High Pressure processing of avocado products

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PROJECT REPORT

HIGH PRESSURE PROCESSING OF AVOCADO PRODUCTS

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Report for:
Horticulture Australia Limited
Project AV05001

Prepared by: Dr Mala Gamage *et al*
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Project Details

AV05001

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Project Aim

The objective of this study was to investigate extension of shelf life of fresh-cut avocado halves, slices and puree by HPP and determine the feasibility of using refrigerated storage for 4 weeks.

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MEDIA SUMMARY

Avocado products were the first international success story for high pressure processing (HPP), and they remain a highlight use of the technology. Hydrostatic pressures of up to 700 MPa (100,000 psi or 7000 bars) are used to inactivate bacteria without affecting the food's delicate flavour notes and texture, which can be negatively impacted by thermal processing.

However, the technical know-how required for effective, safe manufacture and distribution of avocado products is not readily available in the public domain. This project investigated essential technical aspects including the microbiological, physical and processing properties of HPP avocado products.

The experiments concentrated on:

- Optimal texture of raw material for HPP-treated Hass avocado pulp, halves and slices
- Packaging material selection
- Processing temperature's effect on the product's final quality and storage stability
- A microbiological challenge study with *Listeria monocytogenes*
- Optimised treatments and processing conditions; and understanding the effect of processing condition on the microbiological, sensory, physical and chemical properties of the processed products during storage at 4°C for up to four weeks
- Processing and evaluation of the Shepard variety under the previously optimised processing conditions for the Hass variety.

This study found that high pressure can be used successfully to process avocado products with the right processing and storage conditions to give products of excellent quality and sufficient shelf-life under good refrigeration. HPP inactivated high levels of *L. monocytogenes,* the major pathogen of concern for refrigerated products, in selected inoculated avocado products challenged with this pathogen. Good temperature control of the product at all times, in conjunction with the proper application of a HACCP plan, and good hygienic practices, are essential to minimise food safety risk and achieve four weeks shelf life. Pre-treatments, selected packaging and HPP could maintain the colour and texture of avocado products at a desirable level for 4 weeks at 4°C. Similar conditions may be used for the Hass and Shepard varieties.

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TECHNICAL SUMMARY

High pressure processed (HPP) avocado products are a highlight of HPP food products in international markets. However, the technical know-how related to the successful processing and distribution of avocado products is not readily available in the public domain. Hence, this project was conducted to obtain the essential technical knowledge on the processing, microbiological and physical properties of high pressure processed avocado products.

Using the Hass variety, the experiments conducted during this project concentrated on:

- The optimisation of texture requirements of raw material for HPP treated avocado halves and slices;
- Packaging material selection
- The effects of processing temperature on the final quality and storage stability of the product.
- Microbiological challenge study with *Listeria monocytogenes*
- Optimisation of the processing conditions and to identify the effects of processing condition on the microbiological, sensory, physical and chemical properties of the processed products during storage at 4°C for 4 weeks.
- Furthermore, the Shepard variety was processed and evaluated under the best conditions determined for the Hass variety.

The findings from this study showed:

- That ripening avocado fruits to a texture ≥7.0 N (with peel texture readings) is necessary to withstand the peeling, halving, packaging and HPP treatments and to give a final product with a desirable firmness.
- The importance of using packaging materials which have an oxygen transmission rate of <5 cc/m^2/24hr (at 23°C, 50% RH) before and after HPP (at 600 MPa for 3min at 22°C vessel and water temperature before pressurisation). In this study Amcor retort pouches were successfully used for packaging of avocado pulp, and Winpack 7000 packs for slices and halves prior to HPP processing.
- Detectable differences were not observed in the quality of avocados when processed at 4°C and 22°C.
- It was found that HPP (600 MPa for 3 min) of both vacuum packaged avocado pulp and quarters (acidified to pH 5 or unacidified at the natural pH of about 6.5) provided an initial >6 $\log_{10}$ cfu/g reduction of *L. monocytogenes* added to these products in challenge assessment trials. No growth or recovery of viability of *L. monocytogenes* was observed in the pressure treated products after storage for 7 days at 4°C.
- Process optimisation trials (HPP at 600 MPa for 1, 3 and 5 min) revealed that both acidified and unacidified avocado pulp, slices and halves had very low standard plate and yeast and mould counts during storage for 4 weeks at 4°C and no growth of *enterobacteriaceae* or lactic acid bacteria were observed for the first 3 weeks. Differences in microbial counts between the evaluated treatment combinations (acidification x HPP time) were not observed.
- In pack colour measurements (L, a*, b*) and Instron texture measurements also revealed no differences in material properties between the treatment combinations evaluated in this study.
- The small scale consumer type sensory tests found that the acidification reduced the acceptability of avocado pulp and gave a noticeable but acceptable acidic taste to slices and halves. Effects of HPP exposure times were not observed on the sensory parameters.

The sensory assessment found that acidification with ascorbic acid was most effective in minimising the browning of HPP treated avocado slices and pulp.

HPP has been shown to be effective in enhancing the shelf stability of avocado products with minimal impact on the quality of the fruit. Findings of this study indicated that HPP (600 MPa for 3 min) could be successfully used in combination with acidification of avocado pulp (at pH 5 only) for the commercial manufacture of high quality, avocado products stored at 4°C for up to 4 weeks**.**

Un-acidified pulp and only externally acidified avocado slices and halves HPP processed may be kept below 3°C, or if this temperature cannot be assured, should not be kept for more than 10 days between 3 and 8°C, according to the C&CFRA authorative international code of practice.

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1.0 INTRODUCTION

This project was initiated by the Avocado Marketing Co-Operation. According to a market study conducted by AVOMAN, the internal quality of ripe whole avocados was alarmingly poor. Fourteen to twenty five percent of the fruits had flesh browning, internal rots and chilling injury which were unable to be detected from the external appearance of the fruit (Ledger & Barker, 1993). If processed avocado products that exhibit fresh-like characteristics were available in the marketplace, they would generate strong consumer demand for avocado products for general household as well as use by the food service industries. Australia has a year round supply of avocados and other perishable produce suitable for high pressure processing (HPP). Several Australian groups have shown a strong commitment to commercializing HPP once the technical, marketing and logistical issues are resolved, thereby providing a commercially viable package. Overseas experience of HPP processing has shown that over 50% of new operations were successful, which then rapidly expanded.

The key technology to be explored in this study is HPP, with a commercially viable pressure level of up to 600 MPa (93,000 Psi). At this pressure, bacterial cells, yeasts and moulds are inactivated; however, bacterial spores are not affected. Enzymes such as polyphenol oxidase, which causes browning of cut products, are often not completely inactivated by HPP. High pressure treated avocado products have become very successful in overseas markets for refrigerated distribution. However, the details of the process used to achieve reproducible quality, shelf-life and food safety are not available and need to be developed and validated before any national commercial production can be considered.

2.0 LITERATURE REVIEW

Avocado (*Persea americana* Mill.) is a highly caloric fruit containing high levels of unsaturated fat (Werman and Neeman1986; Swisher 1988; Kaisier and Wolstenholme, 1994). Moreover, avocado is rich in lipo- and water-soluble vitamins, especially A and C (Batista *et al*. 1993). Avocado ranks the highest in the beta-sitosterol (76 mg/100g) content among the 20 most commonly consumed fruits in USA. Beta –sitosterol is a predominant phytosterol that could reduce the intestinal cholesterol absorption and decrease hepatic cholesterol synthesis (Duester 2001).

Avocado is a highly perishable fruit with a high metabolic rate resulting in a shelf-life of only 3–4 weeks when stored at optimum temperature and relative humidity (Yahia and Gonzalez-Aguilar 1998). Therefore avocados are processed in to different forms such as halves, slices, chunks and pulp to extend the shelf life of ripe fruit. The major problems encountered in avocado processing are:

1. Enzymatic browning
2. Degradation of chlorophyll
3. Microbial growth due to fairly high pH which leads to spoilage and potential health hazards.
4. Deterioration of the mild flavor due to the application of preservation treatments.

Generally treatments such as heat and/or acidification are applied to prevent browning and microbial growth in fruit products. However, these treatments result in the breakdown of chlorophyll and also change the flavor of fresh-like avocado products (Soliva-Fortuny *et al.* 2002; El-Al 1994; L´opez *et al.* 1994; Grajales-Lagunes *et al.* 1995). Freezing is used in combination with anti browning agents. However, Iturriaga *et al.* (2002) reported microorganisms could survive at frozen state (-18°C) for long time such as 58 weeks.

Consumers are increasingly demanding fresh-like, convenient and healthier foods with fewer preservatives which are known as minimally processed foods. The demand for minimally processed avocado products has urged food manufacturers to use mild preservation techniques such as refrigeration, modified-atmosphere packaging and nonthermal processing techniques or a

combination of these techniques (Leistner and Gorris, 1995; L´opez-Malo *et al*. 1998). Lopez-Malo *et al*. (1999) reported the use of high pressure processing for preservation of colour in avocado puree.

However, minimally processed foods are a good media for growth of microorganisms and represent a potential health risk. The microbiological risks vary substantially with the type of product and the process used for preservation (Alzamora *et al.* 1998).

2.1 Enzymatic Browning:

Avocados are highly susceptible to enzymatic browning. Enzymatic browning reactions are catalyzed by polyphenol oxidase (PPO) activity. Enzymatic browning reactions affect the color of avocado pulp upon halving, slicing and pureeing (Dorantes *et al.* 1998). A direct relationship between the browning susceptibility of avocado fruit and PPO activity was found by Kahn (1975). PPO is a copper-containing enzyme which, in the presence of oxygen, catalyzes the oxidation of phenolic substrates into quinones (Escribano *et al*. 1997). Quinones in turn, are polymerized to brown, red or black pigments (Esp´ın *et al.* 1996). Golan *et al.* (1977) reported that the browning rate of avocados is not only correlated with PPO activity but also with total phenol content of the fruit. Some studies have been carried out on kinetic models to describe avocado PPO activity (Dizik and Knapp 1970).

2.1.1 Prevention Of Browning:

Control of enzymatic browning during processing and storage is important to preserve the original appearance of fruit products. Several methods have been used to inhibit the PPO activity in fruits and vegetables. Addition of chemicals (Janovitz-Klapp *et al.*1990; Lozano *et al.* 1994; Fujita *et al*. 1995), pH adjustment (Wesche-Ebeling and Montgomery, 1990; Siddiq *et al*. 1992), exclusion of oxygen (Paulson *et al*. 1980), refrigeration (Lozano *et al.* 1994) and thermal treatments (Vamos-Vigyazo 1981; Silva and Nogueira 1983) are among the most effective. Traditional preservation technologies rely on using 'extreme conditions' like heat treatments or the addition of chemicals for both microbial and enzymatic stabilization. These preservation techniques extensively damage the organoleptic, nutritional and physico-chemical properties of the food (Chirife, 1993; Hayakawa and Timbers 1997). It has been reported that heating leads to the development of bitter off-flavors in avocado products (Ben-et *et al*. 1973). Heating in the presence of acids leads to the breakdown of chlorophyll in heat treated avocado products.

In order to overcome heat related problems the application of a combination of treatments such as a slight reduction of pH, reduction of water activity, addition of preservatives at low concentration, refrigeration and/or modified atmosphere packaging have been used with avocado products. The combined effect of these hurdles are reported to assure the microbial stability as well as the original sensorial and nutritional characteristics of the food, making it more convenient and healthy (Leistner and Gorris, 1995; Alzamora *et al*. 1998).

L´opez-Malo *et al*.(1998) investigated the effects of high hydrostatic pressure and low pH on PPO and color of avocado puree. They concluded that by combining hurdles such as HPP, low pH and refrigerated storage it is possible to obtain an acceptable color even with a considerably high (45%) residual PPO activity. Weemaes, *et al*. (1999) studied the kinetics of avocado PPO inactivation by combined use of pH, high pressure and thermal treatments.

Different organic acids such as malic, phosphoric, tartaric, citric and ascorbic have been used for lowering of pH as well as for inhibition of PPO activity. Inhibition of PPO activity is reported to be due to the lowering of pH below the optimum range (Almeida and Nogueira 1995) and the chelation of the copper ion in the prosthetic group of the PPO enzyme (Ponting 1960). The inhibitory effect of ascorbic acid is ascribed to the reduction of the *o*-quinones, generated by the action of the PPO, back to the phenolic substrate (Hsu *et al*. 1988).

In a study where anti browning (ascorbic and EDTA) and antimicrobial compound (sorbic acid) were evaluated in combination with different packaging atmospheres (air, N2 and vacuum), the behavior of color changes have been described with a first-order fractional conversion model for

L, a* values and color difference (ΔE), resulting from ΔL and Δa*. The rate constants for L, a* and ΔE and color stabilization values (non zero value of the parameter upon storage) were strongly dependent on the added antioxidant. The packaging atmosphere and the addition of sorbic acid also entailed a significant effect on kinetic constants, but especially on browning stabilization values defined by the first-order model. (Soliva-Fortuny *et al.* 2002).

2.2 Chlorophyll Degradation:

According to Watada *et al.* (1990) the changes in colour of avocado puree could be due to the enzymatic browning (catalyzed by PPO enzymes) and the destruction of chlorophylls by degenerative enzymes such as chlorophyll oxidase, chlorophyllase, or lipolytic acid hydrolase from the bruised cells. It is reported that some additives cause changes to the characteristic green color of avocado puree to olive brown color due to the replacement of magnesium ions for hydrogen ions in chlorophylls (Guzman *et al.* 2002).

2.3 Micro Flora Of Avocado Products:

Yeast and mould populations predominate the indigenous microflora of avocado. *Colletotrichum gloesporioides* is reported to be a common fungi found on ripening avocado fruits (Madi *et al.* 2003).

Soliva-Fortuny *et al.* (2004) reported the presence of aerobic mesophilic microorganisms in avocado purees and the reported initial counts were in the range of 3 $\log_{10}$(CFU/g). It was mentioned that the majority of these organisms were yeast and mould. Palou *et al*. (2000) reported initial yeast and mould counts of < 1.0 $\log_{10}$cfu/g and standard plate of 4.0 $\log_{10}$cfu/g in guacamole samples prior to processing (Soliva-Fortuny *et al*. 2002).

It has been reported that certain stimuli such as inoculation with fungi, exposure to ethylene and carbon dioxide, low temperature and wounding can enhance the production of antifungal diene compounds in avocado fruits (Madi *et al* 2003). The effect of these diene compounds on the microbial flora of processed products is not reported.

2.4 High Pressure Processing

Consumer demand for minimally processed, microbiologically safe, stable food products that are additive free, has stimulated the interest of food companies in high-pressure processing (Hendrix *et al.* (1998).

High pressure processing (HPP) is a nonthermal method of food preservation that has the ability to inactivate microorganisms while maintaining the fresh-like qualities of many food products. HPP, also described as high hydrostatic pressure (HHP), or ultra-high pressure (UHP) processing. During HPP food products are subjected to pressures between 100 and 800 MPa, in packaged or unpackaged forms. Typically pressures of 300 to 700 MPa are utilised to extend the shelf life and improve the safety of foods (Hoover *et al.* 1989).

Process temperature during pressure treatment could be range from below 0°C to above 100°C. Commercial exposure times could range from a millisecond pulse to a treatment time of over 1200 s (20 min).

HPP acts instantaneously and uniformly throughout a mass of food, independent of its size, shape, and composition. Thus, package size, shape, and composition are not considered important factors in process determination. The work of compression during HPP treatment will increase the temperature of foods through adiabatic heating approximately 3°C per 100 MPa, depending on the composition of the food. In foods that contains a significant amount of fat, such as butter or cream, the temperature rise can be larger. Foods cool down to their original temperature on decompression if no heat is lost to or gained from the walls of the pressure vessel during the hold-time at pressure (FDA/CFSAN 2000). Lower processing temperatures could be used to overcome the effect of adiabatic heating on heat sensitive food products.

While the temperature of a homogenous food (one with less than 25% fat) will increase uniformly due to compression, the temperature distribution in the mass of food during the holding period at

pressure can change due to heat transfer to or from the walls of the pressure vessel. The pressure vessel must be held at a temperature equal to the final food temperature increase from compression for truly isothermal conditions. Temperature distribution must be determined in the food and reproduced at/with each treatment cycle if temperature is an integral part of the HPP microbial inactivation process specification (FDA/CFSAN 2000).

2.5 Summary Of Critical Process Factors In HPP

The critical process factors in HPP include pressure, time at pressure, time to achieve treatment pressure, decompression time, treatment temperature (including adiabatic heating), product initial temperature, vessel temperature distribution at pressure, product pH, product composition, product water activity, packaging material integrity, and concurrent processing aids (FDA/CFSAN 2000).

During HPP treatment volume of material decreases as a function of the imposed pressure and an equal expansion occurs on decompression. And so the packaging used for HPP-treated foods must be able to accommodate up to a 15% reduction in volume, and return to its original volume, without loss of seal integrity and barrier properties (FDA/ FSCAN 2000).

2.6 Effect Of HPP On Quality Of Food

HPP has several benefits over thermal processing of food, including uniform and instantaneous transmission of pressure throughout the food, so that it is evenly treated (Smelt 1998).

