EFFECT OF HIGH HYDROSTATIC PRESSURE (HHP) ON SOME QUALITY PARAMETERS AND SHELF-LIFE OF FRUIT AND VEGETABLE JUICES

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

SANER DEDE

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE IN
FOOD ENGINEERING

AUGUST 2005

Approval of the Graduate School of Natural and Applied Sciences.

Prof.Dr. Canan Özgen
Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

Prof.Dr. Levent Bayındırlı
Head of Department

This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

Prof.Dr. Alev Bayındırlı
Co-Supervisor

Assoc.Prof.Dr. Hami Alpas
Supervisor

Examining Committee Members

Prof.Dr. Faruk Bozoğlu	(METU, FDE)	
Assoc.Prof.Dr. Hami Alpas	(METU, FDE)	
Prof.Dr. Alev Bayındırlı	(METU, FDE)	
Assoc.Prof.Dr. Dilek Sivri	(Hacettepe Uni, FDE)	
Inst.Dr. Deniz Çekmecelioğlu	(METU, FDE)	

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name: Saner Dede

Signature:

ABSTRACT

EFFECT OF HIGH HYDROSTATIC PRESSURE (HHP) ON SOME QUALITY PARAETERS AND SHELF-LIFE OF FRUIT AND VEGETABLE JUICES

DEDE, Saner
M.Sc., Department of Food Engineering
Supervisor : Assoc. Prof. Dr. Hami Alpas
Co-Supervisor : Prof. Dr. Alev Bayındırlı

August 2005, 70 pages

The quality and shelf-life of pressure processed (150, 200 and 250 MPa at 25 and 35°C for 5, 10 and 15 minutes) orange, tomato and carrot juices were compared to fresh and thermally pasteurised (60°C for 5, 10 and 15 minutes and 80°C for 1 minute) juices.

Treatments were capable of microbial inhibition of juices to non-detectable levels. The change in ascorbic acid content of HHP treated juices was not statistically significant ($p>0.05$). Both heat treatments at 60 and 80°C, displayed a significant loss and induced a decrease in the free radical scavenging activity but was not affected by HHP treatments.

Pressurization at 250 MPa at 35°C for 15 minutes and thermal pasteurization at 80°C for 1 minute and stored at 4 and 25°C for shelf-life analysis.

HHP treated juices showed a small loss of antioxidants (below 10%) at both storage temperatures whereas the loss is higher (about 30%) in the heat treated

juices through shelf life (30 days). The pressurized juices, stored at 25°C, contained ascorbic acid better than heat treated ones after 30 days.

The total color changes were minor (ΔE=10) for all pressurized juices but for heat pasteurized samples, higher as a result of insufficient antioxidant activity. The pH of juices was not affected by treatment, storage temperature or time.

HHP yielded a better product, regarding the studied parameters of the juices compared to the conventional pasteurization. Therefore, HHP treatment (250 MPa, 35°C for 15 minutes) can be recommended for industrial production of fresh fruit and vegetables.

Keywords: High hydrostatic pressure, Heat treatment, Orange Juice, Tomato Juice, Carrot Juice

ÖZ

YÜKSEK HİDROSTATİK BASINCIN (YHB) MEYVE VE SEBZE SULARININ BAZI KALİTE PARAMETRELERİ VE RAF-ÖMÜRLERİ ÜZERİNE ETKİSİ

DEDE, Saner
Yüksek Lisans, Gıda Mühendisliği Bölümü
Tez Yöneticisi : Doç. Dr. Hami Alpas
Ortak Tez Yöneticisi : Prof. Dr. Alev Bayındırlı

Ağustos 2005, 70 sayfa

Basınç uygulanmış (150, 200 ve 250 MPa, 25 ve 35°C'de 5, 10 ve 15 dakika) portakal, domates ve havuç sularının kalite ve raf ömürleri, taze ve ısıl pastörizasyon (60°C'de 5, 10 and 15 dakika ve 80°C'de 1 dakika) uygulanmışlarla karşılaştırılmıştır.

Uygulamalar örneklerin mikrobiyel yükünü ölçülemeyen seviyelere kadar indirmiştir. Askorbik asit içeriğindeki değişim YHB uygulanmış örneklerde istatistiksel olarak önemli bulunmamıştır ($p>0.05$). 60 ve 80°C'deki ısıl uygulamalar, önemli kayıp göstermiş ve serbest radikal bağlama aktivitesinde kayba neden olmuş fakat YHB uygulamasında buna rastlanmamıştır.

250 MPa, 35°C'de 15 dakika basınç uygulanan ve 80°C'de 1 dakika ısıl işlem gören örnekler, raf ömrü analizleri için 4 ve 25°C'de saklanmıştır.

Raf ömrü boyunca basınç uygulanmış meyve suları her iki depolama sıcaklığında da antioksidan miktarında ufak düşüş (%10 un altında) göstermiştir, fakat ısı uygulanmışlarda kayıp daha yüksek (%30) olmuştur. 25°C depolamada

basınç uygulanmış portakal suyunda, ısı uygulanmışa göre daha iyi askorbik asit miktarı görülmüştür.

Renk değişimleri basınçlanmış meyve suları için çok küçük (ΔE=10) olmuş, ama antioksidan aktivitesi yetersiz olduğu için ısı uygulanmışlarda daha yüksek görülmüştür. Örneklerin pH'ları işlemlerden, depolama sıcaklığından ya da süresinden etkilenmemiştir.

YHB uygulaması, çalışılan parametreler bakımından, kullanılmakta olan ısıl pastörizasyonla karşılaştırıldığında daha iyi portakal, domates ve havuç suyu elde edilmesini sağlamıştır. Bu sonuçlar ışığında, YHB uygulaması (250 MPa, 35°C, 15 dakika) meyve ve sebze suyu endüstriyel üretimi için önerilebilir.

Anahtar Kelimeler: Yüksek hidrostatik basınç, Isıl işlem, Portakal suyu, Domates suyu, Havuç suyu.

to my parents…

ACKNOWLEDGEMENTS

There are many people who deserve my deep acknowledgements since they had contributions, help and encouragements.

I deeply appreciate my family for their patience, all the support they gave and sacrifices they made to make me the man I am today. I also thank to my relatives who were very supportive all my life.

I would like to thank to all my undergraduate friends especially my dearest ones Selim Yumak, Özgür Güneş and Engin Aydın for their friendship and moral supports after and during my undergraduate life.

My special thanks go to all my friends at ÖYP programme; Aysu Acar, Neslihan Altay, Cem Baltacıoğlu, Hande Baltacıoğlu, Işıl Barutçu, Mete Çevik, Erkan Karacabey, B. Gökçen Mazı, Mutlu Pilavtepe, Nadide Seyhun and Özge Şakıyan for their patience, extensive support, friendship and accompany. My M.S. life would not be this enjoyable and fun without them. I can never repay you.

I would like to thank all the research assistants and other graduate students of the Food Engineering Department, especially to Sencer Buzrul. I would also have to all other members of the department from whom I needed help or advice. Everybody has been really helpful and kind.

I am also grateful to Prof.Dr. Alev Bayındırlı for her valuable comments and professional help with this study. Her suggestions and her extensive experience and knowledge guided me to the completion of this study.

And, last but not least, I would like to express my sincere appreciation to my advisor, Assoc.Prof.Dr. Hami Alpas for his academic support that kept me focused and made this study a success. It had been a real honor and pleasure for me to work with him.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Recent Developments in Food Processing and High Hydrostatic Pressure (HHP)

Over the last decade, consumer demand has increasingly required processed foods to have more 'natural' flavor and color, with a shelf life that is sufficient for distribution and a reasonable period of home storage before consumption. This can be achieved by minimal processing methods that preserve foods but also retain to a greater extend their nutritional quality and sensory characteristics by reducing the reliance on heat as the main preservative action. Traditionally, fermented foods have many of these characteristics; irradiation has been adopted in some countries as a minimal method of food preservation; and chilling and controlled or modified atmospheres are now widely adopted to suppress the microbial growth. There has also been increasing interest in developing other combinations of existing and novel methods to achieve mild preservation. Such novel minimal processing methods as, pulsed electric fields, high pressure processing, high intensity light and ultrasound destroy microorganisms, and in some cases enzymes, and there are no substantial increases in product temperature. There is therefore little damage to pigments, flavor compounds or vitamins and, in contrast to heat processing, the sensory characteristics and nutritional value of foods are not degraded to a significant extent. The resulting products have higher quality and consumer appeal in markets where the retention of nutritional sensory characteristics can command premium prices (Fellows, 2000).

1.1.1 History of General Use of HHP in Food Products

HHP is being used for years in other industries such as ceramics, carbon graphite, diamond, steel/alloy and plastics (Tewari et al., 1999). High pressure treatment to kill bacteria was first described by Roger (Smelt, 1998). The first use of high pressures as a method of food processing was in 1899 in the West Virginia University in the USA, where the experiments were conducted using high hydrostatic pressures to preserve milk, fruit juice, meat and a variety of fruits. It was demonstrated that microorganisms in these products could be destroyed by pressures of 658 MPa (6500 atm) in 10 minutes. In the early years of the twentieth century, other researches showed that protein structure in egg-white could be altered by high pressures. However, these early researchers found that the potential is limited because the enzymes were largely unaffected, particularly in milk. They were also constrained by difficulties in the manufacture of high pressure units and inadequate packaging materials to contain the foods during processing, and research was discontinued. Advances in the design of presses together with rapid advances in packaging materials during the 1970s, enabled research to begin again on high pressure processing in the 1980s, focused mainly in Japan (Fellows, 2000).

By 1990 the first commercial products produced by high pressure processing went on sale in Japan. One company introduced a range of pressure-processed jams, including apple, kiwi, strawberry and raspberry in flexible sealed plastic packs, and two other companies started production of bulk orange juice and grape fruit juice. The jams had a shelf life of two months under chilled storage, which is required to prevent enzyme activity. Other products included fruit jellies, sauces, fruit yoghurts and salad dressings. The products currently sell at three to four times the cost of conventional products, but the higher quality, particularly flavor and texture of the fruit, has so far ensured sufficient demand for commercial viability. Similar products have recently reached the US and Europe markets (Mermelstein, 1997), because of the very high investment and processing costs of high pressure processing as well as regulatory problems in some regions (Jongen, 2002).

1.1.2 General Principle and Mechanism of HHP

Two principles describe the effect of HHP. Firstly, the principle of Le Chatelier, according to which any phenomenon (phase transition, chemical reaction, change in molecular configuration) accompanied by a decrease in volume can be enhanced by pressure. Secondly, pressure is instantaneously and uniformly transmitted to the food independent of size and geometry i.e., the food will be compressed by a uniform pressure from every direction and then return to its original shape when the pressure is released. This is known as isostatic pressure (Palou, 1998; Trujillo et al., 2000; Alpas, 2000; Tewari et al., 1999; Smelt, 1998; Gervilla et al., 1999; Gaucheron et al., 1997). The principle of isostatic processing is briefly presented in Figure 1.1.

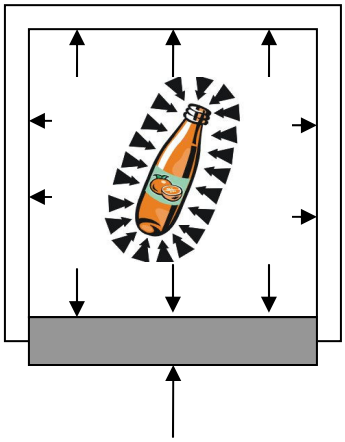

Figure 1.1 The principle of isostatic processing.

At present it is known that the high pressures only affect non-covalent chemical bonds. (i.e. ionic, hydrogen and hydrophobic bonds), leaving covalent bonds intact. This permits destruction of microbial activity without significantly affecting food molecules that contribute to the texture or flavor of the food (Fellows, 2000).

1.1.3 HHP Equipment and Operation

The main components of high pressure system are:

- a pressure vessel and its closure
- a pressure generating system
- a temperature control device
- a materials handling system

Most pressure vessels are made of a high tensile steel alloy 'monoblocks' (forged from a single piece material), which can withstand pressures of 400-600 MPa. For higher pressures, pre-stressed multilayer or wire-wound vessels are used (Mertens, 1995). Vessels are sealed by a threaded steel closure, a closure having an interrupted thread, which can be removed more quickly, or by a sealed frame that is positioned over the vessel. In operation, after all air has been removed, a pressure transmitting medium (either water or oil) is pumped from a reservoir into the pressure vessel using a pressure intensifier until the desired pressure is reached. This is termed 'indirect compression' and requires static pressure seals. Another method, termed 'direct compression' uses a piston to compress the vessel, but this requires dynamic pressure seals between the piston and internal vessel surface, which are subject to wear and are not used in commercial applications (Fellows, 2000).

Temperature control in commercial operations can be achieved by pumping a heating/cooling medium through a jacket that surrounds the pressure vessel. This is satisfactory in most applications as a constant temperature is required, but if it is necessary to regularly change the temperature, the large thermal inertia of the vessel and relatively small heat transfer area make this type of temperature control very slow to respond to changes. In such situations, an internal heat exchanger is fitted.

There are two methods of processing foods in high pressure vessels: in-container processing and bulk processing. Because foods reduce in volume at the very high pressures used in processing (for example water reduces in volume by approximately 4% at 100 MPa, 7% at 200 MPa, 11.5% at 400 MPa and 15% at 600 MPa at 22°C), there is considerable stress and distortion to the package and

the seal when in-container processing is used because the pressure is uniform. Bulk handling is similar, requiring only pumps, pipes and valves.

Pressure chambers for food processing, for cost reasons, have a practical limitation at 600 MPa, which is sufficient for most applications. For technical reasons, all available units are batch systems; however semi-continuous production can be achieved (Jongen, 2002). Semi-continuous processing is highly energy efficient although at present the capital costs of equipment remain high. It is possible that such liquid foods could also be used as the pressurizing fluid by direct pumping with high pressure pumps. Such systems would reduce the capital cost of a pressure vessel and simplify materials handling. If liquids were also rapidly decompressed through a small orifice, the high velocity and turbulent flow would increase the shearing forces on microorganisms and thus increase their rate of destruction (Earnshaw, 1992). Knorr (1995) reported that developments include combined freeze concentration, pressure freezing and high pressure blanching. Initial results suggest that pressure blanched fruits are dried more rapidly than those treated by conventional hot water blanching.

1.1.4 Uses in Food Science

1.1.4.1 Effect on Microorganisms

At moderate pressure, growth and reproduction rate of vegetative bacteria is retarded while at higher pressures, inactivation occurs. Although pressure stability is largely dependent on the type of microorganism, the species and the medium conditions, it is generally admitted that pressures between 200 and 600 MPa at room temperature are sufficient to cause a substantial reduction of viable vegetative cells. Vegetative forms such as yeasts and moulds are most pressure sensitive and inactivated by pressures between 200 and 300 MPa. Gram-negative bacteria can be inactivated by pressures about 300 MPa and are, in their turn, less pressure stable than Gram-positive bacteria, for which pressures higher than 400 MPa are required for inactivation (Tressler, 1961). However, numerous exceptions to these general statements can be found. Some very pressure-resistant strains of *E. coli* O157:H7 were found by Benito et al. (1999), for example. In addition, in

contrast to laboratory conditions, microorganisms are often more stable in actual food products. In general, the protective effect of real food products has been attributed to the presence of proteins and sugars. On the other hand, synergistic effects between pressure and acidification or addition of anti microbial substances can be exploited to lower the pressure resistance of microorganisms (Hauben et al., 1997, Garcia-Graells et al., 1998).

1.1.4.2 Effect on Proteins and Enzymes

Protein molecules can be denaturized by high pressure. It is a complex phenomenon depending on the protein structure, pressure range, temperature, pH and solvent composition (Palou, 1998). The secondary, tertiary and quaternary structures can be significantly affected by HHP since they are non-covalent bonds. So HHP can result in novel functional properties because tertiary structure is important in determining protein functionality (Tewari et al., 1999). The main targets of pressure are the electrostatic and hydrophobic bonds in protein molecules. Protein denaturation becomes irreversible beyond a given pressure threshold which depends on protein (Palou, 1998).

Some key enzymes in fruit and vegetable processing include:

- polyphenoloxidase (PPO) which is responsible for enzymatic browning
- lipoxygenase (LOX) which induces changes in flavor, color and nutritional value
- pectinmethylesterase (PME) which is responsible for cloud destabilization and consistency changes
- peroxidase (POD) which gives rise to unfavorable flavors.

PPO is not very heat resistant (Lourenço et al., 1990; Yemenicioglu et al., 1997; Weemaes et al., 1998a). Upon pressurization, in contrast, PPO may display, depending on its source, either enhancement of catalytic activity or inactivation. Pressures needed to induce substantial inactivation of PPO vary between 200 and 1000 MPa, depending on the enzyme origin and micro environmental conditions such as medium composition or pH (Weemaes, 1998).

