This study was undertaken to determine the sensory differences in high pressure vs. heat processed food systems after storage at ambient and refrigerated temperatures as determined by a trained sensory panel. Spanish rice and spaghetti with meat sauce were prepared and treated with heat and with high pressure processing (HHP). A citrus fruit mix consisting of pieces of orange, grapefruit, and pineapple was processed by mild heat and HHP, and heat alone.

One day after processing, treated products were tested along with untreated controls. Products were stored at either 22°C or 3°C, and tested at 10, 30, 60, 90, and 120 days. Sensory testing was done by a panel trained in a QDA-type method, and data was analyzed by univariate and multivariate methods.

For spaghetti with meat sauce, significant differences (p>0.05) were found between processing methods stored at the same temperature in appearance and texture attributes, with the high pressure processed samples closer to unstored product than those treated by heat. Differences in treatments first appeared in ‘dry appearance’ at 10 days, and by 120 days there were differences in ‘tomato integrity’, ‘pasta integrity’, ‘brightness of color’,
and ‘firmness of pasta’ as well. Most of these differences were due to the stickiness caused by the extra amylose leaking out of the heat treated pasta over time.

For Spanish rice, there were no statistically significant differences between samples processed by the two methods and stored at the same temperature. The Spanish rice was formulated with parboiled rice, which allows very little amylose leakage, so it did not show amylose-related effects as the spaghetti with meat sauce did.

The fruit mix processed with HPP and mild heat had significantly higher ratings in appearance attributes ‘brightness of color’ and piece integrity’, and lower ratings in ‘cooked’ descriptors than product treated with heat alone stored at the same temperature.
Sensory Changes in High Pressure Processed vs. Heat Processed Food Systems over Time.

by

Andrea M. Rodakowski

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APPROVED:

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Major Professor, representing Food Science and Technology

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Chair of Department of Food Science and Technology

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Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

__________________________
Andrea M. Rodakowski, Author
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CONTRIBUTION OF AUTHORS

Marcia Walker developed the products used in this study and helped prepare the samples for this research project. Dr. N. Scott Urquhart assisted in the data analysis. Dr. Dan Farkas identified the need for this research, defined its objectives, and secured funding for the project.
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Sensory Changes in High Pressure Processed vs. Heat Processed Food Systems over Time

Introduction

There are several basic underlying methods of preserving food, variations of which have been used by humans for millennia to extend their food supply. These are heat, chemical preservation, drying, and refrigeration or freezing. In the 20th century, food scientists have come up with two more fundamental technologies for preserving food, irradiation and high pressure processing (HPP). Irradiation is slowly gaining acceptance for some food uses, but in the US this method is hampered by a public that identifies “radiation” with “death”. High pressure processing is the latest technology to be seriously researched. High pressure processing for food preservation is defined as pressures from 100-700 MPa (mega-pascals) (Cheftel, 1992). HPP extends shelf life by injuring or killing microbes that would spoil the food, and does this with much less change to the food system than heat, drying, or freezing.

In 1899, Bert Hite of the West Virginia Agricultural Experiment Station, published the first article on ultra high pressure to preserve food, The Effect of Pressure in the Preservation of Milk. This is a remarkable paper, and both this publication and Hite’s 1914 paper show him to have been a very creative thinker and resourceful problem solver. He came up with the idea of using HPP as a possible alternative to heat pasteurization of milk; he wanted a method that would not impart a cooked flavor, and wouldn’t require refrigeration after processing.

At first Hite was a bit skeptical of the idea of using pressure, thinking that if it were a viable alternative, it would have been explored already. Then he started thinking
about laboratory equipment, and realized that there was no machinery to apply pressure for scientific work. This struck him as odd, but also suggested why high pressure had not been studied yet; there was not the equipment to do it. Much of his two papers on high pressure research deal with the working out of laboratory methods and designs of machinery for subjecting experimental quantities to pressures in the 200-900 MPa range. Unfortunately, many of his equipment engineering problems are the same ones researchers are struggling with 100 years later.

After Hite, there was no substantial or sustained work on high pressure as a food preservation method until the 1980’s. Other fields of study included high pressure research, however, and some of this was important in HPP. Materials science pushed forward the technology needed to build HPP machines as it developed ways to mold ceramic powder into engine parts for commercial production. Oceanic research discovered life forms living at pressures of up to 100 MPa in the deepest parts of the oceans, and so research began on how high pressure affected cellular processes. This work also influenced another application of HPP to food science, that of using it to tenderize meat after slaughter.

In 1974, Wilson, who worked for FMC (Food Machinery Corporation) presented a paper at IFT on using pressure up to 138 MPa in conjunction with heat pasteurizing temperatures of 82-103°C to sterilize low-acid food. However, this research was never formally published, and there is no record of it directly influencing later research.

It wasn’t until the 1980’s that sustained research in high pressure food processing was published. In 1986 researchers from the University of Delaware presented work on the effect of high pressure on *Salmonella enteritis* in chicken meat at a symposium on
high pressure biology (Metrick, et al, 1986). In 1987 the proceeding of the Third Conference for Food Protection was published, and it included *Novel Processes--Ultra High Pressure Processing* by Dan Farkas, one of the authors of the Delaware study. He speculated on the use of HPP for food preservation and pointed to areas that would have to be researched in order for the method to be considered a true processing option.

Another paper proposing using high pressure for preservation and processing was published in 1987 by Japanese researcher, Rikimaru Hayashi, who also became one of the leaders in the field (Hayashi, 1987.)

The future of HPP looks bright. Diets and tastes are shifting to fresher, less processed foods with fewer additives. HPP is uniquely able to extend the shelf-life of many food products with minimal changes during processing. Judging by what has been published, there is obviously interest in exploring and spreading this technology.

Research started in Japan and the United States, and is now being done in Germany, Canada, Spain, England, France, and the Scandinavian nations as well. Test marketing of HPP food is being done in Japan, Spain, and the United States. Research that will further the understanding and refinement of HPP is being done in almost every facet of food science. Many researchers have commented on HPP's ability to preserve food with minimal changes, but very little research using accepted sensory methodology has been done to objectively assess this. This study's aim was to compare sensory changes in HPP vs. heat processed food systems after up to 120 days storage at ambient or refrigerated temperatures.
Descriptive Analysis

Descriptive analysis methods allow both qualitative and quantitative measurement of all sensory dimensions of a product. The most commonly profiled dimensions are aroma, flavor by mouth, texture, and aftertaste (Meilgaard, et al, 1991).

These are the major applications of descriptive analysis (Stone and Sidel, 1993(a)):

1. Product development--either to profile prototypes, or to correlate with sensory consumer data.

2. Quality control--define characteristics for the control product to be used for ongoing quality control tests.

3. Relate to instrumental/chemical analysis--identify specific product differences that can be related to specific instrumental and chemical analyses.

4. Storage testing--track a product's sensory changes over time to understand changes that take place in storage under different conditions, or with different packaging.

Descriptive analysis grew out of the tradition of certain fields using experts. The brewmaster, perfumist, and similar experts would evaluate products to better specify raw ingredients and to identify needed changes in the production analysis (Stone and Sidel, 1993(a)). The idea was to monitor the product to make sure that it was suitable for the company to sell under its name to consumers. The expert evaluated the product, and compared it to his/her own internal standard, which may or may not have had much to do with the tastes of the consuming public. This method worked reasonably well when
competition was mostly local, and ingredient and production choices were limited. In this century, the rapid increase in scientific knowledge affected the production process, and better transportation and marketing affected both the procurement of raw ingredients and increased the choices available to consumers, and the expert alone was no longer enough. Sensory science started developing in response to a demand for more precise, unbiased, and quantifiable information about products (Stone and Sidel, 1993(a)). The most popular descriptive analysis methods are summarized below. The method used on this project was a variant of the QDA \(^\text{®}\) method.

There have been many methods of descriptive analysis developed in the past 40 years, but the most widely known are those developed and marketed by several consultants. All methods address similar concerns: 1) selection and training of panelists, 2) development of language, 3) evaluation sessions, and 4) data analysis and interpretation (Rubico, 1993).

**Flavor Profile\(^\text{®}\)**

The Flavor Profile method (FPM) was developed by the Arthur D. Little Co. in the late 1940’s (Cairncross and Sjostrom, 1950). Prospective panelists are screened for basic aroma and taste discrimination, and intensity discrimination. A personal interview is done to assess ability to work in a group. Training is done with a wide variety of standards providing examples of the product, ingredients, and processing variations. The panel develops a vocabulary and common frame of reference on a seven-point scale. After evaluating a product for about an hour, the panel has a discussion about the product led by the panel leader, who then writes a consensus report on the product. This method, although still widely used, shows its age in its lack of statistical analysis. The small
number of panelists may lead to inconsistency and un reproduceable results, and the panel (and therefore the results) can be dominated by the panel leader or a senior panel member (Meilgaard, et al 1991).

**Texture Profile®**

The Texture Profile method was developed by Szczesniak and colleagues at General Foods Corp. (Szczesniak, 1963; Brandt et al, 1963) in the 1960’s, and builds on the principles of using standards and developing a common vocabulary. The panel is made up of subjects screened on the ability to discriminate on attributes known to be important to the class of product to be evaluated, and by an interview to determine attitude. During training, the panelists are introduced to underlying rheological properties, and subsequently the descriptors generated are based on these underlying textural principles. This knowledge base lets panelists avoid redundant terms and pick the ones that are technically the most appropriate (Meilgaard, et al, 1991). Many scaling techniques can be used, including category, line, and magnitude estimation. The final report can be done with the consensus method used with Flavor Profile, but statistical treatment is now much more common.

**Quantitative Descriptive Analysis (The QDA Method)®**

Dissatisfaction with the lack of statistical rigor in descriptive analysis led Tragon Corp., partly in collaboration with the Department of Food Science at UC Davis, to develop Quantitative Descriptive Analysis, QDA® in the early 1970’s (Meilgaard, et al, 1991). The underlying principles are a behavioral orientation, a consensus approach to language development, use of replication for assessing subject and attribute sensitivity,
identifying specific product differences, and using defined statistical analysis (Stone and Sidel, 1993(b)).

Panelists for a particular project are selected on their ability to differentiate between variations in the product to be evaluated. An interview for availability and group skills is also given to first-time applicants. A pool of applicants is maintained as not all people will have equal sensitivity to all products. It is also for psychological reasons that every panelist isn't on every panel, including elitism and test fatigue that can result by overusing panelists. Not less than 10 panelists are recommended, as the overall contribution of each subject to the total variability increases accordingly, and too much dependence is then placed on too few subjects. Every sensory modality is evaluated, as there is much interaction of sensory information in the high centers of the brain (Stone and Sidel, 1993(b)). Not including aroma in a beverage, for example, could lead to it's influencing judgements on taste. References are used during training to help stimulate term generation. The panel leader functions strictly as a facilitator, and is not to influence panel decisions; descriptors and judgements are not predetermined, by the panel leader or anyone else. Training usually only lasts 7-10 hours, and revolves around developing a consistent terminology and an agreed-on evaluation procedure. With the use of ANOVA and repeated trials, scale location differences among panelists “come out in the wash” and this is not addressed. A 15 cm line scale is used, with word anchors 1.5 cm from each end. This scale was developed to produce results with the least variability, the most sensitivity, and to avoid number bias (Stone and Sidel, 1993(b)). Testing is done in individual booths to reduce influence from other panelists and the group leader. Multiple products are usually evaluated in a test session. Four test replications are
recommended for most products. Data analysis is done with ANOVA, both to locate product differences, and for panel tracking and monitoring. Data is presented graphically as well as tabularly, and this method introduced the spiderweb (also known as radar) graphing technique to sensory analysis (Stone et al, 1974).

Stone and Sidel (1993(b)) point out that QDA’s testing of multiple samples in one session capitalizes on the fact that humans are very good judges of relative differences and poor judges of absolute differences. This is certainly true, but it also points out the major criticism leveled at this method. Because of its lack of intensity scales for descriptors, the only result is relative differences between the set of products tested. Stone and Sidel (1993(b)) also point out that it is cost-effective to judge many products at one sitting, but having to analyze each four times isn’t.

This method is effective when there is a large group of products to be tested, and there are relatively large differences in them. QDA data is excellent for correlating with consumer test data to determine what product attributes are driving consumer acceptance of the sensory aspects of a product. In such a task as this, very fine differences in products are likely to matter little, and very fine differences will probably not be picked up by a panel with little training. It is also good if a client with very little money wants a one-time description of a set of products.

**Spectrum™**

The Spectrum™ method of descriptive analysis was developed by Gail Civille in the mid-1980’s through the work of her company, Sensory Spectrum. Civille worked with Szczesniak at General Foods, and this method is similar in some respects, notably in its use of intensity standards and training panelists on scientific principles underlying
sensory modalities. Spectrum claims to fit a descriptive program to a client's particular needs. A panel is trained to evaluate a certain class of products, but only the sensory information the client needs is collected; a panel on dry pet food may only evaluate appearance and aroma, for example, and only down to the level of specificity of descriptors deemed necessary. The scaling method is chosen to complement the objectives (Meilgaard, et al, 1991).