Pressures used in the HPP of foods appear to have little effect on covalent bonds (Tauscher 1998; 1999) thus, foods subjected to HPP treatment at or near room temperature would not undergo significant chemical transformations due to the pressure treatment. As a result many of the nutrients and flavour compounds of the food are unaffected or minimally affected by high-pressure processing at room temperature, resulting in a product that often has a superior taste, nutritional value and quality compared to thermally processed counterparts (Baxter 2002; Ludikhuyze *et al.* 2002; Yen and Lin 1996; Kimura *et al.* 1994; Cheftel 1991). HPP may be combined with heat to achieve an increased rate of inactivation of microbes and enzymes. Chemical changes in the food generally will be a function of the process temperature and time selected in conjunction with the pressure treatment (FDA/CFSAN 2000).

Conventional thermal processing ensures safety and extends the shelf life of food; however it is often severe and may lead to detrimental changes in the organoleptic and nutritional qualities of the product. High pressure processing (HPP) is a promising nonthermal process because the pressure treatments required to inactivate bacterial cells, yeasts and moulds have a minimal effect on the sensory qualities associated with 'fresh-like' attributes (Mermelstein 1997). Of particular interest are those that tend to prolong the shelf life of ready to eat refrigerated foods by inactivating spoilage and pathogenic microorganisms. Most such products are acid foods, such as fruit juices and jams.

High hydrostatic pressures also can cause structural changes in structurally fragile foods such as strawberries or lettuce. Cell deformation and cell membrane damage can result in softening and cell serum loss. Usually these changes are undesirable because the food will appear to be processed and no longer has fresh-like attributes.

Several foods are currently available on the international market, including pressurised sliced ham in Spain; guacamole, salsa, juices, ready-to-eat meats and oysters in the USA; oysters in Australia; jellies and jams in Japan; and juice and fruit smoothie products in several European countries (Hendrickx 1998: Grant *et al.* 2000). However, the effects of high pressure variables on some of these products have not been reported in detail.

HPP is currently used by a range of companies such as Hormel Foods, Perdue Farms, Avomex, Clearwater Seafoods, Winsom's, Calavo, Motivatit Seafood, Lovitt Farms, Joey Oyster, Jumex and Leahy Orchards (Nutraingredeints 2007).

2.7 Effect Of HPP On PPO Inactivation:

According to Seyderhelm (1996) high pressure can be used as an alternative to high temperature for the irreversible inactivation of enzymes. The pressure needed strongly depends on the enzyme. Some enzymes can be inactivated at room temperature by a few hundred MPa, while others can withstand 1000MPa (Hendrickx 1998). Because of the extreme pressure stability of some food quality enzymes, combined processes (e.g. pressure and temperature) might be necessary for enzyme inactivation at industrially relevant pressures (Farr 1990). In some HPP treated food the flavor and color deterioration during storage were attributed to residual enzyme activity (Horie *et al.* 1991; Cano *et al*. 1997).

PPO has been reported as an extremely pressure-resistant enzyme (Knorr, 1995; Cano *et al.* 1997; Weemaes *et al.* 1998; L´opez-Malo *et al.* 1999; Palou *et al.* 1999). Mushroom and potato PPO are very pressure stable, since treatments at 800 - 900MPa are required for activity reduction (Esthangi *et al.* 1994; Weemases *et al.* 1997). Grape, strawberry, apricot and apple PPO seem to be more pressure sensitive. Apricot, strawberry and grape PPO could be inactivated by pressures exceeding 100, 400 and 600MPa, respectively (Hendrickx 1998). Depending on pH, pressures of 100-700MPa were needed for the inactivation of apple PPO (Anese *et al.* 1995).

For several PPO enzymes, it has been reported that pressure-induced inactivation proceeds faster at lower pH. In addition to pH, pressure inactivation is influenced by the addition of salts, sugars or other compounds. The pressure inactivation of apple PPO was enhanced by the addition of CaCl2 and that of mushroom PPO was enhanced in the presence of 50mM benzoic acid or 5mM glutathione (Weemaes *et al.*1997). It has been demonstrated that the efficiency of high pressure microbial and enzyme inactivation can be improved by applying pressure cycles (Hendrickx *et al*. 1998; Palou *et al*.1998 a,b). Enzyme activity retention after a multi-cycle process was lower than that of a single-cycle process with the same total duration Ludikhuyze *et al*. 1997).

L´opez-Malo *et al.* (1998) investigated the effects of high hydrostatic pressure and pH on PPO and color of avocado puree. Pressure treatment at 689 MPa for 30 min could reduce the PPO activity to below 20% in avocado pulp with a pH of 4.1. They concluded that by combining these preservation factors it is possible to obtain an acceptable color even when the residual PPO activity was considerably high (45%).

Weemaes *et al.* (1999) studied the pressure inactivation of avocado PPO (pH 5.0) in the absence or presence of 10 mM ethylene diaminetetraacetic acid (EDTA), NaCl, or benzoic acid or 0.05 mM 4-hexylresorcinol or glutathione at room temperature (25°C). The inactivation kinetics was described by a fractional conversion model. There appeared to be a resistant enzyme fraction (~10% to 20%), necessitating about 825 MPa for inactivation in the absence of anti-browning agents. EDTA addition resulted in sensitization of the pressure-sensitive enzyme fraction, which was attributed to the acid effect. Benzoic acid or NaCl addition resulted in a marked stabilization, whereas addition of glutathione resulted only in a minor stabilization of the pressure-sensitive enzyme fraction. 4-Hexylresorcinol displayed a sensitizing effect below and a stabilizing effect above 700 MPa. The pressure dependency of the inactivation rate constant was altered ($p < 0.05$) by addition of 4-hexylresorcinol or benzoic acid.

A detailed study of the combined effect of pressure (0.1- 900 MPa) and temperature (25 -77.5°C) on avocado PPO, was conducted by Weemas *et al.* (1997). The inactivation of the enzyme at room temperature was observed at 800-900MPa and there was an antagonistic effect of pressure and temperature at pressures below 250MPa and temperatures exceeding 62.5°C.

However, in a few cases, enzyme activation due to pressure treatment alone has been observed for apple, onion, pear and strawberry PPO (Anese *et al.* 1995; Cano *et al.* 1997).

2.8 Effect Of HPP In Other Enzymes In Avocado Products.

The effects of HPP on chlorophyll oxidase, chlorophyllase, or lipolytic acid hydrolase of avocado is not reported.

Endo-polygalacturonase (PG, EC 3.2.1.15) catalyzes the hydrolytic cleavage of α-(1,4)-galacturonan linkages, and polyuronide degradation, particularly depolymerization, in ripening avocado fruits. It is suggested that the pattern of polyuronide hydrolysis in avocado fruit is strongly regulated by PME (methyl ester levels), and PGs (Wakabayashi *et al*. 2000).

It is reported that PG in tomato is effectively inactivated by HPP (Crelier *et al*. 2001). The activity of PME is reported to be activated by HPP in some commodities. Crelier *et al* (2001) reported that the selective activation of PME and inactivation of PG could be achieved by selecting the right HPP treatment combinations and could result in some advantage in texture retention in tomato products.

2.9 Effect Of HPP On Microorganisms:

In general the efficacy of a preservation technology is influenced by a number of microorganism-related factors. These include the type and form of the target microorganism; the genus, species and strain of microorganism; growth stage; environmental stress selection mechanisms; and sub-lethal injury. Each of these factors influences the resistance of a microorganism to a preservation process, independently of the apparent inactivation capacity of that particular process (FDA/SCAN 2000).

Vegetative cells, including yeasts and moulds, are reported to be rather pressure sensitive; i.e. they can be inactivated by pressures of 300-600MPa (Knorr 1995; Patterson *et al*. 1995). Bacterial spores, on the other hand, are highly pressure resistant, since pressures exceeding 1200 MPa may be needed for their inactivation (Knorr 1995).

Experience with acid foods suggests that shelf-stable (commercially sterile) products, having a water activity close to one, and pH values less than 4.0, can be preserved using a pressure of 580 MPa and a process hold-time of 3 min. This treatment has been shown to inactivate 10^6 cfu/g of *E. coli* O157:H7, *Listeria* spp., *Salmonella* spp., or *Staphylococcus* spp. in salsa and apple juice (FDA/ FSCAN 2000).

Acid foods between the pH values of 4.0 and 4.5 can be made commercially sterile using a pressure of 580 MPa and a hold-time of 15 min. Products would have an initial temperature in the range of 22°C. A HACCP plan is essential to insure that ingredients entering the process have low counts of pathogens and spoilage microbes. Actual hold-time values must be determined from challenge packs and storage studies perhaps twice the length of the intended shelf-life of the product (FDA/ FSCAN 2000). According to Lechowich (1993) preservation of acid foods is, the most likely application of high-pressure processing.

Sterilization of low-acid foods (pH > 4.6), on the other hand, will most probably rely on a combination of high-pressure processing and other (mild) treatments. For both pasteurization and sterilization processes, combined pressure and temperature treatments are frequently regarded as most appropriate (FDA/ FSCAN 2000). Low-acid products could be pasteurized by HPP, so that the treated products are rendered free of pathogens normally associated with the product.

Iturriaga *et al.* (2002) evaluated the potential ability of *L. monocytogenes* to grow or survive in avocado pulp and processed guacamole stored at 22, 4 to 7 and -18°C. Populations of *L. monocytogenes* in avocado pulp stored at 22°C increased from 2 to 6 and 9 log CFU/g after 24 and 48 h, respectively. At 4 to 7°C, the growth rate of *L. monocytogenes* in avocado pulp was greatly decreased; generation time was 8.2 h, in contrast with 1.35 h observed at 22°C. *L. monocytogenes* populations did not increase in processed guacamole either at 22°C for 48 h or at 4 to 7°C for 15 days. The bacteriostatic effect in processed guacamole may have resulted from the presence of added substances, especially citric acid and disodium dihydrogen pyrophosphate.

Aerobic plate counts and coliforms increased in avocado pulp and processed guacamole stored at ambient temperature and under refrigeration (Iturriaga *et al.* 2002). However, these increments did not affect the growth of the pathogen. *L. monocytogenes* (50,000 most probable number [MPN]/g) survived at least 58 weeks in both products stored frozen at -18°C; the final population was 335 MPN/g in avocado pulp and 23 MPN/g in processed guacamole. Although the

composition of avocado fruit differs significantly (high content of lipids and scarcity of simple carbohydrates) from that typical of most fruits, these results underline avocado pulp as a potential vehicle of human listeriosis and indicate that freezing should not be used as the sole mechanism to control this pathogen. This suggests that even frozen avocado products need treatment to reduce the initial microbial load.

Raghubeer *et al* (2000) studied the effects of high hydrostatic pressure (HPP; 545 MPa) on strains of *Escherichia coli* O157:H7, *Listeria monocytogenes,* enterotoxigenic *Staphylococcus aureus,* and nonpathogenic microorganisms in tomato-based salsa at pH 3.9. Products were evaluated for the survival of the inoculated pathogens following HPP treatment and after storage at 4°C and 21 to 23°C for up to 2 months. Inoculated samples without HPP treatment, stored under the same conditions, were also evaluated to determine the effects of the acid environment of salsa on the survival of inoculated strains. None of the inoculated pathogens were detected in the HPP-treated samples for all treatments throughout the storage period. Inoculated pathogens were detected in the non–HPP-treated samples stored at 4°C after 1 month, with *L. monocytogenes* showing the highest level of survivors. In the non–HPP-treated samples stored at 21 to 23°C, *E. coli* and *S. aureus* were not detected after 1 week, but *L. monocytogenes* was detected in low levels. Studies with nonpathogenic strains of the pathogens were conducted at Oregon State University using HPP treatments in a semicontinuous production system. The nonpathogenic microorganisms (*E. coli, Listeria innocua, Listeria welshimeri,* and nonenterotoxigenic *S. aureus*) were inoculated together into a feeder tank containing 100 liters of salsa. Microbiological results of samples collected before HPP treatment and from the aseptic filler were similar to those obtained for the pathogenic strains. No survivors were detected in any of the HPP-treated samples.

2.10 Microorganisms With Greatest Pressure Resistance

The most pressure-resistant pathogenic vegetative cell populations appear to be those of *E. coli* O157:H8 with a D-value of 6 min (k= 0.384/min) at 600 MPa, and *S. aureus* with a D-value of 7.14 min (k = 0.323/min) at 600 MPa (FDA/SCAN 2000).

The most pressure-resistant spores appear to be *C. sporogenes* with a D-value of 16.772 min (k = 0.138/min) at 600MPa (T = 90°C) and *C. botulinum* Type A 62-A with a D-value of 6.7 min (k = 0.344/min) at 827 MPa (T = 75°C). The pressure coefficient z(P) of 1524 MPa at 75°C for *C. botulinum* Type A 62-A constitutes an additional indication of the pressure resistance of the spore populations. A recent report shows little if any inactivation after 30 min of *C. botulinum* 17B and Cap 9B exposure to 827 MPa at 75°C (FDA/SCAN 2000).

As indicated by the D values bacterial endo-spores are the most pressure-resistant life forms known. The most heat-resistant pathogen, and one of the most lethal to human beings, is *C. botulinum*, primarily types A, B, E, and F. As such, *C. botulinum* heads the list of most pressure-resistant and dangerous organisms faced by HPP. Spore suspensions of strains 17B and Cap 9B tolerated exposures of 30 min to 827 MPa and 75°C. Among the spore formers of concern, *Bacillus cereus* has been the most studied because of its facultatively anaerobic nature and very low rate of lethality (FDA/SCAN 2000).

Generally, gram-positive vegetative bacteria are more resistant to environmental stresses than vegetative cells of gram-negative bacteria. This observation commonly applies to pressure resistance as well. Among the pathogenic non-sporeforming gram-positive bacteria, *Listeria monocytogenes* and *Staphyloccocus aureus* are the two most well-studied regarding the use of HPP processing. *Staphyloccocus aureus* appears to have a high resistance to pressure (FDA/SCAN 2000).

There appears to be a wide range of pressure sensitivity among the pathogenic gram-negative bacteria. Patterson *et al.* (1995) have studied a clinical isolate of *E. coli* O157:H7 that possesses pressure resistance comparable to spores. Some strains of *Salmonella* spp. have demonstrated relatively high levels of pressure resistances. Due to the high pressure resistances of these organisms and their importance in food safety, *E. coli* O157:H7 and *Salmonella* spp. are

considered as the key concern in the development of effective HPP food treatments (FDA/FSCAN 2000).

3.0 MATERIALS AND METHODS:

3.1 Avocado

Experiment 1: Effect of packaging material on in pack browning of avocado halves during storage.

Australian grown Sheppard avocados were obtained from Safeway, Werribee.

Experiments 2-5: Evaluation of suitable firmness, pH adjustment of pulp, suitable texture for HPP acid types.

Avocado fruits of Hass variety (class A) were obtained from the SunFresh Avocados in Queensland. Fruits were held at room temperature for ripening. After ripening to the required firmness, fruits were used in the experiments at room temperature.

Experiment 6: Microbial challenge test.

Hass avocados were obtained from the Exotic Fruit Traders at Sydney Markets and held for 24 hours at 20°C prior to treatment.

Experiment 7: Effect of acidification and HPP exposure time at 600 MPa on the microbiological quality, pH, texture and colour of Hass avocado products during 4 weeks storage at 4°C.

Hass avocados (class A) were obtained from SunFresh Avocados in Queensland. Avocados were ripened at room temperature without any ethylene treatment. Hardness of the avocado fruits was measured with peel as described in Section 3.4.5. When the firmness of avocado was ≥7.0 N with peel they were used for experiments.

Experiment 8: Validation of results obtained in experiment 7 using Sheppard variety

Sheppard avocados (class A) were obtained from Sunfresh Avocados in Queensland and were ripened. Texture was measured and used in experiment 8 when the avocado firmness was as ≥7.0 with peel.

3.2 Cleaning Of Avocado Fruit

Experiments 1 -5 and 7-8

Fruits (at room temperature, 22°C) were washed in 200 ppm chlorine solution at 20°C set at pH 11.00 (Milton, Australia) for 5 minutes before each experiment to remove any adhering extraneous matter and to reduce the initial microbial load.

Experiment 6

The fruit, at 20°C, were sanitized in 100 ppm of Tsunami® at 20°C for 5 min prior to halving, peeling and stone removal.

3.3 Preparation Of Avocado Products

The preparation steps for experiments 1-6 are presented in Section 10.1.

3.3.1 Experiment 6

Preparation Of Avocado Pulp

Avocado flesh was pureed with a hand-held stick blender for 3 to 5 minutes until a smooth consistency was reached. The resulting puree with a pH of 6.5 +/- 0.08 was subsequently divided into 4 portions. The portions were then blended with, ascorbic acid, malic acid or equal proportions of ascorbic and malic acid until pH 5 was achieved as per the experiment outline. The remaining portion was left as a control (unacidified).

Preparation Of Avocado Quarters

The avocados were peeled and stoned the halves were cut into quarters and a 5 x 3 cm section was taken from the broadest section of the quarter. This was to enable the produce to fit into the 2L

unit HPP vessel at Food Science Australia, North Ryde, without damaging the structure of the avocado pieces. Avocado pieces were treated with a dip of either 2% w/v ascorbic acid, 2% w/v malic acid or a 1% w/v ascorbic and 1% w/v malic acid solution. A control was prepared by dipping in distilled water. All pieces were dipped for 1 minute and subsequently drained for 3 minutes.