For LOX, thermal stability at atmospheric pressure largely varies with the enzyme source and medium (Indrawati, 2000). Detailed studies of pressure inactivation have been performed for tomato, soybean, green bean and pea LOX. Threshold pressures for inactivation in a narrow range between 400 and 600 MPa have been reported (Heinisch et al., 1995; Ludikhuyze et al., 1998; Tangwongchai et al., 1999; Indrawati et al., 1999; Indrawati et al., 2000).

PME demethylates pectin resulting in low-methoxy pectin, which may then form insoluble complexes with calcium ions, leading to precipitation of the pectins and cloud loss (Goodner et al., 1998; Basak and Ramaswamy, 1996). The required heat treatment (90°C for 1 min) to inactivate the heat-stable isoenzymes may result in flavor and aroma changes that reduce the 'fresh-like' attributes of the juice and result in non-enzymatic browning (Reynolds, 1963), hence there is much interest in the use of non-thermal processing technologies for the inactivation of PME in citrus fruit juices.

PME from different fruits has been reported to be quite thermo resistant: temperatures between 80 and 95 °C are required to induce significant inactivation and even then PME remains active (Van Den Broeck, 2000). This resistance was ascribed to the presence of heat labile PME isozymes (Versteeg et al., 1980; Wicker and Temelli, 1988; Van Den Broeck et al., 2000). Pressure stability has mainly been investigated for orange PME and to a lesser degree for grapefruit, guava and tomato PME. Threshold pressures for inactivation at room temperature of PME from different sources have been reported to vary largely from 150 to 1200 MPa, depending on the origin and the medium in which the inactivation is carried out (Van Den Broeck, 2000).

POD, which is generally considered to be the most heat stable vegetable enzyme, is at least in some cases also extremely pressure resistant. In green beans, a pressure treatment of 900 MPa merely induced slight inactivation at room temperature, while in combination with elevated temperature enhanced the inactivation effect 600 MPa (Quaglia et al., 1996).

1.1.4.3 Effect on Vitamins

Ascorbic acid and carotenoids are the important indicators of nutritional quality. Ascorbate is present in most vegetables and fruits and is a powerful reducing agent which plays a key role in human nutrition, acting as an electron donor in O_2 depending reducing reactions, and promoting iron and copper in their reduced states by its antioxidant function.

HPP can be used to avoid the detrimental effects, including vitamin losses, of traditional high temperature pasteurization of many foods (Hayashi, 1995). Traditional thermal processing of orange juice causes vitamin losses, including loss of vitamin C (Farnworth et al., 2001) and changes in carotenoids (important to the color and the nutritional value of the juice). Several studies have shown that ascorbic acid and Beta-carotene to be minimally affected by HPP (Butz et al., 2002; Fernandez et al., 2001; Nienaber and Shellhammer, 2001; Sancho et al., 1999). Pressure treatments of orange juice had minimal effect on other quality parameters of orange juice, including pH and °Brix, during extended refrigerated storage (Parish, 1998a).

Bignon (1996) observed that vitamin A, C, B_1, B_2 and E content of fruit and vegetable products is not significantly affected by pressure treatment in contrast to thermal treatment. Besides, in the case of strawberries and guava puree, the decrease in vitamin C content during storage after pressure treatment (400-600 MPa, 15-30 min, 20°C) was found to be much lower compared to the fresh products (Sancho et al., 1999). A more detailed kinetic study of pressure-temperature stability of ascorbic acid in buffer, orange juice and tomato juice was performed by Van den Broeck et al. (1998). They found only significant degradation of ascorbic acid when pressures of about 850 MPa was combined with temperatures between 60 and 80°C, and more in tomato and orange juice than in buffer.

1.1.4.4 Effect on Color

For many fruit and vegetable products such as fruit jam, strawberries, tomato juice, guava puree, avocado puree and banana puree, high pressure

treatment was noted largely to preserve fresh color (Watanabe et al., 1991; Poretta et al., 1995; Donsi et al., 1996; Yen and Lin, 1996; Lopez-Malo et al., 1998). The brightness (L-color value) and redness/greenness (a-color value) of pressure-treated products were found to be superior compared with their thermally treated counterparts. However, during storage of guava and banana puree, the green color gradually decreased because of browning as a result of residual PPO activity (Lopez-Malo, 1998; Palou et al., 1999). The longest acceptability storage time was achieved by using high pressure, low pH and refrigerated storage.

1.1.4.5 Effect on Antioxidants

A great number of antioxidants are naturally present in orange juice, being responsible for the potential protective action of orange juice against certain degenerative diseases. According to recent epidemiological studies, high consumption of orange juice is associated with a reduced risk for free radical related oxidative damage and diseases such as different types of cancer, cardiovascular or neurological diseases (Diplock, 1994; Hollman et al., 1996; Vinson et al., 2002).

Recent studies showed that HHP treated juices has better antioxidant retention compared to thermally processed ones (Polydera et al., 2004, Scalzo et al. 2004). Different constituents contributed to the total antioxidant activity of orange juice. L-Ascorbic acid constituted the most important antioxidant compound reacting instantaneously with free radicals, while the reaction of other antioxidants of orange juice (mainly flavonoids and other polyphenolic compounds) was time dependent, being described by the sum of two exponential decay functions of different rate. A high pressure treatment of 600 MPa at 40°C for 4 min led to a better retention of antioxidant activity during post processing storage of orange juice at 0–30°C compared to conventional thermal pasteurization (80°C, 60 s), mainly due to lower ascorbic acid degradation rates (Polydera et al. 2004). It was evident that the thermal treatments induced a decrease in the free radical scavenging activity and were contemporarily responsible for the degradation of ascorbic acid in blood orange juice (Scalzo et al. 2004). Sanchez-Moreno et al. (2003) and de Ancos et al. (2002) also reported that HHP treatments of 50-250

MPa, 30-60°C, 15-30 minutes did not affect the antiradical scavenging of orange juices significantly.

Even if we assume that a case-by-case evaluation remains indispensable, a general approach is nevertheless attractive, and should be intrinsically relevant.

1.2 History of Fruit and Vegetable Processing

1.2.1 Microbiology of Fruit and Vegetable Juices

Microbial spoilage of juice products may lead to off flavors, odors, turbidity and gas production (Jay & Anderson, 2001). A limited range of yeasts, moulds and aciduric bacteria are capable of growth at the low pH of orange juice, typically pH 3.3–4.0 (Bracket, 1997). To extend shelf life, mild heat treatments (60–65°C) are required to destroy yeasts and most fungal spores, while higher temperatures (89–95°C) are required for inactivation of lactic acid bacteria. However, *Alicyclobacillus* spp. and the ascospores of some heat resistant moulds may still not be inactivated at these higher temperatures (Hocking & Jensen, 2001). Historically, acid foods such as fruit juices have been considered safe, however, recent foodborne disease outbreaks attributed to unpasteurised juices contaminated with pathogens. Such as *Salmonella* spp. and *Escherichia coli* O157:H7 have demonstrated that unpasteurised juice can be a vehicle for food-borne illness (Australian Department of Health and Ageing, 1999; Cook et al., 1998; Centers for Disease Control and Prevention, 1996). While these and other acid-tolerant pathogens may not be able to grow in the juice, their low infectious dose, especially for sensitive consumers, highlights the need to include pathogen control measures during juice manufacture.

There are three types of spoilage: a material may go moldy; or it may be fermented with production of carbon dioxide and alcohol; or it may be acetified as in production of vinegar. These changes are usually due to three different kinds of microorganisms; mold fungi, yeasts and bacteria. To produce a juice with no danger of spoilage a process of 5.5 log microbial reduction is needed (Tressler, 1961; Tressler, 1971; Cemeroğlu, 2001).

Bacteria are frequently found in fruit and vegetable juices. The so-called lactic acid, acetic acid and butyric acid bacteria deserve special consideration for spoilage and also some other bacteria because they are related to food poisoning. Lactic acid bacteria are the most important bacteria, because they cause frequent spoilage in the juices many fruit and vegetable juices. Strains of *Lactobacillus* and *Leuconostoc* occur regularly on the fruits and vegetables. They can multiply vigorously when air is absent. They can grow in vacuum equipment or in juice stored with CO_2 under pressure. They are fairly acid-tolerant. Therefore lactic acid bacteria are a danger for acid-poor juices.

Acetic acid bacteria, like lactic acid bacteria, are common on fruit and vegetable. They are much less common agents of spoilage but they are very tolerant to high acidity. Butyric acid bacteria often develop butyric acid that has a particularly objectionable smell. Yeasts carry out alcoholic fermentation, producing ethanol and carbon dioxide from sugars. Most yeasts produce organic acids, especially acetic.

A wide variety of mold fungi take part in the rotting of fruit and vegetables, and are therefore likely to appear in fruit and vegetable juices. Fungi growth leads to defects in the juice like; off-flavors and sometimes difficulty in fermentation. They attack pectin and cause clarification of the juice. They change acid composition of the juice leading to more flavor defects.

1.2.2 Fruit and Vegetable Processing

1.2.2.1 Orange Juice Processing

Orange juice is the predominant juice manufactured by the beverage processing industry worldwide with a share of approximately 50% of the total fruit juice trade (UN Food and Agriculture Organization, 1991). Concentrated orange juice provides an easily stored and transported product accepted by many customers. However, recent emphasis by consumers on fresh, natural and

unmodified foods has seen the demand for fresh orange juice increase (Tillotson, 2000).

Upon reaching the processing plant the fruit goes through inspection lines, where bruised or broken fruit are removed, and then is conveyed to washer. The fruit is soaked briefly in water containing a detergent, scrubbed by revolving brushes and rinsed with clean water. The fruit is inspected again to remove damaged fruit missed before or subsequently bruised or broken. The fruit is then separated into sizes automatically and enters the juice extractors.

By using in-line juice extractors, fruit is processed into juice. From the extractor the juice passes to a finisher where excess pulp is removed. After finishing, the juice flows to large stainless steel tanks where it is checked for acidity and soluble solids. Sugar is added, if needed.

Pasteurization of citrus juices accomplishes two things. First it destroys microorganisms which would otherwise cause fermentation in the can and second, it inactivates enzymes which would otherwise cause cloud loss and other changes in the juice. Generally, higher temperatures are needed for enzyme inactivation than destruction of microorganisms. So a treatment of 80°C for 1 minute is used for PME inactivation (Versteeg et al., 1980).

Deoilers were developed in order to be able to control the peel oil level in canned citrus juices. Deoilers are essentially small vacuum evaporators in which the juice is heated to about 52°C and from 3 to 6% of the juice evaporated.

Oxidation has long been considered as a mechanism of flavor deterioration in citrus juices and the tendency has been to recommend that the oxygen level be kept low. So deaerators are widely used to control oxygen in juice production.

Juice can be filled in bottles or cans while hot. Filling time should be short to minimize flavor loss (Fig. B.1).

1.2.2.2 Tomato Juice Processing

Because of the nature of mechanical harvesting and bulk handling of tomatoes, more care must be exercised to prevent bacterial buildups. The first soaking wash should contain up to 200 ppm of available chlorine and subsequent flood-washing should maintain a minimum of 5 ppm of residual chlorine. The tomatoes receive some field-sorting on the harvester or in a central sorting operation where loads of tomatoes can be graded by washing, sorting and inspection.

There are many combinations of crushing, chopping or even slicing which can be combined with different heat treatment for enzyme inactivation. When crushing the vegetable a treatment of 80°C for 15 seconds is used for PME inactivation.

Juice extractors are used in the tomato industry. Since, heating tomato juice containing dissolved or occluded air impairs the retention of vitamin C, some producers employ deaerators in which the product is vacuum-deaerated.

The juice can be salted, just for taste concerns, in batches at this stage or added to the individual cans by means of dry salt or salt tablet dispensers. The sodium chloride is added to tomato juice from 0.5% to 1.25% by weight.

In order to retard or prevent settling and separation as much as possible, tomato juice sometimes homogenized or viscolized in machines of the type used for milk and other dairy products.

Although tomato juice is an acid product, it has been subject to frequent outbreaks of spoilage by *B. coagulans*. A treatment of F_0=0.7 should be used to sterilize the juice (Fig. B.2).

1.2.2.3 Carrot Juice Processing

A good carrot juice may be made by pressing in hydraulic press carrots which have been blanched 15 minutes in boiling water. The carrots are washed, water blanched at 90°C for five minutes, trimmed and then ground with an ordinary stainless steel hammer mill. The extracted juice is acidified with citric acid and processed. Acidification is needed to lower the pH of the juice. And the product has a pH of 5.5-6.5 and food products at this pH range should be sterilized (Fig. B.3).

1.3 Objectives of the Study

Since HHP offers an alternative way to preserve food, its effects on orange, tomato and carrot juices were studied to compare the quality parameters with heat pasteurization application. The treatments employed in this study were chosen according to previously reported studies on enzyme inactivation, microbial inactivation and applicability by the industry considering economical dimension.

The main objective of this study was to investigate the effects of HHP on quality parameters and shelf life of orange, tomato and carrot juices in comparison to heat pasteurization. The parameters studied were total aerobic bacteria, antiradical scavenging capacity, ascorbic acid content, color and pH. Shelf life was studied at 4 and 25^{o}C for a period of 30 days.

Another objective was to propose a HHP treatment condition to help the industry to supply healthier, fresh-like, reputable juice products in terms of the parameters studied.

CHAPTER 2

MATERIALS AND METHODS

2.1 Samples

Oranges, tomatoes and carrots were purchased from a local market (Ankara, Turkey). They were all harvested in mid-season in Finike (Antalya, Turkey). Oranges, tomatoes and carrots were freshly processed right after purchase.

2.2 Sample Preparation and Processing

Oranges, tomatoes and carrots were juiced using a home type fruit processor (Moulinex, Spain). All equipment used was sanitized prior to usage with %60 ethanol (Merck, Germany) followed by a sterile water rinse. Samples were filtered using sterile cheese cloth. After the treatments, samples were held in ice and all the measurements were done within 1h.

2.3 Treatments

2.3.1 HHP Application

HHP treatments were performed in a designed and constructed lab-scale unit (capacity: 30 cm^3, maximum P: 350 MPa) (Fig. 2.1). The rate of pressure increase and pressure release was approximately 5-10 seconds for the designed system, respectively. Water was used as the pressure transmitting medium. The equipment consists of a pressure chamber of cylindrical design, two end closures, a

means for restraining the end closures, a pressure pump, and a hydraulic unit to generate high pressure for system compression and also a temperature control device. The pressure vessel was made of hot galvanized carbon steel and piston was hard chrome plated and polished to mirror finish (steel type heat treated special K) which was processed into the required sizes at Electrical and Electronic Engineering Department of Middle East Technical University, Ankara, Turkey. The liquid was heated prior to pressurization to the desired temperature by an electrical heating system surrounding the chamber. Pressurization time reported in this study did not include the pressure increase and release times.

Figure 2.1 HHP unit

Samples were pressurized at 150, 200 and 250 MPa at 25 and 35°C for 5, 10 and 15 minutes. The treatments employed in this study were chosen according to previously reported studies on enzyme inactivation, microbial inactivation and applicability by the industry considering economical dimension. Freshly squeezed fruit juices were dispensed in 2 mL portions in sterile cryovials (Simport Plastic,

Canada), avoiding as much air as possible and placed inside the pressurization chamber. The chamber was fully filled with water and kept for 1-2 minutes for temperature equilibration before pressurization; this temperature and time relation for equilibration had been determined earlier. Immediately after pressurization, the vials were removed and cooled in an ice bath. Unpressurized samples were used as controls. Experiments and measurements were duplicated on separate days.

2.3.2 Heat Treatment

Samples were heat treated in a water bath at 60°C for 5, 10 and 15 minutes (representative of the mildest pasteurization treatment used by industry) and at 80°C for 1 minute (industrial pasteurization application) (Scalzo et al., 2004). 60°C treatments were applied for better monitoring the effect of heat treatment on parameters studied.

Two glass tubes containing the same amount (2 mL) of sample were put in the water bath that was set to the desired temperature. When the samples reach to the desired temperature, one of the tubes was taken out from the water bath and immediately cooled in an ice bath. Other tube with the sample was kept at the desired temperature for the reported process time. By doing this, come-up effect is determined and subtracted from the total effect. All experiments and measurements were replicated twice on separate days.

After HHP or heat treatment, samples were analyzed for microbiological and chemical analysis within 1h. The experimental layout is summarized in Table 2.1.