The Spectrum method tests prospective panelists on basic aroma and taste discrimination as well as on discrimination of attributes of the product class. The panel leader plays an active role in determining the selection of terms, and guiding panelists' responses. Training also aims to teach panelists about the underlying dimensions of the characteristics, and to provide a similar frame of reference in terms of scaling and terminology (Munoz and Civille, 1992). Stress is placed on defining and describing terms exactly; vanilla and vanillin would never be confused, for example, and each term would need its own set of references. Besides descriptor references, there are also intensity reference scales. Panelists learn not only to define attributes very precisely, but also to rate them the same as the rest of the panel, as reducing panelist variability is an important aspect of training in this method. Training with this method can take 80 hours. There is also considerable time spent on preparing the standards. For testing, several replications are done in individual testing booths (Harper, 1993). Analysis methods and panelist tracking is similar to that developed for the QDA method, though Spectrum doesn't use spiderweb plots, saying that they are easily misunderstood by clients trained in other disciplines (Meilgaard, et al 1991).
Criticisms of this method center on its heavy influence by the panel leader, and its dependence on intensity standards. Most standards are commercially available products, and it has been pointed out that reformulation of a brand or uneven quality control of a standard product could throw the whole scale off.

This method is excellent when small differences need to be found, and/or the class of products will have to be evaluated repeatedly. It has wide application in quality control situations.

Most sensory analysts use methods that could be characterized as blends of the QDA and Spectrum approaches in terms of panel leadership, reference use, and training time, due to the demands of the project.

**Free Choice Profiling**

This is a method developed by Williams and Arnold (1984) at the Agricultural and Food Council in UK. It was a response to the problem of consumers having different definitions for the same descriptor, leading to inaccurate consumer tests (Meilgaard, et al, 1991). Williams and Langeron (1984) applied the method to descriptive analysis. The developers were after a method that would compensate for the variability that will always occur when working with human subjects. This includes the fact that different people have different physiological makeups and therefore are not going to perceive stimuli in exactly the same way, nor are they going to describe them in the same way. This method was not developed by consultants, and so is not nearly as rigidly defined as the previously discussed methods, and researchers are adapting it to their own needs. The only two constant features are that all panelists develop their own list of descriptors, and the data are analyzed by a multivariate method called Generalized Procrustes Analysis (GPA), the
results of which are then analyzed by Principle Components Analysis (PCA). The original paper (Williams and Langeron, 1984) reported spending only an hour for training, to introduce panelists to the task, and to explain how to use the scale. No training time was used for individuals to develop terms for their ballots, probably because seven of the ten judges were already expert tasters of the product (port wine), and this also meant there was no need for standards. When the panelists are not experts, researchers have found it necessary to have some standards available and to spend time introducing panelists to the range of products to be evaluated to help stimulate term generation (Vaia, 1995; Hartwig, 1994). Still, no attempt is made to force panelists to accept a given definition for a descriptor, and it is accepted that the same term will mean different things to different panelists; this variation is taken care of by GPA.

This method is questioned primarily on it's use of GPA for analysis, and that the final decision on what differences mean is left to the analyst. GPA is still regarded as an experimental procedure by many, and Huitson (1989) was able to find “differences” in a data set produced by a random number generator. Also, it is up to the sensory scientist to determine what the differences mean; this method substitutes the influence of the panel leader on the panel’s terms during training, with influence from the analyst at the end by assigning terms to the differences found. In practice, however, this argument doesn’t seem to stand up; most sensory scientists will list out all terms used by panelists that define the principle component axes, and related terms usually group together over panelists. The PCA charts for FCP look very much like those produced by PCA for other descriptive analysis methods. Researchers have found FPC to give results similar to traditional descriptive analysis methods (Williams and Arnold, 1985; Rubico, 1993).
FCP is useful in two very different situations. First, when panelists are experts in their field, they often have their own personal lexicons for products, and are reluctant to change their definitions. Dumont (1994) used FCP for this reason when evaluating Oregon Pinot Noirs with a panel of winemakers. The second is when time for training is very limited. Stone and Sidel (1993) have attacked the use of FCP as the method of choice for time-limited projects, pointing out that it usually takes no less time to train a panel with their QDA method than it does with FCP. This is true, but FCP is a logical alternative to QDA when the descriptors are especially hard to learn or reach consensus. Our lab used FCP very successfully with an aroma-only panel with a short deadline.

From a psychological standpoint, QDA was a great improvement because it eliminates bias from the panel leader. FCP’s improvement in this area is that it eliminates the bias to reach group consensus on terms. Such pressure can interfere with critical thinking, and has been labeled “group think” (Whetten and Cameron, 1984). Certainly, an aware and well-trained panel leader can minimize this pressure, but the basic nature of the task means it’s always there. However, since FCP’s training is focused on helping individuals define their own terms, this isn’t an issue. One possible area for further research in descriptive analysis is an exploration as to whether sources of bias are introduced with different descriptive methods, and how they affect panel performance.

Summary

All the methods reviewed are currently being used in the industry, and provide slightly different types of information. It is the role of the sensory scientist to assess a project, and determine the most reasonable method (or, more likely, blend of methods)
that is going to result in the best information for the end users under the given resource constraints.

**Heat Processing**

*Introduction*

Heat had been used to make food safer, more palatable, and easier to digest for tens of thousands of years. Cooking by itself rarely allows foods to be kept longer, as recontamination from microorganisms after cooking will lead to spoilage (Potter, 1986). Using heat to preserve food for any length of time is a modern idea, dating only to 1809 and Nicholas Appert’s invention of canning.

The lowest level of processing a heat-treated product should receive is based on the minimum necessary to guarantee freedom from pathogens and toxins (Potter, 1986). Treatment beyond this minimum may well be selected to further shelf life, but the more heat applied, the more severe the changes in the product. Although heat treatment can enhance a food’s texture by softening cell walls, and develop various wanted flavors, heat effects are not always welcome.

**Chemical Actions of Heat**

Heat brings about changes in food by two means: denaturing physical structures, and breaking covalent bonds. When a molecule is denatured, the secondary, tertiary, and quaternary structures can be disrupted, and these changes can effect functional and nutritional properties. Heat, unlike high pressure processing, can affect molecules by forming or breaking covalent bonds. Heat provides the activation energy needed to allow
many chemical reactions to go forward, forming new compounds in foods (Lindsay, 1985).

**Proteins**

Heat denatures proteins and can change their covalent bonds. Their susceptibility to denaturation by heat depends on many factors, such as the nature of the protein, protein concentration, water activity, pH, ionic strength, and the types of ions present. Heat denaturation often leads to a decrease in solubility, due to exposure of the hydrophobic groups and the aggregation of the unfolded protein molecules (Cheftel, et al, 1985).

One of the most troublesome of the covalent bond reactions is nonenzymatic browning (Maillard reaction or carmelization). This complex series of reactions between amino acid side chains and reducing sugars can produce new flavor compounds and black and brown pigments (Cheftel, et al, 1985). Sometimes these reactions are controlled and helpful, such as when they are used to produce toasted flavors in baked goods, but often they occur when not wanted. Heat during processing and storage can enhance nonenzymatic browning as many of the reactions in that sequence have a high energy of activation, which is reached when the food system is heat processed (Cheftel, et al, 1985).

**Enzymes**

Heat works to rid food of the effect of enzymes by denaturing them. Destruction of the quaternary, tertiary, and secondary structures means that the enzyme no longer has the unique conformation necessary to function as a catalyst, so all further ripening or degradative reactions they catalyze are stopped (Richardson and Hyslop, 1985).
Unfortunately, vitamins can be broken down and are no longer available, so nutritional value can suffer with heat treatment (Richardson and Hyslop, 1985).

**Microorganisms**

Heat kills microorganisms by disrupting membrane functions and denaturing proteins (Ketchum, 1988). With increased temperature, the cell membrane becomes more fluid, and it loses its selective permeability (Pelczar, et al, 1993).

Whether the heat is moist or dry is important in determining its lethality to microorganisms. Moist heat causes denaturation and coagulation of vital proteins, and the activation energy of these reactions is fairly low (Pelczar, et al, 1993). Dry heat kills by oxidizing the organic constituents of the cell, and since the activation energy of oxidation reactions is much higher than that of denaturation reactions, more heat is needed (Pelczar, et al, 1993).

The medium in which the microorganism exists also influences the effectiveness of the heat treatment. Fats and oils, and sugar in high concentration provide a protective effect on microorganisms, and proteins and starches do to a lesser degree (Potter, 1986). Acid conditions help in killing microorganisms.

**Spores**

Spores are often highly resistant to environmental changes, including extreme heat. The heat-resistant sporeformer of most concern to food technologists is *Clostridium botulinum*. *C. botulinum* spores can technically be killed by boiling at 100°C, but it takes 300-530 minutes, so foods that support the growth of this organism are processed in steam under pressure at 121°C for 2-15 minutes (the exact times depends on the particular type of food and how it is packaged) (Potter, 1986).
The exact mechanism of spore heat resistance is not known. During sporulation, most of the water is expelled from the spore, and this may contribute to its heat resistance (Pelczar, et al, 1993). Another factor may be the presence of dipicolinic acid (DPA), a unique substance found in all spores, but not in vegetative cells (Pelczar, et al, 1993).

Vegetative Bacterial Cells

Vegetative bacterial cells, which are much more sensitive to heat than spores, can be killed by 5 to 10 minutes of moist heat at 60 to 70°C under experimental conditions in the laboratory (Pelczar, et al, 1993). Killing these cells is the goal of canning high acid foods (pH below 4.6) (Potter, 1986). Sporesformers can’t grow in an acid environment, and the long-term, ambient temperature storage of canned foods means these microorganisms must be killed. When elimination of these microorganisms is the goal of food processing several minutes in a boiling water bath is often used (Potter, 1986).

Yeast and Molds

With the exception of molds responsible for aflatoxins and the mold Claviceps purpurea which causes the ergot infection in rye and other cereals, yeast and most molds are major causes of food spoilage but are not threats to public health (Potter, 1986). Under experimental conditions yeast and molds are killed by 5 to 10 minutes of moist heat at 50 to 60 degrees (Pelczar, et al, 1993).

Starch

In food systems, starch can function as a bulking agent, thickener, gelling agent, water absorber, and anti-stick/sticky agent (Eliasson and Gunmundsson, 1996). Nutritionally it is important as an energy source. Starch from cereals occurs in nature in
granules which are composed of approximately 25% amylose and 75% amylopectin (Eliasson and Gunmundsson, 1996), embedded in a protein matrix (Dexter, et al, 1977; Miller, et al, 1973; Chabot, et al, 1976). Amylose molecules are linear and less bulky than the branched amylopectin. During cooking, water enters through holes in the starch protein matrix, is absorbed by the starch granules, and a great deal of stress is placed on the protein matrix. The matrix starts to rupture, allowing linear amylose to leak out. The longer the cooking time, the greater the breakdown of the protein matrix, and the greater the amount of amylose leakage (Dexter, et al, 1977). This amylose leakage is necessary for gelation, but in many food systems can cause problems. It is the cause of stickiness in pasta and potato flakes (Eliasson and Gunmundsson, 1996; Dexter, et al, 1977).

**Pigments**

Food colors serve as an important gauge of food quality for producers, processors, and final consumers (Potter, 1986). Degradation of pigments can point to processing and storage abuse (Potter, 1986). It has long been known that people will not select foods if the color doesn’t fall in the range they consider proper for that food.

**Anthocyanins**

Anthocyanins are a class of flavanoids that are responsible for red and red-purple hues in many fruits and vegetables, most notably the cane berries, strawberries, grapes, and cranberries (Wrolstad, 1994). These water-soluble pigments are heat labile. The exact mechanism of heat destruction is unclear, but it is thought that heat hydrolyzes the glycosidic side chain, leaving a very reactive molecule, which then polymerizes (Wrolstad, 1994). Polymeric anthocyanins are much less highly colored than monomeric anthocyanins. The amount of heat processing anthocyanins can take without affecting
the color varies widely, with those in cranberries holding up very well, for example, and those in strawberries being notoriously unstable (Wrolstad, 1994).

Carotenoids

These lipid-soluble pigments break down with oxidation, but are very heat stable (Bauernfeind, et al, 1971). They provide many of the yellow and red pigments in vegetables and fruits, including tomatoes, carrots, and citrus fruits.

Chlorophylls

Chlorophylls are the green and green-yellow pigments involved in photosynthesis. Chlorophyll has a magnesium ion in the center of the molecule, and when this is replaced by hydrogen ions, its color changes from bright green to olive brown. This reaction is called pheophytinization, and happens when chlorophyll is in an acid medium. When plant tissues are heated, cell walls break down, the natural acids in the plant can migrate into the areas of chlorophyll granules, and pheophytinization then takes place (Wrolstad, 1994). This is one of the fastest, most visible, and troublesome pigment reactions food scientists must deal with, as processed vegetables with pheophytinization are very unappealing to consumers (Wrolstad, 1994).

Flavor

Heat causes dramatic changes in flavor. Some of these changes are wanted, even crucial to producing an acceptable product, like the toasted flavors in baked goods. Others are simply tolerated as the price of having shelf-stable food, such as the disappearance of most green and floral notes in canned fruits and vegetables. There are three main ways heat changes food flavors: it can denature the enzymes that produce the
flavoring compounds, boil off volatile compounds, and it can bring about covalent bond reactions creating new flavor compounds.

Denaturing enzymes that catalyze creation of flavors can be both positive and negative. Denaturing the enzyme that causes sugars to link into starch after corn is picked, diminishing its sweet taste, is a benefit of heat treatment. However, enzymes also produce esters and aldehydes that supply fruity, floral, green, and sweet aromas that we associate with fresh produce (Lindsay, 1985), and those are lost with heat treatment. Part of the appeal of sushi is the delicate taste and aroma of very fresh raw fish, which is due to enzymes that manufacture those particular aldehydes, alcohols, and ketones (Lindsay, 1985).