3.3.2 Experiments 7-8

Preparation Of Avocado Halves

Fruits were manually halved and the seed was removed. Peel was removed manually and the peeled halves were used with and without dipping in malic acid solution as outlined in Table 3. Four halves were dipped in 500 ml of acid solution for 5 min. Halves were removed from the solution and placed inside Winpack 7000 bags. Each bag contained two avocado halves.

Preparation Of Avocado Slices

Peeled halves were prepared as for the preparation of avocado halves. Each half was cut into 4 slices manually. Dipping treatments were applied to slices from each half separately as outlined under halves. For slices ascorbic acid treatments were used for dipping treatments. Dipping was conducted as described for halves and placed in Cryovac B471 bags.

Preparation Of Avocado Pulp

Fruits were manually halved and the seed was removed. The pulp was scooped out with a spoon. Two kg batches of pulp were used for each treatment combination. Pulp was blended in a Hobart mixer (Hobart Corporation, USA) using a dough mixing paddle. The required amount of ascorbic acid and malic acid mix (2:1) was used to adjust the pH to 4.4 or 5.00. Pulp was weighed (200g) in to Amcor retort packs.

3.3.3 Packaging Material

The packaging materials used in this study are listed in Table 1.

Table 1 – Packaging material types, properties and suppliers.

Type/ Name	OTR (cc/m2/24 hr @ atm. Pressure)	WVTR (g/m2/24hr @ atm. pressure	Thickness	Composition	Supplier
Holmes	<8 *	<6	70µm	NYLON/EVOH/PE	Cryovac
B471 (Cheese bags)	30	10	58 µm	LLDPE, EVA and PVDC	Cryovac
Retort pouches	<3	<6	70µm	PET (12um) ALOX / ADH / OPA (15um) / ADH / CLEAR PP (70um)	Amcor
WINPACK 7000	0.1#	0.65#	3 mils	mPE-tie-Nylon-EVOH-Nylon-tie-mPE	WINPACK

* at 23ºC/75% RH; ** post retort at 23°C/50% RH ; # cc/100 in^2/24 hr

3.3.4 Packaging

All bags containing avocado products were individually sealed in a Webomatic (Webomatic, Germany) vacuum packaging machine. Vacuum was held at -1.0 bars for 5 seconds. Samples were stored for less than 1hr at 4°C pending HPP treatment.

3.3.5 High pressure Processing Treatment

If not specifically specified, all products were pressure treated at 600 MPa for the duration specified under each treatment at 22°C (sample, HPP vessel and water temperature) in a 35 L high pressure unit (Avure Technologies, Inc., USA). After processing all samples were stored at 4°C for 28 days.

3.4 Analysis Of Avocado Products

3.4.1 Microbiology Tests

Total Plate Counts: Total Plate Count was performed as described in Australian Standard AS5013.1-2004 Food Microbiology Method 1- Examination for specific organisms: Standard plate counts were performed on plate count agar medium (Oxoid, Australia) incubated at 30 ± 1°C for 72 ± 2 hours. Detection limit for this test was 1 Log_{10} cfu/g.

Yeast and mould counts: Yeast and mould count was performed as described in Australian Standard AS1766.2.2-1997 Food Microbiology Method 2.2 - Examination for specific organisms: Colony count of yeasts and moulds on Dichloran Rose Bengal Chloramphenicol medium (DRBC, Oxoid, Australia). Plates were incubated upright at 25 ± 1°C for 5 days. Yeast colonies confirmed by microscopic examination. Detection limit for this test was 2 Log_{10} cfu/g.

Lactic acid bacteria count: Count of lactic acid bacteria was performed on MRS agar (De Man, Rogosa, Sharpe). Plates were incubated under anaerobic conditions at 30°C ± 2°C for 72 hours. Detection limit for this test was Log_{10} cfu/g.

Enterobacteriaceae count: Enumeration of *Enterobacteriaceae* was performed using Violet Red Bile Glucose agar (Oxoid, Australia). Plates were overlaid with the same agar to suppress the growth of non-fermentative gram negative bacteria and incubated at 37°C ± 2°C for 24hours. Detection limit for this test was 1 Log_{10} cfu/g.

3.4.2 Objective Measurement Of Colour

Three replicates of each treatment combination was used to measure the colour (CIE L, a* and b* values) using a Minolta chromameter (CR 300, Japan). According to Wikipedia

> "CIE L*a*b* (CIELAB) is the most complete colour model used to describe all the colours visible to the human eye. The three parameters in the colour model represent the lightness of the colour (L*, L*=0 yields black and L*=100 indicates white), its position between magenta and green (a*, negative values indicate green while positive values indicate magenta) and its position between yellow and blue (b*, negative values indicate blue and positive values indicate yellow)."

3.4.3 Measurement of pH:

The pH of avocado pulp was measured by using an Activon pH meter (Activon, model 210, Australia) and surface pH probe (Sensorex 450CD, USA) after calibrating with buffers at pH 4.0 and 7.0 (Metrohom, Australia) daily before use.

3.4.4 Measurement Of Texture With Penetrometer

Hand-held penetrometer (Fruit Pressure Tester, FT 327, Italy) was used to measure the texture of avocado fruit after removing a thin slice of the peel using an 8 mm probe.

3.4.5 Measurement Of Texture With Instron

The texture of whole fruit with or without skin was determined using an Instron Texture measuring device at a cross head speed of 20 mm per minute with a 100 N weight. The force required to depress a 10 mm spherical probe 2 mm into the fruit with and without skin intact was recorded. Four readings around the fruit at the highest diameter were measured at 3-4 day intervals in five fruits per sample set.

3.4.6 Sensory Analysis

For sensory analysis sample packs were opened and samples were labeled with a random 3 digit number. Labeled samples were presented to the panelists on an open tray in a random order.

Informal sensory analyses were conducted on day 1 using 10 panellists. The panel recorded their results using the form given in section 10.3.

3.5 Experiment 6 - Microbiological Challenge Test

Table 2 - Experimental matrix for challenge testing of avocado products with *Listeria monocytogenes* cocktail.

	Ascorbic acid	Malic acid	Ascorbic & malic acid	Control water
Pulp	Blended with pulp to pH 5	Blended with pulp to pH 5	Blended with pulp to pH 5	Untreated pulp
Quarters	Dipped in 2% solution for 1 min	Dipped in 2% solution for 1 min	Dipped in 2% solution for 1 min (1% ascorbic + 1% malic)	Dipped in distilled water for 1 min

3.5.1 Preparation Of Listeria Monocytogenes Inoculum Cocktail

Strains were selected based on their use in previous HPP experiments and for their known resistance to HPP, and/ or their source being a fruit-based product. One loop full of each *L. monocytogenes* strain (FSA culture numbers 2542, 2655, 2343 and 2345) was resuscitated from glycerol stock (-80°C) in 10 mL TSB with yeast extract (TSBYE) for 24 h at 30°C, aerobically. TSBYE cultures were then subcultured (100µL) into 10mL of TSBYE with 1% total glucose (TSBYEG), and incubated for 24h at 30°C aerobically, in order to acid adapt the strains. To determine the number of viable cells a 1mL aliquot was taken from the final subculture of each strain and serially diluted and spread plated. The counts from these plates were used to calculate the initial inoculum concentration. Five mL was taken from each of the final subcultures for each strain and combined to form a mixed cocktail inoculum.

3.5.2 Inoculation Of Avocado

Following acidulation, 20g +/- 0.5g of avocado pulp or pieces were packed into high oxygen barrier bags (FS7155 Cryovac®). Each bag was individually inoculated with 200µl of the *L. monocytogenes* cocktail so that a concentration of 7.5 $\log_{10}$ cfu/g was achieved. A total of three replicates were prepared for each treatment and each assessment period.

The bags were then double heat-sealed with a mild vacuum expelling as much air as possible in the sealing process. The bags containing avocado pulp were homogenised for 1 min in a stomacher lab blender. All the inoculated bags were then placed into bags containing freshly prepared 0.2% proxitane (active ingredient is peroxyacetic acid) to protect the high pressure unit from contamination in the event that a bag leaked during HPP, and then finally placed in another protective bag.

3.5.3 High Pressure Processing Of Inoculated Avocado

Bags were processed at ambient temperature (~15 to 20°C) in a 2 L high pressure unit (Avure Technologies, USA); at 600 MPa for 3 minutes. Following processing the vacuum sealed bags were removed from the two protective outer bags and dried prior to storage at 4°C. Assessments for recovery of *L. monocytogenes* were conducted two days and seven days after processing.

3.5.4 Recovery Of Listeria From HPP Avocado

L. monocytogenes colonies were recovered using a standard spread plate method. Immediately prior to each assessment period, the bags containing the inoculated avocado were homogenised in a Stomacher for 1 minute. An initial 1 in 4 dilution was made of 5 g +/- 0.1 g of the HPP avocado. Two further 1 in 10 dilutions were then prepared from this initial dilution. The diluent used was TSBYE. Aliquots, 100 µL, of each dilution were spread plated onto duplicate tryptone soya agar (TSA) plates and incubated at 30°C, aerobically for 72 hours. The plates were then examined and counts made of any colonies present and the cfu/g calculated. The limit of detection of the method was 20 cfu/g (1.3 Log cfu/g).

3.6 Experiment 7 – Effect Of Acidification And HPP Exposure Time At 600 MPa On The Microbiological Quality, pH, Texture And Colour Of Hass Avocado Products During 4 Weeks Storage At 4°C.

Experiment plan for was obtained from the statistician to evaluate the effects of

- 3 pH levels (6.50 (+1), 5.00 (0), 4.39 (-1)) and
- 3 HPP exposure times at 600MPa (5 min (+1), 3 min (0), 1 min(-1)).

Table 3: Treatment matrix used to evaluate the effect of % acid used for dipping of Hass avocado halve and slices or pH of the avocado pulp and HPP exposure time.

Day	Run	% acid / pH	HPP time
1	1	-1	-1
1	2	-1	1
1	3	1	-1
1	4	1	1
1	5	0	0
2	6	-1	0
2	7	1	0
2	8	0	-1
2	9	0	1
2	10	0	0
3	11	-1	-1
3	12	-1	1
3	13	1	-1
3	14	1	1
3	15	0	0

This design was used to conduct experiments on 3 avocado products with modifications listed in Table 4.

Table 4: Treatments used in experiment 7 with Hass avocado as per matrix in Table 3.

Product type	% acid / pH levels	HPP time	Packaging used
Halves	No acid added (+1) 1% Malic acid (0) 2% Malic acid (-1)	5.0 min (+1), 3.0 min (0), 1.0 min(-1)	Winpack 7000
Slices	No acid added (+1) 1% Ascorbic acid (0) 3% Ascorbic acid (-1)	5.0 min (+1), 3.0 min (0), 1.0 min(-1)	Cryovac B471
Pulp*	6.5 (+1) 5.0 (0) 4.3 (-1)	5.0 min (+1), 3.0 min (0), 1.0 min(-1)	Amcor retort packs

*Ascorbic acid: malic acid 2:1 was used to adjust the pH of pulp

Samples were analysed as outlined in Table 5.

Table 5: Test and test frequency of Hass avocado products of experiment 7 during 4 weeks storage at 4°C

Test	Storage intervals
Microbiology (TPC, Y&M, Lactic count, *Enterobacteriaceae*)	0, 1, 2 , 3 & 4 weeks 3 replicates/ treatment/week
Colour (L*, a*, b*)	3 replicates/ treatment Same sample were repeatedly measured over 4 weeks
pH and texture	0, 1, 2, 3 & 4 weeks 3 replicates/ treatment /week
Sensory analysis	Day 1 (ten panellists)

Statistical analysis of data: The analysis of sensory data on week 0, pH on week 0 and 4 were conducted using the following steps:

1. Examine whether the day effect needs to be in the model.
2. Perform an analysis with a 3 x 3 factorial treatment structure, and
3. Examine the predicted/adjusted treatment means and their SEDs.
4. Perform a response surface analysis.

3.7 Experiment 8 – Validation Of Results Obtained In Experiment 7 Using Sheppard Variety

Fruits were cleaned as in section 3.2 and samples were prepared as in section 3.2. Slices, halves and pulp samples were subjected to the treatments outlined in Table 6.

Table 6: Treatments used in experiment 8 with Sheppard avocado.

Product type	pH levels	HPP time	Packaging used
Slices	Control 3% Ascorbic acid dip* Control + HPP 3% ascorbic acid dip + HPP	- - 3.0 min 3.0 min	Cryovac B471
Halves	Control 1% Malic acid dip** Control + HPP 1% Malic acid dip + HPP	- - 3.0 min 3.0 min	Winpack 7000
Pulp	Control pH 5.0 pulp*** Control + HPP pH 5.0 pulp + HPP	- - 3.0 min 3.0 min	Amcor retort packs

* The 1% malic acid concentration was selected due to unpleasant flavour observed at higher concentrations. Also the pH difference in 1% and 2%solutions were not contributing to a remarkable change the final surface pH of the treated halves.
** Ascorbic acid at 3% level results a surface pH of 5.00 and provided the maximum protection with minimum flavour change.
*** Ascorbic: malic = 2:1 was used to adjust the pH of pulp

4.0 RESULTS

Results of experiments 1-5 were presented to HAL and Avocado Marketing Co-operative in Milestone Report 1. They are presented in section 10.1 of this report.

4.1 Experiment 6:: Microbiological Challenge Test

This experiment was conducted to identify the effect of acidification and the application of HPP (600 MPa for 3 min) on *L. monocytogenes* inoculated at a initial microbial load of 7.5 $\log_{10}$ cfu/g. Following storage for two and seven days at 4°C, no viable colonies of *L. monocytogenes* were recovered from the non acidulated control samples or any of the acid treated avocado pulp or pieces (Table 7 and Table 8).

Table 7: Recovery of *Listeria monocytogenes* from avocado quarters acidulated with ascorbic or malic acid and HPP treated for 3 min at 600 MPa.

Acidulation treatments	Replicates	$\log_{10}$ cfu/g reduction 2 days after HPP**	$\log_{10}$ cfu/g count (recovery or growth) 7 days after HPP
Control (water)	1	>6.2	<1.3
	2	>6.2	<1.3
	3	>6.2	<1.3
Ascorbic acid 2%	1	>6.2	<1.3
	2	>6.2	<1.3
	3	>6.2	<1.3
Malic acid 2%	1	>6.2	<1.3
	2	>6.2	<1.3
	3	>6.2	<1.3
Ascorbic acid 1% plus Malic acid 1%	1	>6.2	<1.3
	2	>6.2	<1.3
	3	>6.2	<1.3

* Initial concentration = 7.5 $\log_{10}$ cfu/g ** Limit of detection = 1.3 $\log_{10}$ cfu/g

Table 8: Recovery of *Listeria monocytogenes* from avocado pulp acidulated with ascorbic and malic acid mixture (2:1) to pH 5.0 and HPP treated for 3 min at 600 MPa.

Acidulation treatments	Replicates	$\log_{10}$ cfu/g reduction 2 days after HPP**	$\log_{10}$ cfu/g count (recovery or growth) 7 days after HPP
Control (no addition)	1	>6.2	<1.3
	2	>6.2	<1.3
	3	>6.2	<1.3
Ascorbic acid to pH 5.0	1	>6.2	<1.3
	2	>6.2	<1.3
	3	>6.2	<1.3
Malic acid to pH 5.0	1	>6.2	<1.3
	2	>6.2	<1.3
	3	>6.2	<1.3
Ascorbic and malic acid to pH	1	>6.2	<1.3
	2	>6.2	<1.3
	3	>6.2	<1.3

* Initial viable count = 7.5 $\log_{10}$ cfu/g ** Limit of detection = 1.3 $\log_{10}$ cfu/g

4.2 Experiment 7: Effect Of Acidification And HPP Exposure Time At 600 MPa On The Microbiological Quality, pH, Texture And Colour Of Hass Avocado Products During 4 Weeks Storage At 4°C.

This experiment was conducted to identify the effect of acidification and the application of HPP on the microbiological parameters, sensory properties, colour, texture and the pH fluctuations of the product during storage at 4°C.

4.2.1 Pulp

Microbiological Parameters

Table 9: Standard plate counts of avocado pulp acidified with or without ascorbic acid + malic acid (MA) mix, packed in Amcor bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 4 weeks.

Treatment	Storage time (weeks)				
	0	1	2	3	4
pH 4.4 + HPP 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 4.4 + HPP 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 4.4 + HPP 5 min	<1.0	<1.0	<1.0	<1.0	2.5
pH 5.0 + HPP 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 5.0 + HPP 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 5.0 + HPP 5 min	<1.0	<1.0	<1.0	<1.0	1.4
pH 6.5 + HPP 1 min	<1.0	<1.0	<1.0	<1.0	2.1
pH 6.5 + HPP 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 6.5 + HPP 5 min	<1.0	<1.0	<1.0	<1.0	1.6
pH 4.4 + No HPP	2.4	NA	NA	NA	NA
pH 5.0 + No HPP	3.4	4.6	NA	NA	NA
pH 6.5 + No HPP	3.3	3.4	NA	NA	NA

NA – not assessed : Minimum detectable level – <1.0 $\log_{10}$ cfu/g

At the planning stage it was not planned to asses the control samples that had no HPP treatment after 1 week of storage.