For self life analysis, duplicate samples were pressurized at 250 MPa at 35°C for 15 minutes and also heat treated at 80°C for 1 minute (Table 2.2). Pressure and heat treated samples were stored at 4 and 25°C in the dark for one month. The samples were taken at 2-day intervals. New cryovials were opened each time. Untreated samples were used as controls.

Table 2.1 The treatment conditions and parameters studied for orange, tomato and carrot juices for HHP and heat treatment

	HHP Treatment				Heat Treatment	
	25°C			35°C	60°C	80°C
Parameters Studied	**150 MPa 5-10-15 min**	**200 MPa 5-10-15 min**	**250 MPa 5-10-15 min**	**250 MPa 5-10-15 min**	**5-10-15 min**	**1 min**
Total Aerobic Count	+	+	+	+	+	+
Antioxidant Activity			+	+	+	+
Ascorbic Acid			+	+	+	+

Table 2.2 The treatment conditions and parameters studied for orange, tomato and carrot juices for shelf life analysis

	HHP Treatment 35°C 250 MPa	Heat Treatment 80°C
Parameters Studied	**15 min**	**1 min**
Total Aerobic Count	+	+
Antioxidant Activity	+	+
Ascorbic Acid	+	+

2.4 Microbiological Analysis

Serial dilutions of HHP and heat treated juices were performed in 0.1% peptone (Merck, Germany) water. Total aerobic bacterial count was performed by the spread plate technique on tryptic soy agar (TSA) (Merck, Germany). Duplicate agar plates were used for each sample and incubated at 37°C ± 1°C for 48 hours. Plates containing 25-250 cfu/mL were selected for counting.

2 separate cryovials filled with same juice were pressurized together. Dilutions were taken twice from each cryovial and 2 sterile Petri plates were prepared by spread plate method from each dilution. This way 8 parallel set-up is achieved (Fig 2.2).

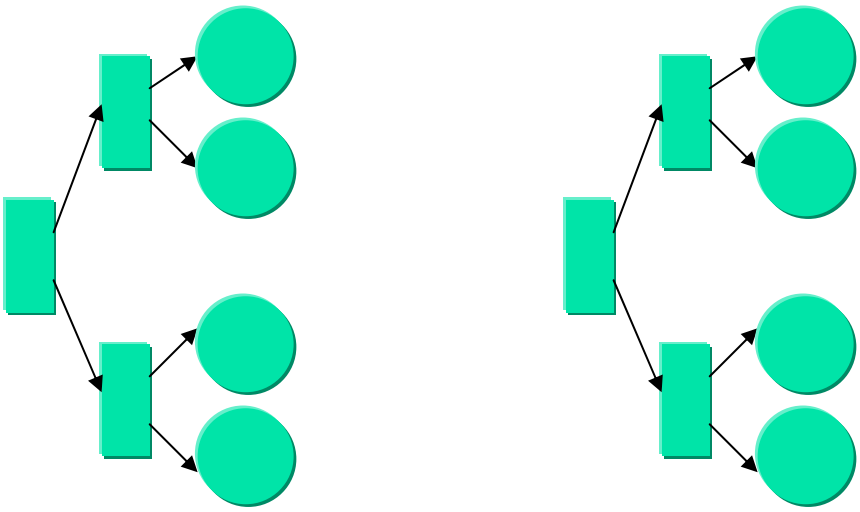

Figure 2.2 2 cryovials x 2 petri dishes x 2 replica = 8 parallel set-up

2.5 Physical and Chemical Analysis

2.5.1 Antioxidant Scavenging Activity

Antioxidant scavenging activity was evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) (SIGMA, Germany) radical. The model of scavenging DPPH$^{\bullet}$ in a homogenous organic phase is a simple method to evaluate the activity of antioxidants. Antioxidants serve hydrogen to free radicals and scavenge radicals (Rapisarda et al. 1999, Wang et al. 1999).

Scavenging activity of juices was measured by DPPH$^{\bullet}$ radical quenching (Brand-Williams et al. 1995). 1 mL of juice sample was added to 4 mL of methanol (Riedel-de Haën, Germany) and homogenized in a refrigerated centrifuge (Sorvall RC5C, Du Pont, Wilmington, USA) at 20.000 rpm for 5 minutes. 0.1 mL of this product is added to 2 mL of methanolic solution containing DPPH$^{\bullet}$ (0,025g DPPH$^{\bullet}$ / L methanol) in a spectrophotometer cuvette. The reaction mixture was shaken and left to stand for 15 minutes at room temperature in dark. The absorbance values were measured by spectrophotometer (Pharmacia LKB, Novaspec II, England) at 517 nm against a blank of methanol without DPPH$^{\bullet}$.

The DPPH• concentration in the reaction medium was calculated from the following calibration curve (Fig. 2.3), determined by linear regression:

$$Abs_{517nm} = (30.59 \times DPPH) - 0.0386$$

Where Abs_{517nm} is the absorbance read at 517 nm and DPPH• is the amount of free radical the solution contains.

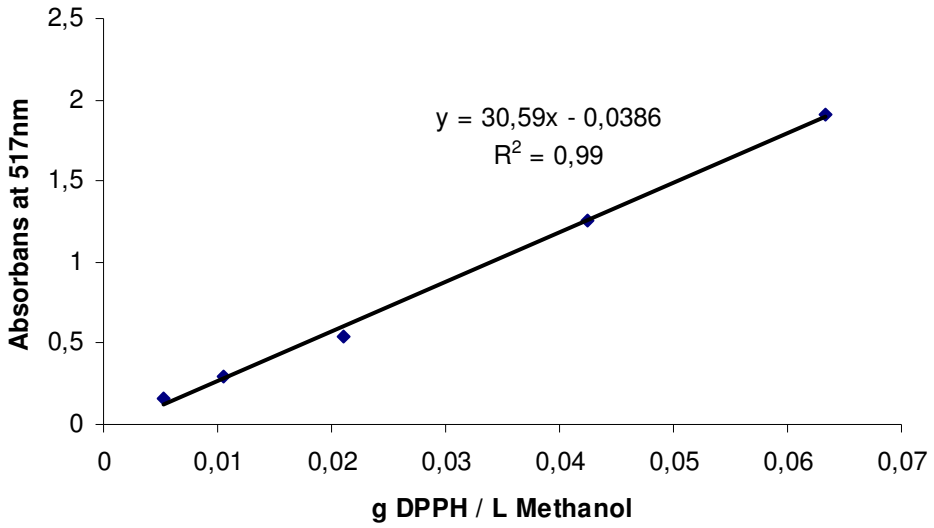

Figure 2.3 Standard curve for DPPH• in methanol

Data was evaluated with the change in DPPH• concentration. Results are given using the formula below.

$$A = (DPPH_{t=0\,\mathrm{min}}) - (DPPH_{t=15\,\mathrm{min}})$$

Where A is the amount of antioxidant, $DPPH^{\bullet}_{t=0min}$ is the amount of DPPH• at the beginning of the reaction and $DPPH^{\bullet}_{t=15min}$ is the DPPH• amount 15 min after the reaction starts.

For the shelf life analysis, antioxidant activity is reported as % of the initial antioxidant amount where marked as 100%, using the following formula:

$$A_R\,\% = \frac{A_{t=anytime}}{A_{t=0}} \times 100$$

Where A_R% is the remaining antioxidant percent, $A_{t=anytime}$ is the amount of antioxidant during the shelf life period and $A_{t=0}$ is the amount of antioxidant at the beginning of shelf life.

2.5.2 Ascorbic Acid

Ascorbic acid content of the samples were analyzed according to the 2,6-dichlorophenolindophenol titrimetric method (AOAC Official Method 967.21). The basic principle is ascorbic acid (Riedel-de Haën, 33034, Germany) reduces oxidation-reduction indicator dye, 2,6-dichlorophenolindophenol (Merck, Germany), to a colorless solution. At the end point, excess unreduced dye is rose pink in acid solution. Ascorbic acid is extracted and the titration is performed in the presence of acetic acid (Carlo Erba Reagenti, Italy) and meta-phosphoric acid (Riedel-de Haën, Germany) solution in order to maintain proper acidity for the reaction and to avoid auto-oxidation of ascorbic acid at high pH.

2.5.3 pH

pH of the samples were determined by pH-meter (WTW 537 pH-meter) at 20°C.

2.5.4 Color Measurement

Color of the samples was analyzed by Avantes spectrophotometer (Avantes, Avaspec-2048, The Netherlands) with a light source set on D65. L, a and b values were measured and ΔE is calculated using the formula below (Billmeyer and

Saltzman, 1981). Where L_0, a_0 and b_0 values are the color values for standard white solution (0.05 g TiO_2/ 100 mL water).

$$\Delta E = \left[(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2 \right]^{1/2}$$

2.5.5 Analysis of Results

The results of HHP and heat treatment were submitted to one-way Analysis of Variance (ANOVA). For the shelf-life analysis a three-way ANOVA was used with treatments, storage time and temperature as factors. Significant differences between means were tested using Duncan's multiple range test with a probability level fixed at $p<0.05$. Differences at $p<0.05$ were considered to be significant. Statistical treatments were carried out with SPSS 12.0 for Windows.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Effects of Treatments

3.1.1 Effects on Total Aerobic Bacteria

Log_{10} reduction values calculated from total aerobic counts (Fig. 3.1, 3.2 and 3.3) determines the microbial quality of orange, tomato and carrot juices after high pressure processing, heat treatment or no treatment. The initial microbial load of orange, tomato and carrot juices were 3.5, 4.5 and 5.5 log_{10}cfu/mL respectively. Pressure treatment of 250 MPa, 35°C, 15 min and heat treatment at 80°C, 1 min were enough to reduce the population levels below the detection limit.

In case of orange juice, 60°C, 10 and 15 min and 80 °C, 1 min treatments were statistically insignificant ($p>0.05$). Likewise 60°C, 15 min and 80°C, 1 min treatments were statistically insignificant ($p>0.05$) for tomato juice.

Increasing treatment intensities displayed an increasing effect of reduction of microbial load on all of the juices. In case of pressurization, increasing the pressure, temperature and time variables and incase of heat treatment, increasing the temperature and time variables, individually increased the microbial reduction for all three juices significantly ($p<0.05$). The time of either pressurization or heat treatment at 60^{o}C was the least effective among the parameters. In general pressure increase was more effective on reduction of aerobic bacteria than other variables.

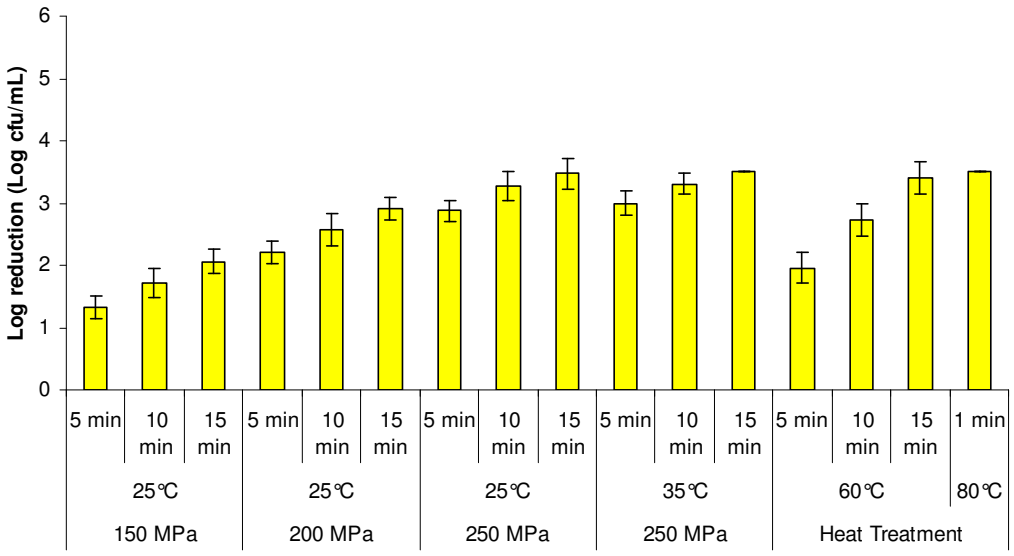

Figure 3.1 Mean total aerobic bacteria reduction of heat and high pressure treated orange juice (3.5 log mean initial microbial load). The error bars denote the standard deviation.

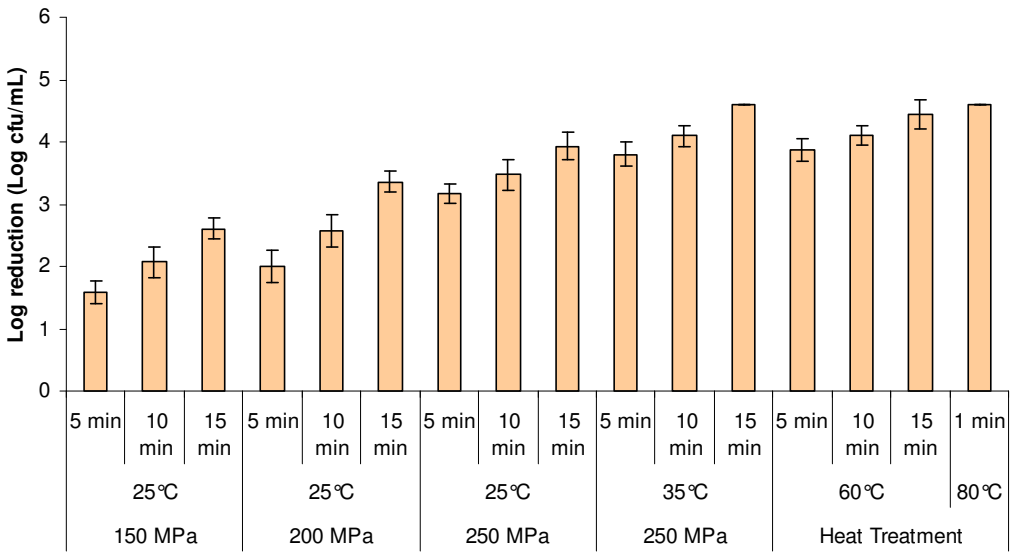

Figure 3.2 Mean total aerobic bacteria reduction of heat and high pressure treated tomato juice (4.5 log mean initial microbial load). The error bars denote the standard deviation.

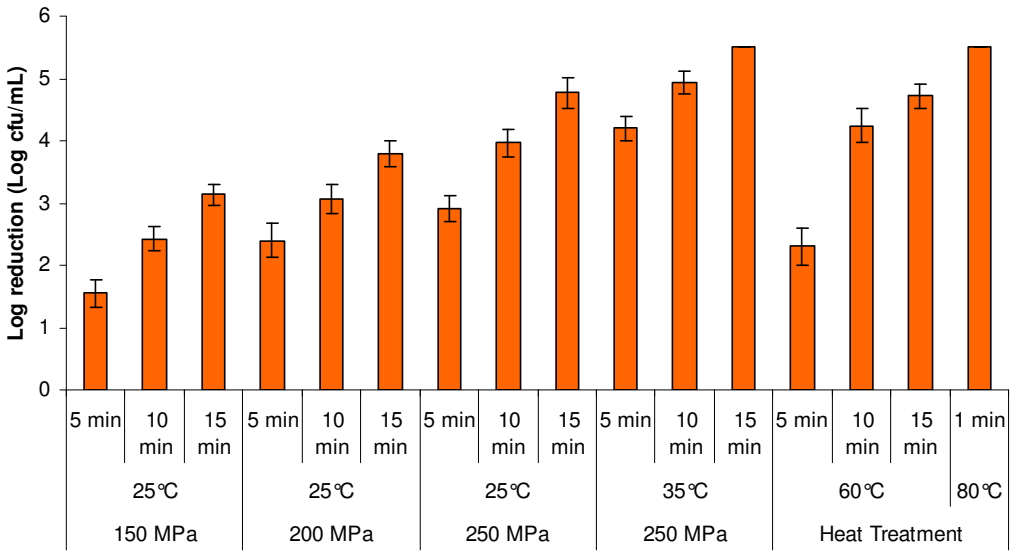

Figure 3.3 Mean total aerobic bacteria reduction of heat and high pressure treated carrot juice (5.5 log mean initial microbial load). The error bars denote the standard deviation.

Orange juice has a lower pH (3.5) than tomato (4.5) and carrot (6.0) juices. Low pH juices have a lower initial microbial population and results in higher stress condition under pressure than a juice with a higher pH. This effect was analyzed with yeast and molds by Bull et al. 2004 and more pressure and acidity resistant microorganisms like *Alicyclobacillus acidoterrestris* (Alpas et al. 2003). HHP inactivation of bacteria in fruit juices have been reported to be affected by the pH of the juice, decreasing acidity resulted with more inactivation of microorganisms (Linton et al. 1999).