Covalent bond reactions occur when temperatures are high enough to overcome the activation energy of the reaction, causing it to go forward. Many of these reactions create flavors in foods. Two primary classes of these reactions are the autoxidation of lipids, and non-enzymatic browning.

Autoxidation of lipids can occur during heat processing. In small concentrations, these compounds may be highly desirable additions to the food’s taste, but in excess produce aldehydes and ketones that give painty, metallic, cardboard, and tallow-like flavors and aromas (Lindsay, 1985).

The class of reactions most commonly thought of as heat-induced flavor reactions are non-enzymatic browning reactions. In general, the flavors produced add toasted, burnt, caramel, nutty, meaty, and floral notes to foods (Lindsay, 1985). Non-enzymatic browning is a term that covers both carmelization reactions and the Maillard reaction. As the name carmelization indicates, these reactions can produce yellow, brown and black
pigments (Whistler and Daniel, 1985). Carmelization takes place when carbohydrates, especially sugars, are heated; the reaction is helped by the addition of acid and certain salts (Whistler and Daniel, 1985). The Maillard reaction involves an amino-bearing compound, a reducing sugar, and water (Whistler and Daniel, 1985). Both reaction types are very complex, with the formation of many intermediate compounds. Both are characterized by dehydration and condensation reactions. There are many possible stopping points for these reactions, depending on temperature, availability of reactants, pH, and water activity. The Maillard reaction, for example, doesn’t go far enough to produce a color change if the pH is lower than 6.0, and does best with an intermediate water activity (Whistler and Daniel, 1985).

**High Pressure Processing**

*Introduction*

It has been known since the turn of the century that ultra-high pressure can be used for preserving food (Hite, 1899). Hite used high pressure processing (HPP) of up to 600 MPa preserving milk, vegetables, fruit, and fruit juices. He also did experiments with time and pressures needed to kill pure cultures of microorganisms in different media (Hite, et al, 1914). After Hite, there was no sustained work done on HPP until the 1980’s. Some of Hite’s major findings verified by researchers 80 years later are: 1) HPP allows increased shelf life without the degradative effects of heat. Color, flavor, and texture suffer less than with heat preservation. 2) Pressures above 200 MPa must be used to generate a preservative effect. 3) Preservation is mostly brought about through damaging or killing microorganisms that cause spoilage.
**Processing With UHP**

For HPP, food is sealed in a flexible container plastic bags and flexible molded plastic bowls that will withstand the change in volume during compression. The container is placed in a chamber filled with water or oil and pressure is applied by a hydraulic pump which introduces additional liquid until the desired pressure is reached. The pressure is applied isostatically; it is transmitted uniformly and instantly to the contents of the chamber, independent of the volume, composition, or geometry of the sample (Farkas, 1987; Cheftel, 1992). After the desired pressure has been reached, it takes no more energy to hold that pressure for an extended period of time.

Water volume decreases 4% at 100 MPa of pressure, and 15% at 600 MPa (at 22° C) and food has about the same compressibility as water (Farkas, 1987; Hayashi, 1989; Cheftel, 1992). When hearing of HPP for the first time, many wonder if the preservative effects are not the result of a temperature increase brought about by the decrease in volume, but the temperature increase is not large enough to cause significant changes. When water is compressed 300 MPa, its temperature increases by 8° C if the compression was adiabatic and instantaneous (Cheftel, 1992). In practice, it takes time for the hydraulic pump to build up the desired pressure and heat can be exchanged between the contents of the chamber and its thick metal walls.

**Proteins**

The effect of HPP on proteins has been sporadically investigated since 1914 when Bridgman reported that ovalbumen coagulated at pressures of 500 MPa, although it wasn’t until 1941 that Grant determined that this was due to the denaturation of proteins.
Most of the effects of pressure on biological samples stem from Le Chatelier’s principle: any phenomenon (phase change, molecular transconformation, chemical reaction) accompanied by a decrease in volume is enhanced by an increase in pressure (Cheftel, 1992). Following Le Chatelier’s principle, when HPP is applied to formation of hydrogen bonds, disruption of hydrophobic interactions, and the separation of ion pairs would be expected, as all these reactions are accompanied by a decrease in volume (Balny, et al, 1989). However, opposite effects have sometimes been reported, such as the enhancement of hydrophobic interactions above 300-400 MPa, due to the higher compressibility of free water as compared to that of bound water (Ohmiya et al, 1989). The interactions possible are very complex, and the formation and disruption of non-covalent bonds, the conformation changes of the polypeptide backbone, and solvation changes of solvent-exposed active groups all contribute to the final outcome (Masson, 1992).

**Microorganisms**

HPP effectively inactivates most of the non-sporeforming microorganisms responsible for food spoilage, resulting in much-improved shelf life for acid foods. Although many studies have been done on the effects of pressure on given bacteria, little is know about the exact mechanisms of bacterial destruction. Various morphological changes are observed with increasing pressure; compression of gas vacuoles, cell lengthening, separation of the cell membrane from the cell wall, modification of the nucleus and of intracellular organelles, and release of intracellular material into the extracellular spaces (Cheftel, 1992). Microbial death may result from ATPase inhibition,
or from the crystallization of membrane phospholipids, with consecutive irreversible changes in cell permeability and ion exchanges.

Yeasts and molds are very sensitive to pressure, while spores can survive pressures above 1000 MPa (Cheftel, 1992). As with heat, gram positive bacteria are more resistant than gram negative ones. Formulating foods with a pH below 4.5 can overcome the problem of pressure-resistant spores (Walker and Farkas, 1995; Farkas and Walker, 1993). Also, combining HPP with mild heat treatment can be used to destroy spores (Hayashi, 1992).

**Enzymes**

The effect of HPP on enzymes is mixed; it may enhance or inhibit enzyme activity. The effect of HPP on a particular enzyme depends on the positive or negative value of the reaction (or activation) volume (Cheftel, 1992; Hoover, 1993). Although some enzymes in some media have been reduced by 90% at 600 MPa (Ogawa, et al, 1992) there is not the 100% inactivation that can be accomplished with heat. It is generally recommended that a mild heat treatment and/or refrigeration be used in conjunction with HPP to overcome this deficit (Hoover, et al 1989; Ogawa, et al, 1992; Morris, 1993). Enzymatic browning reactions in some fruits or cell-free extracts of those fruits, appear to be enhanced by HPP, as polyphenoloxidase activity seems to be enhanced (Asaka and Hayashi, 1991). It has been noted that composition of media influences the rate of enzyme inactivation, and those with a high sugar content have a protective effect (Ogawa, et al, 1992; Knorr, et al, 1992).
**Flavor and Texture**

Many researchers investigating HPP's effects on food note how little the process seems to affect flavor (Hite, 1899, 1914; Farkas, 1987; Hayashi, 1989; Ogawa, 1992). It is thought that HPP does not disrupt covalent bonds as heat does, and this is what largely accounts for the preservation of a treated food's sensory characteristics (Hayashi, 1989). Since temperature rises are negligible or nonexistent (depending on whether the HPP processor has a cooling mechanism) with HPP, there are none of the flavor changes associated with heat. Heat encourages non-enzymatic browning reactions which produce flavors and colors which are often unwanted, and these are absent with HPP preservation. Natural color pigments are not altered. There is no heat to accelerate the polymerization of anthocyanins, so they retain their color-producing form. Cell walls stay intact, so acid can’t leak into cell structures containing chlorophyll, and pheophytinization cannot occur, so vegetables maintain their bright green color. There is no heat to evaporate aroma volatiles, so many low molecular weight green and floral aromas remain.

Since, as outlined above, HPP may have either inhibitory or enhancing effects on enzymes, flavors and aromas controlled by enzyme action may be affected, though no instances of this have yet been reported in the literature. It has been observed that grapefruit juice treated with HPP for the Japanese market has a flavor that is less harsh and bitter than untreated juice (Farkas, 1997). Perhaps HPP has an effect on one or more of the enzymes involved with bittering compounds. Naringen is the principal compound associated with bitterness in grapefruit, and naringenase, an enzyme in citrus pectin debitters naringen (Lindsay, 1985). Limonin, associated with bitterness in oranges but also found in grapefruit, is formed by enzymatic hydrolysis (Lindsay, 1985). Lowered
bitterness in HPP grapefruit juice could by explained by HPP enhancing the first reaction, or inhibiting the second.

Texture changes in HPP citrus fruits have been noted. The gas vacuoles are severely and irreversibly compressed, due to the high compressibility of gases. This problem is greatly reduced if the natural fruit sections are cut into pieces first, allowing the vacuum packing to remove most of the gas before processing (Aleman, 1996; Farkas, 1997).

**Shelf Life Testing**

An Institute of Food Technologists’ working group defined shelf life (also called stability testing or storage testing) as “the period between manufacture and retail purchase of a food product during which the product is of satisfactory quality” (IFT, 1974). Gnanasekharan and Flores (1993) defined it as the length of time a packaged food can be stored before the onset of detectable and undesirable changes occur. Shelf life may need to be determined because the food is of a product class that legally requires an expiration date to be sold (Stone and Sidel, 1993). Even if not legally mandated, competitive and business practices often dictate having an expiration date (Stone and Sidel, 1993). Sensory testing, along with microbiological, and sometimes chemical testing is often done as well, to make sure the product is sensorially acceptable and safe. Shelf life testing may be done to study the effect of specific factors such as storage temperature, packaging materials, or additives in new or existing products (Gacula, 1975).

Using sensory science in shelf life testing originated at the Armed Forces Food and Container Institute in the early 1950’s (Peryam, 1964). Peryam’s methodology used a nine-point hedonic scale (1=dislike extremely, 9=like extremely). Peryam stressed that
testing be done in a controlled environment, and that naive consumers be used. Thirty-two to 40 panelists were used in each test. These simple principles of sensory testing were not employed almost 25 years later in a long article discussing use of sensory testing in shelf life determination (Labuza and Schmidl, 1988) where the authors report regularly using 8-12 company employees for hedonic testing.

Gacula published statistical procedures to analyze shelf life studies in the mid 1970's (Gacula, 1975; Gacula and Kubala, 1975). These papers are built on the premise that the shelf life of individual units of product will vary considerably, and deals with finding the best storage end point if this is true. Later writers have complained these methods are too theoretical and impractical (Stone and Sidel, 1993).

Dethmers published the most comprehensive explanation of theory and practice of shelf life testing (Dethmers, 1979), noting that shelf life testing may involve scientists from quality assurance, food chemistry, microbiology, statistics, and sensory. Dethmers identifies eight things the sensory scientist should be aware of when testing a product, including the purpose of the experiment; formulation, processing, packaging, experimental design, and basic sensory evaluation procedures.

Dethmers is also the first to mention using descriptive analysis as well as affective (hedonic) testing. Affective testing is used because the goal of shelf life testing usually is to see when the product becomes unacceptable (or noticeably different) to the consumer. To see when this happens, consumers must be tested. However, there may also be situations when it is useful to know what specific changes in sensory attributes occur over time, and for that a trained descriptive panel is necessary. The descriptive and consumer data may then be correlated to see what attribute changes decrease consumer liking as the
product ages. Descriptive analysis is also especially useful for new product shelf life
determination (Dethmers, 1979). Stone and Sidel recommend using both descriptive and
effective testing (Stone and Sidel, 1993).

The exact number of tests and the exact testing times can’t be absolutely
determined ahead of time (Stone and Sidel, 1993; Labuza and Schmidl, 1988; Dethmers,
1979; Gacula, 1975). Food may deteriorate faster or slower than initially thought, and it
may make sense to move testing times, or do more or less testing. The key point is to be
flexible, and plan ahead so you can be flexible. For example, extra units should always
be put into storage so that if it is decided later that more testing farther out is warranted,
there is enough sample to do so (Stone and Sidel, 1993).

Dethmers (1979) and Stone and Sidel (1993) discuss the problems of finding a
control (zero-time sample) to be used at each test time. In many cases, product can be
frozen at zero-time, and then used as a control. This will not work for all products,
however. Using freshly made product as a control is questionable, as there can be batch
to batch variation.

Accelerated shelf life testing, storing sample under conditions of high temperature
and sometimes high humidity for shorter periods of time instead of regular storage
conditions, is mentioned by many researchers (Peryam, 1964; Gacula, 1975; Dethmers,
1979;). Others (Gnanasekharan and Flores, 1993; Stone and Sidel, 1993) recommend
against this, noting that the results of accelerated and regular storage studies are often
different, as accelerated conditions can initiate degradative mechanisms not normally
present (Gnanasekharan and Flores, 1993).
To try to reduce the time and expense of sensory testing to determine shelf life, research has been done on mathematical modeling of shelf life (Labuza and Schmidl, 1988; Gnanasekharan and Flores, 1993). Such models must take into account the mechanics of food degradation, the effect of the environment, and the packaging specifics. The numerous combinations of these factors makes generalized modeling infeasible, and therefore models are specific to certain foods or classes of foods (Gnanasekharan and Flores, 1993).
Sensory Changes in High Pressure Processed vs. Heat Processed Spaghetti with Meat Sauce and Spanish Rice over Time

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Abstract

Two starch based entrees, spaghetti with meat sauce and Spanish rice, were preserved by heat or high pressure processing (HPP) then tested by a trained descriptive panel after storage at 22°C or 3°C. Testing was done after 1, 10, 30, 90, and 120 days storage. Over time spaghetti with meat sauce processed with HPP was closer in appearance and texture attributes to untreated product than heat processed product. The differences are due to amylose leakage from the heat treated pasta. There were no significant differences (p<0.05) between treatments of Spanish rice stored at the same temperature because it was made with parboiled rice, which leaks little amylose.