Standard plate counts: After pressure treatment SPC was not detected in stored samples until week 3. At week 4 low counts of <2.6 $\log_{10}$ cfu/g were observed in treatments pH 4.4 + HPP 5 min, pH 5.0 + HPP 5 min, pH 6.5 + HPP 1 min and pH 6.5 + HPP 5 min (Table 9). Control samples showed an increase in viable count after week 1 storage.

Yeast and mould counts: Recovery in the viable count of yeasts and moulds after pressure treatment was only observed at very low levels (≤ 2.0 $\log_{10}$ cfu/g) in samples throughout the storage period (Table 10).

Table 10: Yeast and mould counts of avocado pulp acidified with or without ascorbic acid + malic acid (MA) mix, packed in Amcor bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 4 weeks

Treatment	Storage time (weeks)				
	0	1	2	3	4
pH 4.4 + HPP 1 min	2.3	<1.0	<1.0	<1.0	2.0
pH 4.4 + HPP 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 4.4 + HPP 5 min	<1.0	1.1	<1.0	<1.0	1.7
pH 5.0 + HPP 1 min	<1.0	1.4	<1.0	<1.0	<1.0
pH 5.0 + HPP 3 min	<1.0	1.4	<1.0	<1.0	<1.0
pH 5.0 + HPP 5 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 6.5 + HPP 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 6.5 + HPP 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 6.5 + HPP 5 min	<1.0	1.1	<1.0	<1.0	2.0
pH 4.4 + No HPP	2.3	NA	NA	NA	NA
pH 5.0 + No HPP	2.4	2.5	NA	NA	NA
pH 6.5 + No HPP	3.3	3.5	NA	NA	NA

NA – not assessed : Minimum detectable level – <2.0 $\log_{10}$ cfu/g

Table 11: Enterobacteriaceae counts of avocado pulp acidified with or without ascorbic acid + Malic acid (MA) mix, packed in Amcor bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 4 weeks.

Treatment	Storage time (weeks)				
	0	1	2	3	4
pH 4.39 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 4.39 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 4.39 5 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 5.00 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 5.00 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 5.00 5 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 6.50 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 6.50 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 6.50 5 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 4.39 - No HPP	2.2	NA	NA	NA	NA
pH 5.00 - No HPP	2.8	1.0	NA	NA	NA
pH 6.50 - No HPP	2.6	2.97	NA	NA	NA

NA – not assessed : Minimum detectable level – <1.0 $\log_{10}$ cfu/g

Enterobacteriaceae counts: Treatment with pressure resulted in an initial reduction of *Enterobacteriaceae* to below the detection limit and no recovery of viability was observed throughout the 4 week storage period (Table 11). The viable counts of the unacidified control samples doubled within one week of storage.

Lactic acid bacteria: No recovery in the viable count of Lactic acid bacteria was observed after pressure treatments, throughout the 4 week storage period (Table 12). The viable counts of the unacidified control samples doubled within one week of storage.

Table 12: Lactic acid bacteria counts of avocado pulp acidified with or without ascorbic acid + malic acid (MA) mix, packed in Amcor bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 4 weeks.

Treatment	Storage time (weeks)				
	0	1	2	3	4
pH 4.4 + HPP 1 min	3.6	<1.0	<1.0	<1.0	<1.0
pH 4.4 + HPP 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 4.4 + HPP 5 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 5.0 + HPP 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 5.0 + HPP 3 min	2.6	<1.0	<1.0	<1.0	<1.0
pH 5.0 + HPP 5 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 6.5 + HPP 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 6.5 + HPP 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 6.5 + HPP 5 min	<1.0	<1.0	<1.0	<1.0	<1.0
pH 4.4 + No HPP	2.2	NA	NA	NA	NA
pH 5.0 + No HPP	3.2	1.7	NA	NA	NA
pH 6.5 + No HPP	2.9	3.2	NA	NA	NA

NA – not assessed : Minimum detectable level <1.0 $\log_{10}$ cfu/g

In Pack Colour

L* value (lightness): L* value showed an increase, indicating an increase in lightness, up to 3 weeks and then showed a decreasing trend (Figure 1). Acidified pulps showed higher L values (lightness) than unacidified pulp up to week 3. On week 4 all samples showed L values in the same range. Difference between HPP treatment times were not observed in acidified or unacidified pulp.

a* values (greenness): The a* values showed a gradual increase over the storage period indicating a decrease in greenness of treated and control samples. Samples acidified to pH 4.4 showed an increase in a* value which is higher than at the pH 5.00 and unacidified samples. Difference between HPP treatment times were not observed in acidified or unacidified pulp.

b* values (yellowness): At 0 and 1 week b* values were higher (indicating more yellowness) than at week 2 – 4 (Figure 3). Particular trend in b* value was not observed in general for the whole experiment or for individual treatment combinations. Differences in b* value due to HPP treatment time or acidification was not observed.

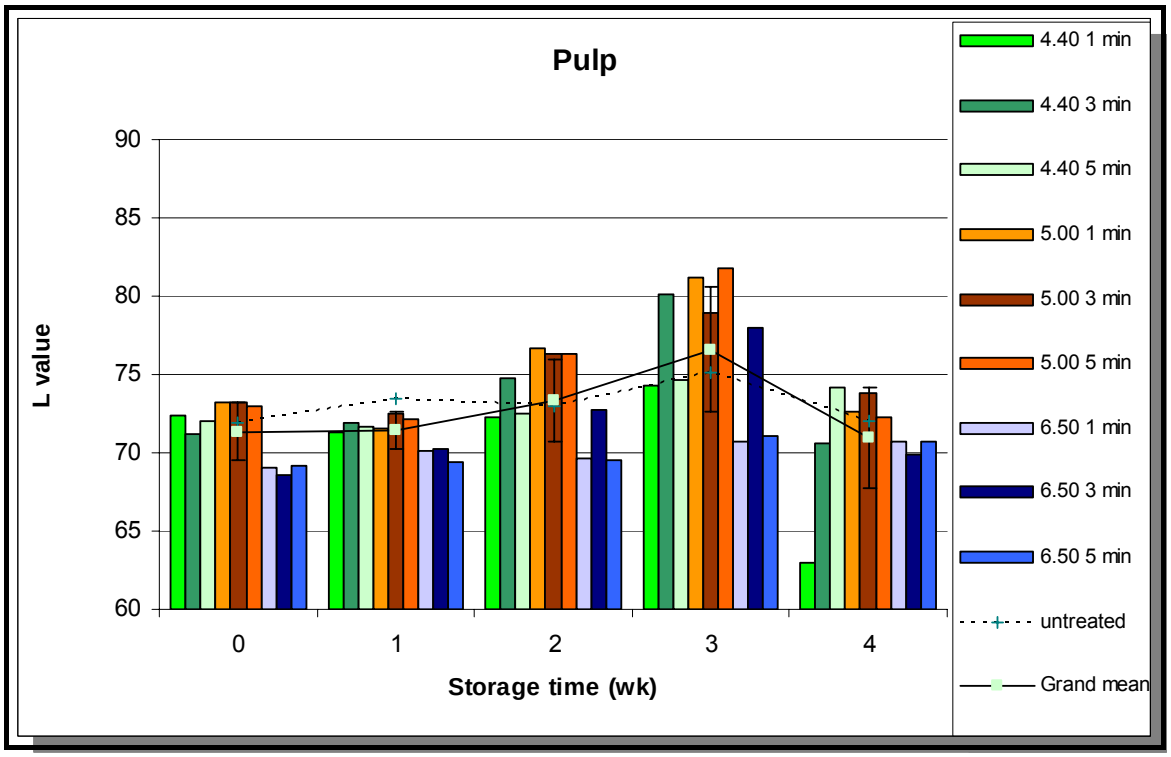

Figure 1: L* values of avocado pulp acidified with or without ascorbic acid + Malic acid (MA) mix, packed in Amcor bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 4 weeks (L*=0 yields black and L*=100 indicates white; Grand mean – average of all treatments; Untreated – No HPP and no acidification).

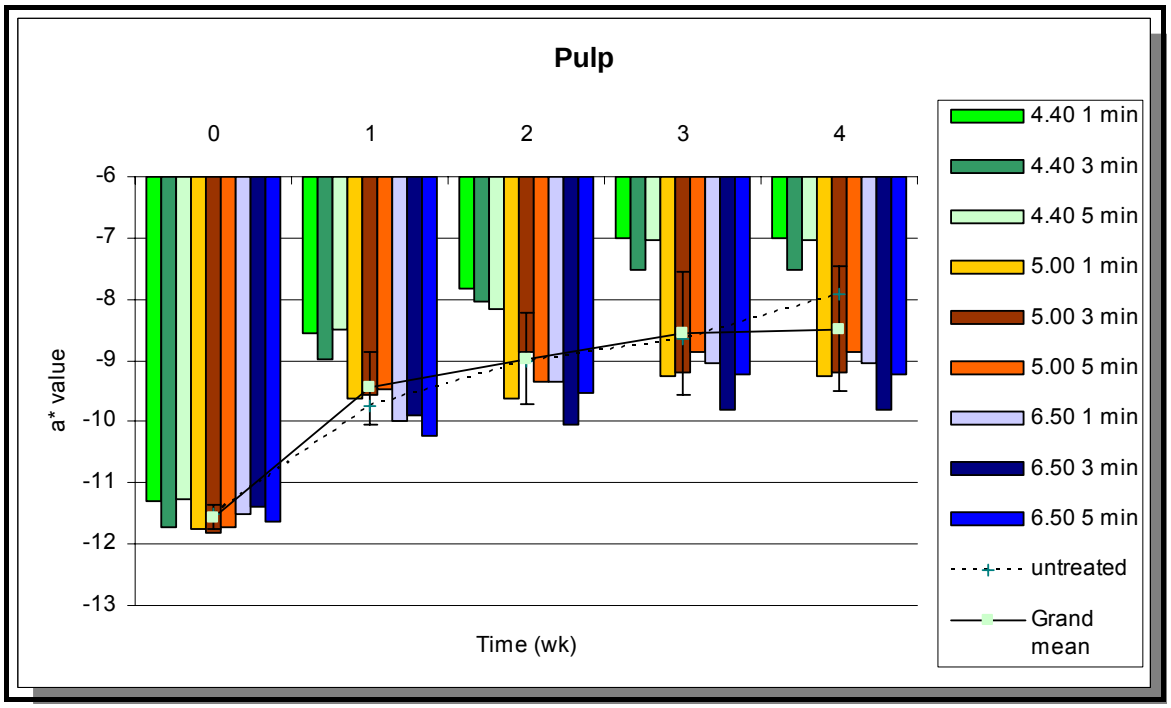

Figure 2: – a* values of avocado pulp acidified with or without ascorbic acid + Malic acid (MA) mix, packed in Amcor bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 4 weeks (-a = indicates green : +a = indicates magenta; Grand mean – average of all treatments; Untreated – No HPP and no acidification).

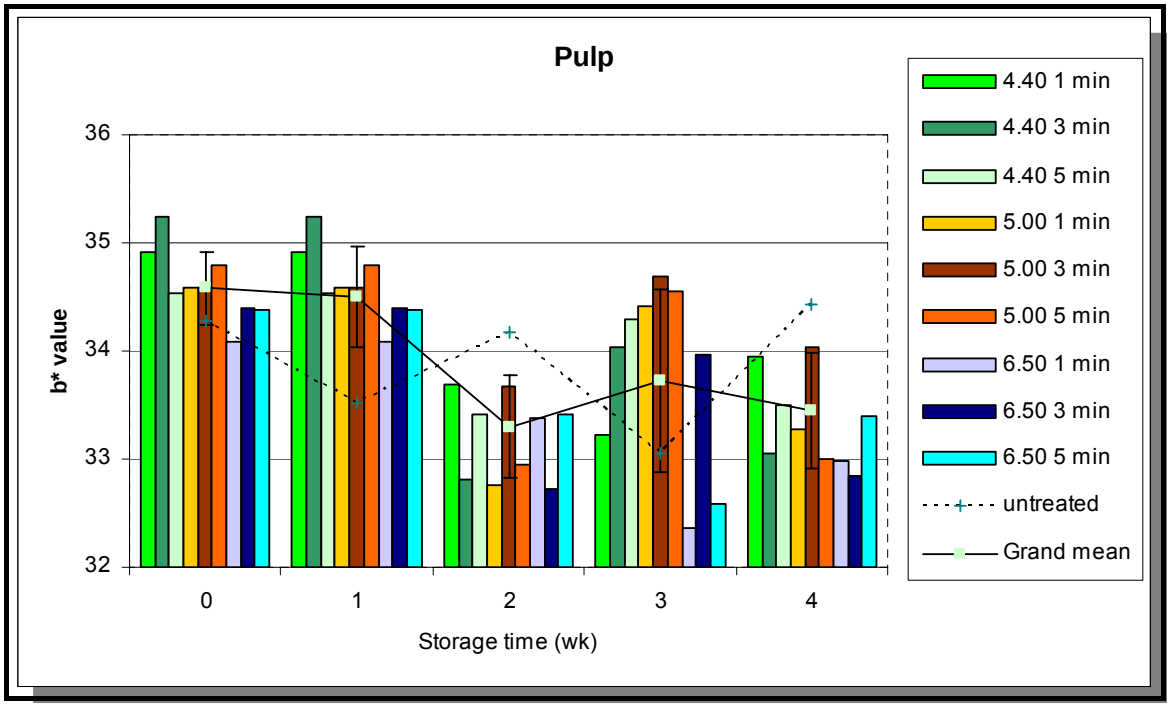

Figure 3: – b* values of avocado pulp acidified with or without ascorbic acid + Malic acid (MA) mix, packed in Amcor bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 4 weeks (- b*= indicate blue : + b* = indicate yellow; Grand mean – average of all

treatments;Untreated – No HPP and no acidification).

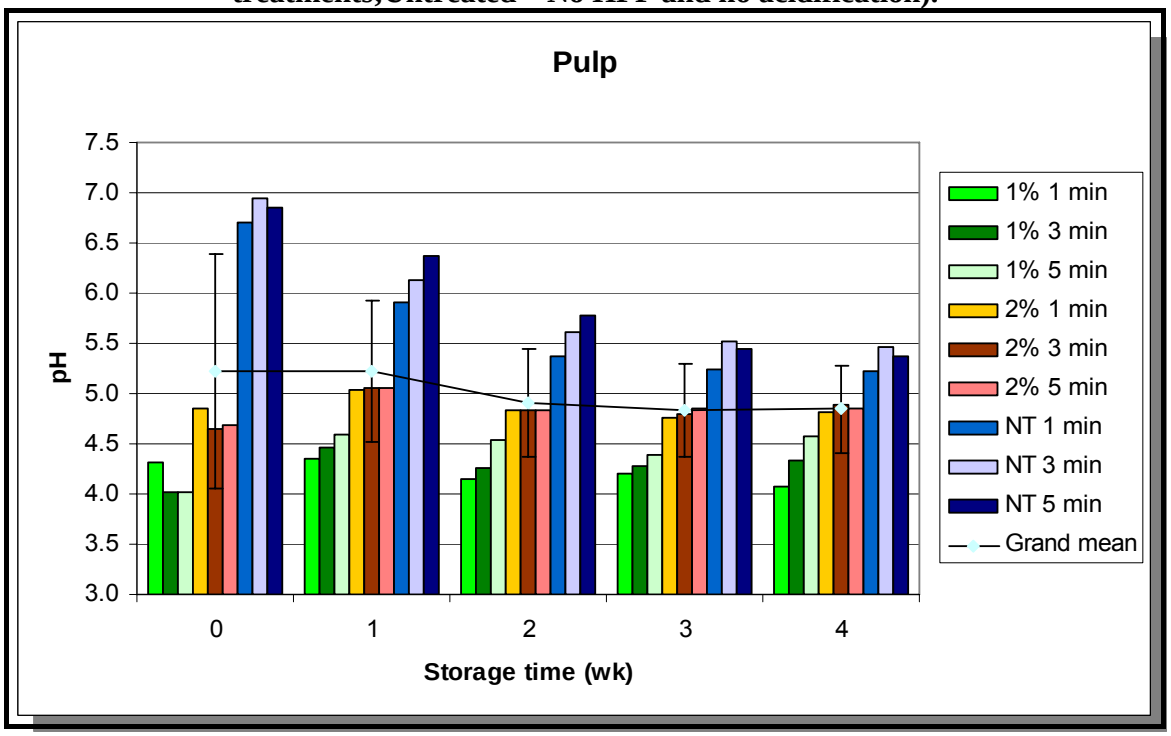

Figure 4: pH values of avocado pulp acidified with or without ascorbic acid + Malic acid (MA) mix, packed in Amcor bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 4 weeks (Grand mean – average of all treatments).

Table 13: Statistical significance of main effects and interaction effect on the final pH of avocado pulp acidified with or without ascorbic acid (AA) + malic acid (MA) mix, packed in Amcor bags, HPP treated at 600 MPa 1-5 min and stored at 4°C on 0 and 4 weeks.