3.1.2 Effects on Ascorbic Acid

The concentration of vitamin C is the most important indicator of nutritional quality in fruit juices, especially in orange juice. Figures 3.4, 3.5 and 3.6 shows that the change in ascorbic acid content of HHP treated juices was not statistically significant ($p>0.05$). On the contrary, both heat treatments at 60 and 80°C, displayed a significant loss of vitamin C. Polydera et al. (2003) reported that

ascorbic acid loss of high pressurized orange juice had higher temperature dependence than thermally treated one and immediately after processing, high pressurized orange juice also better retained the flavor of untreated reconstituted juice.

As opposed to HHP treatment, the heat treatment at 60°C reduced the ascorbic acid content of all juices significantly ($p<0.05$), except carrot juice treated for 5 min. High temperature short time heat treatment (80°C, 1 min) displayed a better retention of vitamin C for all juices, insignificant ($p>0.05$) to no treatment and pressurization for orange and carrot juices.

Since ascorbic acid is constructed of covalent bonds, it is not a surprising result that HHP application has no effect on ascorbic acid. Sancho et al. (1999) reported that minor variations were found among the vitamins (B_1, B_6 and C) after pressurization (200, 400, 600 MPa for 30 minutes at 20°C), where ascorbic acid was not affected by the intensity of high hydrostatic pressure applied.

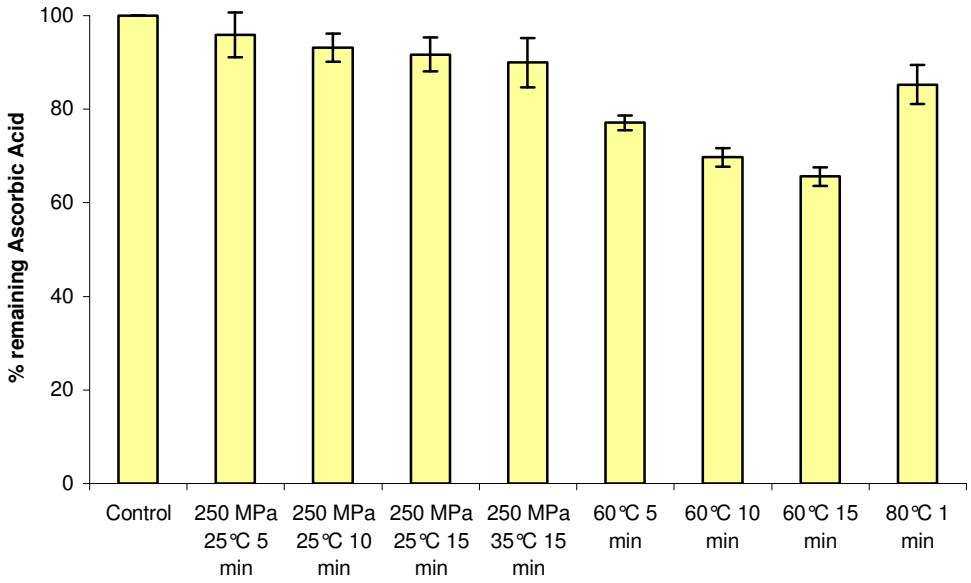

Figure 3.4 Variation of ascorbic acid in orange juice with heat and pressure. The error bars denote the standard deviation.

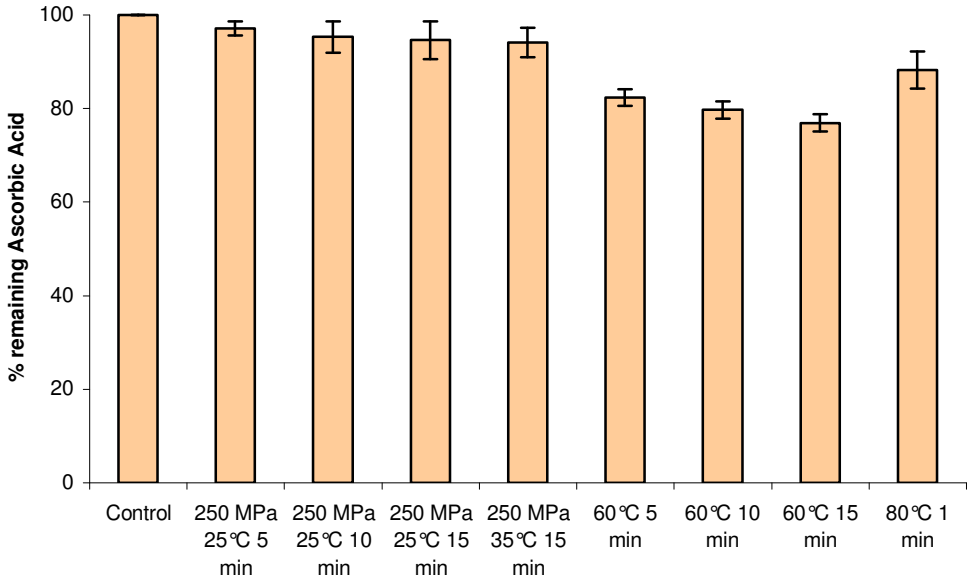

Figure 3.5 Variation of ascorbic acid in tomato juice with heat and pressure. The error bars denote the standard deviation.

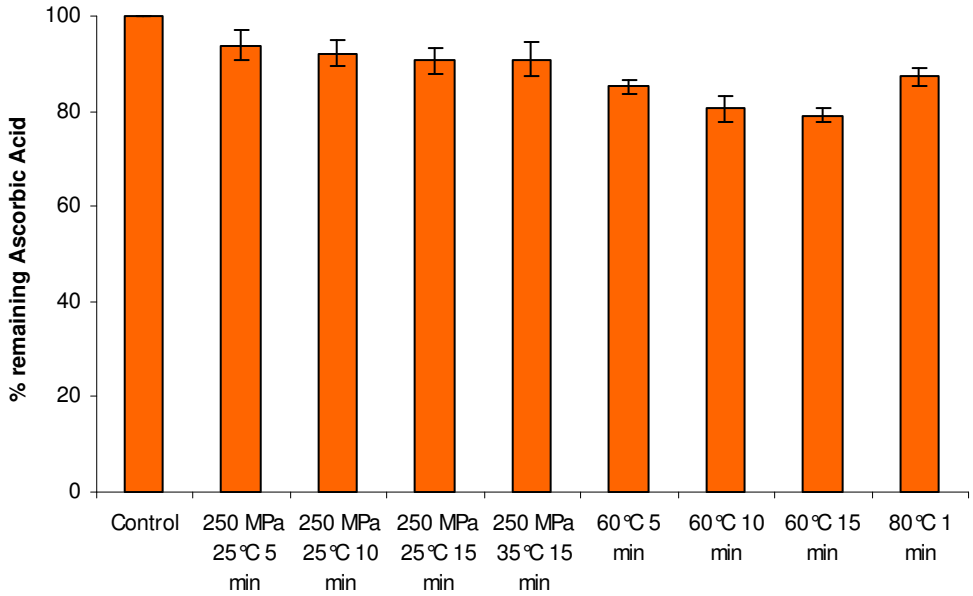

Figure 3.6 Variation of ascorbic acid in carrot juice with heat and pressure. The error bars denote the standard deviation.

3.1.3 Effects on Antioxidant Activity

The antioxidant activity, measured right after treatment, was not affected by HHP treatments (Fig. 3.7, 3.8 and 3.9). However, for heat treatments the activity decreases as the treatment gets harsher (longer process time, higher process temperature). In a study conducted by Scalso et al. (2004) with blood orange juice, samples were treated with blanching (80°C for 6 minutes), pasteurization (80°C for 1 minute) and blanch-pasteurization (subjected to blanching and then pasteurization) and compared to the non-treated juice samples for radical scavenging activity. It is reported that the thermal treatments induced a decrease in the free radical scavenging activity.

For carrot and tomato juices, it is seen that a significant difference between 250 MPa, 35°C, 15 min pressure treatment and 80°C, 1 min heat treatment does not exist ($p<0.05$). This is not a surprising result for pressure application; likewise high temperature short time heat treatment did not affect those compounds responsible for antiradical scavenging.

It was expected to see the increase of antioxidants which would result in over 100% remaining antioxidant amount in processed juices, by the positive effect of heat on phenolic substances (anthocyanins and hydroxycinnamates). However, Scalso et al. (2004) also reported that the thermally-induced antioxidant and free radical scavenging activities of blood orange juices showed opposite trends in relation to the assay. In particular, the inhibition of enzymatically-mediated linoleic acid peroxidation was increased by thermal treatments, while the scavenging effect toward $OH^{\bullet}$ and $DPPH^{\bullet}$, decreased.

The first point is sustained by the amounts of some phenolic substances with antioxidant action (anthocyanins and hydroxycinnamates). The increase of these components in thermally treated substrates is known in some vegetables such as carrot, rosemary and sage. This phenomenon is probably caused by two concomitant events; the thermally induced extraction of antioxidant molecules previously complexed or polymerized and the retention of active principles caused by the inactivation of the enzymes involved in their catabolism (Gazzani et al. 1998, Pizzocaro 1989, Pizzocaro et al. 1991, Pizzocaro et al. 1995, Pizzocaro et al. 1997).

As for the second point, it was evident that the thermal treatments induced a decrease in the free radical scavenging activity and were contemporarily responsible for the degradation of ascorbic acid in blood orange juice (Scalso et al. 2004). It is clear that the phenolic compounds (anthocyanins and hydroxycinnamates) were able to protect against the oxidation of lipophilic substances and ascorbic acid, a water-soluble component, acts as an $OH^{\bullet}$ or $DPPH^{\bullet}$ scavenger.

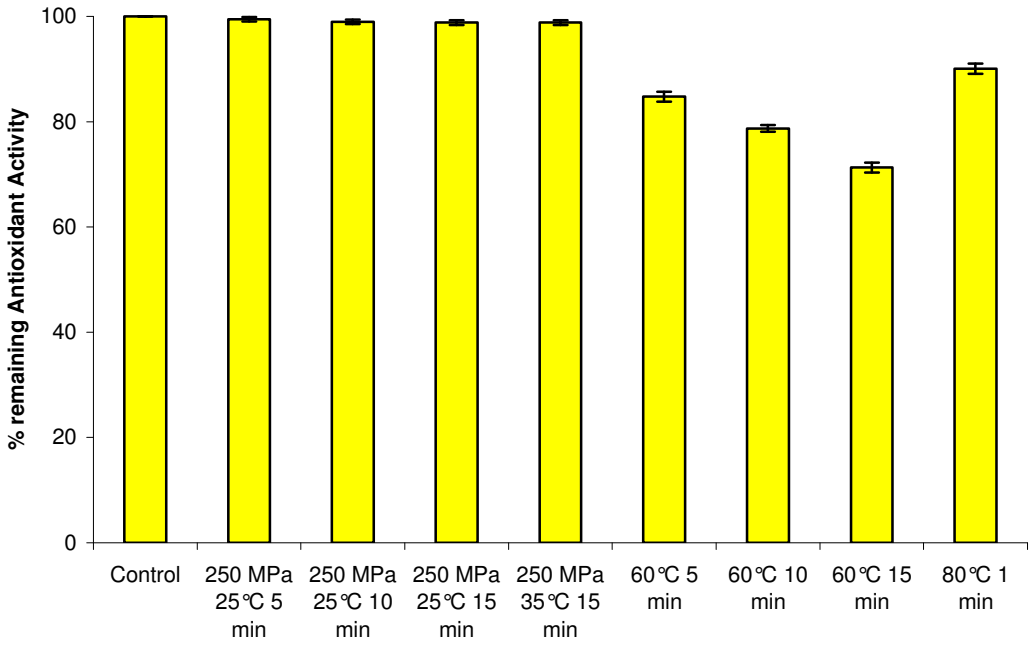

Figure 3.7 Variation of anti-oxidant activity in orange juice with heat and pressure. The error bars denote the standard deviation.

3.1.4 Summary of Treatment Effects

Table 3.1 summarizes the best treatment conditions for the studied parameters for orange, tomato and carrot juices for either pressure or heat treatments.

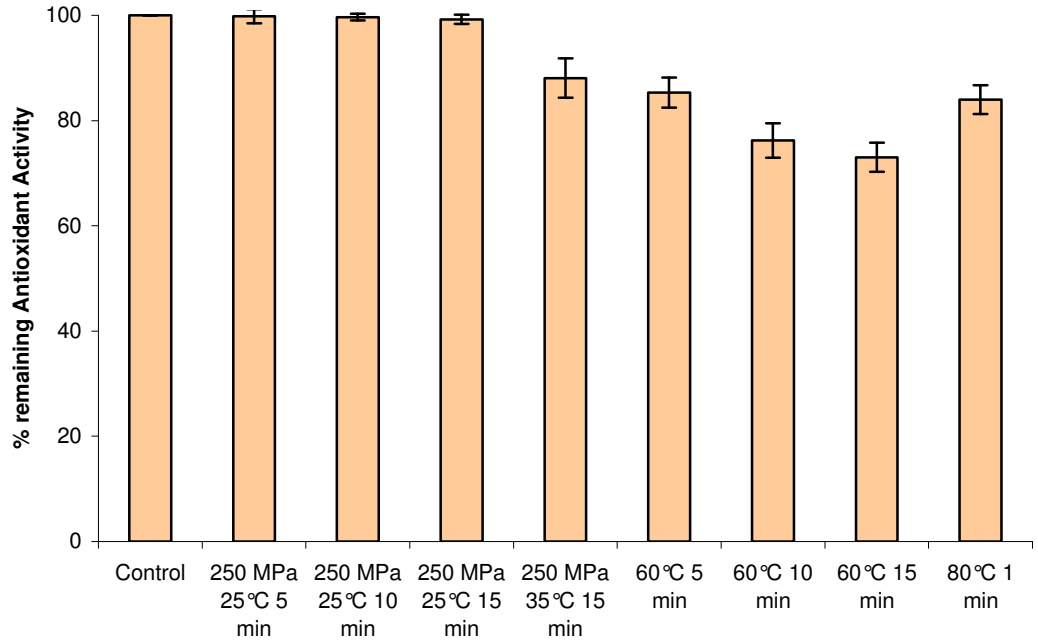

Figure 3.8 Variation of anti-oxidant activity in tomato juice with heat and pressure. The error bars denote the standard deviation.

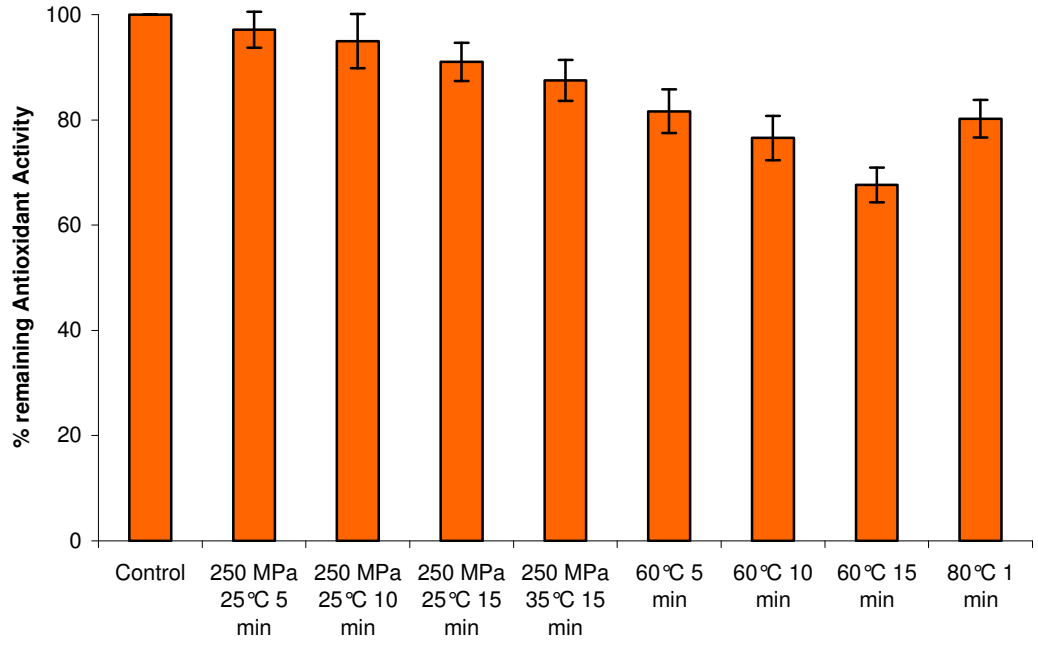

Figure 3.9 Variation of anti-oxidant activity in carrot juice with heat and pressure. The error bars denote the standard deviation.