Introduction

Since the turn of the century it has been known that high pressure can be used for preserving food (Hite, 1899). Hite used high pressure processing (HPP) to preserve milk, meat, and fruit juices with much less change in flavor than with heat processing (Hite, 1899; 1914). However, it wasn’t until the 1980’s that additional HPP food preservation research was published. In 1986 researchers from the University of Delaware presented work on the effect of Salmonella enteritis in chicken meat at a symposium on high pressure biology (Metrick, et al, 1986). Farkas, (1987) speculated on the use of high pressure for food preservation, pointing to areas that would have to be researched in order for the method to be considered a true processing option. Another paper proposing use of high pressure for preservation and processing was published in 1987 by Japanese researcher Rikimaru Hayashi.

Ultra-high pressure for food preservation uses pressures from 100-700 MPa (Cheftel, 1992). Food is sealed in a flexible container plastic bags or flexible molded
plastic bowls, placed in a chamber filled with water or oil, and pressure is applied to the chamber’s contents by a hydraulic pump forces additional liquid into the chamber until the desired pressure is achieved. After the desired pressure has been reached, it takes no more energy to hold it for an extended period of time. Pressure is applied isostatically; it is transmitted uniformly and instantly to the contents of the chamber, independent of the volume, shape, or composition of the sample (Farkas, 1987; Cheftel, 1992). It is thought that HPP does not disrupt covalent bonds as heat does, and this is what largely accounts for the preservation of color, flavor, and texture in treated food (Hayashi, 1989). The Maillard and carmelization reactions, and hence the flavors and colors that are generated with heat preservation, are absent with HPP. Natural color pigments are preserved. Cell walls stay intact, so acid can’t leak into cell structures containing chlorophyll so pheophytinization cannot occur, and vegetables maintain their bright green color. There is no heat to evaporate aroma volatiles, so many low molecular weight green and floral aromas remain.

Despite many researchers noting how little the process seems to affect flavor (Hite, 1899, 1914; Farkas, 1987; Hayashi, 1989; Ogawa, et al, 1992) there have been few studies that use established sensory methodology to examine effects of HPP on food flavor (Szczawinski, et al, 1998; Young, et al, 1997; Walker, et al, 1996; Walker, et al, 1995). The objective of this study is to examine differences in sensory attributes of heat vs. HPP spaghetti with meat sauce and Spanish rice over time.

These products were chosen to examine changes that would take place in starch-based, processed food systems common in the U.S. diet. Spanish rice (Appendix 1 lists specifications) is a system composed of rice, canned tomatoes, various dried spices, and
fresh green bell peppers. Spaghetti with meat sauce (Appendix 2 lists specifications) is composed of pasta, ground beef, canned tomato products, and various dried spices.

**Materials and Methods**

Spanish rice and spaghetti with meat sauce were prepared according to the formulations outlined in Appendices 1 and 2. Three separate batches (11.4 kg/batch) of each product were prepared and processed. Each batch was processed on a separate day. GMP’s were followed during the preparation and processing of the food systems. Products were prepared in the Department of Food Science and Technology pilot plant facility at Oregon State University.

**Experimental Design**

The experiment was designed to investigate changes in sensory properties of food processed using heat and HPP methods and held at ambient (22°C) and refrigerated storage (3°C). A randomized complete block design was used, with batches serving as blocks. After processing, products were evaluated by descriptive analysis at six time points: 1, 10, 30, 60, 90, and 120 days after processing. At the first evaluation (one day storage) three treatments were tested: an untreated control, a heat treated sample, and a pressure treated sample. After 10, 30, 60, 90, and 120 days of storage four treatments were tested: heat treated stored at 3°C, heat treated stored at 22°C, pressure treated stored at 3°C, and pressure treated stored at 22°C. Each of the three batches was tested once at each time point.
Sample Preparation and Storage

Products were put into heat sealed bags made of Saran coated nylon 3.25 ml thick (Kapak KSP 410-1MB, Minneapolis, MN). The bags measured 6.5 cm x 28 cm, and held about 230 g of product. Forty-eight bags of product were produced from each batch. Two bags were used for each testing.

An untreated control was used only for day 1 testing. After the sample was prepared, it was sealed in the Saran-coated nylon bags and held at 4°C until testing the next day.

For product treated with heat, sealed Saran-coated nylon bags were placed in a 100°C water bath for ten minutes, then immediately cooled in ice water for 10 minutes. This was to achieve commercial sterility as in a canned acidified product. Bags of product to be pressure treated were put in an outer bag of Saran and nylon 8 cm x 30 cm. and the outer bag filled with tap water and heat sealed. The outer bag was to insure hydraulic fluid from the HPP machine did not contaminate the sample. Pressure treated bags were processed at 345 MPa for 30 minutes at ambient temperature (22°C) in an Autoclave Engineers IP-2-22-60 isostatic press (Erie, PA) with a pressure chamber 55.9 cm deep and 5.1 cm diameter filled with water containing 2% hydraulic fluid (Hydrolubic 142, Houghton and Co., Valley Forge, PA). After HPP treatment bags of product were removed from the outer bags. Bags from both heat and HPP methods were then labeled with the production date and processing method, and randomly assigned to 3°C or 22°C dark storage rooms.
Microbiological Testing

As this was a feeding study, every care was taken to make sure product was safe for human consumption. Two days prior to sensory testing, two bags of heat treated and two of pressure treated product of the appropriate batch were randomly selected from each storage room. A sample was taken from each bag for plate counts, and the bags immediately resealed and held at 4°C until descriptive testing. Standard plate count and yeast and mold counts were performed on all pouches of product that were to be tested by panel members. The microbial cutoff for acceptability was 1000 CFU (colony forming units) per gram for the standard plate count, and any growth for the yeast and mold count.

Sensory Evaluation

Descriptive analysis was done by a trained panel of ten volunteers, nine of whom had prior descriptive panel experience, recruited from the students and staff of the Oregon State University Department of Food Science and Technology. A preliminary ballot for each product was prepared by the formulation developer and three sensory scientists. The panel started training with the preliminary ballots. For each entree, the panel spent 6 training sessions learning the descriptors and finalizing the list of descriptors on the ballot. The ballots used a 0-15 point intensity scale for rating descriptors, with 0=none, 7=moderate, and 15=extreme. Training covered aroma, flavor, texture, and appearance descriptors. Final ballots are given in Appendices 3 and 4. Aroma standards were used during training and available during testing, and are given in Appendix 5.

Panelists were seated in individual booths with red lights when testing for aroma, flavor, and texture. Appearance was evaluated last, in a separate room with white
incandescent lighting, to assure that ratings for appearance didn’t affect ratings for other descriptors.

**Presentation of Samples**

Panelists were served 35 g of samples in 100 ml plastic containers (VRW medium weighing boats, VRW, Seattle). Samples were labeled with three-digit random numbers. Panelists evaluated either the spaghetti samples or the Spanish rice samples during a testing session. Serving order was randomized across trays and panelists. All samples from one batch were served at one session at each time period. All portions of samples to be served to a single panelist during that day’s session were placed on a small serving tray, covered with a paper towel to prevent splattering, and heated in an 800 watt microwave oven for 45 sec. to 65°C. The paper towel was removed and the tray was covered with aluminum foil and held under a 250 watt infrared heating lamp (2LOR, Keating of Chicago, Chicago, IL) for no longer than 10 min. until served to a panelist. The temperature at serving was not lower than 55°C.

**Statistical Analysis**

All analysis was done with SPSS version 8.0 (SPSS Inc., 1997). Data were analyzed per descriptor using a three-way analysis of variance (ANOVA), using the GLM module. Where appropriate, LSD on pairwise comparisons (p≤0.05) was used to determine significant differences between the treatments. The ANOVA model was composed of panelist, treatment, batch, panelist x treatment, and batch x treatment. Panelist and batch (and their interaction terms) were treated as random effects, to increase the scope of inference that could be drawn from the study (Lundahl and McDaniel, 1988). The data set for each time period was analyzed independently.
Panelist x treatment interaction p-values were examined for each attribute to determine the panel’s consistency in rating attributes. Panelist x treatment interaction plots were constructed to determine which panelist(s) were rating samples differently from the panel when $p \leq 0.05$. These panelist’s data were then removed, and the data reanalyzed. In no case where this was done did treatment effects that were nonsignificant become significant. Usually, a panelist x session interaction is also analyzed to assess panelist consistency over sessions. This can only be done if the samples come from the same batch of product. Since the size of the experimental HPP equipment severely limited batch size, batches large enough to supply more than one session could not be processed.

Complete data sets (responses to aroma, flavor, appearance and texture attributes for one time period) were analyzed using principal axis factoring (principal factor analysis) on the residuals from a one-way ANOVA of panelist; this was done in an effort to take out the variability associated with different panelists using different parts of the scale. Factors were rotated with a varimax rotation to aid in interpretability. Results were averaged across panelists for each treatment and for each batch. Separation of treatments was then determined using the same ANOVA model used in the univariate analysis.

**Results and Discussion**

**Spaghetti With Meat Sauce**

Univariate Analysis

HPP treated products differed significantly from heat treated in appearance and texture after storage; HPP treated products showed less degradation, and therefore were
closer to what products were like before storage. Table 1 contains means, significant
differences, and standard deviations for spaghetti with meat sauce. Most striking is the
difference in the ‘dry appearance’ attribute between processing methods; the heat treated
samples are rated higher than HPP samples at both 3°C and 22°C storage temperatures at
10, 30, 60, 90, and 120 days storage. The pasta in the heat treated product clumped
together more, and the sauce clung to the pasta in little globules instead of smoothly
coating it. HPP product was rated significantly higher in ‘brightness of color’ and ‘pasta
integrity’ than heat treated product when stored at 3°C at 90 days. At 120 days, HPP
samples were rated higher than heat processed in ‘brightness of color’, ‘pasta integrity’,
and ‘tomato integrity’ at both 3°C and 22°C storage temperatures. At 120 days, ‘pasta
firmness’ is higher in HPP than heat processed product stored at 3°C. ‘Pasta firmness’
showed differences only between storage temperatures, not processing methods, at 60
and 90 days with the refrigerated samples firmer than those stored at ambient
temperature.

Differences in the HPP and heat-treated samples are due to the effect the extra
heat treatment had upon the pasta. The HPP pasta does not undergo as much amylose
leakage as heat processed pasta. Dexter, et al (1977) reported that the longer spaghetti is
cooked, the more the filamentous protein network opens. A more open protein network
allows more amylose to escape over time. Greater protein matrix breakdown in the heat
treated samples would cause the decrease in ratings for the appearance of integrity of the
pasta, and pasta firmness. The amylose that escapes from the protein matrix will engage
water on its hydrogen-bonding sites, making the product more viscous and dryer,
explaining the increase in the dryness attribute, and decrease the integrity of the tomato pieces by drawing water out of them.

Multivariate Analysis

The increased stability of the appearance and texture attributes of HPP samples is revealed even more clearly through factor analysis. Figures 1-4 are factor analysis plots for spaghetti with meat sauce at different time periods, with data for all descriptors used in the analyses. Pressure-processed samples rated significantly higher than heat treated samples on factors composed of appearance and texture attributes at 30, 60, 90 and 120 days.

There were significant differences between batches on some factors. When this happened, the first batch always differed from the others. This was probably due to a training effect; during the first testing session, panelists were becoming re-acquainted with the products and attributes since the previous evaluation (usually 30 days earlier). Holding warm-up sessions would have avoided this problem. When there were differences between batches as well as treatments (60 and 90 days), all sessions are plotted (Figs. 2 and 3). When there was no batch effect (30 and 120 days), the session average is plotted (Figs. 1 and 4).

Figure 1 contains a plot of factor 2 vs. factor 3, where significant differences between treatments were found, from the 30 days data set. There were no significant treatment differences in factor 1. Single-line enclosures denote different significance levels among the treatments on the x-axis (factor 2), and double-line enclosures denote different significance levels among the treatments on the y-axis (factor 3). At 30 days, differences caused by the amount of amylose leakage began to show. Pressure treatments
Table 1. Spaghetti with meat sauce: Means and standard deviations of sensory descriptors across storage times from the trained descriptive panel.

<table>
<thead>
<tr>
<th>Descriptors</th>
<th>1 Day</th>
<th>10 Days</th>
<th>30 Days</th>
<th>60 Days</th>
<th>90 Days</th>
<th>120 Days</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td>9.2</td>
<td>(2.7)</td>
<td>7.6</td>
<td>(2.8)</td>
<td>8.1</td>
<td>(3.0)</td>
</tr>
<tr>
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<td>8.6</td>
<td>(2.7)</td>
<td>7.4</td>
<td>(2.9)</td>
<td>6.8</td>
<td>(3.1)</td>
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<tr>
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<td>5.9</td>
<td>(2.8)</td>
<td>6.5</td>
<td>(3.2)</td>
<td>5.5</td>
<td>(3.2)</td>
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<tr>
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</tr>
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<td>0.6</td>
<td>(2.1)</td>
<td>0.8</td>
<td>(2.5)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td>(2.4)</td>
<td>6.5</td>
<td>(2.6)</td>
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<td>5.4</td>
<td>(2.8)</td>
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<td>(2.3)</td>
</tr>
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<td>(2.3)</td>
<td>6.8</td>
<td>(2.5)</td>
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<td>(2.1)</td>
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<td>(2.9)</td>
<td>5.9</td>
<td>(2.5)</td>
<td>5.3</td>
<td>(2.7)</td>
</tr>
<tr>
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<td>7.4</td>
<td>(2.6)</td>
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<td>(2.6)</td>
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<td>(2.7)</td>
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<td>(2.6)</td>
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<td>1.1</td>
<td>(0.8)</td>
<td>1.2</td>
<td>(0.8)</td>
</tr>
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1Scale = 0-15, 0 = none and 15 = extreme. Ratings with the same or no letter superscript show no significant difference (p ≤ 0.05).
Table 1, continued.