Storage time	Main effect of Initial pH level	Main effect of HPP exposure time	Interaction effect of pH X HPP exposure time	Probability
pH 0 weeks	Significant pH 4.4 = 4.04 pH 5.0 = 4.70 pH 6.5 = 6.83 LSD = 0.24	Ns	Ns	$P_{pH}<0.001$
4 Weeks	Significant pH 4.4 = 4.35 pH 5.0 = 4.84 pH 6.5 = 5.35 LSD = 0.24	Ns	Ns	$P_{pH}<0.001$

LSD – Least significant difference ; Ns – Not significant

pH

Final pH of the pulp after processing was dependent on the level of acidification applied initially. With storage, pH decreased gradually in un acidified samples. The change in pH in acidified treatments were minimal (Figure 4).

The pH of samples just after processing and at week 4 was analyzed statistically (Table 13). Both analysis of variance (ANOVA) and response surface analysis showed significant effects of acidification on the final pH at 0 and 4 weeks after storage. Acidified samples showed a

significantly lower pH than unacidified samples. The response surface models for the prediction of pH, on 0 and 4 weeks after processing are outlined in Table 14.

Response surface diagrams were not created because the pH after processing and after 4 weeks storage were influenced only by initial pH.

Table 14: Response surface models for of avocado pulp acidified with or without ascorbic acid + Malic acid (MA) mix, packed in Amcor bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day and 4 weeks.

Storage time	Response surface model
0 weeks	pH after processing = -1.75 + 1.31 pH_0
4 weeks	pH after 4 weeks = –4.80 + 3.17 p H_0– 0.248 $(pH_0)^2$

pH_0 - Initial pH

Table 15: Statistical significance of main effects and interaction effect on sensory parameters of avocado pulp acidified with or without ascorbic acid + malic acid (MA) mix, packed in Amcor bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for one day.

Parameter	Main effect of Initial pH level (pH_0)	Main effect of HPP exposure time	Interaction effect of pH X HPP exposure time	Probability
Acceptability (A)	Significant pH 4.4 = 3.10 pH 5.0 = 4.53 pH 6.5 = 5.95 LSD = 1.98	Ns	Ns	P_{pH} <0.001
Acidic Taste (AT)	Ns	Ns	Ns	
Out of pack browning (B)	Significant pH 4.4 = 4.24 pH 5.0 = 4.87 pH 6.5 = 5.23 LSD = 0.94	Ns	Ns	P_{pH} <0.001
Consistency (C)	Ns	Ns	Ns	

LSD – Least significant difference; Ns – Not significant

Sensory Parameters

For sensory analysis sample packs were opened and samples were presented to the panelists together with a fresh avocado piece. However, the scores obtained for the fresh sample was not included in the statistical analysis because it was not included in the statistical design.

Acceptability: Both ANOVA and response surface analysis showed that the acceptability of pulp is significantly influenced by the initial pH level used (Table 15). The acceptability of pulp at pH 4.4 was significantly lower than the unacidified pulp. The acceptability of pulp at pH 5.0 was not significantly different from the untreated pulp with a pH of 6.5. HPP and the interaction factors were not significantly influencing the acceptability. Therefore the acceptability values in Table 17 were not significantly different. The response surface model for acceptability is given in Table 16. Since acceptability is only influenced by pH_0 response surface curve was not plotted

Table 16: Response surface models for sensory parameters of avocado pulp acidified with or without ascorbic acid + malic acid (MA) mix, packed in Amcor bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day.

Sensory parameter	Response surface model
Acceptability	$A = -2.26 + 1.28\ pH_0$
Out of pack browning	$B = -1.01 + 0.709\ pH_0 - 0.863\ HPP + 0.139\ HPP^2$
Consistency	$C = -21.4 + 9.41\ pH_0 - 0.821\ pH_0^2$

pH_0 – Initial pH; HPP – HPP exposure time

Table 17: Acceptability scores of avocado pulp acidified with or without ascorbic acid + malic acid (MA) mix, packed in Amcor bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day (pH X HPP - interaction effect).

Initial pH	HPP exposure time (min)		
	1	3	5
4.4	3.55	3.15	2.60
5.0	4.17	4.51	4.92
6.5	5.98	5.92	5.93

Table 18: Acidic taste scores of avocado pulp acidified with or without ascorbic acid + malic acid (MA) mix, packed in Amcor bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day (pH X HPP - interaction effect).

Initial pH	HPP exposure time (min)		
	1	3	5
4.4	5.11	7.33	5.00
5.0	4.26	4.99	4.02
6.5	4.10	1.65	3.86

Acidic taste: Surprisingly according to both ANOVA and response surface analysis, acidic taste (Table 18) of the pulp was not significantly influenced by the initial pH, HPP treatment time or the interaction of these two factors (Table 15) Table 18 shows the mean values of pulp treated under different conditions. The LSD was 10.22.

Table 19: Out of pack browning scores of avocado pulp acidified with or without ascorbic acid + malic acid (MA) mix, packed in Amcor bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day (pH X HPP - interaction effect).

Initial pH	HPP exposure time (min)		
	1	3	5
4.4	1.17	1.01	1.02
5.0	2.43	1.24	2.05
6.5	2.59	2.62	2.64

Out of pack browning: According to both ANOVA and response surface analysis, browning was significantly affected by the acidification of pulp (Table 15). Pulp at pH 4.4 was significantly less brown than the unacidified pulp. The HPP exposure time and interaction effect was not significant. In general the browning of all samples was very low and was between 1.01 and 2.64 (Table 19). The non linear response surface model for browning is given in Table 16. This model is based on both pH and HPP exposure time parameters. According to the response surface curve

(Figure 5) browning decreased with acidification. Minimum level of browning in pulp was observed when HPP treatment time was 3 min and pH at 4.4

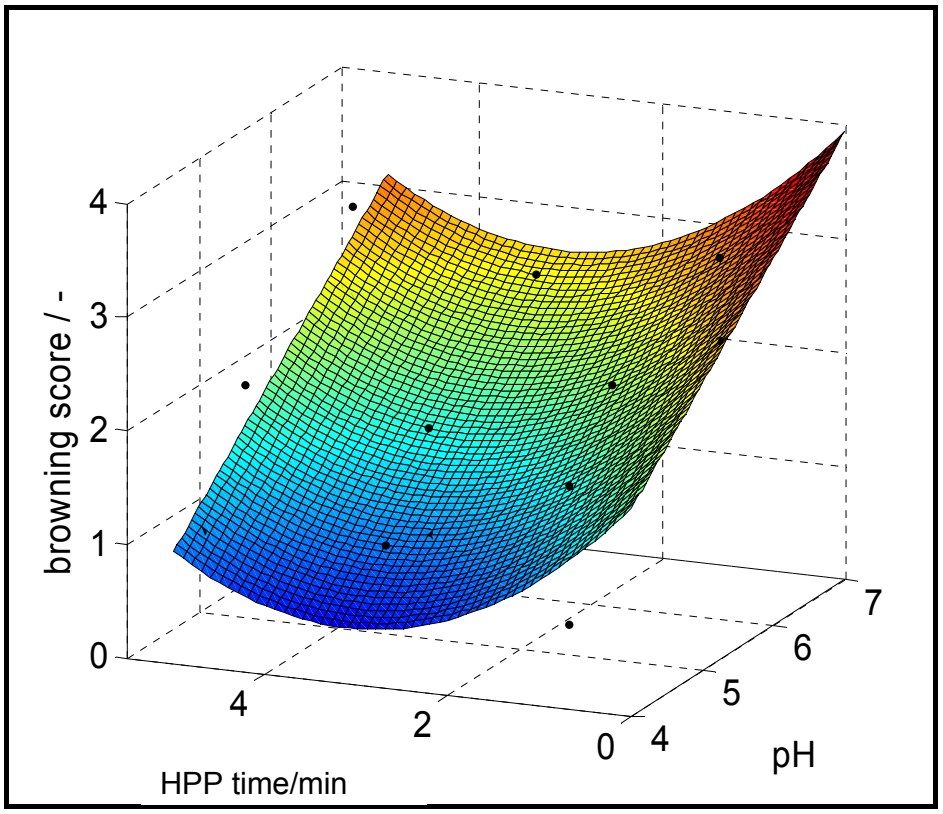

Figure 5: Response surface curve on browning scores of avocado pulp acidified with or without ascorbic acid + Malic acid (MA) mix, packed in Amcor bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day

Table 20: Consistency scores of avocado pulp acidified with or without ascorbic acid + Malic acid (MA) mix, packed in Amcor bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day (pH X HPP - interaction effect).

Initial pH	HPP exposure time (min)		
	1	3	5
4.4	4.23	3.93	4.37
5.0	4.27	5.32	5.02
6.5	5.80	4.61	5.29

Consistency: ANOVA analysis did not show any significant effects of initial pH, HPP time or the interaction of these two factors on the consistency (Table 15). The response surface analysis showed significant effect of pH component. The quadratic equation for this relationship is given in Table 16. Consistency scores were between 3.93 and 5.80 (Table 34).

4.2.2 Halves

Microbiological Parameters

Table 21: Standard plate counts of avocado halves treated with or without malic acid (MA), packed in Winpack 7000 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 4 weeks.

Treatment (MA% + HPP time)	Storage time (weeks)				
	0	**1**	**2**	**3**	**4**
2% + 1 min	1.2	1.0	1.0	<1.0	**<1.0**
2% + 3 min	<1.0	<1.0*	1.6	<1.0	**<1.0**
2% + 5 min	1.9	<1.0	<1.0	<1.0	**1.2**
1% + 1 min	<1.0	<1.0*	<1.0	<1.0	**1.9**
1% + 3 min	<1.0	<1.0	1.8	<1.0	**1.4**
1% + 5 min	<1.0	<1.0*	1.3	<1.0	**1.0**
0% + 1 min	1.8	<1.0	1.5	<1.0	**1.3**
0% + 3 min	<1.0	<1.0	<1.0	<1.0	**1.3**
0% + 5 min	<1.0	<1.0	1.5	<1.0	**1.3**

Minimum detectable level <1 $\log_{10}$ cfu/g ; * counts less than <1.0 $\log_{10}$ cfu/g; was present.

Standard Plate Count: All pressure treatments resulted in very low SPC (<2.0 $\log_{10}$ cfu/g) over the entire storage period tested (Table 21).

Table 22: Yeast and mould counts of avocado halves treated with or without malic acid (MA), packed in Winpack 7000 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 4 weeks.

Treatment (MA% + HPP time)	Storage time (weeks)				
	0	**1**	**2**	**3**	**4**
2% + 1 min	<1.0	1.7	<1.0	<1.0	1.7
2% + 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
2% + 5 min	1.7	1.8	<1.0	<1.0	1.7
1% + 1 min	<10	<1.0	<1.0	<1.0	1.0
1% + 3 min	<1.0	<1.0	1.5	<1.0	<1.0
1% + 5 min	<1.0	<1.0	<1.0	<1.0	1.0
0% + 1 min	1.8	<1.0	1.7	<1.0	1.7
0% + 3 min	<1.0	<1.0	<1.0	<1.0	2.0
0% + 5 min	<1.0*	<1.0	1.7	<1.0	2.0

NA – not assessed; Minimum detectable level <1.0 $\log_{10}$ cfu/g; * counts less than <1.0 $\log_{10}$ cfu/g; was present in one replicate

Yeast and mould count: Sporadic yeast and mould counts were observed in 5 treatments but were maintained at very low levels ($\leq$2.0 $\log_{10}$ cfu/g;) over the entire storage period tested (Table 22).

Table 23: Enterobacteriaceae counts of avocado halves treated with or without malic acid (MA), packed in Winpack 7000 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 4 weeks.

Treatment (MA% + HPP time)	Storage time (weeks)				
	0	1	2	3	4
2% + 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
2% + 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
2% + 5 min	<1.0	<1.0	<1.0*	<1.0	<1.0
1% + 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
1% + 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
1% + 5 min	<1.0	<1.0	<1.0	<1.0	<1.0
0% + 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
0% + 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
0% + 5 min	<1.0	<1.0	<1.0	<1.0	<1.0

Minimum detectable level <1.0 $\log_{10}$ cfu/g: * One replicate contained 1.0 $\log_{10}$ cfu/g

Enterobactereacea count: Except for in one replicate of 2% MA + 5 min HPP all the other samples did not show any growth of *Enterobactereacea* bacteria (Table 23).

Table 24: Lactic acid bacteria counts of avocado halves treated with or without malic acid (MA), packed in Winpack 7000 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 4 weeks.

Treatment (MA% + HPP time)	Storage time (weeks)				
	0	1	2	3	4
2% 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
2% + 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
2% + 5 min	<1.0	<1.0	<1.0	<1.0	<1.0
1% + 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
1% + 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
1% + 5 min	<1.0	<1.0	<1.0	<1.0	<1.0
0% + 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
0% + 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
0% + 5 min	<1.0	<1.0	<1.0	<1.0	<1.0

Minimum detectable level <1.0 $\log_{10}$ cfu/g

Lactic acid bacteria count: Lactic acid bacteria growth was not observed in avocado halves treated with 0-2% MA and HPP treated for 1-5 min (Table 24).

In-Pack Colour

Visually it was difficult to observe any color change except when the seed cavity was not fully evacuated during the vacuum sealing step or when a pouch was leaking due to improper sealing. In avocado halves and slices the green side and the yellow side were measured separately because the rate of browning was usually different on these two sides.

L* value (lightness): As expected the yellow side of the avocado halves showed a higher L* value indicating a lighter colour than the greenside (Figure 6 and Figure 7). On both sides of the avocado halves lightness gradually decreased indicating darkening. However, this decrease in lightness was not very obvious to the naked eye. After the first week L* values of all treatments were in a narrow range of 56 – 45 on green side and 67-64 on yellow side. Generally the yellow side is more prone to browning and shows a decrease in L* value if avocado halves are subjected to browning. The untreated samples also recorded L* values within the same range as the treated samples. Hence the data was not subjected to statistical analysis. Distinct differences were not

observed between treatments except for the halves not subjected to acid dipping and 5 min + HPP treatment which showed a decreasing trend in L value of the yellow side (Figure 7).

a* value (greenness): As expected the green sides showed lower a* values indicating more greenness than yellow sides (Figure 8 and Figure 9). Non acid dipped samples showed a lower a* value indicating more greenness than in acid dipped samples. The untreated samples also showed an increase in a* value indicating the loss of green colour. The untreated sample showed a trend of continuous increase in the a* value whereas the HPP treated samples showed an increase in a* value up to 3 weeks and a slight decrease again by week 4 (see the trend lines untreated and grand mean of all the treatments).

With browning generally a* values of yellow side of avocado halves increased and if browning was visible, a* values changed to positive values. Unlike on the green side of avocado halves, the acid dipped samples showed a lower a* value than the undipped samples (Figure 9) indicating less browning. The trend of a* values during storage was similar in both HPP treated and untreated samples.

b* values (yellowness): Green side showed lower b* values indicating less yellowness than the yellow side (Figure 10 and Figure 11). On both green and yellow sides the yellowness slightly decreased around weeks 2 and 3 and then increased. Untreated samples also showed a similar trend. Treatment (Acid dip + HPP time) related trends were not observed.

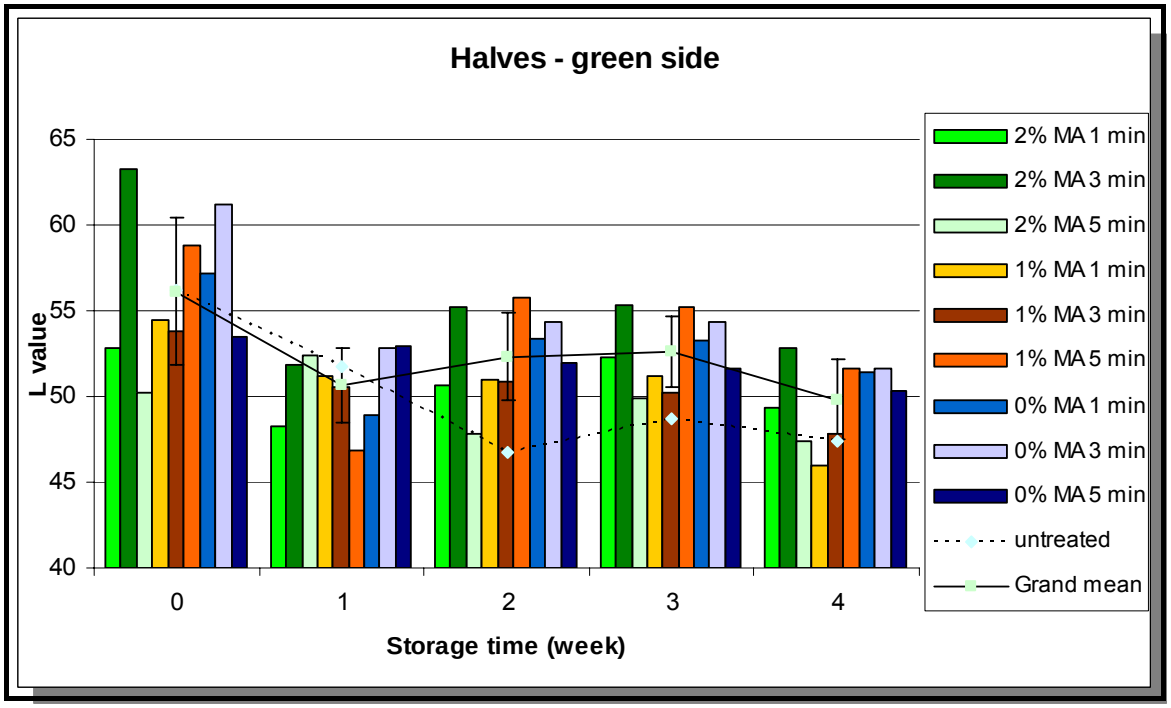

Figure 6: Changes in L values of the green side of avocado halves treated with 0-2% malic acid (MA), vacuum packed in Winpack 7000 bags, treated with HPP at 600MPa for 1 – 5 min and stored at 4°C for 4 weeks. (L*=0 yields black and L*=100 indicates white).