For pressurization, 250 MPa, 35°C, 15 min and for heat treatment 80°C, 1 min combinations were the best in case of microbial inactivation, for all three juices.

Pressure application of 250 MPa, 25°C, 5 min retained vitamin C better with a value of 93% after treatment. And antioxidant amount was higher than other HHP combinations. Likewise, heat application of 80°C, 1 min did have a better vitamin C value than treatments done at 60°C. But for antioxidant amount, 80°C, 1 min treatment was better for orange juice. However, 60°C, 5 min gave a better result for tomato and carrot juices. This may be explained by the high amount of vitamin C in orange juice.

Table 3.1 The best parameter values after treatment for three juices.

		Control	Pressure	Heat
Orange Juice	**T.A.C (log cfu/mL)**	3.5	ND* (250MPa/35℃/15min)	ND (80℃/1min)
	Antioxidant (%)	100	99,46 (250MPa/25℃/5min)	90.05 (80℃/1min)
	Ascorbic Acid (%)	100	95,89 (250MPa/25℃/5min)	85.29 (80℃/1min)
Tomato Juice	**T.A.C (log cfu/mL)**	4.65	ND (250MPa/35℃/15min)	ND (80℃/1min)
	Antioxidant (%)	100	99,83 (250MPa/25℃/5min)	85.33 (60℃/5min)
	Ascorbic Acid (%)	100	97,14 (250MPa/25℃/5min)	88.24 (80℃/1min)
Carrot Juice	**T.A.C (log cfu/mL)**	5.5	ND (250MPa/35℃/15min)	ND (80℃/1min)
	Antioxidant (%)	100	97,12 (250MPa/25℃/5min)	81.63 (60℃/5min)
	Ascorbic Acid (%)	100	93,88 (250MPa/25℃/5min)	87.27 (80℃/1min)

* ND not detected

Total microbial inactivation was achieved by HHP (250 MPa, 35°C, 15 min) and thermal treatments (80°C, 1 min) both. On the overall, HHP applications gave better antioxidant and ascorbic acid values than heat treatments studied.

Pressurization application of 250 MPa, 35°C, 15 minutes can be adopted by industry for its high level of microbial inactivation capacity. Among thermal treatment applications, 80°C, 1 minute treatment makes sense, where highest

microbial inactivation is achieved and has suitable ascorbic acid retention (and antioxidant retention for orange juice) levels. However, HHP treated products are expected to be more acceptable due to higher nutritional, physical and health promoting values.

3.2 Shelf Life

3.2.1 Total Aerobic Bacteria During Shelf Life

After HHP (250 MPa, 35°C, 15 min) or heat (80°C, 1 min) treatments, total aerobic microorganisms were reduced to non-detectable levels in orange, tomato and carrot juices. Mean initial populations were 3.00, 4.50, and 5.50 $\log_{10}$ cfu/mL, respectively. All juices were microbiologically stable (microbial counts were below the detection level of 25 cfu/mL) through the shelf life experiments (30 days) (Table 3.2).

3.2.2 Antioxidant Amount During Shelf Life

HHP treated juices showed a small (20%) loss of antioxidants at both storage temperatures (4°C and 25°C) whereas the loss is higher (80%) in the heat treated juices through shelf life (Fig. 3.10, 3.11). Ancos et al (2002) reported that after 30 days of storage at 4°C the untreated orange juice showed a significant decrease of 18% in free radical-scavenging capacity and orange juices treated at 350 MPa, 30°C showed approximately 20% inhibition at different time combinations.

Table 3.2 Total aerobic counts (log cfu/ml) during 30 day storage shelf life (days).

80°C/1min	control	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
Orange Juice (4°C)	3.00	ND*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Orange Juice (25°C)	3.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tomato Juice (4°C)	4.50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tomato Juice (25°C)	4.50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Carrot Juice (4°C)	5.50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Carrot Juice (25°C)	5.50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

250MPa/35°C/15min	control	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
Orange Juice (4°C)	3.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Orange Juice (25°C)	3.00	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tomato Juice (4°C)	4.50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tomato Juice (25°C)	4.50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Carrot Juice (4°C)	5.50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Carrot Juice (25°C)	5.50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

* ND: not detected

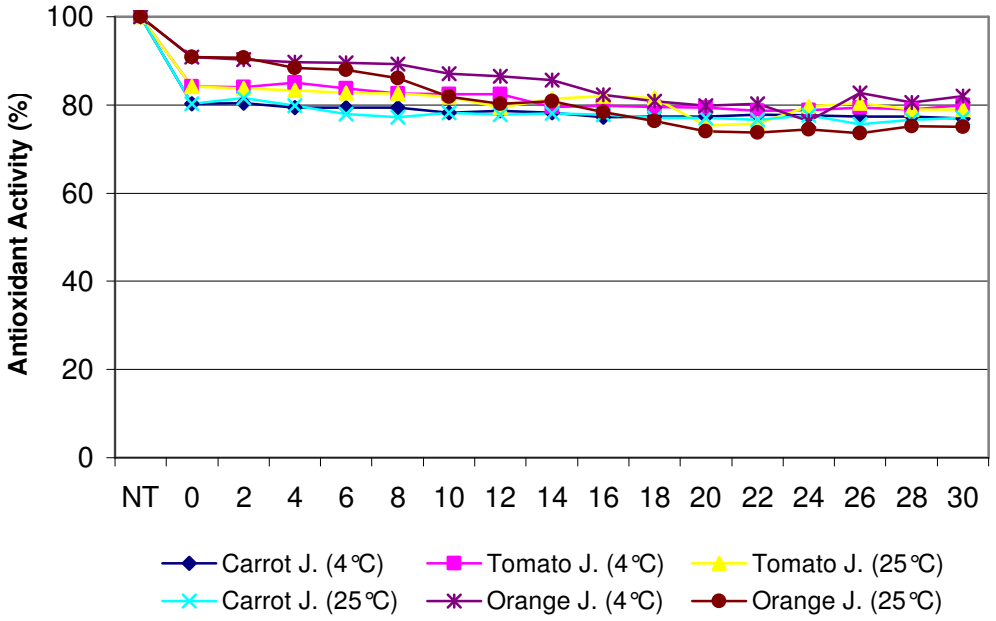

Figure 3.10 Antioxidant amount through shelf life for HHP treated (250 MPa, 35°C, 15 min) juices.

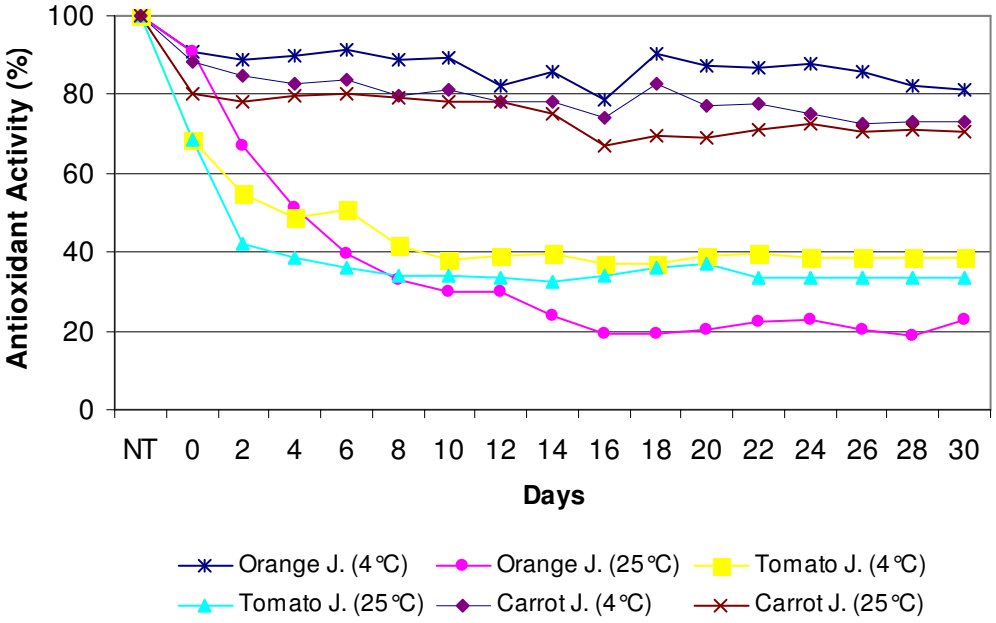

Figure 3.11 Antioxidant amount through shelf life for heat treated (80°C, 1 min) juices.

Increasing the storage temperature resulted in a small decrease in total antioxidant activity of pressure treated juices. However, for heat treated juices the effect was considerably high. Compared to conventional pasteurization, high pressure treatment led to higher total antioxidant activity immediately after processing (time 0 of storage) as well as during storage at both 4 and 25°C.

Similar results were also reported by Polydera et al (2005) in a kinetic study of orange juice, where the antioxidant activity was described mathematically as a function of storage time and temperature conditions.

Sanchez-Moreno et al (2003) also reported that high pressure treatments (100 MPa, 60°C, 5 min; 350 MPa, 30°C, 2,5 min and 400 MPa, 40°C, 1 min) did not affect the bioactive compounds responsible for the radical scavenging capacity of freshly squeezed orange juices, either immediately after treatment or during the chilled storage period. The kinetics of antioxidants from orange juice in the reaction towards radical $DPPH^{\bullet}$ seemed not to be influenced by the high pressure treatment assayed. Where Fig. 3.10 gives corrects this result. It is clear that radical scavenging capacity is steady throughout the shelf life period for all juices studied at both storage temperatures.

3.2.3 Ascorbic Acid Change During Shelf Life

According to the Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Union, ascorbic acid content has to be more than 20 mg/100 ml orange juice at the expiration date (Polydera et al., 2003). This vitamin C content was used to estimate the end of the shelf life period of orange juice in this study. The shelf life of orange juice was estimated, in accordance with this limitation, as the time period in which there is a 70% ascorbic acid loss, since initial ascorbic acid concentration of the orange juice studied was about 70 mg/100 mL. The results for both high pressure and heat processed orange juice when stored at different temperatures are illustrated in Table 3.3.

Table 3.3 Shelf life (days) of high pressurize and thermally pasteurized orange juice when stored at 4° and 25°C (based on vitamin C loss).

Storage Temperature(°C)	Shelf life (days)	
	High pressurized juice (250MPa, 35°C, 15min)	Thermally Pasteurized juice (80°C, 1min)
4	>30	>30
25	>30	8

For pressurized orange juice, storage at 4°C resulted in ascorbic acid content over 75% after 30 days. Even at 25°C storage, 50% of the initial vitamin C amount remained after 18 days (Fig. 3.12, 3.13). For heat treated orange juice, storage at 4°C resulted in a low degradation of vitamin C (about 20%). However storage at 25°C had a devastating effect as the % of vitamin C dropped below 80% at the 4^{th} day of storage. The shelf life is determined to be 8 days due to the ascorbic acid degradation, reaching to a 70% loss which would lead to an unacceptable product.

As the ascorbic acid levels of tomato and carrot juices are lower than orange juice, the trend differs. Vitamin C content of pressurized tomato and carrot juices, at both 4 and 25°C storage, remained over 70% and 45% after 30 days of storage respectively. However, tomato and carrot juices treated at 80°C for 1 min displayed a rapid vitamin C drop at both 4 and 25°C storage. Carrot juice had no value of vitamin C content after 18 and 16 days for 4 and 25°C storage, respectively. Where tomato juice had only around 15% for both storage temperatures.

The slower vitamin C loss rates during storage of high pressurized orange juice led to a significant extension of its shelf life compared to that of the conventionally pasteurized juice. For the same storage period, high pressure treated juices was judged of superior quality than the conventionally thermally processed ones, retaining more flavor of the untreated juices.

No microbial growth was observed during storage of either high pressure or heat treated juices during shelf life. Therefore spoilage from microorganisms was

not a major factor for the determination of shelf life of orange, tomato and carrot juices studied.

In case of storage conditions of high pressurized orange juices, storage at 4°C always had higher vitamin C content than storage at 25°C for all juices.

Among compounds exhibiting antioxidant activity in juices, vitamin C is the most important antioxidant accounting for 65-90% of the total antioxidant activity of orange juice (Miller and Rice-Ewans, 1997, Gardner et al. 2000). The reaction between L-ascorbic acid and DPPH$^{\bullet}$ radical occurs instantly. On the contrary, the reaction between most flavonoids and the radical is time dependent; resulting in continuously decreasing absorbance with time (Polydera et al. 2005). Therefore, a better retention of the antioxidant activity of the juices due to ascorbic acid was observed for high pressurized juices in our study.

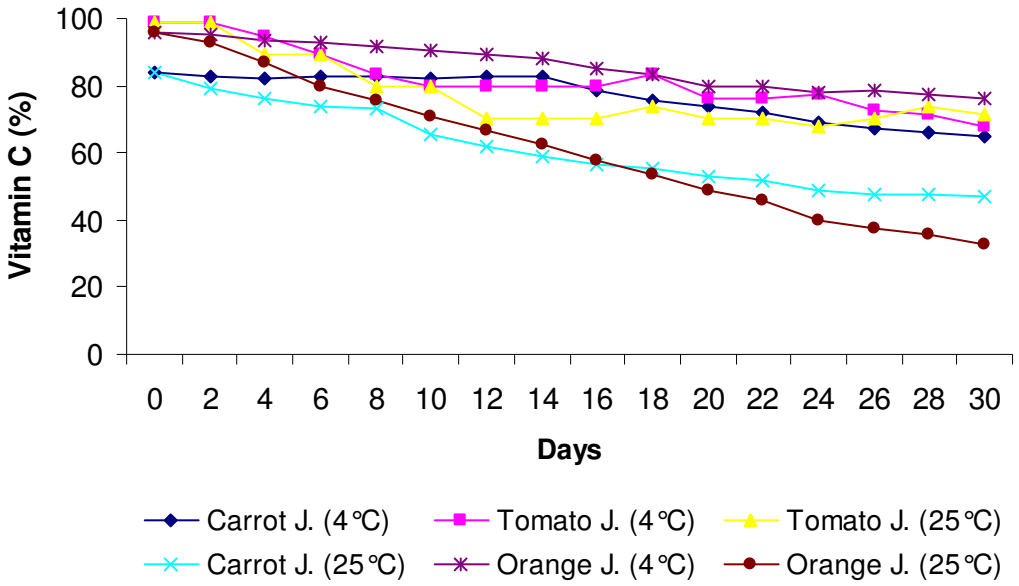

Figure 3.12 Vitamin C content through shelf life for HHP treated (250 MPa, 35°C, 15 min) juices.

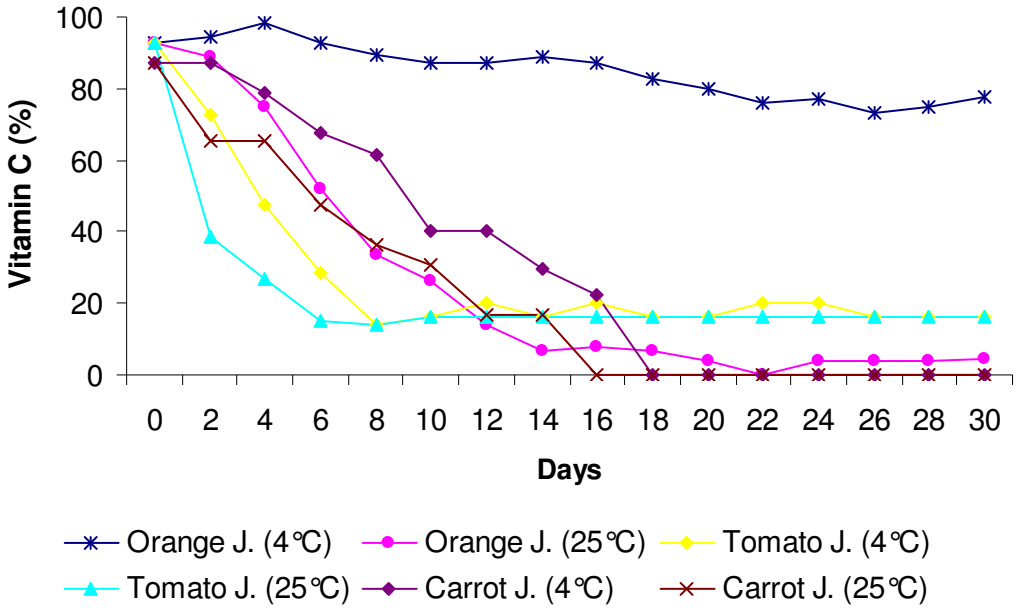

Figure 3.13 Vitamin C content through shelf life for heat treated (80°C, 1 min) juices.