<table>
<thead>
<tr>
<th>Descriptors</th>
<th>I Day</th>
<th>10 Days</th>
<th>30 Days</th>
<th>60 Days</th>
<th>90 Days</th>
<th>120 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3°C stor.</td>
<td>22°C stor.</td>
<td>3°C stor.</td>
<td>22°C stor.</td>
<td>3°C stor.</td>
<td>22°C stor.</td>
</tr>
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<td>(1.7)</td>
<td>(2.0)</td>
<td>(2.2)</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>9.6</td>
<td>9.2</td>
<td>(2.3)</td>
<td>(2.2)</td>
<td>(2.5)</td>
</tr>
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<td>Integrity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
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<td>8.6</td>
<td>7.7</td>
<td>(3.0)</td>
<td>(3.0)</td>
<td>(2.4)</td>
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</tr>
<tr>
<td>Dryness</td>
<td>Not collected</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Texture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasta</td>
<td>8.9</td>
<td>8.2</td>
<td>7.4</td>
<td>(2.4)</td>
<td>(2.0)</td>
<td>(1.7)</td>
</tr>
<tr>
<td>Firmness</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Scale = 0-15, 0 = none and 15 = extreme. Ratings with the same or no letter superscript show no significant difference (p ≤ 0.05).

2 Descriptor was added after day 1 testing.
had significantly higher ratings than the heat treatments in factor 2, composed primarily of appearance attributes ‘integrity of pasta’, ‘brightness’, and ‘integrity of tomato’. Both pressure treated samples are in quadrant I, and both heat treated samples are in quadrant IV. In factor 3, which was most influenced by ‘dry appearance’ at the negative end of the y-axis, P 3°C treatment was significantly higher than the P 22°C and H 3°C treatments, which were significantly higher than the H 22°C treatment. The effect of refrigerated vs room temperature storage is also visible here, with the 22°C samples in both cases plotting to the bottom and left of the 3°C stored samples processed using the same method.

Appearance attributes ‘brightness of color’ and ‘integrity of pasta’ dominated the third factor at 60 days (Fig. 2), where P 3°C treated product was significantly higher than the H 3°C treated, and P 22°C was significantly higher than H 22°C. Again, the 22°C samples plotted to the left of the 3°C stored samples preserved using the same method, suggesting the effect on appearance of storage at the higher temperature, even though the difference was not significant. This may be due to the significant batch effect in factor three, with the first batch pulling to the negative end of factor 3. As mentioned above, this is probably due to lack of a warm-up session before resuming testing after a break of about three weeks.

At 90 days (Fig. 3), the P 3°C sample was significantly higher than the H 3°C sample on factor 1, composed primarily of the appearance and texture attributes ‘brightness’, ‘integrity of pasta’, ‘dry appearance’, and ‘firmness of pasta’. There were no significant differences between P 22°C, H 22°C, and H 3°C samples. This time period had the least separation between samples. Besides the batch effect pulling the responses
from the first batch to the positive end of the scale of the first factor, the 90 day testings were done in December, and there were several panelists absent for several sessions. This missing data would make it harder to detect differences.

At 120 days (Fig. 4), pressure samples were rated significantly higher than heated samples at both 3°C and 22°C storage temperatures on the first factor, representing appearance and texture, with the main constituents being attributes ‘brightness of color’, ‘integrity of pasta’, ‘integrity of tomato’, ‘dry appearance’, and ‘firmness of pasta’. It is interesting to note that after four months, the H 3°C product is essentially the same as the P 22°C product.

Table 2 contains the sensory modalities that dominate the first three factors and the p-values of those factors. Over time, the significance of factors representing appearance and texture increases, going from p=0.300 at 0 days storage, to p=0.000 at 120 days (Table 2) as appearance and texture differences become more noticeable. Also, factors that were principally appearance and texture were the only ones to have a p-value <0.05, indicating that aroma and flavor did not vary strongly between treatments at any time.

Table 2. Predominate sensory modalities and p-values in factor analysis of spaghetti.

<table>
<thead>
<tr>
<th>Time</th>
<th>Factor 1</th>
<th>p</th>
<th>Factor 2</th>
<th>p</th>
<th>Factor 3</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>Flavor</td>
<td>.611</td>
<td>Appearance</td>
<td>.304</td>
<td>Aroma</td>
<td>.685</td>
</tr>
<tr>
<td>10 days</td>
<td>Aroma, flavor</td>
<td>.131</td>
<td>Flavor</td>
<td>.283</td>
<td>Appearance</td>
<td>.100</td>
</tr>
<tr>
<td>30 days</td>
<td>Aroma, flavor</td>
<td>.858</td>
<td>Appearance</td>
<td>.041</td>
<td>Appearance</td>
<td>.025</td>
</tr>
<tr>
<td>60 days</td>
<td>in transition</td>
<td>.099</td>
<td>Flavor</td>
<td>.510</td>
<td>Appearance</td>
<td>.007</td>
</tr>
<tr>
<td>90 days</td>
<td>Appearance, texture</td>
<td>.016</td>
<td>Flavor</td>
<td>.102</td>
<td>Aroma, flavor</td>
<td>.098</td>
</tr>
<tr>
<td>120 days</td>
<td>Appearance, texture</td>
<td>.000</td>
<td>Aroma</td>
<td>.485</td>
<td>Flavor, aroma</td>
<td>.103</td>
</tr>
</tbody>
</table>
Figure 1. Consensus plot of principal axis factoring of all descriptors for spaghetti with meat sauce after 30 days storage: factor 2 vs. 3. Significance at the $p<0.05$ level for factor 2 shown by single line enclosure, for factor 3 by double line enclosure.

Figure 2. Consensus plot of principal axis factoring for spaghetti with meat sauce after 60 days storage: factor 3 vs. 1. Significance at the $p<0.05$ level for factor 3 shown by single line enclosure.
Figure 3. Consensus plot of principal axis factoring for spaghetti with meat sauce after 90 days storage: factor 1 vs. 2. Significance at the p<0.05 level for factor 1 shown by single line enclosure.

![Figure 3](image1)

Figure 4. Consensus plot of principal axis factoring for spaghetti with meat sauce after 120 days storage: factor 1 vs. 2. Significance at the p<0.05 level for factor 1 shown by single line enclosure.

![Figure 4](image2)
**Spanish Rice**

Table 3 contains means, standard deviations, and significant differences for the sensory descriptors of Spanish rice. There were no differences between heat and HPP samples held at the same temperature at any of the test periods. There were no statistically significant differences between treatments when multivariate analysis was performed on the factors.

The only significant differences in treatment were those caused by differences in storage temperature. Samples held at 3°C had significantly higher ratings than those held at 22°C in ‘brightness of color’ and ‘green pepper integrity’ after 60, 90, and 120 days, and in ‘onion flavor’, ‘tomato integrity’, ‘green pepper flavor’, and ‘onion flavor’ after 90 and 120 days, and in ‘tomato flavor’, ‘rice integrity’ and ‘green pepper crunchiness’ after 120 days. Samples held at 3°C had significantly lower ratings than those held at 22°C in ‘off-aroma’ and ‘off-flavor’ at 120 days. Food continues to change in storage, and temperature is one of the key variables that determine the changes. The heat in storage forms and breaks down volatiles and flavor compounds to change aroma and taste; it breaks down cell walls, leading to changes in appearance and texture. The lower values for ‘brightness’ are the result of non-enzymatic browning reactions, specifically, caramelize in the rice. Caramelize takes place when carbohydrates are subjected to elevated temperature over time, and is aided by the addition of acid and certain salts. It proceeds far enough to produce a color change when the pH is below 6.0 (Whistler and Daniel, 1985). After 60 days storage, the samples stored at 22°C had received considerably more heat than those stored at 3°C, which aided the development of yellow and brown colorants in the rice. The descriptor used in the study was ‘brightness’, but as the rice stored at 22°C began to change in color, the ‘brightness’ of those samples
Table 3. Spanish rice: Means and standard deviations of sensory descriptors across storage times from the trained descriptive panel.

<table>
<thead>
<tr>
<th>Descriptors</th>
<th>1 Day</th>
<th>10 Days</th>
<th>30 Days</th>
<th>60 Days</th>
<th>90 Days</th>
<th>120 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3°C stor.</td>
<td>22°C stor.</td>
<td>3°C stor.</td>
<td>22°C stor.</td>
<td>3°C stor.</td>
<td>22°C stor.</td>
</tr>
<tr>
<td><strong>Aroma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green pepper</td>
<td>7.1</td>
<td>7.4</td>
<td>7.1</td>
<td>7.1</td>
<td>6.6</td>
<td>6.6</td>
</tr>
<tr>
<td>Tomatoes</td>
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<td>7.0</td>
<td>6.9</td>
<td>6.9</td>
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<td>6.8</td>
</tr>
<tr>
<td>Onions</td>
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<td>5.9</td>
<td>5.8</td>
<td>5.8</td>
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<td>4.6</td>
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<td>4.6</td>
</tr>
<tr>
<td>Off-aroma</td>
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<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
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<td><strong>Flavor</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sour</td>
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<td>6.2</td>
<td>6.2</td>
<td>6.2</td>
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<td>6.2</td>
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<td>Sweet</td>
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<td>5.0</td>
<td>5.0</td>
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<tr>
<td>Green pepper</td>
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</table>

1 Scale = 0-15, 0 = none and 15 = extreme. Ratings with the same or no letter superscript show no significant difference (p ≤ 0.05).
Table 3, Continued.

<table>
<thead>
<tr>
<th>Descriptors</th>
<th>1 Day</th>
<th>10 Days</th>
<th>30 Days</th>
<th>60 Days</th>
<th>90 Days</th>
<th>120 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
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</tr>
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<td>(2.5)</td>
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<td>(2.5)</td>
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<tr>
<td>Texture</td>
<td>8.5</td>
<td>8.3</td>
<td>6.9</td>
<td>9.1</td>
<td>8.6</td>
<td>7.6</td>
</tr>
<tr>
<td>Firmness of rice</td>
<td>(2.5)</td>
<td>(2.5)</td>
<td>(1.9)</td>
<td>(1.9)</td>
<td>(2.1)</td>
<td>(2.2)</td>
</tr>
<tr>
<td>Crunch of rice</td>
<td>8.1</td>
<td>7.7</td>
<td>6.9</td>
<td>7.9</td>
<td>7.3</td>
<td>6.7</td>
</tr>
<tr>
<td>Gr. pepper</td>
<td>(2.5)</td>
<td>(2.3)</td>
<td>(2.4)</td>
<td>(2.6)</td>
<td>(2.9)</td>
<td>(2.6)</td>
</tr>
</tbody>
</table>

Scale = 0-15, 0 = none and 15 = extreme. Ratings with the same or no letter superscript show no significant difference (p ≤ 0.05).
decreased, and at 120 days the color of those products had a distinct brown cast. Samples held at 3°C had significantly lower ratings than those held at 22°C in ‘off-aroma’ and ‘off-flavor’ at 120 days. Parboiled rice, which is what was used in the Spanish rice formulation, does not swell during cooking nearly as much as regular rice. Since it does not swell, the starch granules do not split, and there is little amylose leakage (Bhattacharya and Ali, 1985; Damir, 1985; Lee, and Singh, 1991). Without the amylose leakage and the stickiness it causes, the Spanish rice did not show the appearance and texture changes that happened to the spaghetti with meat sauce.

**Conclusion**

HPP produces a spaghetti with meat sauce with a much longer shelf life than heat preservation. The appearance and firmness of the pasta of the HPP product will be much more like that of fresh than heat preserved product after 30 and up to 120 days storage. It may be that this improvement would also hold for other pasta entrees, but research confirming this would have to be done. Spanish rice made with parboiled rice will not see an improvement in shelf life by using HPP instead of heat preservation.

**References**


Hayashi, R., 1987. Possibility of high pressure technology for cooking, sterilization, processing and storage of foods. Shokuhin to Kaihatsu 22 (7): 55


Sensory Changes in High Pressure Processed vs. Heat Processed Citrus Fruit Mix over Time

Andrea M. Rodakowski, Marcia K. Walker, Daniel F. Farkas, N. Scott Urquhart, and Mina R. McDaniel

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Abstract

A citrus fruit mix of oranges, grapefruit, and pineapple pieces was processed with heat alone, or high pressure processing (HPP) combined with mild heat to reduce enzyme activity. Products were stored at 3°C and 22°C, and tested periodically by a trained descriptive panel to determine sensory differences between treatments. Products preserved with HPP and mild heat were rated higher on appearance attributes and lower on ‘cooked aroma’ and ‘cooked flavor’ than the product treated with heat alone. Citrus fruit mix treated with HHP and mild heat had a longer shelf life than those treated with heat alone.

