurance, including



deductibles, will be your responsibility. Financial compensation for lost wages; disability, pain or discomfort is not available. This does not mean that you are giving up any legal rights you may have. You may contact Janice Harte with any questions (355-8474x105). Your response is confidential and we will protect your confidentiality to the full extent of the law. You are free to not answer any question you choose, but please try to answer every question. We are not able to use incomplete responses nor are we able to provide the incentive for incomplete responses.

If you have any questions during your reading this consent form, or during or after your participation, please do not hesitate to contact the on-site sensory evaluation leader and/or the principle investigator. Feel free to contact Dr. Janice Harte, the principle investigator, via phone at 517-355-8474, ext. 105. She also can be reached via email at [harteja@msu.edu](mailto:harteja@msu.edu) for any inquiry you might have due to your participation in the study.

In case you have questions or concerns about your role and rights as a research participant, please feel free to contact Dr. Peter Vasilenko, Michigan State University's Chair of University Committee on Research Involving Human Subject (UCRIHS) by phone: (517) 355-2180, fax: (517) 432-4503, email: [ucrihs@msu.edu](mailto:ucrihs@msu.edu) or regular mail: 202 Olds Hall, East Lansing, MI 48824.

PLEASE NOTE UPON YOUR SIGNING THIS CONSENT FORM, YOU VOLUNTARILY AGREE TO PARTICIPATE IN OUR STUDY. YOUR SIGNATURE INDICATES YOU HAVE READ THE INFORMATION PROVIDED ABOVE AND THAT YOU HAVE HAD AN ADEQUATE OPPORTUNITY TO DISCUSS THIS STUDY WITH THE PRINCIPLE INVESTIGATOR AND HAVE HAD ALL YOUR QUESTIONS ANSWERED TO YOUR SATISFACTION. YOU WILL BE GIVEN A COPY OF THIS CONSENT FORM WITH YOUR SIGNATURE FOR YOUR RECORDS UPON YOUR REQUEST.

SIGNED \_\_\_\_\_ DATE \_\_\_\_\_

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