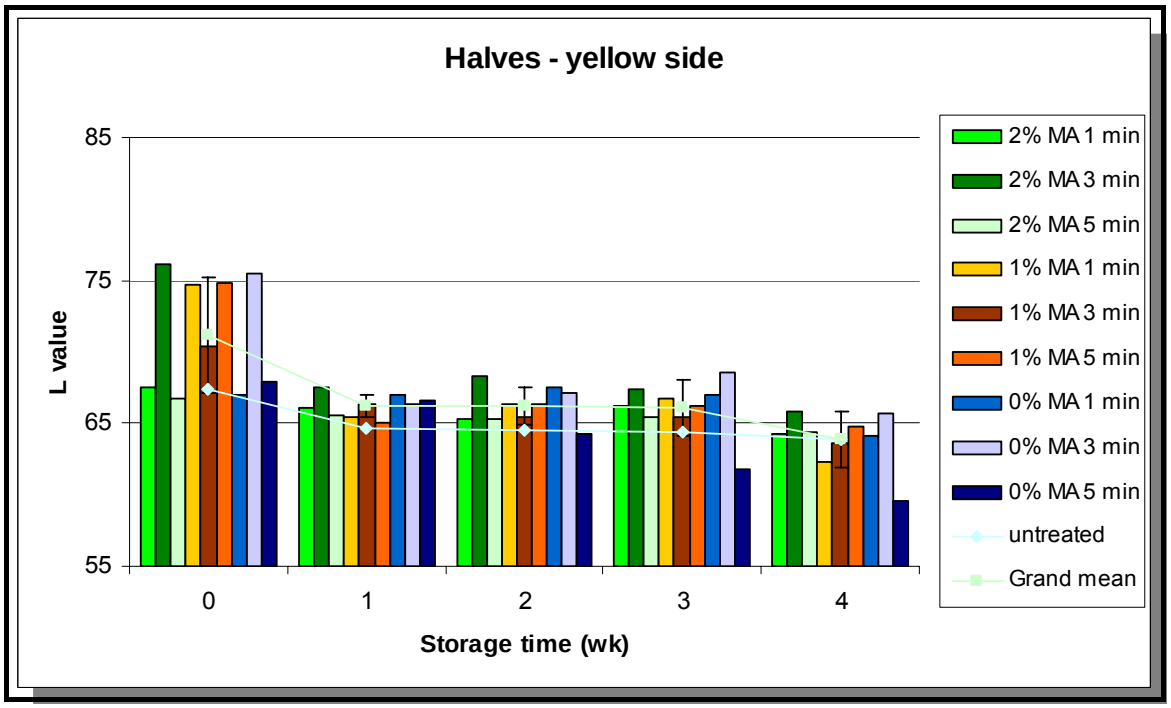

Figure 7: Changes in L values; Grand mean – average of the yellow side of avocado halves treated with 0-2% malic acid (MA), vacuum packed in Winpack 7000 bags, treated with HPP at 600MPa for 1 – 5 min and stored at 4°C for 4 weeks. (L*=0 indicates black ; L*=100 indicates white; Grand mean – average of all treatments; Untreated – No acidification and no HPP).

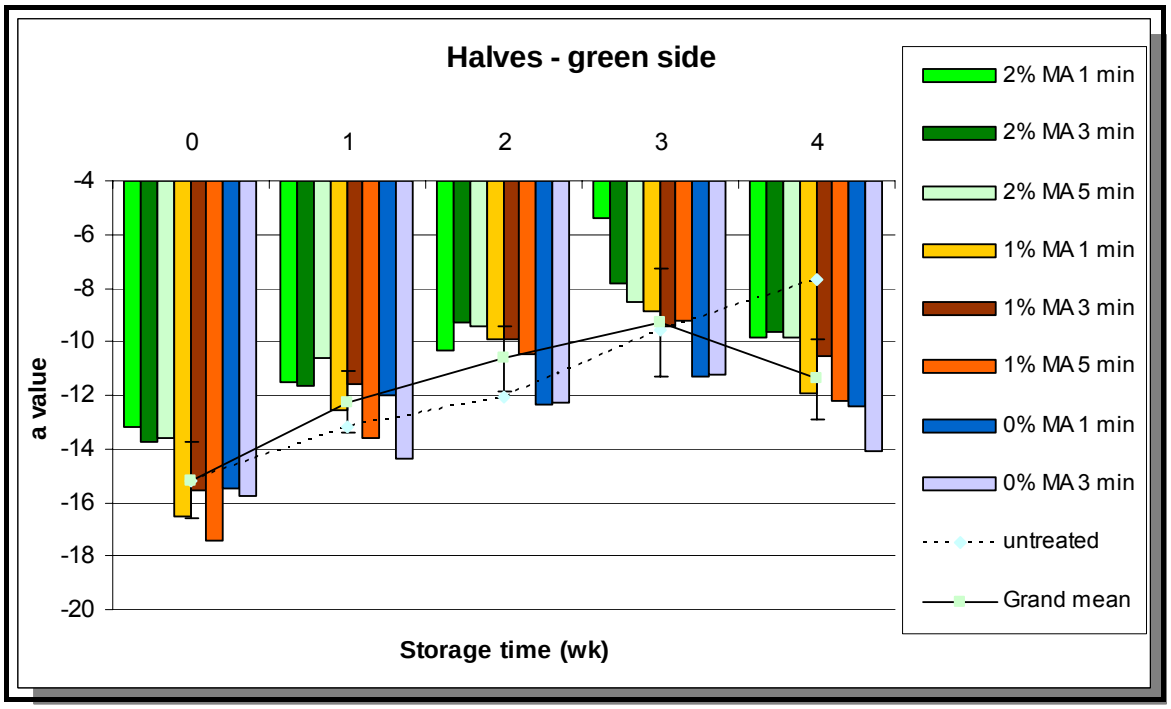

Figure 8: Changes in a* values of the green side of avocado halves treated with 0-2% malic acid (MA), vacuum packed in Winpack 7000 bags, treated with HPP at 600MPa for 1 – 5 min and stored at 4°C for 4 weeks(-a = indicates green : +a = indicates magenta: Grand mean – average of all treatments; Untreated – No acidification and no HPP).

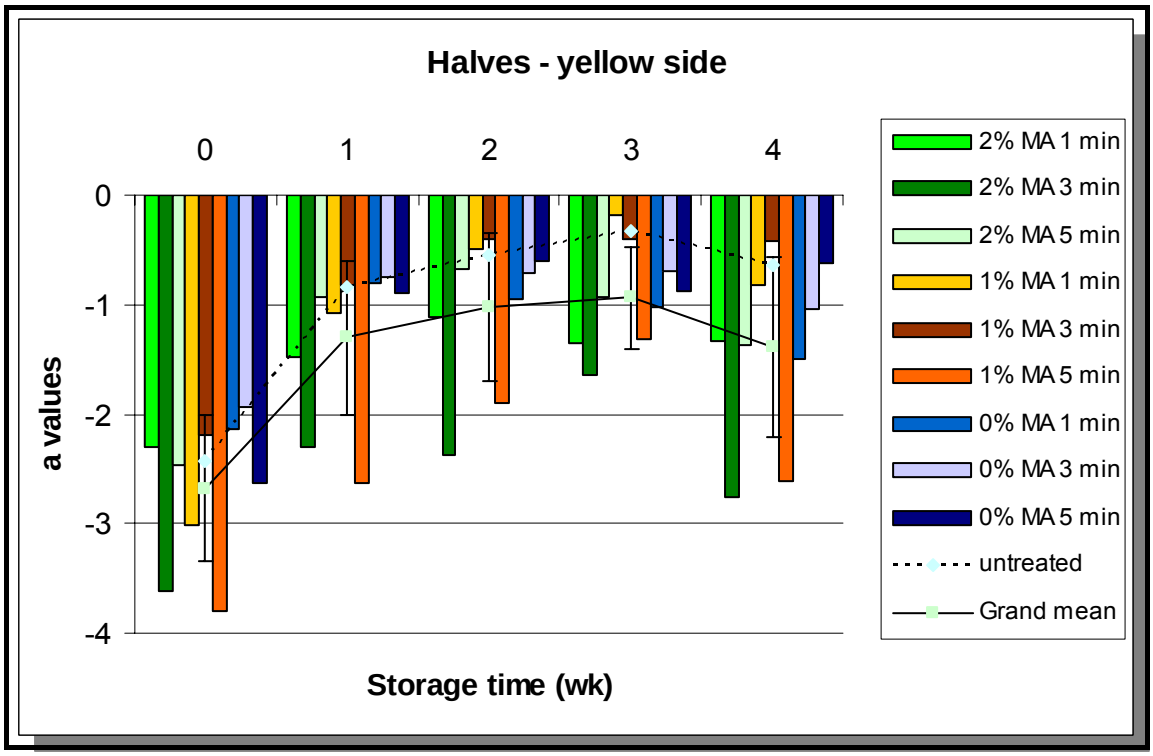

Figure 9: Changes in a* values of the yellow side of avocado halves treated with 0-2% malic acid (MA), vacuum packed in Winpack 7000 bags, treated with HPP at 600MPa for 1 – 5 min and stored at 4°C for 4 weeks. (-a = indicates green : +a = indicates magenta; Grand mean – average of all treatments; Untreated – No HPP and no acidification).

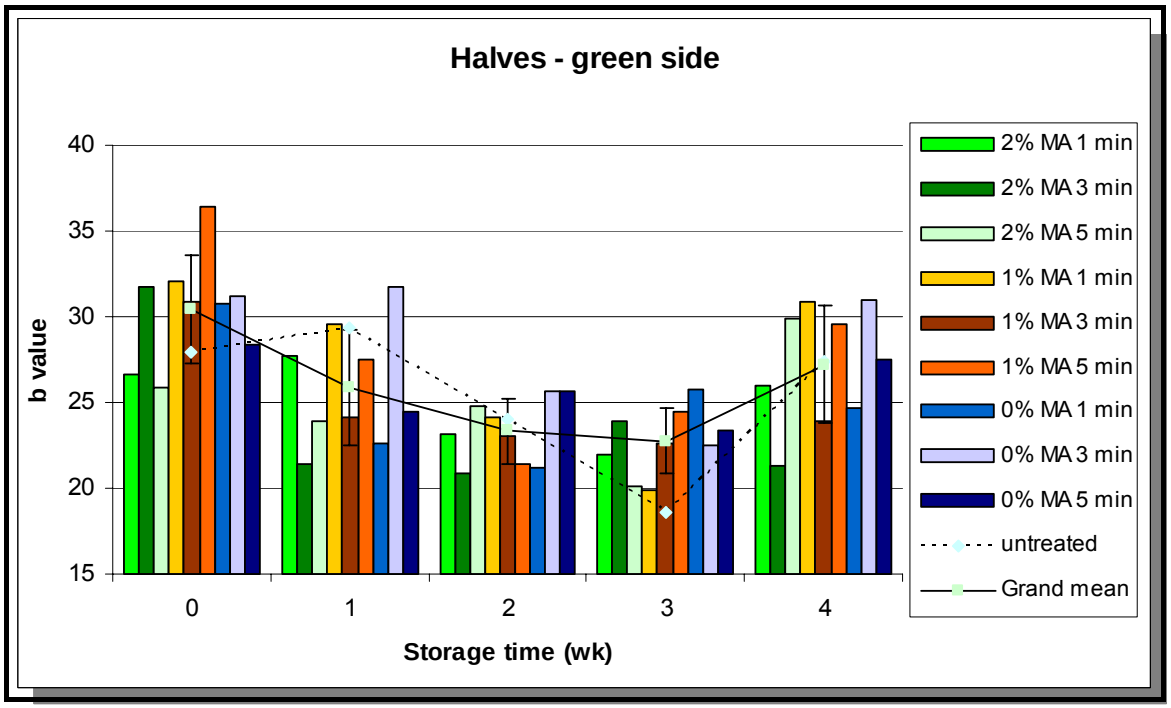

Figure 10: Changes in b* values of the green side of avocado halves treated with 0-2% malic acid (MA), vacuum packed in Winpack 7000 bags, treated with HPP at 600MPa for 1 – 5 min and stored at 4°C for 4 weeks (- b*= indicate blue : + b* = indicate yellow; Grand mean – average of all treatments; Untreated – No HPP and no acidification).

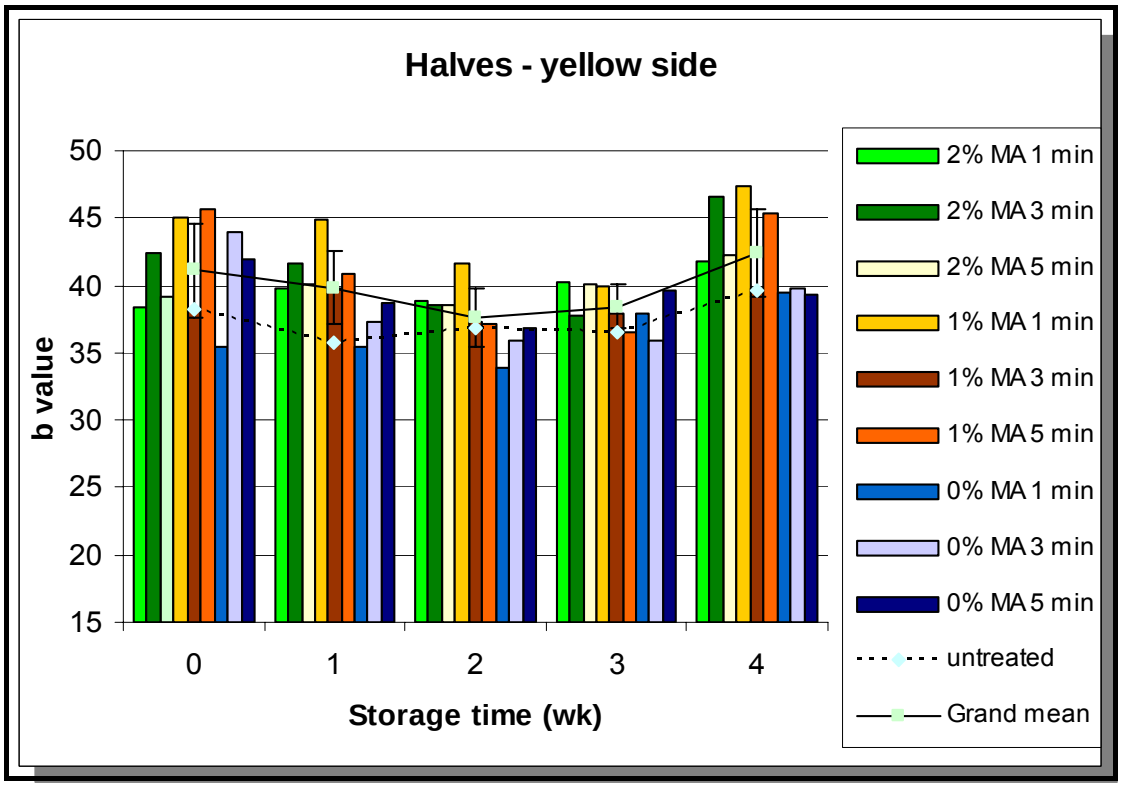

Figure 11: Changes in b* values of the yellow side of avocado halves treated with 0-2% malic acid (MA), vacuum packed in Winpack 7000 bags, treated with HPP at 600MPa for 1 – 5 min and stored at 4°C for 4 weeks(- b*= indicate blue : + b* = indicate yellow; Grand mean – average of all treatments; Untreated – No HPP and no acidification).