Introduction

There are several basic methods of preserving food, variations of which have been used by humans for millennia to extend their food supply. These are heat, chemical preservation, drying, and temperature reduction (refrigeration or freezing). In the 20th century, food scientists have developed two more fundamental technologies for preserving food, irradiation and high pressure processing. High pressure processing is the latest technology to be seriously researched. High pressure processing for food preservation is defined as pressures from 100-700 MPa (Cheftel, 1992). High pressure processing (HPP) extends shelf life by injuring or killing microbes that would spoil food, and does this with much less change to the food system than heat, drying, or freezing. HPP does not impact aroma, flavor, appearance and texture as much as other methods, especially heat (Hite, 1899, 1914; Eshtiaghi, et al, 1994; Farkas, 1987, 1993; Hayashi, 1989; Ogawa, 1992).
In 1899, Bert Hite of the West Virginia Agricultural Experiment Station, published the first article on ultra high pressure food preservation. In 1914 he published a report on HPP’s effects on fruit, fruit juices, vegetables, and charts of pressure death time curves for various microbes in different media (Hite, 1914).

After Hite, there was no substantial or sustained work on high pressure as a food preservation method until the 1980’s. Other fields of study carried out high pressure research, however, and some of this was important in HPP. Materials science pushed forward the technology needed to build HPP machines as it developed ways to mold ceramic powder into engine parts for commercial production. Oceanic research discovered life forms living at pressures of up to 100 MPa in the deepest parts of the oceans, and began investigating how high pressure affected cellular processes. This work also influenced another application of HPP in food science, that of using it to tenderize meat after slaughter.

In 1986 researchers from the University of Delaware presented work on the effect of *Salmonella enteritis* in chicken meat at a symposium on high pressure biology (Metrick, et al, 1986). Farkas (1987) speculated on the use of HPP and pointed to areas that would have to be researched in order for the method to be considered a true processing option. Another paper proposing using high pressure for preservation and processing was published in 1987 by Japanese researcher, Rikimaru Hayashi, who also became one of the leaders in the field (Hayashi, 1987.)

Food to be preserved with HPP is put into a flexible container such as a plastic pouch or bowl to accommodate the decrease in volume during HPP treatment. Water volume decreases 4% under 100 MPa of pressure, and 15% under 600 MPa (at 22° C);
food has about the same compressibility as water (Farkas, 1987; Hayashi, 1989; Cheftel, 1992). Exact mechanisms of microbial death are not known, but may result from ATPase inhibition, or from crystallization of membrane phospholipids, with irreversible changes occurring in cell wall permeability when volume is decreased (Cheftel, 1992).

Spores are largely resistant to destruction by HPP (Farkas and Hoover, 1990), with spores of Bacillus subtilis surviving pressures of 1200 MPa (Timson and Short, 1965). It is recommended that foods to be high pressure processed have a pH below 4.5 to prevent germination of spores (Walker and Farkas, 1995).

Enzymatic reactions may be enhanced or inhibited by HPP, depending on the positive or negative value of the reaction (or activation) volume (Cheftel, 1992; Hoover, 1993). Enzymatic browning reactions in some fruits or fruit juices appear to be enhanced by HPP, as polyphenoloxidase action seems to increase (Asaka and Hayashi, 1991). It has been noted that media composition influences the rate of enzyme inactivation, and those with a high sugar content protect enzymes from deactivation (Ogawa, et al, 1992; Knorr, et al, 1992). Although pectinesterase in juices is reduced by 80-90% at 600 MPa (Ogawa, et al, 1992) there is not the 100% inactivation of enzymes with HPP that can be accomplished with heat. It is generally recommended that a mild heat treatment or refrigeration be used in conjunction with HPP to overcome this deficit (Hoover, et al 1989; Ogawa, et al, 1992; Hayashi, 1992; Morris, 1993).

The objective of this study was to compare the sensory characteristics of a citrus fruit mix processed by heat with one processed by a combination of low heat to partially inactivate enzymes, and HPP. Samples were evaluated after storage. As samples were to be stored for up to four months at ambient and refrigerated temperatures, a mild heat
and HPP combination was used as HPP alone was felt to be inadequate to insure enzyme inactivation. A citrus fruit mix of orange, pineapple, and grapefruit pieces was chosen to build on work done earlier at Oregon State University on pineapple preservation by HPP (Aleman, et al 1994), and to extend the scope by including other commercially important citrus fruits.

Materials and Methods

A citrus fruit mix was prepared according to the formulation in Appendix 6. Three separate batches (11.4 kg/batch) were prepared and processed. Due to the limited capacity of the HPP machine, it took one full day to finish one batch. GMP’s were followed during the preparation and processing of the food system. The fruit mix was prepared in the Department of Food Science and Technology pilot plant facility at Oregon State University.

Experimental Design

After processing, products were evaluated by descriptive analysis at six time points: one day after processing, 10 days, 30 days, 60 days, 90 days, and 120 days. At the first evaluation (one day storage) three treatments were tested; an untreated control, a heat treated sample, and a pressure treated sample. After 10, 30, 60, 90, and 120 days of storage four treatments were tested; heat treated stored at 3° C, heat treated stored at 22° C, pressure plus mild heat treated stored at 3° C, and pressure plus mild heat treated stored at 22° C. Each of the three batches was tested once at each time point.
Sample Preparation

Product was put into heat sealed pouches made of Saran coated nylon 3.25 ml thick (Kapak KSP 410-1MB, Minneapolis, MN). The pouches measured 6.5 cm x 28 cm, and held about 230 g of product. Forty-eight pouches of product were produced from each batch.

An untreated control was used only for the 1 day storage testing. After the sample was prepared, it was sealed in the same pouches in which processed product was sealed, and held at 4°C until tested the next day. For heat treatment only, sealed pouches were placed in a 100°C water bath for 10 minutes, then cooled in ice water for 10 minutes. For HPP and mild heat treatment, pouches of product were put into an outer pouch 8 cm x 30 cm of Kapak, then filled with tap water and heat sealed. The outer pouch was to insure hydraulic fluid from the HPP machine did not contaminate the product. Samples were processed at 345 MPa for 30 minutes at ambient temperature (22°C) in an Autoclave Engineers IP-2-22-60 isostatic press (Erie, PA) with a pressure chamber 55.9 cm deep and 5.1 cm diameter filled with water containing 2% hydraulic fluid (Hydrolubic 142, Houghton and Co., Valley Forge, PA). The outer bag was cut open, the pouches removed and put in an 80°C water bath for 10 minutes, then an ice water bath for 10 minutes. All pouches were labeled with production date and processing method, and randomly assigned to 3°C or 22°C dark storage rooms.

Microbiological Testing

As this was a feeding study, every care was taken to make sure product was safe for human consumption. Two days prior to the 10, 30, 60, 90, and 120 day evaluations, two pouches of each treatment of fruit mix of the appropriate batch were randomly
selected from each storage room and tested for microbial contamination, then resealed and held at 4°C until testing. Standard plate count and yeast and mold counts were performed on all pouches of product that were to be tested by panel members. The microbial cutoff for acceptability was 1000 CFU (colony forming units) per gram for the standard plate count, and any growth for the yeast and mold count.

**Presentation of Samples**

Pouches were removed from the 4°C refrigerator 30 minutes before the sensory panel and divided into approximately 35g portions in 100 ml white plastic dishes (VRW medium weighing boats, VRW, Seattle). Serving temperature was 16°C. Samples were labeled with random three-digit codes.

**Sensory Evaluation**

Descriptive analysis was done by a trained panel of ten volunteers, nine of whom had prior descriptive panel experience, recruited from the students and staff of the Department of Food Science and Technology. A preliminary ballot was prepared by the formulation developer and three sensory scientists. Panel training started with the preliminary ballot; it took six one-hour training sessions to train the panel and develop the final ballot. Training covered aroma, flavor, texture, and appearance terms. A 16-point intensity scale was used for rating descriptors, with 0=none, and 15=extreme. The final ballot is given in Appendix 7. Descriptor standards (Appendix 8) were used during training and available during testing.

Panelists were seated in individual booths with red lights when testing for aroma, flavor, and texture. Appearance was evaluated last, in a separate room with white
incandescent lighting, to assure that ratings for appearance didn’t affect ratings for other descriptors.

**Statistical Analysis**

Data were analyzed per descriptor using a three-way analysis of variance (ANOVA). Where appropriate, LSD multiple comparison procedures (p≤0.05) were used to determine significant differences between the treatments. The ANOVA model was composed of panelist, treatment, batch, panelist x treatment, and batch x treatment. Panelist and batch (and all their interaction terms) were treated as random effects, to increase the scope of inference that could be drawn from the study (Lundahl and McDaniel, 1988). The data set for each time period was analyzed independently.

Panelist x treatment interaction p-values were examined for each attribute to determine the panel’s consistency in rating attributes. When p≤0.05, interaction plots between treatment and panelist were constructed to determine which panelist(s) were rating samples differently from the panel. These panelists were then removed, and the data reanalyzed. In no case where this was done did nonsignificant treatment effects become significant. Usually, a panelist x session is also done to assess panelist consistency over sessions. This can only be done if the samples come from the same batch of product. Since the size of the experimental HPP equipment severely limited batch size, batches large enough to supply more than one session could not be processed.

**Results and Discussion**

HPP plus mild heat treatment resulted in a product that was closer to a fresh citrus mix than product preserved with heat alone. HPP plus mild heat treatment received
significantly higher ratings than heat treatment alone on appearance attributes ‘piece integrity’ and ‘brightness’. ‘Cooked flavor’ and ‘cooked aroma’ descriptor scores were significantly lower for HPP plus mild heat treatment samples than those treated with heat alone.

After 60 days storage, the fruit mix produced by both processes and stored at 22°C was not evaluated by the panel due to browning, off-taste, and off-aroma. These samples were clearly outside consumer acceptability, so sensory evaluation was no longer needed. Only samples stored at 3°C were tested at 60 and 90 days. At 120 days, the 3°C sample had unacceptable browning, so there was no testing at that time period. Multivariate analysis was performed on all fruit data sets. They did not add to understanding quality changes with this food system, and so are not presented.

Table 4 contains means, standard deviations, and significance levels of descriptor ratings of the fruit mix at all test periods. In testing done one day after processing, HPP with mild heat treated product was closer to the untreated control than product treated with heat alone. HPP with mild heat treated product was rated significantly lower than product with heat treatment alone in ‘cooked aroma’ and ‘cooked flavor’ descriptors, and higher than the untreated control. Samples treated by HPP with mild heat and those treated by heat alone were not significantly different from each other, and were significantly different from the control sample in many descriptors. The control was rated higher than the other two treatments in ‘aroma’, ‘pineapple flavor’, ‘pineapple sweetness’, ‘orange flavor’, ‘orange sweetness’, ‘grapefruit flavor’, ‘toughness of orange membrane’, ‘toughness of grapefruit membrane’, ‘piece integrity’, and ‘brightness’. It was lower in ‘cooked flavor’, ‘metallic flavor’, and ‘cooked aroma’.
Table 4. Fruit Mix: Means\(^1\) and standard deviations of sensory descriptors across storage times from the trained descriptive panel.

<table>
<thead>
<tr>
<th>Descriptors</th>
<th>1 Day</th>
<th>10 Days</th>
<th>30 Days</th>
<th>60 Days</th>
<th>90 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3° storage</td>
<td>22° storage</td>
<td>3° storage</td>
<td>22° storage</td>
<td>3° storage</td>
</tr>
<tr>
<td>Pineapple</td>
<td>9.1(^b)</td>
<td>5.6(^a)</td>
<td>6.3(^a)</td>
<td>5.6</td>
<td>5.1</td>
</tr>
<tr>
<td>Orange</td>
<td>8.3</td>
<td>7.8</td>
<td>7.1</td>
<td>6.0</td>
<td>4.9</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>7.6</td>
<td>6.7</td>
<td>6.0</td>
<td>5.1</td>
<td>4.3</td>
</tr>
<tr>
<td>Cooked</td>
<td>2.0(^a)</td>
<td>5.5(^b)</td>
<td>8.8(^c)</td>
<td>4.6</td>
<td>3.7</td>
</tr>
<tr>
<td>Off-aroma</td>
<td>0.4</td>
<td>1.6</td>
<td>2.1</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Flavor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pineapple</td>
<td>10.3(^b)</td>
<td>6.0(^a)</td>
<td>6.5(^a)</td>
<td>6.4(^ab)</td>
<td>5.5(^a)</td>
</tr>
<tr>
<td>intensity</td>
<td>3.0</td>
<td>2.4</td>
<td>3.2</td>
<td>2.9</td>
<td>2.1</td>
</tr>
<tr>
<td>Sour</td>
<td>3.2</td>
<td>4.5</td>
<td>4.3</td>
<td>4.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Sweet</td>
<td>6.4(^b)</td>
<td>3.5(^a)</td>
<td>4.6(^c)</td>
<td>3.9</td>
<td>3.5</td>
</tr>
<tr>
<td>Bitter</td>
<td>1.0</td>
<td>2.7</td>
<td>2.6</td>
<td>4.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Orange</td>
<td>9.4 (^b)</td>
<td>6.6(^a)</td>
<td>6.0(^a)</td>
<td>6.8</td>
<td>5.6</td>
</tr>
<tr>
<td>intensity</td>
<td>3.4</td>
<td>2.7</td>
<td>2.5</td>
<td>2.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Sour</td>
<td>2.3</td>
<td>3.0</td>
<td>2.6</td>
<td>3.1</td>
<td>3.7</td>
</tr>
<tr>
<td>Sweet</td>
<td>6.3(^b)</td>
<td>3.9(^a)</td>
<td>4.4(^c)</td>
<td>4.4(^b)</td>
<td>3.4(^a)</td>
</tr>
<tr>
<td>Bitter</td>
<td>1.0</td>
<td>2.1</td>
<td>2.8</td>
<td>2.8</td>
<td>3.0</td>
</tr>
</tbody>
</table>

\(^1\)Scale = 0-15, 0 = none and 15 = extreme. Ratings with the same or no letter superscript show no significant difference (p ≤ 0.05).
Table 4, continued.