In summary, the decrease of total antioxidant activity of studied juices during storage (Figures 3.10, 3.11) can be mainly attributed to ascorbic acid loss (3.12, 3.13). Furthermore, the higher overall antioxidant activity of high pressure treated juices compared to thermally treated ones during storage (4 and 25°C) was the result of better retention of ascorbic acid, which was observed for high pressurized juices.

3.2.4 Color Change During Shelf Life

The color difference of juices is another way of correcting the antioxidant activity change. As ascorbic acid and other antioxidant compounds oxidize, the color is affected. So as can be easily seen by looking at the antioxidant and vitamin C changes of juices (Fig. 3.10, 3.11, 3.12, 3.13), HHP application has a small effect in

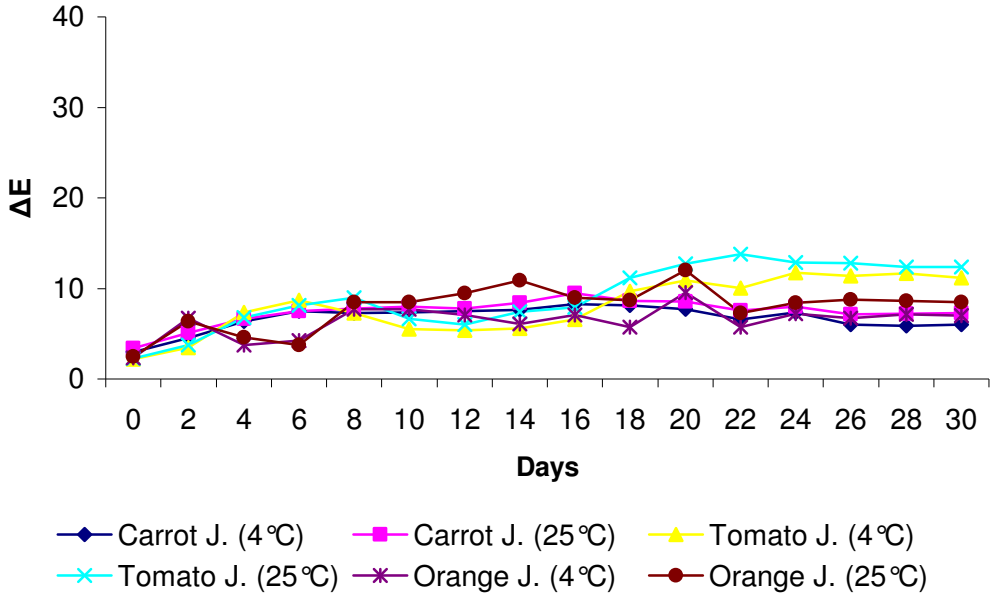

Figure 3.14 Color change through shelf life for HHP treated (250 MPa, 35°C, 15 min) juices.

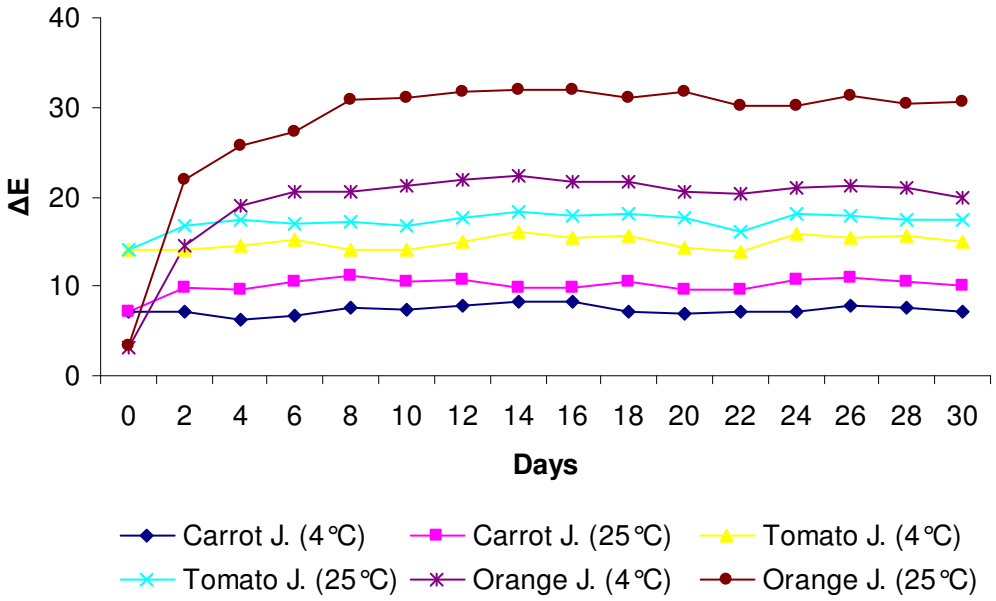

Figure 3.15 Color change through shelf life for heat treated (80°C, 1 min) juices.

color change than thermally treated ones through the shelf life period (Fig. 3.14, 3.15). This small change was predicted and can be attributed to effects of high pressure on the release of carotenoids from protein associates or disruption of chloroplasts (de Ancos et al., 2000).

For pressurized samples, the color changes were about or lower than 10 for orange, tomato and carrot juices. But for heat pasteurized samples, color changes were more intense and higher as a result of insufficient antioxidant activity.

This high level of change in color by heat may also be explained by the positive effect on phenolic substances (anthocyanins and hydroxycinnamates) (Scalzo et al. 2004).

To summarize, HHP processed juices displayed more natural and fresh-like color properties than thermally treated ones. Today, the consumers are more likely to select processed products with better natural properties.

3.2.5 pH During Shelf Life

pH of juices was not affected by treatment, storage temperature or storage time. It was also stable with no significant change during the shelf life period (Fig. 3.16, 3.17).

3.2.6 Effect of Variables

Effects of variables (treatment, storage temperature and time) on studied parameters (antioxidant, vitamin C, color and pH) are reported in Table 3.4. Except pH, all parameters were significantly affected by treatment, storage time and temperature.

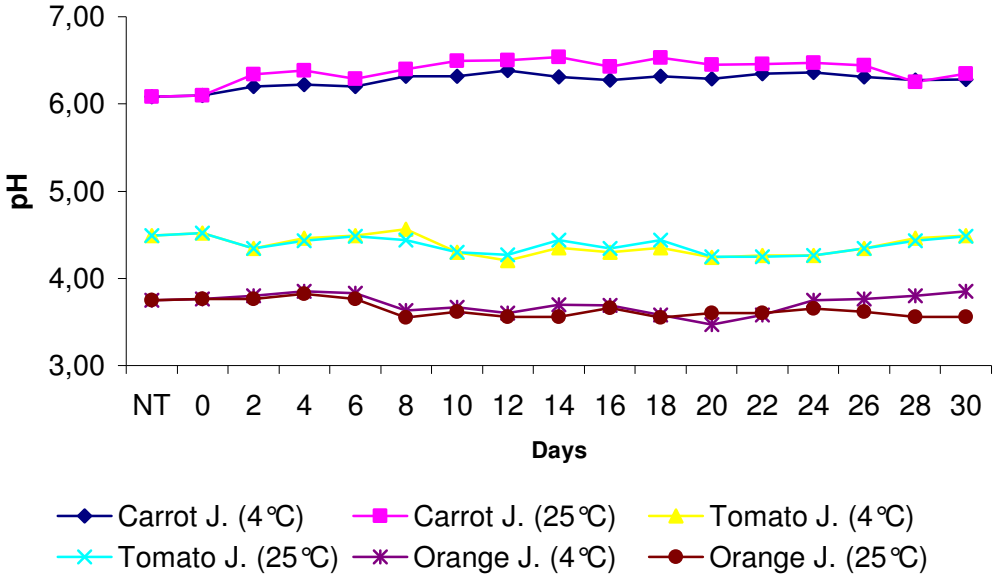

Figure 3.16 pH through shelf life for HHP treated (250 MPa, 35°C, 15 min) juices.

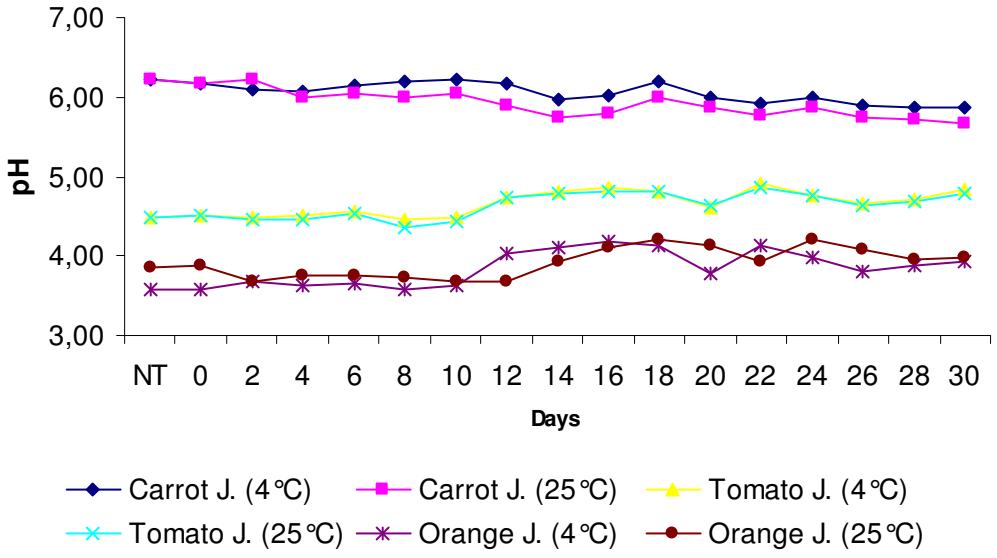

Figure 3.17 pH through shelf life for heat treated (80°C, 1 min) juices.

For tomato juices, interaction of storage time and temperature, and treatment, storage time and temperature did not affect the parameters antioxidant and pH. Vitamin C was not affected by interaction of treatment and storage temperature.

Results of carrot juice analysis showed that interactions of storage time and temperature and treatment, storage time and temperature did not affect the parameter antioxidant.

All interactions were effective on all parameters for orange juice analysis.

Table 3.4 Effects of variables

Orange J.	Treatment(a)	Storage Temp(b)	Storage Time(c)	axb	axc	bxc	axbxc
Antioxidant	✓	✓	✓	✓	✓	✓	✓
Vitamin C	✓	✓	✓	✓	✓	✓	✓
Color	✓	✓	✓	✓	✓	✓	✓
pH	✓		✓	✓	✓	✓	✓

Tomato J.	Treatment(a)	Storage Temp(b)	Storage Time(c)	axb	axc	bxc	axbxc
Antioxidant	✓	✓	✓	✓	✓		
Vitamin C	✓	✓	✓		✓	✓	✓
Color	✓	✓	✓	✓	✓	✓	✓
pH	✓		✓	✓	✓		

Carrot J.	Treatment(a)	Storage Temp(b)	Storage Time(c)	axb	bxc	axc	axbxc
Antioxidant	✓	✓	✓	✓		✓	
Vitamin C	✓	✓	✓	✓	✓	✓	✓
Color	✓	✓	✓	✓	✓	✓	✓
pH	✓		✓	✓	✓	✓	✓

* significance at $p<0.05$

✓ means the parameter is effected by the variable

CHAPTER 4

CONCLUSIONS and RECOMMENDATIONS

In food industry, fruit and vegetable juice have an important place since products of this market are consumed extensively. One of the reasons is the product's high amount of nutritional and health promoting value, like vitamin C and other antioxidants.

Since HHP offers an alternative way to preserve food, its effects on orange, tomato and carrot juices were studied to compare the quality parameters with heat pasteurization application.

HHP conditions studied were found not to affect ascorbic acid content, antiradical scavenging activity, pH and color of fruit and vegetable juices significantly ($p<0.05$) in comparison with untreated freshly squeezed ones. Both HHP and heat treatment applications were able to produce microbiologically stable products with a mean bacterial reduction of 5.5 $\log_{10}$ cfu/mL is needed by the industry, on the plus side, more fresh-like product is achieved for HHP treatment.

During a storage period of 30 days, the radical scavenging activity, ascorbic acid content, color and pH of pressure treated juices were not significantly different from fresh, untreated juice. These parameters did not change significantly over the storage time at 4°C storage temperature ($p>0.05$).

All juices were microbiologically stable through the shelf life experiments. As ascorbic acid content is not affected by HHP application, it was not a surprise that antiradical scavenging activity is stable through shelf life since vitamin C is the main compound responsible for antioxidation in fruit and vegetable juices. And as a second effect, HHP treated juices showed a little color change but heat treated ones did had a very high and rapid color change shift. This is also affected by oxidation reactions.

High-pressure treatment could be an efficient processing method for preserving fruit and vegetable juices as freshly squeezed for over 30 days from the point of sensory (color) and nutritional (vitamin C) quality attributes.

A healthier, more fresh-like, so a product with consumer attractive, can be achieved by HHP implementation into the fruit and vegetable juice industry.

As a recommendation, to understand the effect of HHP on fruit and vegetable juices, different fruits and vegetables can be studied, along with other product types like jams and purees. To analyze the nutritional value and shelf life determination, important enzymes like PME, present in the juices, should be analyzed.

REFERENCES

Alpas, H. 2000. Effect of temperature, pH and bacteriocins on the viability of selected food pathogens in food systems during high hydrostatic pressure (HHP) processing. Ph. D. Thesis, Middle East Technical University, Ankara.

Alpas, H., Alma, L. and Bozoğlu, F. 2003. Inactivation of Alicyclobacillus acidoterrestris vegetative cells in model system, apple, orange and tomato juices by high hydrostatic pressure. World Journal of Microbiology and Biotechnology, 19:619-623.

Ancos B., Sgroppo S., Plaza, L. and Cano, P. 2002. Possible nutritional and health-related value promotion in orange juice preserved by high-pressure treatment. Journal of the Science of Food and Agriculture, 82:790-796.

AOAC Official Method 967.21. Ascorbic acid in vitamin preparations and juices, AOAC Official Methods of Analysis (1995), 45.1.14.

Australian Department of Health and Ageing. 1999. Salmonellosis outbreak, South Australia. Communicable Diseases Intelligence, *23*(3):73.

Basak, S. and Ramaswamy, H.S. 1996. Ultra high pressure treatment of orange juice: a kinetic study on deactivation of pectin methyl esterase. Food Research International, 29:601 –607.

Benito A., Ventoura G., Casadei M., Robinson, T. and Mackey, B. 1999. Variation in resistance of natural isolates of Escherichia coli O157 to high hydrostatic pressure, mild heat and other stresses. Appl. Environ. Microbiol., 65:1564-1573.

Bignon, J. 1996. Cold pasteurizers hyperbar for stabilization of fresh fruit juices. Fruit processing, 2:46-54.

Billmeyer, F. W. and Saltzman, M. 1981. Principles of color technology. New York: John Wiley and Sons. 25–66, 67–110.

Bracket, R. E. 1997. Fruits, vegetables and grains. In M. P. Doyle, L. R. Beuchat, T. J. Montville, Food microbiology fundamentals and frontiers (pp. 121). Washington, DC: ASM Press.

Brand-Williams, W., Cuvelier, M. E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. Lebensmittel Wissenschaft und Technologie, 28:25-30.

Bull, K., Zerdin, K., Howe, E., Goicoechea, D., Raramanandhan, P., Stockman, R., Sellahewa, J., Szabo, E., Johnson, R. and Stewart, C. 2004. The effect of high pressure processing on the microbial, physical and chemical properties of Valencia and Navel orange juice. Innovative Food Science and Emerging Technologies, 5:135–149.

Butz, P., Fernandez, G. A., Fister, H., Merkel, C. and Tauscher, B. 2002. Changes in functional properties of vegetables induced by high pressure treatment. Food Research International, 35:295–300.

Cemeroğlu, B. and Karadeniz, F. 2001. Meyve ve Sebze İşleme Teknolojisi 2: Meyve Suyu Üretim Teknolojisi. Gıda Teknolojisi Derneği Yayınları, No: 25, Ankara.

Centers for Disease Control and Prevention (CDC). 1996. Outbreak of *Escherichia coli* O157:H7 infections associated with drinking unpasteurised commercial apple juice-British Columbia, California, Colorado, and Washington. Morbidity and Mortality Weekly Report, 45:975.

Cook, K. A., Dobbs, T. E., Hlady, W. G., Wells, J. G., Barrett, T. J., Puhr, N. D., Lancette, G. A., Bodager, D. W., Toth, B. L., Genese, C. A., Highsmith, A. K., Pilot, K. E., Finelli, L. and Swerdlow, D. L. 1998. Outbreak of *Salmonella* serotype Hartford infections associated with unpasteurized orange juice. Journal of American Medical Association, 280(17):1504 –1509.