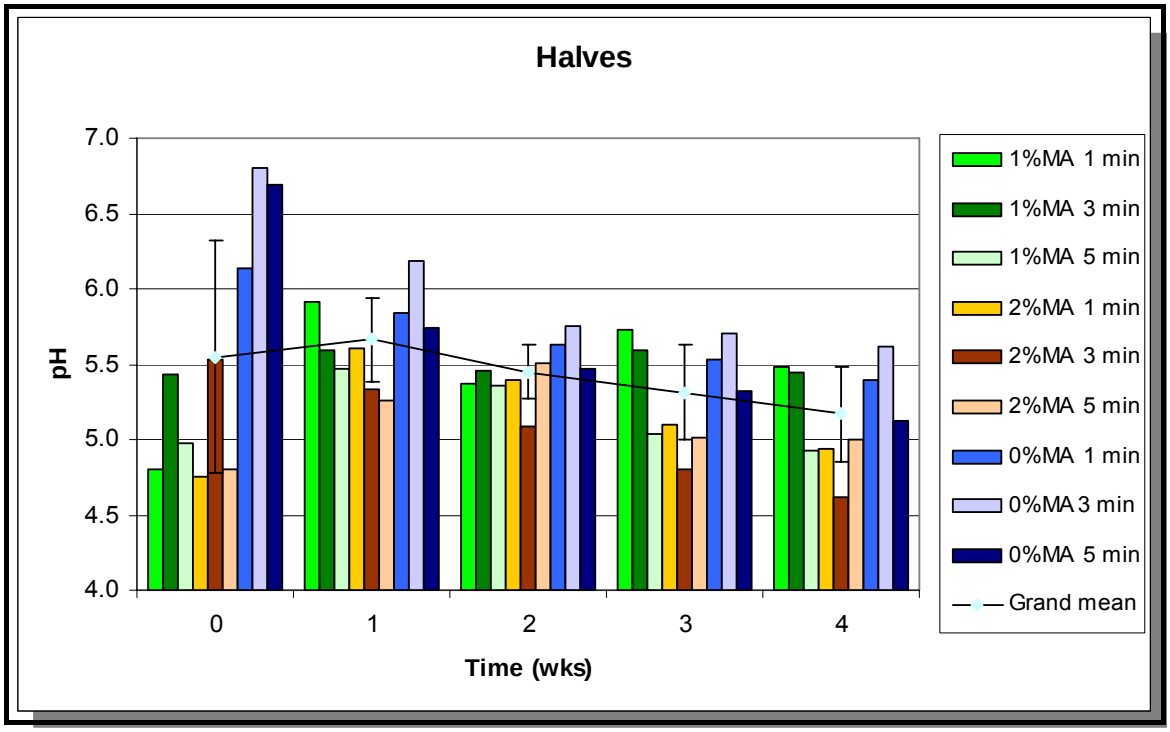

Figure 12: Changes in surface pH values of avocado halves treated with 0-2% malic acid (MA), vacuum packed in Winpack 7000 bags, treated with HPP at 600MPa for 1 – 5 min and stored at 4°C for 4 weeks (Untreated – No HPP and no acidification).

Surface pH

Avocado halves showed a gradual decrease in pH during storage (Figure 12). The surface pH of acid dipped avocado halves were lower than the avocado halves that were not subjected to dipping treatments. This difference became less prominent with storage time.

Table 25: Statistical significance of main effects and interaction effects on surface pH values of avocado halves treated with or without malic acid (MA), packed in Winpack 7000 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 0 and 4 weeks.

Storage time	Main effect	Main effect	Interaction effect	Probability
	% Malic acid (MA)	HPP exposure time	% MA X HPP exposure time	
pH 0 weeks	Significant 0%MA = 6.54 1%MA = 5.07 2%MA = 5.03 LSD =0.53	Significant HPP1 min = 5.23 HPP 3 min = 5.92 HPP 5 min = 5.49 LSD =0.53	Ns	P_{pH} <0.001 P_{HPP} = 0.029
pH 4 Weeks	Significant*	Significant*	Significant See Table 20	P_{pH} <0.001 $P_{\%MA}$ = 0.012 $P_{pH\ X\ \%MA}$ <0.001

LSD – Least significant difference; Ns – Not significant; * - Main effect means are not presented since interaction effect means are presented in Table 20.

The statistical analysis of the data showed that on week 0 there were significant differences between the dipping treatments and HPP treatment times (Table 25). A non-linear relationship was obtained by response surface analysis to calculate the final pH on day 0, by using the % MA and HPP exposure time (Table 27). Figure 13 show that the surface pH was lowest when the acid was added at the highest level and no HPP treatment was applied.

On week 4 the interaction of acid dip treatment and HPP treatment was significant (Table 25). Avocado halves dipped in 2% malic acid and pressure treated for 3 min and halves dipped in 1% malic acid and HPP treated for 5 min at 600 MPa showed significantly lower pH than the other treatments, after 4 weeks of storage (Table 26). A quadratic relationship was obtained by response surface analysis to calculate the final pH on week 4, by using the % MA and HPP exposure time (Table 27). Figure 14 shows that the pH has stabilized over the storage period. The pH of avocado halves were low in acidified samples and no difference between HPP exposure times could be seen.

Table 26: Surface pH of avocado halves treated with or without malic acid (MA), packed in Winpack 7000 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 4 weeks (pH X HPP interaction).

% Malic acid	HPP exposure time (min)		
	1	3	5
0	5.40	5.61	5.12
1	5.47	5.44	4.91
2	4.94	4.61	5.00

Least significant difference = 0.32

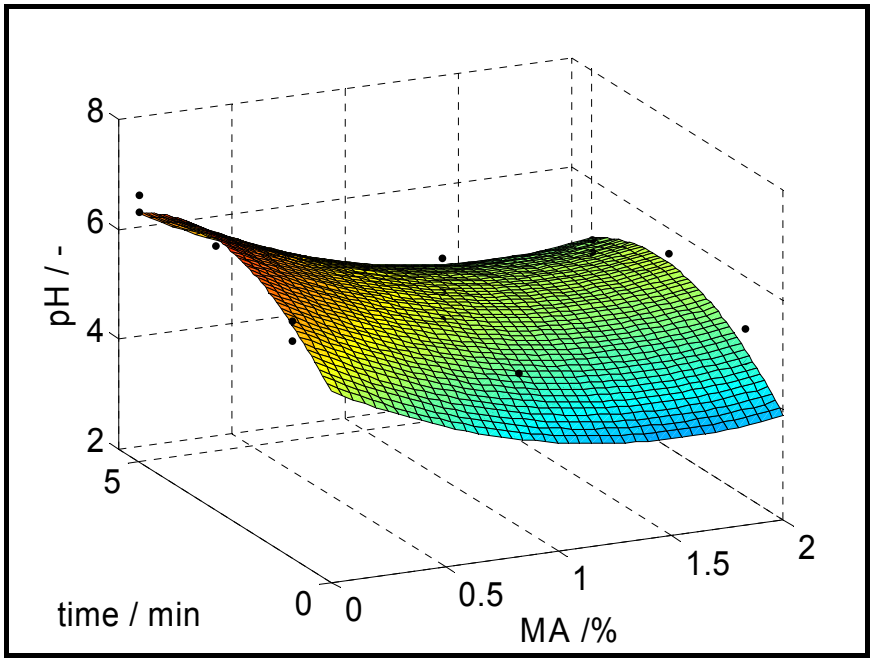

Figure 13: Response surface curve for the surface pH after treatment and after 0 weeks storage of avocado halves treated with or without malic acid (MA), packed in Winpack 7000 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C.

Table 27: Response surface model for the surface pH after treatment and after 4 weeks storage of avocado halves treated with or without malic acid (MA), packed in Winpack 7000 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C.

Storage time	Response surface model
0 weeks	pH after treatment = 5.48– 2.22 MA% + 0.716 $(MA\%)^2$ + 0.905 HPP – 0.140 HPP^2
4 weeks	pH after storage = 5.46 + 0.278 MA% -0.240 $(MA\%)^2$ -0.0431 HPP

MA% - Malic acid %; HPP – HPP exposure time

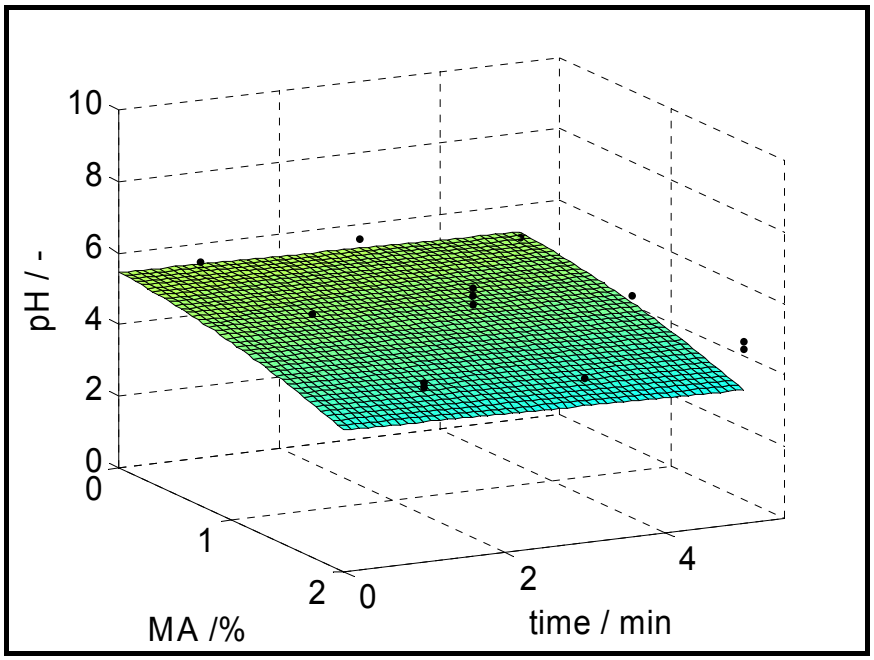

Figure 14: Response surface curve for the surface pH after treatment and after 4 weeks storage of avocado halves treated with or without malic acid (MA), packed in Winpack 7000 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C.

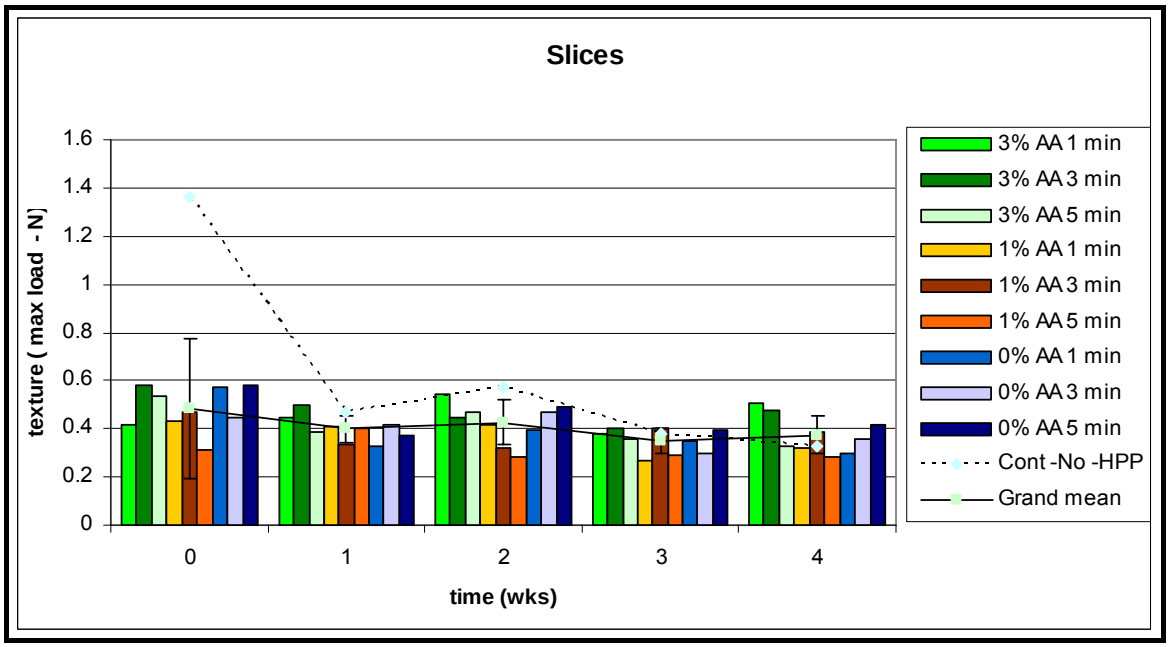

Figure 15: Changes in texture of avocado halves treated with 0-2% malic acid (MA), vacuum packed in Winpack 7000 bags, treated with HPP at 600MPa for 1 – 5 min and stored at 4°C for 4 weeks (Grand mean – average of all treatments; Untreated – No HPP and no acidification).

Texture

The texture of the HPP treated avocado pieces ranged between 0.2–0.8 N (Figure 15). The untreated sample showed 1.2 N reading on week 0 and this decreased to the level of 0.4 N in the first week. Then the texture of the untreated sample and the HPP treated samples were in the same range. After first week of storage there was no trend observed for each treatment combination over the storage period. Texture results were not statistically analyzed.

Table 28: Statistical significance of main effects and interaction effects on sensory data of avocado halves treated with or without malic acid (MA), packed in Winpack 7000 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day.

Parameter	Main effect % Acid dip	Main effect HPP exposure time	Interaction effect pH X HPP exposure time	Probability
Acceptability (A)	Ns	Ns	Ns	-
Acidic Taste (AT)	Significant 0% MA = 5.25 1% MA = 2.46 2% MA = 2.25 LSD = 0.60	Significant HPP 1 min = 3.06 HPP 3 min =3.53 HPP 5 min = 3.36 LSD = 0.60	Ns	P_{pH} <0.001 P_{HPP} = 0.003
Out of pack browning (B)	Ns	Ns	Ns	
Firmness (F)	Ns	Significant HPP 1 min = 5.84 HPP 3 min = 4.06 HPP 5 min = 5.91 LSD = 1.70	Ns	P_{HPP} <0.001

LSD – Least significant difference; Ns – Not significant

Table 29: Results of response surface analysis on sensory properties of avocado halves treated with or without malic acid (MA), packed in Winpack 7000 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day.

Sensory property	Response surface model
Acidic taste	$AT = 1.13 - 0.674\ MA\% + 1.09\ MA\%^2 + 0.884\ HPP - 0.130\ HPP^2$
Out of pack browning	$B = 4.43 - 0.306\ MA\% - 0.136\ HPP$
Firmness	$F = 8.39 - 3.12\ HPP + 0.512\ HPP^2$

MA% - Malic acid %; HPP – HPP exposure time

Sensory Parameters

Acceptability: Sensory analysis was conducted only on day 1 after processing. The main effects of % acid in dipping solution, HPP exposure time or their interaction effect were not statistically significant with acceptability of avocado halves (Table 28). The acceptability values ranged between 6.14 – 3.09 (Table 29). The response surface analysis of data did not indicate a significant relationship between acceptability and % acid dip or HPP exposure time.

Table 30: Acceptability scores of avocado halves treated with or without malic acid (MA), packed in Winpack 7000 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day of storage at 4°C (pH X HPP - interaction effect).

% Malic acid	HPP exposure time (min)		
	1	3	5
0	5.11	6.14	4.99
1	4.06	4.98	3.09
2	4.85	3.84	4.89

Acidic taste: The acid dip and HPP exposure time main effects were significantly contributing to the acidic taste development in treated avocado halves (Table 28). Acidic taste increased with the increase of %acid in the dipping solution and with the increase of HPP exposure time. The interaction effect or the combined effect of both acid treatment and HPP was not significant (Table 31). Acidic taste scores observed in this study ranged from 2.02 to 5.67.

The response surface analysis was performed on acidic taste values, the pH component and the HPP components were significant. The equation in Table 29 could be used to predict the acidic taste of the treated avocado halves by using the % acid in the dipping solution (within 0–2 % MA range). Figure 16 show that minimum acidic taste was observed when there were no acidification and HPP treatments applied. Acidic taste also increased with the increase of HPP exposure time.

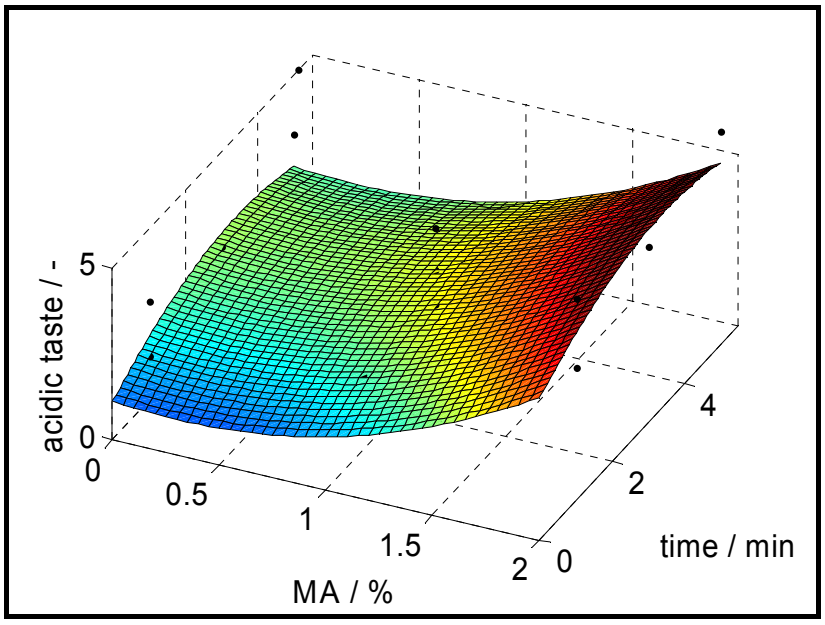

Figure 16: Response surface diagram on acidic taste of avocado halves treated with or without malic acid (MA), packed in Winpack 7000 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day.

Table 31: Acidic taste scores of avocado halves treated with or without malic acid (MA), packed in Winpack 7000 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day of storage at 4°C (pH X HPP - interaction effect).