<table>
<thead>
<tr>
<th>Descriptors</th>
<th>1 Day</th>
<th>10 Days</th>
<th>30 Days</th>
<th>60 Days</th>
<th>90 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavor, cont.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grapefruit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>intensity</td>
<td>10.7b</td>
<td>7.8a</td>
<td>7.9a</td>
<td>7.7b</td>
<td>7.8b</td>
</tr>
<tr>
<td>Sour</td>
<td>5.5</td>
<td>5.2</td>
<td>5.2</td>
<td>4.4b</td>
<td>4.5b</td>
</tr>
<tr>
<td>Sweet</td>
<td>3.2</td>
<td>2.6</td>
<td>2.5</td>
<td>3.2</td>
<td>3.0</td>
</tr>
<tr>
<td>Bitter</td>
<td>4.8</td>
<td>4.3</td>
<td>5.0</td>
<td>4.1</td>
<td>4.0</td>
</tr>
<tr>
<td>Cooked</td>
<td>1.2a</td>
<td>5.1b</td>
<td>7.1c</td>
<td>4.2</td>
<td>4.5</td>
</tr>
<tr>
<td>Metallic</td>
<td>0.5a</td>
<td>3.0b</td>
<td>3.4b</td>
<td>1.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Off-flavor</td>
<td>Not collected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Texture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rupture force</td>
<td>8.2b</td>
<td>6.0a</td>
<td>6.0a</td>
<td>6.5b</td>
<td>6.7b</td>
</tr>
<tr>
<td>orange</td>
<td>(3.7)</td>
<td>(2.6)</td>
<td>(1.7)</td>
<td>(2.5)</td>
<td>(2.7)</td>
</tr>
<tr>
<td>Rupture force</td>
<td>6.9b</td>
<td>4.3a</td>
<td>3.9a</td>
<td>7.6c</td>
<td>7.5c</td>
</tr>
<tr>
<td>grapefruit</td>
<td>(3.6)</td>
<td>(1.9)</td>
<td>(1.5)</td>
<td>(2.6)</td>
<td>(2.5)</td>
</tr>
<tr>
<td>Appearance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Piece</td>
<td>12.5b</td>
<td>8.1a</td>
<td>8.9a</td>
<td>9.3c</td>
<td>8.3bc</td>
</tr>
<tr>
<td>integrity</td>
<td>(1.6)</td>
<td>(1.9)</td>
<td>(2.7)</td>
<td>(2.3)</td>
<td>(2.8)</td>
</tr>
<tr>
<td>Brightness</td>
<td>11.8b</td>
<td>9.6a</td>
<td>10.3a</td>
<td>8.4b</td>
<td>8.0b</td>
</tr>
<tr>
<td>Spots on pine.</td>
<td>2.2</td>
<td>1.6</td>
<td>1.8</td>
<td>2.4</td>
<td>2.4</td>
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<tr>
<td>Translucency</td>
<td>5.3</td>
<td>6.2a</td>
<td>5.5</td>
<td>4.5</td>
<td>4.7</td>
</tr>
<tr>
<td>of pineapple</td>
<td>(3.2)</td>
<td>(2.4)</td>
<td>(2.2)</td>
<td>(2.4)</td>
<td>(2.8)</td>
</tr>
</tbody>
</table>

1Scale = 0-15, 0 = none and 15 = extreme. Ratings with the same or no letter superscript show no significant difference (p ≤ 0.05).
2Descriptor was added after day 1 testing.
The intensities of the texture terms, 'force needed to rupture the sectional membrane' of the orange and grapefruit pieces, had significant differences between treatments at all testing times (Table 4). There was an inverse relationship between total amount of heat, as expressed in time at a given temperature above 0°C, a sample receives in both processing and storage, and the force needed to rupture the outer membranes of the sectional fruits. Testing after one day storage showed no significant difference between the force needed to tear outer membranes of sectional fruits of HPP plus mild heat treatment and heat treatment alone (Table 4). The untreated control was rated significantly higher than the other two treatments. After 10 days storage, samples stored at 22°C needed less force to rupture outer membranes of sectional fruits than those stored at 3°C, and within a storage time heat only treated products needed less force to rupture outer membranes than HPP with mild heat treatment. Heat breaks down the membrane, rendering it easier to rupture.

Heat breakdown of membranes also explains the higher scores for HPP plus mild heat treated product in 'piece integrity'; the softening of sectional membrane and juice sacs inside lead to a more ragged and deformed piece of fruit. Figure 5 contains the 'piece integrity' ratings of the attribute. After 10 days storage, the HPP plus mild heat treatment sample stored at 22°C was rated significantly higher than heat treatment alone stored at 22°C. The results of different storage temperatures are clear. Product stored at 3°C rated higher than that stored at 22°C. At 30 days, the P 22°C rated significantly higher than the H 22°C, but overall the results are less clear for this time period. The ratings for P 3°C and H 3°C are slightly higher than those of P 22°C, but not to a
significant degree. After 60 and 90 days, P 3°C was rated significantly higher than H 3°C, with the gap between them increasing.

Intensity ratings for the piece integrity (Figure 5) of P 3°C go down between 10 to 30 days, then increase at the 60 and 90 day tests. This often happens with descriptive data when panelists are not trained extensively with intensity standards; panelists had no absolute intensities to refer to, so ratings are influenced primarily by the relative intensities of the samples they are testing at any one time. Because of this, comparing ratings for different time points against each other must be done with caution.

The results for ‘brightness’ were similar to those for ‘piece integrity’, with the HPP plus mild heat treatment rated significantly higher than heat treatment alone after 10 days at 22°C and 60 and 90 days at 3°C (Figure 6). There were no significant differences between treatments at 30 days, although the pattern of the responses was very similar to that of 10 days. The loss of brightness in color is probably due to carmelization reactions happening over time. Carotenoids, the colorants in citrus fruits, are extremely stable, not degrading with blanching, retorting, or freezing (Borenstein and Bunnell, 1966). In storage, citrus juice undergoes carotenoid degradation under two conditions: if oxygen is not removed before processing, and in response to light (Bauernfeind, J. C., et al, 1971). As the fruit mix in this study was vacuum packed and stored in the dark, carotenoid degradation was not the source of the color change. Carmelization takes place when carbohydrates, especially sugars, are subjected to heat, and is aided by the addition of acid and certain salts. It proceeds far enough to produce a color change when the pH is below 6.0 (Whistler and Daniel, 1985). The samples treated by heat alone received more heat than those treated with HPP and mild heat; this extra heat could have pushed the
Figure 5. Intensity ratings and significance levels for piece integrity descriptor in fruit mix descriptive data.

Figure 6. Intensity ratings and significance levels for brightness descriptor in fruit mix descriptive data.
Figure 7. Intensity ratings and significance levels for cooked flavor descriptor in fruit mix descriptive data.

Figure 8. Intensity ratings and significance levels for cooked flavor descriptor in fruit mix descriptive data.
reaction farther along the path to eventual color production than the HPP with mild heat treatment.

HPP plus mild heat treatment stored at 3°C was rated lower in ‘cooked flavor’ after 60 and 90 days than heat treatments stored at 3°C, and lower in ‘cooked aroma’ after 90 days. Figures 7 and 8 are ratings of the ‘cooked flavor’ and ‘cooked aroma’ descriptors. There were very clear differences between the HPP with mild heat, heat alone, and the unprocessed control at the one day testing, but the difference between the HPP with mild heat and the heat treatment alone seem to vanish by the 10 day testing. Even though it isn’t a descriptor used in standard industry quality assessment, sensory research has reported a ‘cooked’ note in orange juice before. Mohonas and Shaw (1989) reported a cooked or prune-like flavor noted by panelists in their storage study of aseptically packaged pasteurized orange juice. Shah (1998) found cooked aroma and flavor higher in heat pasteurized orange juice than fresh using descriptive analysis, and determined that cooked notes were present in fresh juice, but were suppressed by volatiles driven off during pasteurization. The descriptors of the peaks of the aroma active compounds identified in the gas chromatography-olfactometry part of that study include many that have ‘off’ aromas that may contribute to the ‘cooked’ aroma of orange juice. Perhaps changes in the fruit mix are the result of the interaction of several compounds, the mix of which changes with time, hence the changes in perception of intensity over time. If some of these compounds started breaking down or volatilizing after storage but others did not, that would explain the drop in the heat treated samples between the 1 day and 10 day testings. If other compounds were formed or increased over time, perhaps as a result of a non-enzymatic browning reaction that was influenced by the extra heat
received in processing by the heat treated samples, that would explain the significant
difference at the end of the study.

**Conclusions**

Treating citrus fruit mix with a combination of HPP and mild heat instead of heat
treatment alone will result in a product closer to fresh fruit, with better appearance
attributes and less 'cooked' aroma and flavor. Product treated with HPP and mild heat
can be kept for up to 30 days at ambient temperature and 90 days refrigerated, while the
heat processed can only be kept for less than 10 days at ambient temperatures or 30 days
refrigerated.

**References**


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Conclusions

High pressure processing plus mild heat treatment of a citrus fruit mix and HPP of spaghetti with meat sauce gave products that were rated higher in sensory descriptors over time at ambient and refrigerated temperatures than the same products processed with heat. These HPP products were more like untreated products in their sensory profile than heat treat products were. This can translate into longer shelf lives for producers and retailers and products with a less processed flavor profile for consumers. The fruit mix treated with HPP and mild heat can be kept for up to 30 days at ambient temperature and 90 days refrigerated, while the heat processed can only be kept for less than 10 days at ambient temperatures or 30 days refrigerated.

There were no statistically significant differences (p<0.05) between treatments of Spanish rice held at the same temperature, so there would be no benefit to HPP processing of this entrée. This may hold true for other food systems with parboiled rice as well, but further research would be necessary for confirmation.
Bibliography


Appendices
Appendix 1. Specification—Spanish rice

1. Ingredients. All ingredients shall be clean, sound, wholesome and free from foreign material, evidence of rodent or insect infestation, extraneous material, off-odors, off-flavors and off-colors.

1.1. Tomatoes, Diced, Canned. Canned tomatoes shall be peeled, cored, mature, diced tomatoes. The use of safe and suitable firming and acidification ingredients and salt is permitted. The tomatoes shall be packed in their own juice. The canned tomatoes shall have not less than 8.0 percent tomato soluble solids and shall possess a red flesh color, normal character, and a good distinct acid sweet tomato flavor and odor. The tomatoes shall be free from extraneous vegetable material and objectionable core material and skins (peel).

1.2. Rice, long grain, parboiled. Rice shall be parboiled, long grain, milled rice, U.S. standards for milled rice. The rice shall contain not more than 2 percent of kernels having white ungelatinized areas. Also the rice shall contain not less than 10 percent nor more than 15 percent moisture.

Uncle Ben's Parboiled, Converted Rice, Uncle Ben’s Inc., Houston, TX has been found to perform satisfactorily in the production of the Spanish rice.

1.3. Sugar, granulated. Sugar shall be white, refined, sucrose, granulated cane or beet or a combination of both.

1.4. Onions, chopped, dehydrated. The dehydrated chopped onions shall be Fancy Grade of the Official Standards and Methods of the American Dehydrated Onion and Garlic Association for dehydrated onions and garlic products.

1.5. Peppers, green, fresh. The green peppers shall be purchased fresh locally. They shall be free from bruises or soft spots that would indicate damage or spoilage. They shall possess a fresh, clean, typical sweet green pepper flavor and odor.

1.6. Salt. Salt shall be free-flowing, white, refined, sodium chloride with or without anticaking agents and shall comply with purity standards for sodium chloride of the Food Chemicals Codex.

1.7. Starch, waxy maize, modified. The starch shall be white, odorless, finely pulverized, modified waxy maize starch for use in processed foods. The modified starch shall demonstrate initial viscosity development in the range of 140 – 170°F and be fully rehydrated at 195°F. The starch shall be bland with essentially no cereal or starch taste.

1.8. Sauce, hot. Hot sauce shall be produced from ground, fermented hot red peppers, distilled vinegar, and salt and may contain stabilizers. The hot sauce shall be a smooth suspension with uniform particle size and possess a pungent peppery odor, and a reddish-orange color.
1.9. Pepper, white, ground. Ground white pepper shall be derived from the dried mature berries of Piper nigrum L. from which the outer and inner coverings have been removed. The pepper shall have a characteristic penetrating odor, a hot biting pungent flavor and a light white-gray color.

1.10. Hickory flavor. Schilling hickory seasoning shall be used to give a characteristic bacon flavor. It shall possess a distinct hickory flavor and odor.