De Ancos, B., Ibanez, E., Reglero, G. and Pilar Cano, M. 2000. Frozen storage effects on anthocyanins and volatile compounds of raspberry fruit. J. Agric. Food Chem., 48:873-879.

Diplock, A.T. 1994. Antioxidants and disease prevention. Molecular Aspects of Medicine, 15:293–376.

Donsi, G., Ferrari, G. and Di Matteo, M. 1996. High pressure stabilization of orange juice: evaluation of the effects of process conditions. Ital. J. Food Sci., 2:99-106.

Earnshaw, R.G. 1992. High Pressure technology and its potential use. In: A. Turner (ed.) Food technology international Europe. Sterling publications international, London, 85-88.

Farnworth, E. R., Lagace´, M., Couture, R., Yaylayan, V. and Stewart, B. 2001. Thermal processing, storage conditions, and the composition and physical properties of orange juice. Food Research International, 34:25 –30.

Fellows, P.J. 2000. Food Processing Technology: Principles & Practice. Woodhead Publishing Limited and CRC Press LLC.

Fernandez, G. A., Butz, P., Bognar, A. and Tauscher, B. 2001. Antioxidative capacity, nutrient content and sensory quality of orange juice and an orange-lemon-carrot juice product after high pressure treatment and storage in different packaging. European Food Research and Technology, 213(4–5):290 –296.

Garcia-Graells, C., Hauben, K.J. and Micheils, C.W. 1998. High pressure inactivation and sublethal injury of pressure resistant *Echerischia coli* mutants in fruit juices. Appl. Environ. Microbiol., 64:1566-1574.

Gardner, P., White, T., McPhail, D. and Duthie, G. 2000. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. Food Chemistry, 68:471-474.

Gazzani, G., Papetti, A., Masolini, G. and Daglia, M. 1998. Anti- and prooxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment. Journal of Agriculture and Food Chemistry, 46:4118–4122.

Gervilla, R., Sendra, E., Ferragut, V. and Guamis, B. 1999. Sensitivity of Staphylococcus aureus and Lactobacillus helveticus in Onive Milk Subjected to high hydrostatic pressure. J. Dairy Sci., 82:1099-1107.

Gaucheron, F., Famelart, M.H., Mariette, F., Raulot, K., Michel, F. and LeGraet, Y. 1997. Combined effects of temperature and high-pressure treatments on physicochemical characteristics of skim milk. Food Chem., 59:439-447.

Goodner, J. K., Braddock, R. J., & Parish, M. E. 1998. Inactivation of pectinesterase in orange and grapefruit juices by high pressure. Journal of Agriculture and Food Chemistry, 46:1997–2000.

Heinish, O., Kowalski, E., Goossens, K., Frank, J., Heremans, K., Ludwig, H. and Tauscher, B. 1995. Pressure effects on the stability of lipoxigenase: Fourier transform-infrared spectroscopy (FT-IR) and enzyme activity studies, Z Lebensm Unters Forsch, 201:562-567.

Hauben, K.J., Wutytack, E.Y., Soontjens, C.C. and Micheils, C.W. 1997. High pressure transient sensitization of Escherichia coli to lysozyme and nisin by disruption of outer membrane permeability. J. Food Technol., 59:350-355.

Hayashi, R. 1995. Use of high pressure in bioscience and in biotechnology (pp.1–7). High pressure bioscience and biotechnology, vol.13. Amsterdam: Elsevier.

Hocking, A. D. and Jensen, N. 2001. Soft drinks, cordials, juices, bottled water and related products. Spoilage of processed foods: causes and diagnosis (pp.93–94). Waterloo NSW: AIFST Inc. (NSW Branch) Food Microbiology Group.

Hollman, P.C., Hertog, M.G.L. and Katan, M.B. 1996. Analysis and health effects of flavonoids. Food Chemistry, 57(1):43–46.

Indrawati, Van Loey, A.M., Ludikhuyze, L. and Hendrickx, M. 1999. Soybean lipoxygenase inactivation by pressure at subzero and elevated temperatures. J. Agric. Food Chem., 47:2468-2542.

Indrawati. 2000. Lipoxygenase inactivation by high pressure treatment at subzero and elevated temperatures: a kinetic study. PhD dissertation, Katholieke Universeit Leuven, Belgium.

Jay, S. and Anderson, J. 2001. Fruit juice and related products. Spoilage of processed foods: causes and diagnosis (pp.189). Waterloo NSW: AIFST Inc. (NSW Branch) Food Microbiology Group.

Jongen, W. 2002. Fruit and Vegetable Processing. Woodhead Publishing Limited and CRC Press LLC.

Knorr, D. 1995. High pressure treatment of food: microbiology. In G.W. Gould (ed.) New methods of food preservation. Blackie academic and professional, London, 159-175.

Linton, M., McClements, J. M. and Patterson, M. F. 1999. Inactivation of Escherichia coli O157:H7 in orange juice using a combination of high pressure and mild heat. Journal of Food Protection, 62(3):277–279.

Lorenço, E.J., De Souza, Leao J. and Neves, V.A. 1990. Heat inactivation and kinetics of polyphenoloxidase from palmito (Enterpe edulis), J. Sci. Food Agric., 52:249-308.

Lopez-Malo, A., Palou, E., Barbosa-Canovas, G.V., Welti-Chanes, J. and Swanson, B.G. 1998. Polyphenoloxidase activity and color changes during storage of high hydrostatic pressure treated avocado puree. Food Res. Int., 31(8):549-556.

Ludikhuyze, L., Indrawarti, Van Den Broeck, I. Weemaes, C. and Hendrickx, M. 1998. Effect of combined pressure and temperature on soybean lipoxygenase: I. influence of extrinsic factors on isobaricisothermal inactivation kinetics. J. Agric. Food Chem., 46:4074-4154.

Mermelstein, N.H. 1997. High Pressure Processing reaches the US Market. Food Technol., 51:95-96.

Mertens, B. 1995. Hydrostatic Pressure treatment of food: equipment and processing. In G.W. Gould (ed.) New methods of food preservation. Blackie academic and professional, London, 135-158.

Miller, J. and Rice-Ewans, C. 1997. The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. Food Chem., 60:331-337.

Nienaber, U. and Shellhammer, T.H. 2001. High-pressure processing of orange juice: combination treatments and a shelf life study. Journal of Food Science, 66:332 –336.

Palou, E. 1998. Food Preservation by High Hydrostatic Pressure, Process Variables and Microbial Inactivation. Ph. D. Thesis, Washington State University, Pullman.

Palou, E., Lopez-Malo, A., Barbosa-Canovas, G.V., Welti-Chanes, J. and Swanson, B.G. 1999. Polyphenoloxidase activity and color of blanched and high hydrostatic pressure treated banana puree. J. Food Sci., 64:42-45.

Parish, M.E. 1998. Orange juice quality after treatment by thermal pasteurization or isostatic high pressure. Lebensmittel Wissenschaft und Tecnhnologie, 31:439–442.

Pizzocaro, F. 1989. Antioxidant and prooxidant activities changes induced in the vegetables by heat inactivation before freezing and by frozen storage. In Proc. Int. Symp. ‘‘Metals and Natural Antioxidants’’, 21–22 November, Milano, Italy.

Pizzocaro, F., Gasparoli, A. and Babbini, G. 1991. Effetto del trattamento termico sull’attivita` antiossidante e sulla qualita` sensoriale delle foglie di rosmarino e della salvia. Annali IVTPAMilano. 22:15–22.

Pizzocaro, F., Gasparoli, A. and Ambrogi, A. 1995. Effetto del trattamento termico sull’attivita` antiossidante dei succhi di specie vegetali eduli. Rivista Italaliana della Sostanze Grasse, 72:289–297.

Pizzocaro, F., Russo Volpe, S., Morelli, R. and Oliva, C. 1997. Modificazioni dell’attivita` antiossidante e antiradicalica in dischi termotrattati di carota. In Atti 3° CISETA Cernobbio, 11–12 September, Pinerolo, Italy: Chiriotti. 423–435.

Polydera, A.C., Stoforos, N.G. and Taoukis, P.S. 2003. Comparative shelf life study and vitamin C loss kinetics in pasteurized and high pressure processed reconstituted orange juice. Journal of Food Engineering, 60:21–29.

Polydera, A.C., Galanou, E., Stoforos, N.G. and Taoukis, P.S. 2004. Inactivation kinetics of pectin methylesterase of greek Navel orange juice as a function of high hydrostatic pressure and temperature process conditions. Journal of Food Engineering, 62:291–298.

Polydera, A.C., Stoforos, N.G. and Taoukis, P.S. 2005. Effect of high hydrostatic pressure treatment on post processing antioxidant activity of fresh Navel orange juice. Food Chemistry, 91:495–503.

Poretta, S., Birzi, A., Ghizzoni, C. and Vicini, E. 1995. Effects of ultra-high hydrostatic pressure treatments on the quality of tomato juice. Food Chem., 52:35-41.

Quaglia, G., Gravina, R., Paperi, R. and Paoletti, F. 1996. Effect of high pressure treatments on peroxidase activity, ascorbic acid content and texture in green peas, Lebensm –Wiss u- Technol., 29:552-557.

Rapisarda, P., Tomaino, A., Lo Cascio, R., Bonina, F., De Pasquale, A. and Saija, A. 1999. Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. Agric. Food Chem., 47:4718-4723.

Reynolds, T.M. 1963. Chemistry of non-enzymic browning. In E.M. Mark, G.F. Stewart, (pp.1–52) Advances in food research, vol.12. London: Academic Press.

Sanchez-Moreno, C., Plaza, L., Ancos, B. and Cano, P. 2003. Effect of high-pressure processing on health-promoting attributes of freshly squeezed orange juice (Citrus sinensis L.) during chilled storage. Eur. Food Res. Technol., 216:18-22.

Sancho, F., Lambert, Y., Demazeau, G., Largeteau, A., Bouvier, J-M. and Narbonne, J-F. 1999. Effect of ultra-high hydrostatic pressure on hydrosoluble vitamins. J. Food Eng., 39:247-253.

Scalzo, R. Lo, Iannoccari, T., Summa, C., Morelli, R. and Rapisarda, P. 2004. Effect of thermal treatments on antioxidant and antiradical activity of blood orange juice. Food Chemistry, 85:41–47.

Smelt, J.P.P.M. 1998. Recent advances in the microbiology of high pressure processing. Trends in Food Sci. and Technol., 9:152-158.

Tangwongchai, R., Ledward, D.A. and Ames, J.M. 1999. Effect of high pressure on lipoxygenase activity in cherry tomatoes. In advances in high pressure bioscience and biotechnology, ed Ludwig H., Springer-Verlag, Berlin and Heidelberg, 435-443.

Tewari, G., Jayas, D.S. and Holley, R.A. 1999. High Pressure Processing of Foods: An Overview. Sci. des Aliments, 19:619-661.

Tillotson, J. 2000. Are your juices and your company ready for the next century? Changing world, changing consumers, changing tastes. Fruit Processing, 7:283-289.

Tressler, D.K. and Joslyn, M.A. 1961. Fruit Juice Processing Technology. The AVI Publishing Company Inc.

Tressler, D.K. and Joslyn, M.A. 1971. Fruit Juice Processing Technology 2nd Edition. The AVI Publishing Company Inc.

Trujillo, A.J., Capellas, M., Buffa, M., Royo, C., Gervilla, R., Felipe, X., Sendra, E., Saldo, J., Ferragut, V. and Guamis, B. 2000. Application of high hydrostatic pressure treatment for cheese production. Food Res. Int., 33:311-316.

U.N. Food and Agriculture Organization. 1991. The world market for fruit juice: Citrus and Tropical. CCP:CI 91/4, Rome.

Wang, M., Jin, Y. and Chi-Tang, H. 1999. Evaluation of resveratrol derivatives as potential antioxidants and identification of a reaction product of resveratrol and 2,2-diphenyl-1-picryhydrazyl radical. Agric. Food Chem., 47:4038-4045.

Watanabe, M., Arai, E., Kumeno, K. and Homma, K. 1991. A new method for producing non-heated jam sample: the use of freeze concentration and high pressure sterilization, Biol. Chem., 55(8):2175-2176.

Weemaes, C. 1998. Temperature and/or Pressure inactivation of polyphenoloxidases for prevention of enzymatic browning in foods: a kinetic study, Ph D Dissertation, Katholieke Universeit Leuven, Belgium.

Weemaes, C., Ludikhuyze, L.R., Van Den Broeck, I., Hendrickx, M.E. and Tobback, P.P. 1998a. Activity, electrophoretic characteristics and heat inactivation of polyphenoloxidases from apples, avocados, grape, pears and plums, Lebensm – Wiss u- Technol., 31:44-53.

Wicker, L. and Temelli, F. 1999. Heat inactivation of pectineasterase in orange juice pulp. J. Food Sci., 53:162-168.

Van Den Broeck, I., Weemaes, C., Ludikhuyze L., Van Loey, A. and Hendrickx M. 1998. Kinetics of isobaric-isothermal degradation of L-ascorbic acid. J. Agric. Food Chem., 46:2001-2006.

Van Den Broeck, I. 2000. Kinetics of temperature and pressure inactivation of pectinesterase from oranges and tomatoes. PhD dissertation, Katholieke Universeit Leuven, Belgium.

Versteeg, C., Rombouts, F.M., Spaansen, C.H. and Pilnil, W. 1980. Thermostability an orange juice cloud destabilizing properties of multiple pectinesterases from orange. J. Food Sci., 45:969-1040.

Vinson, J. A., Liang, X. Q., Proch, J., Hontz, B. A., Dancel, J. and Sandone, N. 2002. Polyphenol antioxidants in citrus juices: In vitro and in vivo studies relevant to heart disease. Flavonoids in Cell Function – Advances in Experimental Medicine and Biology, 505:113–122.

Yemenicioglu, A., Ozkan, M. and Cemeroglu, B. 1997. Heat inactivation kinetics of apple polyphenoloxidase and activation of its latent form. J. Food Sci., 62:508-518.

Yen, G-C. and Lin, H-T. 1996. Comparison of high pressure treatment and thermal pasteurization effects on the quality and shelf-life of guava puree. Int. J. Food Sci. Technol., 31:205-213.

APPENDIX A

ANOVA and DUNCAN TABLES

Table A.1 ANOVA Table for Orange Juice Treatments

		Statistics				
Dependent Variable	**Source**	**SS**	**df**	**MS**	**F**	**Sig.**
Total Aerobic Count	Between Groups	23,644	16	1,478	37,042	,000
	Within Groups	1,356	34	,040		
	Total	25,001	50			
Ascorbic Acid	Between Groups	3446,358	8	430,795	38,709	,000
	Within Groups	200,324	18	11,129		
	Total	3646,682	26			
Radical Scavenging	Between Groups	2753,047	8	344,131	806,872	,000
	Within Groups	7,677	18	,426		
	Total	2760,724	26			

Table A.2 ANOVA Table for Tomato Juice Treatments

		Statistics				
Dependent Variable	**Source**	**SS**	**df**	**MS**	**F**	**Sig.**
Total Aerobic Count	Between Groups	46,267	16	2,892	78,982	,000
	Within Groups	1,245	34	,037		
	Total	47,511	50			
Ascorbic Acid	Between Groups	1670,911	8	208,864	28,596	,000
	Within Groups	131,471	18	7,304		
	Total	1802,383	26			
Radical Scavenging	Between Groups	2695,852	8	336,982	59,929	,000
	Within Groups	101,214	18	5,623		
	Total	2797,066	26			

Table A.3 ANOVA Table for Carrot Juice Treatments

		Statistics				
Dependent Variable	**Source**	**SS**	**df**	**MS**	**F**	**Sig.**
Total Aerobic Count	Between Groups	75,245	16	4,703	110,211	,000
	Within Groups	1,451	34	,043		
	Total	76,695	50			
Ascorbic Acid	Between Groups	1042,668	8	130,333	23,060	,000
	Within Groups	101,735	18	5,652		
	Total	1144,402	26			
Radical Scavenging	Between Groups	2719,439	8	339,930	24,447	,000
	Within Groups	250,282	18	13,905		
	Total	2969,721	26			

Homogeneous Subsets

#	Treatment
1	No Treatment
2	150 MPa, 25°C, 5 min
3	150 MPa, 25°C, 10 min
4	150 MPa, 25°C, 15 min
5	200 MPa, 25°C, 5 min
6	200 MPa, 25°C, 10 min
7	200 MPa, 25°C, 15 min
8	250 MPa, 25°C, 5 min
9	250 MPa, 25°C, 10 min
10	250 MPa, 25°C, 15 min
11	250 MPa, 35°C, 5 min
12	250 MPa, 35°C, 10 min
13	250 MPa, 35°C, 15 min
14	60°C, 5 min
15	60°C, 10 min
16	60°C, 15 min
17	80°C, 1 min

Table A.4 Duncan's Multiple Range Table for Total Aerobic Count for Orange Juice

Duncan[a]

Treatment	N	Subset for alpha = .05						
		1	2	3	4	5	6	7
2	3	1,3200						
3	3		1,7100					
14	3		1,9500	1,9500				
4	3			2,0600				
5	3			2,2000				
6	3				2,5700			
15	3				2,7300	2,7300		
8	3				2,8800	2,8800		
7	3				2,9200	2,9200		
11	3					3,0000	3,0000	
9	3						3,2700	3,2700
12	3						3,3100	3,3100
16	3							3,4000
10	3							3,4700
1	3							3,5000
13	3							3,5000
17	3							3,5000
Sig.		1,000	,150	,157	,056	,139	,080	,231

Means for groups in homogeneous subsets are displayed.
[a] Uses Harmonic Mean Sample Size = 3,000.