1.11. Lemon Concentrate. Shall be made from the juice of fresh lemons. It shall be frozen and packed into five gallon heavy duty plastic pails. The net weight of the pails will be 51.5 lbs. The concentrate is standardized at 395-405 grams/liter. It shall have a pH of 2.8-2.9 and brix of 45-50. It shall contain no preservatives.

Vita-Pakt Citrus Products Co., Covina, CA has been found to be a satisfactory supplier of this product, (818) 332-1101.

2. Preparation and Further Processing

2.1. Ingredients

<table>
<thead>
<tr>
<th>% by Weight</th>
<th>g/batch</th>
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<tr>
<td>Pepper, white, ground</td>
<td>0.02</td>
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</table>

2.2. Soak 10 pounds of rice in 34 pounds of water with approximately 156 grams of lemon concentrate added. The rice is allowed to soak at ambient temperature for 30 minutes.

2.3. Following the soaking period, the rice and lemon water are transferred to a saucepan, and brought to a boil (temp 100° C), covered and the heat reduced to simmer the rice for 7-8 minutes. The rice is then drained and rinsed with cold water to prevent further cooking.

2.4. The starch and just enough cold water to wet the starch is mixed to form a slurry. The onions are also rehydrated in a small amount of water and allowed to set for 5 minutes.
2.5. The tomatoes, sugar, rehydrated onions, salt, hot sauce, hickory flavor and pepper are combined in a jacketed blender or agitated kettle (or saucepan for lab batches) and brought to a temperature of 180-190°F and held for approximately 3-5 minutes with slow agitation. The starch slurry is added and the mixture is held at 180-190°F until thickened (3-5 minutes).

2.6. Fresh green peppers are cut into 3/8” cubes. Approximately 16 lbs. of water is acidified with 217 grams frozen lemon concentrate and brought to a boil. The peppers are submerged in the boiling water, covered and blanched for 2 minutes. The peppers are removed and cooled in ice water for 2 minutes. The pH of the peppers should be below 4.3 and they should test peroxidase negative.

2.7. 14.0 lbs. of the cooled acidified rice is added to the sauce with slow agitation to avoid scorching. The heat in the kettle is turned off.

2.8. The blanched, acidified green peppers are added. Stir until uniformly mixed.
Appendix 2. Specification—spaghetti with meat sauce

1. **Ingredients.** All ingredients shall be clean, sound, wholesome and free from foreign material, evidence of rodent or insect infestation, extraneous material, off-odors, off-flavors and off-colors.

1.1. Tomatoes, canned, crushed or diced. Canned tomatoes shall be peeled, cored, matured, crushed or diced tomatoes. The use of safe and suitable firming and acidification ingredients and salt is permitted. The tomatoes shall be packed in their own juice. The canned tomatoes shall have not less than 8.0 percent tomato soluble solids and shall possess a red flesh color, normal character, and a good distinct acid sweet tomato flavor and odor. The tomatoes shall be free from extraneous vegetable material and objectionable core material and skins (peel).

1.2. Tomato Puree. The tomato puree shall be of U.S. Grade A of the U.S. Standards for Grades of Tomato Puree.

1.3. Sugar, granulated. Sugar shall be white, refined, sucrose, granulated cane or beet or a combination of both.

1.4. Onions, chopped, dehydrated. The dehydrated chopped onions shall be Fancy Grade of the Official Standards and Methods of the American Dehydrated Onion and Garlic Association for dehydrated onions and garlic products.

1.5. Vegetable oil.

1.6. Salt. Salt shall be free-flowing, white, refined, sodium chloride with or without anticaking agents and shall comply with purity standards for sodium chloride of the Food Chemicals Codex.

1.7. Starch, waxy maize, modified. The starch shall be white, odorless, finely pulverized, modified waxy maize starch for use in processed foods. The modified starch shall demonstrate initial viscosity development in the range of 140-170°F and be fully rehydrated at 195°F. The starch shall resist breakdown at low pH, under shear stress, and under conditions of cold storage. The starch shall be bland with essentially no cereal or starch taste.

1.8. Basil, leaves. Basil shall be derived from the dried leaves of Ocimum basilicum L. and possess a sweet, anise-like odor and an aromatic, warm, slightly pungent flavor.

1.9. Oregano, leaves. Oregano shall be derived from the dried leaves of Origanum vulgare L. and shall possess a strong comphoraceous aroma and a pungent, slightly bitter flavor.
1.10. Pepper, black, ground. Ground black pepper shall be derived from the dried mature berries of Piper nigrum L.. The pepper shall have a characteristic penetrating odor, a hot biting pungent flavor and a black-gray color.

1.11. Citric acid. Shall comply with the Food Chemicals Codex.

1.12. Garlic powder. Garlic powder shall be Fancy grade of the Official Standards and Methods of the American Dehydrated Onion and Garlic Association for dehydrated Onion and Garlic powder.

1.13. Fennel, seeds.

1.14. Beef, ground, extra lean. The beef shall be from steers, heifers or cows and shall be derived from any one or any combination of the USDA Certified Institutional Meat Purchase Specifications cuts. Boned and trimmed beef meeting the requirements shall be further trimmed, if necessary so as to assure compliance with the extra lean requirement (<10% fat). The beef shall be ground through a plate having holes of 3/8 inch in diameter. After grinding the beef shall be held for not more than 24 hours at an internal temperature of 28-40°F prior to cooking.

1.15. Spaghetti. The spaghetti shall be enriched and contain 2% by weight of egg albumen. The spaghetti shall conform to the Standard of Identity for Enriched Macaroni Products. The spaghetti shall have a solid cylindrical shape and shall be approximately 2 inches in length.

1.16. Lemon concentrate. Shall be make from the juice of fresh lemons. It shall be frozen and packed into 5 gallon heavy duty plastic pails. The net weight of the pails will be 51.5 lbs. The concentrate is standardized at 395-405 grams/liter. It shall have a pH of 2.8-2.0 and a brix of 45-50. It shall contain no preservatives. Vita-Pakt Citrus Products Co., Covina, CA has been found to be a satisfactory supplier of this, (818) 332-1101.

2. Preparation and Further Processing

2.1. Ingredients by weight.

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<th>Ingredient</th>
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<td>Ingredient</td>
<td>Amount</td>
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<td>--------</td>
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<td>Basil</td>
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<td>Oregano</td>
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<tr>
<td>Black pepper</td>
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</table>

2.2. The onions are reconstituted in hot water. They are then drained of excess water and simmered on medium heat (180°F) in the oil until tender (approximately 3-4 min).

2.3. The tomato products and the seasonings are added to the onions. The sauce shall be heated to 170°F. The pH of the sauce should be 4.0 +/- 0.10.

2.4. Raw ground beef was mixed with 6% by weight lemon concentrate. The raw beef was stuffed loosely into perforated casings. The roll was cooked for 1 hour at 140°F, 1 hour at 160°F, and then cooked at 180°F until the internal temperature of the meat is 170°F. The final pH of the cooked beef must be 4.2 or below.

2.5. The cooked beef is added to the sauce at the percent specified above.

2.6. The 1% lemon concentrate is added to 40 lbs. water in the cookpot. The water is heated to boiling (212°F). The uncooked noodles are added to the water. They are cooked for 8 minutes uncovered and drained. The final pH of the noodles should be 4.0 +/- 0.10.

2.7. The cooked acidified spaghetti noodles are added to the sauce in the weight ratio of 30% noodles to 70% sauce and mixed thoroughly.
Appendix 3. Spaghetti with meat sauce descriptive ballot

Name ___________________________ Date __________

Spaghetti With Meat Sauce Ballot

Please rate the descriptors using a 0-15 intensity scale. 0 = none and 15 = extreme. Please sample the products in the order listed.

Aroma

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<th>Descriptor</th>
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<td>Pasty/starchy/floury</td>
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Flavor

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<tbody>
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<tr>
<td>Sweet</td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td></td>
</tr>
<tr>
<td>Pasta</td>
<td></td>
</tr>
<tr>
<td>Spice blend</td>
<td></td>
</tr>
<tr>
<td>Meat</td>
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Texture

<table>
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<tr>
<th>Descriptor</th>
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Appearance

<table>
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<tr>
<td>Integrity of pasta</td>
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<tr>
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<tr>
<td>Dry appearance; &quot;Clumpiness&quot;</td>
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### Appendix 4. Spanish rice descriptive ballot

Name ________________________  Date ______

**Spanish Rice Ballot**

Please rate the descriptors using a 0-15 intensity scale. 0 = none and 15 = extreme. Please test the samples in the order given.

| Aroma               |  |  |  |  |
|---------------------|  |  |  |  |
| Green Pepper        |  |  |  |  |
| Tomato              |  |  |  |  |
| Onion               |  |  |  |  |
| Rice                |  |  |  |  |
| Off-aroma           |  |  |  |  |

| Texture             |  |  |  |  |
|---------------------|  |  |  |  |
| Firmness of rice    |  |  |  |  |
| Crunchiness of peppers |  |  |  |  |

| Flavor              |  |  |  |  |
|---------------------|  |  |  |  |
| Sour                |  |  |  |  |
| Sweet               |  |  |  |  |
| Salt                |  |  |  |  |
| Tomato              |  |  |  |  |
| Green Pepper        |  |  |  |  |
| Onion               |  |  |  |  |
| Black Pepper        |  |  |  |  |
| Off-flavor          |  |  |  |  |

| Appearance          |  |  |  |  |
|---------------------|  |  |  |  |
| Smoothness of rice grains |  |  |  |  |
| Glossiness          |  |  |  |  |
| Brightness of colors |  |  |  |  |
| Evenness of color over sauce and rice |  |  |  |  |
| Integrity of tomatoes |  |  |  |  |
| Integrity of green peppers |  |  |  |  |
Appendix 5. Aroma standards for spaghetti with meat sauce and Spanish rice

Spaghetti Aroma Standards

All standards placed in 240 ml wine glasses and covered with aluminum lids.

Tomato aroma: 25 g canned diced tomato.
Pasty aroma: 25 g cooked spaghetti.
Meaty aroma: 25 g cooked extra lean ground beef.

Spanish Rice Aroma Standards

All standards placed in 240 ml wine glasses and covered with aluminum lids.

Green pepper aroma: 15 g diced fresh green pepper.
Onion aroma: 10 g dehydrated onions that have been reconstituted in hot water.
Rice aroma: 25 g prepared long grain parboiled rice (Uncle Ben’s Parboiled Converted Rice, Uncle Ben’s Inc. Houston, TX).
Tomato aroma: 25 g canned diced tomato.
Appendix 6. Specification—fruit mix

3. Ingredients. All ingredients shall be clean, sound, wholesome and free from foreign material, evidence of rodent or insect infestation, extraneous material, off-odors, off-flavors and off-colors.

1.1. Pineapple, fresh. Pineapple shall be purchased locally. It will be fresh, whole Hawaiian pineapple. It shall be not be green (underripe) or have soft spots or a fermented smell (overripe).

1.2. Oranges, fresh. Oranges shall be purchased locally. They will be fresh, whole oranges that are free of bruises or soft spots.

1.3. Grapefruit, fresh. Grapefruit shall be purchased locally. They will be fresh whole pink grapefruit that are free of bruises or soft spots.

1.4. Pineapple juice, canned. It shall be canned unsweetened pineapple juice packaged in cans of 46 fl oz/can.

1.5. Preparation and Further Processing

1.6. Ingredients by weight.

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<tr>
<th>Ingredient</th>
<th>% by weight</th>
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</tr>
<tr>
<td>oranges, fresh, pieces</td>
<td>40</td>
</tr>
<tr>
<td>grapefruit, fresh, pieces</td>
<td>20</td>
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</table>

1.7. The pineapple is peeled, cored, and sliced on the small lab sheer. It shall be sliced longitudinally into 1/2” slabs. These slabs are then cut manually into 3/8 – 1/2” cubes.

1.8. The oranges are peeled and the segments are separated. The segments are manually cut into 3/8 – 1/2” pieces.

1.9. The grapefruit are peeled and the segments are separated. The segments are manually cut into 3/8 – 1/2” pieces.

1.10. The vacuum desiccator is filled with canned pineapple juice. The fruit is put into the desiccator and the lid is put on. The fruit is subjected to a vacuum of no less than 29 inches for a minimum of 5 minutes.
Appendix 7. Fruit mix descriptive ballot

Name___________________    Date________

Fruit Mix Ballot

Please rate the samples from left to right. Rate the descriptors using a 0-15 intensity scale. 0 = none and 15 = extreme.

<table>
<thead>
<tr>
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<th>__</th>
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<tr>
<td>Orange</td>
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<tr>
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<table>
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</tr>
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<tr>
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<tr>
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<tr>
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<table>
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Appendix 8. Standards for fruit mix

1. Fresh pineapple in 1/2” cubes put in 240 ml wine glass w/ aluminum lid; for pineapple aroma and flavor.

2. Fresh orange sections cut into 1/2” pieces put in 240 ml wine glass w/ aluminum lid; for orange aroma and flavor.

3. Fresh grapefruit sections cut into 1/2” pieces put in 240 ml wine glass w/ aluminum lid; for grapefruit aroma and flavor.

4. Crushed canned pineapple in 2 oz. cups; for metallic flavor, and cooked citrus aroma and flavor.

5. Canned orange juice, 12 oz. microwaved for two minutes on high, cooled to 22°C, served in 2 oz. cups; for metallic flavor, and cooked citrus aroma and flavor.

6. One 1/2” crosscut slice of fresh pineapple; for pineapple translucency.

7. Thirty-five g of fruit mix in sample dish before it has been vacuum treated; for piece integrity.