Table A.5 Duncan's Multiple Range Table for Total Aerobic Count for Tomato Juice

Duncan[a]

Treatment	N	Subset for alpha = .05					
		1	2	3	4	5	6
2	3	1,5800					
5	3		2,0000				
3	3		2,0700				
6	3			2,5700			
4	3			2,6100			
8	3				3,1700		
7	3				3,3600		
9	3				3,4700		
11	3					3,8000	
14	3					3,8700	
10	3					3,9300	
12	3					4,1000	
15	3					4,1100	
16	3						4,4500
13	3						4,6000
17	3						4,6000
1	3						4,6000
Sig.		1,000	,657	,799	,077	,084	,390

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.6 Duncan's Multiple Range Table for Total Aerobic Count for Carrot Juice

Duncan[a]

Treatment	N	Subset for alpha = .05						
		1	2	3	4	5	6	7
2	3	1,5500						
14	3		2,3000					
5	3		2,4000					
3	3		2,4200					
8	3			2,9000				
6	3			3,0600				
4	3			3,1400				
7	3				3,8000			
9	3				3,9700	3,9700		
11	3					4,2000		
15	3					4,2400		
16	3						4,7200	
10	3						4,7700	
12	3						4,9300	
1	3							5,5000
13	3							5,5000
17	3							5,5000
Sig.		1,000	,508	,188	,321	,139	,249	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Homogeneous Subsets

#	Treatment
1	No Treatment
2	250 MPa, 25°C, 5 min
3	250 MPa, 25°C, 10 min
4	250 MPa, 25°C, 15 min
5	250 MPa, 35°C, 15 min
6	60°C, 5 min
7	60°C, 10 min
8	60°C, 15 min
9	80°C, 1 min

Table A.7 Duncan's Multiple Range Table for Ascorbic Acid for Orange Juice

Duncan[a]

Treatment		Subset for alpha = .05				
	N	1	2	3	4	5
8	3	65,6000				
7	3	69,7000				
6	3		77,1000			
9	3			85,2900		
5	3			89,9800	89,9800	
4	3				92,0333	
3	3				93,1500	
2	3				95,5567	95,5567
1	3					100,0000
Sig.		,150	1,000	,102	,075	,120

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.8 Duncan's Multiple Range Table for Ascorbic Acid for Tomato Juice

Duncan[a]

Treatment		Subset for alpha = .05				
	N	1	2	3	4	5
8	3	76,9000				
7	3	79,7000	79,7000			
6	3		82,3000			
9	3			88,2400		
5	3				94,1200	
4	3				94,6000	
3	3				95,3000	95,3000
2	3				97,1400	97,1400
1	3					100,0000
Sig.		,221	,254	1,000	,225	,058

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.9 Duncan's Multiple Range Table for Ascorbic Acid for Carrot Juice

Duncan[a]

Treatment		Subset for alpha = .05				
	N	1	2	3	4	5
8	3	79,2000				
7	3	80,5000				
6	3		85,1000			
9	3		87,2700	87,2700		
4	3			90,6000	90,6000	
5	3			90,9100	90,9100	
3	3				92,2000	
2	3				93,8800	
1	3					100,0000
Sig.		,512	,278	,091	,137	1,000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.10 Duncan's Multiple Range Table for Radical Scavenging for Orange Juice

Duncan[a]

Treatment		Subset for alpha = .05				
	N	1	2	3	4	5
8	3	71,2800				
7	3		78,7300			
6	3			84,7400		
9	3				90,0500	
5	3					98,8200
4	3					98,8300
3	3					98,9700
2	3					99,4600
1	3					100,0000
Sig.		1,000	1,000	1,000	1,000	,060

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.11 Duncan's Multiple Range Table for Radical Scavenging for Tomato Juice

Duncan[a]

Treatment	N	Subset for alpha = .05		
		1	2	3
8	3	73,0000		
7	3	76,2200		
9	3		84,0000	
6	3		85,3300	
5	3		88,0700	
2	3			99,2000
4	3			99,2400
3	3			99,5600
1	3			100,0000
Sig.		,114	,061	,710

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.12 Duncan's Multiple Range Table for Radical Scavenging for Carrot Juice

Duncan[a]

Treatment		Subset for alpha = .05				
	N	1	2	3	4	5
8	3	67,6400				
7	3		76,5500			
9	3		80,2400	80,2400		
6	3		81,6300	81,6300		
5	3			84,5000		
4	3				91,0100	
3	3				94,9900	94,9900
2	3				97,1200	97,1200
1	3					100,0000
Sig.		1,000	,131	,201	,072	,136

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3,000.

Table A.13 ANOVA table for shelf life Analysis of Orange Juice

Source	D. Var.	SS	df	MS	F	Sig.
Corrected Model	Antioxidant	115401,766(a)	63	1831,774	369,392	,000
	VitaminC	182822,692(b)	63	2901,947	681,952	,000
	Color	18952,752(c)	63	300,837	13999,217	,000
	pH	8,384(d)	63	,133	12,420	,000
Intercept	Antioxidant	975085,358	1	975085,358	196633,767	,000
	VitaminC	805018,621	1	805018,621	189177,889	,000
	Color	45827,790	1	45827,790	2132558,335	,000
	pH	2723,451	1	2723,451	254169,316	,000
Treatment	Antioxidant	24467,779	1	24467,779	4934,123	,000
	VitaminC	15269,257	1	15269,257	3588,247	,000
	Color	13340,001	1	13340,001	620765,914	,000
	pH	2,613	1	2,613	243,892	,000
storagetemp	Antioxidant	39820,148	1	39820,148	8030,051	,000
	VitaminC	82271,736	1	82271,736	19333,706	,000
	Color	1267,627	1	1267,627	58987,968	,000
	pH	,002	1	,002	,187	,666
storagetime	Antioxidant	11506,675	15	767,112	154,694	,000
	VitaminC	48269,896	15	3217,993	756,222	,000
	Color	2550,519	15	170,035	7912,420	,000
	pH	1,457	15	,097	9,068	,000
Treatment * storagetemp	Antioxidant	29143,601	1	29143,601	5877,040	,000
	VitaminC	13680,565	1	13680,565	3214,908	,000
	Color	634,526	1	634,526	29527,126	,000
	pH	,196	1	,196	18,325	,000
Treatment * storagetime	Antioxidant	2498,738	15	166,583	33,593	,000
	VitaminC	3167,165	15	211,144	49,619	,000
	Color	961,594	15	64,106	2983,131	,000
	pH	2,552	15	,170	15,881	,000
storagetemp * storagetime	Antioxidant	4779,233	15	318,616	64,251	,000
	VitaminC	17138,337	15	1142,556	268,499	,000
	Color	140,331	15	9,355	435,346	,000
	pH	,916	15	,061	5,698	,000
Treatment * storagetemp * storagetime	Antioxidant	3185,593	15	212,373	42,827	,000
	VitaminC	3025,737	15	201,716	47,403	,000
	Color	58,155	15	3,877	180,414	,000
	pH	,647	15	,043	4,025	,000
Error	Antioxidant	634,738	128	4,959		
	VitaminC	544,685	128	4,255		
	Color	2,751	128	,021		
	pH	1,372	128	,011		
Total	Antioxidant	1091121,862	192			

Table A.13 ANOVA table for shelf life Analysis of Orange Juice (cont'd).

	VitaminC	988385,998	192
	Color	64783,293	192
	pH	2733,207	192
Corrected Total	Antioxidant	116036,504	191
	VitaminC	183367,378	191
	Color	18955,503	191
	pH	9,756	191

a R Squared = ,995 (Adjusted R Squared = ,992)
b R Squared = ,997 (Adjusted R Squared = ,996)
c R Squared = 1,000 (Adjusted R Squared = 1,000)
d R Squared = ,859 (Adjusted R Squared = ,790)

Table A.14 ANOVA table for shelf life Analysis of Tomato Juice

Source	D. Var.	SS	df	MS	F	Sig.
Corrected Model	Antioxidant	88497,911(a)	63	1404,729	133,263	,000
	VitaminC	192791,845(b)	63	3060,188	698,764	,000
	Color	3896,177(c)	63	61,844	1586,063	,000
	pH	9,608(d)	63	,153	6,957	,000
Intercept	Antioxidant	702795,830	1	702795,830	66672,582	,000
	VitaminC	531630,384	1	531630,384	121392,679	,000
	Color	29111,183	1	29111,183	746590,139	,000
	pH	3885,840	1	3885,840	177250,151	,000
Treatment	Antioxidant	80329,649	1	80329,649	7620,684	,000
	VitaminC	141805,673	1	141805,673	32379,960	,000
	Color	2681,133	1	2681,133	68760,776	,000
	pH	4,638	1	4,638	211,543	,000
storagetemp	Antioxidant	498,875	1	498,875	47,327	,000
	VitaminC	1063,048	1	1063,048	242,737	,000
	Color	138,618	1	138,618	3555,020	,000
	pH	,020	1	,020	,913	,341
storagetime	Antioxidant	4506,410	15	300,427	28,501	,000
	VitaminC	38375,653	15	2558,377	584,181	,000
	Color	618,422	15	41,228	1057,343	,000
	pH	1,410	15	,094	4,287	,000
Treatment * storagetemp	Antioxidant	365,010	1	365,010	34,628	,000
	VitaminC	12,010	1	12,010	2,742	,100
	Color	18,377	1	18,377	471,296	,000
	pH	,099	1	,099	4,516	,035
Treatment * storagetime	Antioxidant	2370,614	15	158,041	14,993	,000
	VitaminC	8970,099	15	598,007	136,549	,000
	Color	403,611	15	26,907	690,071	,000
	pH	2,749	15	,183	8,359	,000
storagetemp * storagetime	Antioxidant	236,409	15	15,761	1,495	,116
	VitaminC	1114,708	15	74,314	16,969	,000
	Color	23,131	15	1,542	39,547	,000
	pH	,391	15	,026	1,190	,287
Treatment * storagetemp * storagetime	Antioxidant	190,943	15	12,730	1,208	,274
	VitaminC	1450,655	15	96,710	22,083	,000
	Color	12,886	15	,859	22,031	,000
	pH	,301	15	,020	,916	,549
Error	Antioxidant	1349,248	128	10,541		
	VitaminC	560,567	128	4,379		
	Color	4,991	128	,039		
	pH	2,806	128	,022		

Table A.14 ANOVA table for shelf life Analysis of Tomato Juice (cont'd).

Total	Antioxidant	792642,990	192
	VitaminC	724982,796	192
	Color	33012,351	192
	pH	3898,254	192
Corrected Total	Antioxidant	89847,159	191
	VitaminC	193352,412	191
	Color	3901,168	191
	pH	12,414	191

a R Squared = ,985 (Adjusted R Squared = ,978)
b R Squared = ,997 (Adjusted R Squared = ,996)
c R Squared = ,999 (Adjusted R Squared = ,998)
d R Squared = ,774 (Adjusted R Squared = ,663)

Table A.15 ANOVA table for shelf life Analysis of Carrot Juice.

Source	D. Var.	SS	df	MS	F	Sig.
Corrected Model	Antioxidant	2745,041(a)	63	43,572	6,132	,000
	VitaminC	191934,460(b)	63	3046,579	826,483	,000
	Color	562,083(c)	63	8,922	180,111	,000
	pH	,195(d)	63	,003	108,026	,000
Intercept	Antioxidant	1149571,209	1	1149571,209	161773,992	,000
	VitaminC	447313,978	1	447313,978	121348,402	,000
	Color	11896,607	1	11896,607	240161,141	,000
	pH	7484,633	1	7484,633	261281729,618	,000
Treatment	Antioxidant	102,127	1	102,127	14,372	,000
	VitaminC	82972,999	1	82972,999	22509,113	,000
	Color	134,570	1	134,570	2716,604	,000
	pH	,004	1	,004	137,618	,000
storagetemp	Antioxidant	285,748	1	285,748	40,212	,000
	VitaminC	7447,873	1	7447,873	2020,477	,000
	Color	144,491	1	144,491	2916,888	,000
	pH	,000	1	,000	,018	,893
storagetime	Antioxidant	1367,605	15	91,174	12,830	,000
	VitaminC	73813,979	15	4920,932	1334,962	,000
	Color	149,350	15	9,957	200,999	,000
	pH	,050	15	,003	115,538	,000
Treatment * storagetemp	Antioxidant	195,677	1	195,677	27,537	,000
	VitaminC	483,544	1	483,544	131,177	,000
	Color	51,047	1	51,047	1030,502	,000
	pH	,004	1	,004	125,255	,000
Treatment * storagetime	Antioxidant	517,052	15	34,470	4,851	,000
	VitaminC	23593,655	15	1572,910	426,703	,000
	Color	57,286	15	3,819	77,096	,000
	pH	,055	15	,004	127,572	,000
storagetemp * storagetime	Antioxidant	112,959	15	7,531	1,060	,400
	VitaminC	1389,501	15	92,633	25,130	,000
	Color	10,882	15	,725	14,646	,000
	pH	,030	15	,002	70,806	,000
Treatment * storagetemp * storagetime	Antioxidant	163,872	15	10,925	1,537	,101
	VitaminC	2232,908	15	148,861	40,383	,000
	Color	14,458	15	,964	19,457	,000
	pH	,053	15	,004	122,268	,000
Error	Antioxidant	909,572	128	7,106		
	VitaminC	471,833	128	3,686		
	Color	6,341	128	,050		
	pH	,004	128	,000		
Total	Antioxidant	1153225,823	192			

Table A.15 ANOVA table for shelf life Analysis of Carrot Juice (cont'd).

	VitaminC	639720,271	192
	Color	12465,031	192
	pH	7484,831	192
Corrected Total	Antioxidant	3654,614	191
	VitaminC	192406,293	191
	Color	568,424	191
	pH	,199	191

a R Squared = ,751 (Adjusted R Squared = ,629)
b R Squared = ,998 (Adjusted R Squared = ,996)
c R Squared = ,989 (Adjusted R Squared = ,983)
d R Squared = ,982 (Adjusted R Squared = ,972)

APPENDIX B

FRUIT AND VEGETABLE PROCESSING

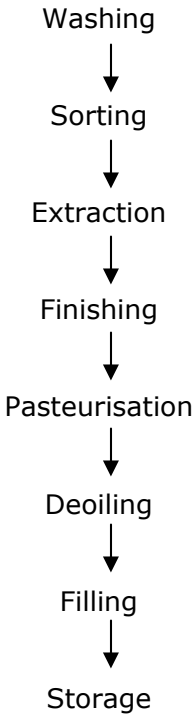

Figure B.1 Orange Juice Processing.

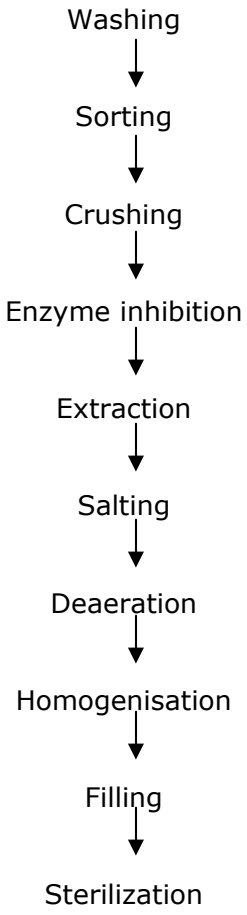

Figure B.2 Tomato Juice Processing.

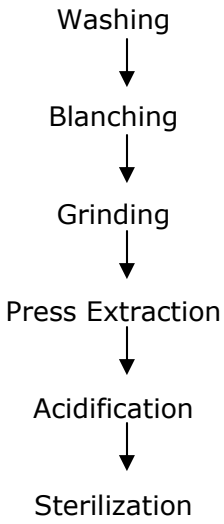

Figure B.3 Carrot Juice Processing.