% Malic acid	HPP exposure time (min)		
	1	3	5
0	2.06	2.29	2.39
1	2.27	3.08	2.02
2	4.85	5.22	5.67

Out of pack browning: The main effects of % acid dip in dipping solution, HPP exposure time or their interaction effect were not statistically significant with browning of avocado halves (Table 28). However, the response surface analysis showed that % acid in dipping solution and HPP exposure time was significant. The equation given in Table 29 could be used to predict browning of the avocado halves. Browning scores ranged between 2.99 and 4.55 in this study (Table 32). Figure 17 indicate that browning was lowest at the highest acid concentration (2%) and at the highest HPP exposure time (5 min).

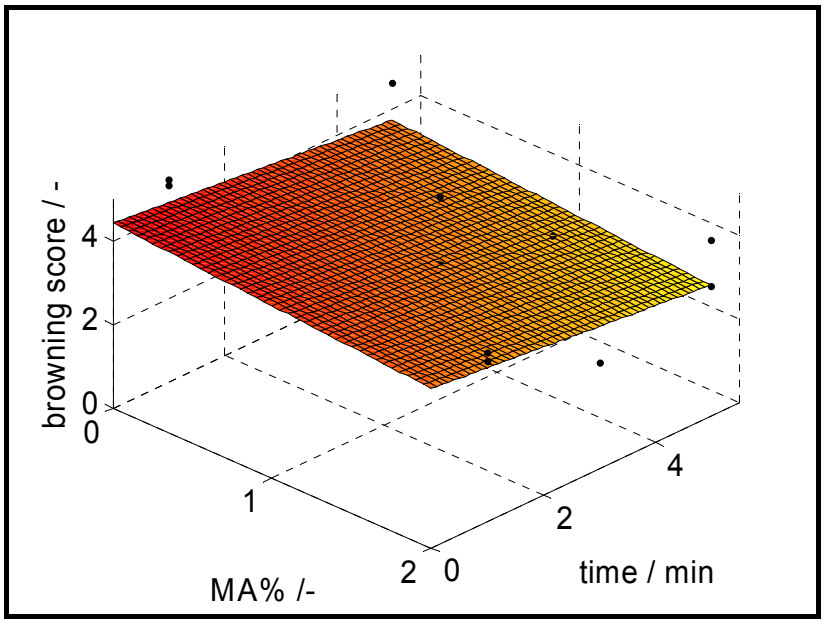

Figure 17: Response surface diagram on out of pack browning scores of avocado halves treated with or without malic acid (MA), packed in Winpack 7000 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day.

Table 32: Out of pack browning scores of avocado halves treated with or without malic acid (MA), packed in Winpack 7000 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day of storage at 4°C (pH X HPP - interaction effect).

% Malic acid	HPP exposure time (min)		
	1	3	5
0	4.55	3.56	3.87
1	3.89	3.66	3.09
2	3.72	2.99	3.45

Firmness: Firmness of avocado halves was influenced by the HPP exposure time and statistically significant differences were observed between the mean values (Table 28). Avocado halves treated at 3 min exposure time recorded a significantly lower firmness than at 1 and 5 min exposure times. However, the HPP treatment means showed a non linear relationship. The response surface analysis showed significant effects for the HPP exposure time and the equation for this relationship is given in Table 29.

The firmness scores were between 3.65 and 8.45 but statistically significant differences were not observed between these mean values (Table 33).

Table 33: Firmness scores of avocado halves treated with or without malic acid (MA), packed in Winpack 7000 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day of storage at 4°C (pH X HPP - interaction effect).

% Malic acid	HPP exposure time (min)		
	1	3	5
0	5.39	4.50	5.14
1	7.10	4.02	8.45
2	5.04	3.65	4.16

4.2.3 Slices

Microbiological Analysis Of Slices

Table 34: Standard plate counts of avocado slices treated with or without ascorbic acid (AA), packed in Cryovac B471 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 4 weeks.

Treatment (AA% + HPP time)	Storage time (weeks)				
	0	1	2	3	4
3% + 1 min	<1.0	<1.0	<1.0	1.2	<1.0
3% + 3 min	<1.0	<1.0	<1.0	<1.0	<10
3% + 5 min	<1.0	<1.0	<1.0	1.3	<1.0
1% + 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
1% + 3 min	<1.0	<1.0	<1.0	<1.0*	<1.0
1% + 5 min	<1.0	<1.0	<1.0	<1.0	<1.0
0% + 1 min	<1.0	<10	2.4	2.8	<1.0
0% + 3 min	<1.0	<1.0	<1.0	<1.0	3.6
0% + 5 min	<1.0	<1.0	2.6	3.6	5.4

NA – not assessed : Minimum detectable level <1.0 $\log_{10}$ cfu/g ; * Counts below 1.0 $\log_{10}$ cfu/g were present in one replicate.

Standard plate count: The standard plate counts were observed in non acidified but HPP treated samples after 2 weeks of storage (Table 34). Counts ranged from 2.4 to 5.4 $\log_{10}$ cfu/g.

Table 35: Yeast and mould counts of avocado slices treated with or without ascorbic acid (AA), packed in Cryovac B471 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 4 weeks.

Treatment (AA% + HPP time)	Storage time (weeks)				
	0	1	2	3	4
3% + 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
3% + 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
3% + 5 min	<1.0*	<1.0	1.7	1.9	<1.0
1% + 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
1% + 3 min	<1.0	<1.0	1.5	<1.0	<1.0
1% + 5 min	<1.0	<1.0	<1.0	<1.0	<1.0
0% + 1 min	1.0	<1.0	1.7	2.0	<1.0
0% + 3 min	<1.0	<1.0	<1.0	<1.0	3.6
0% + 5 min	<1.0	<1.0	1.7	<1.0	<1.0

NA – not assessed: Minimum detectable level <1.0 $\log_{10}$ cfu/g ; * Counts below 1.0 $\log_{10}$ cfu/g were present in one replicate.

Yeast and mould counts: Yeast and mould counts were observed in non acidified but HPP treated samples and 1% AA + 5 min HPP treated samples after 2 weeks of storage. 3% AA+ 5 min HPP treated samples showed yeast and mould counts from 0 weeks to 3 weeks (Table 35). Counts ranged from 1.0 to 3.6 $\log_{10}$ cfu/g.

Table 36: Enterobacteriaceae counts of avocado slices treated with or without ascorbic acid (AA), packed in Cryovac B471 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 4 weeks.

Treatment (AA% + HPP time)	Storage time (weeks)				
	0	1	2	3	4
3% + 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
3% + 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
3% + 5 min	<1.0	<1.0	<1.0	<1.0	<1.0
1% + 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
1% + 3 min	<1.0	<1.0	≤1.0*	<1.0	<1.0
1% + 5 min	<1.0	<1.0	<1.0	<1.0	<10
0% + 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
0% + 3 min	<1.0	<1.0	<1.0	<1.0	3.8
0% + 5 min	<1.0	<1.0	<1.0	<1.0	5.1

NA – not assessed; Minimum detectable level . <1.0 $\log_{10}$ cfu/g ; * One replicate contained 1.3 $\log_{10}$ cfu/g

Enterobactereacea bacteria count: Detectable *Enterobactereacea* counts were observed only in unacidified (pH 6.5) samples when subjected to 600 MPa for 3 or 5 min and stored at 4°C for 4 weeks (Table 36 and Table 37).

Table 37: Lactic acid bacteria counts of avocado slices treated with or without ascorbic acid (AA), packed in Cryovac B471 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 4 weeks.

Treatment (AA% + HPP time)	Storage time (weeks)				
	0	1	2	3	4
3% + 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
3% + 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
3% + 5 min	<1.0	<1.0	<1.0	<1.0	<1.0
1% + 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
1% + 3 min	<1.0	<1.0	<1.0	<1.0	<1.0
1% + 5 min	<1.0	<1.0	<1.0	<1.0	<1.0
0% + 1 min	<1.0	<1.0	<1.0	<1.0	<1.0
0% + 3 min	<1.0	<1.0	<1.0	<1.0	3.6
0% + 5 min	<1.0	<1.0	2.4	1.9	<1.0

NA – not assessed : Minimum detectable level <1.0 $\log_{10}$ cfu/g

Lactic acid bacteria count: Outgrowth of Lactic acid bacteria were only observed in unacidified (pH 6.5 samples) when subjected to 600 MPa for 3 min and stored for 4 weeks or 600 MPa for 5 min and stored for 2- 3 weeks at 4°C. The counts ranged from <1.0 to 2.4 $\log_{10}$ cfu/g (Table 37).

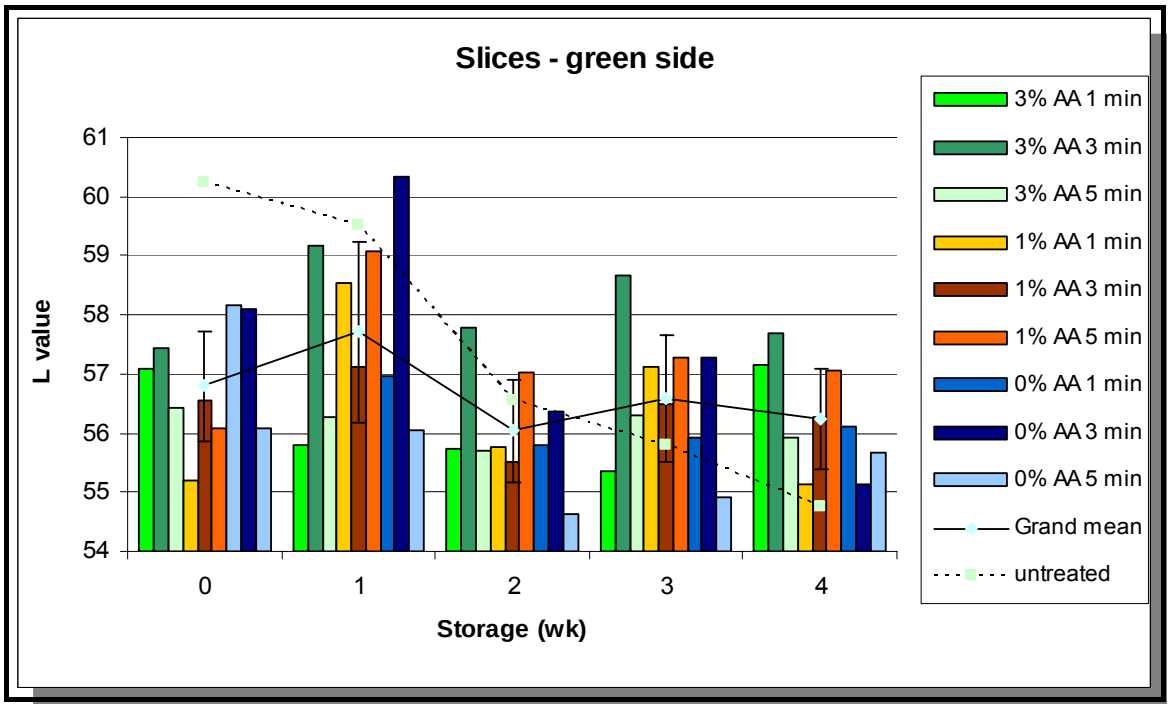

Figure 18: Changes in L values of the green side of avocado slices treated with 0-3% ascorbic acid (AA), vacuum packed in Cryovac B471 bags, treated with HPP at 600MPa for 1 – 5 min and stored at 4°C for 4 weeks. (L*=0 indicates black ; L*=100 indicates white; Grand mean – average of all treatments; Untreated – No HPP and no acidification).

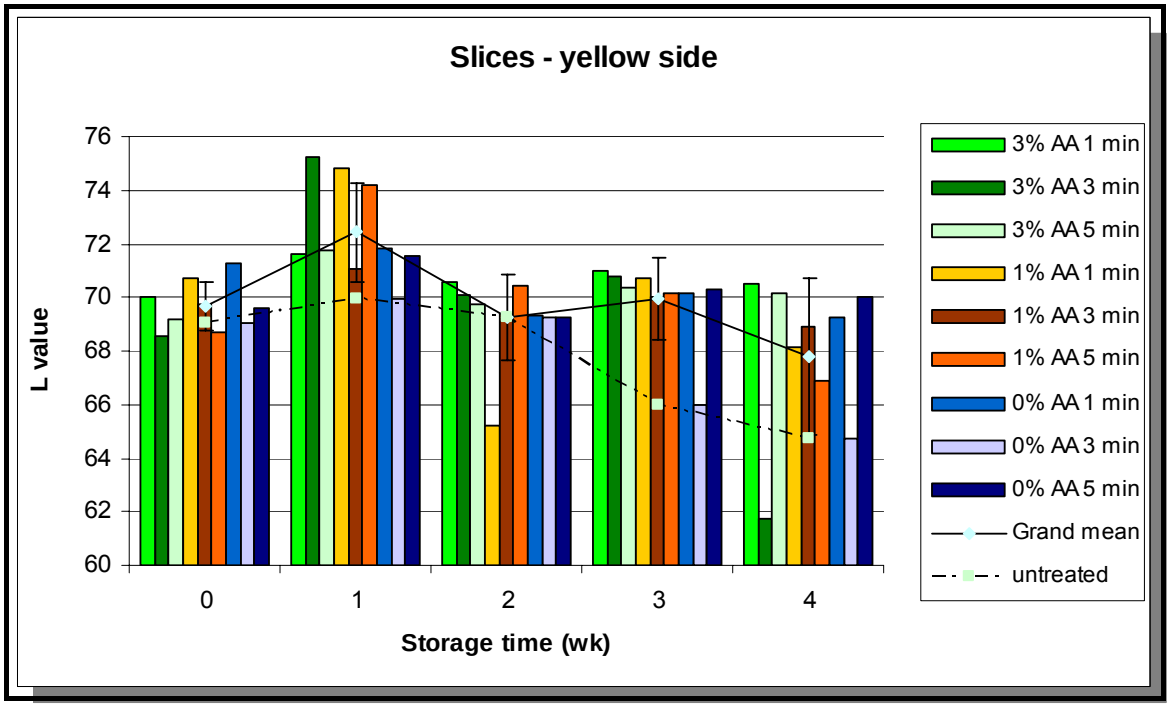

Figure 19: Changes in L values of the yellow side of avocado slices treated with 0-3% ascorbic acid (AA), vacuum packed in Cryovac B471 bags, treated with HPP at 600MPa for 1 – 5 min and stored at 4°C for 4 weeks. (L*=0 indicates black ; L*=100 indicates white; Grand mean – average of all treatments;Untreated – No HPP and no acidification).

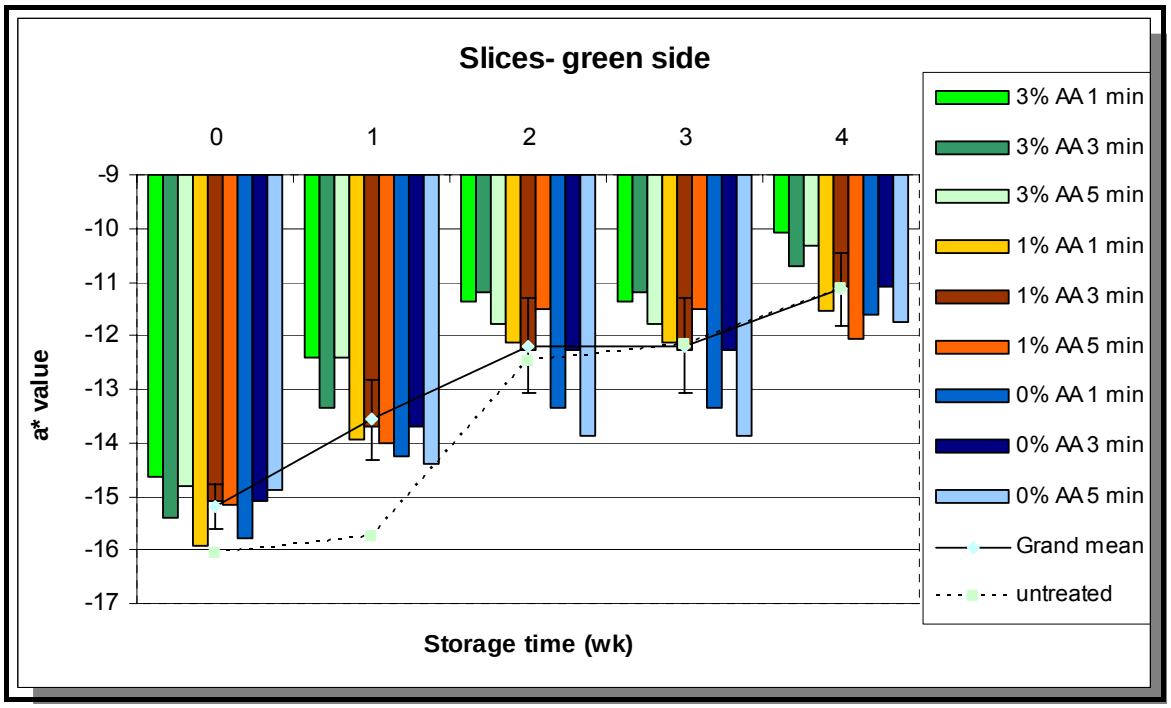

Figure 20: Changes in a* values of the green side of avocado slices treated with 0-3% ascorbic acid (AA), vacuum packed in Cryovac B471 bags, treated with HPP at 600MPa for 1 – 5 min and stored at 4°C for 4 weeks. (-a = indicates green : +a = indicates magenta; Grand mean – average of all treatments;Untreated – No HPP and no acidification).

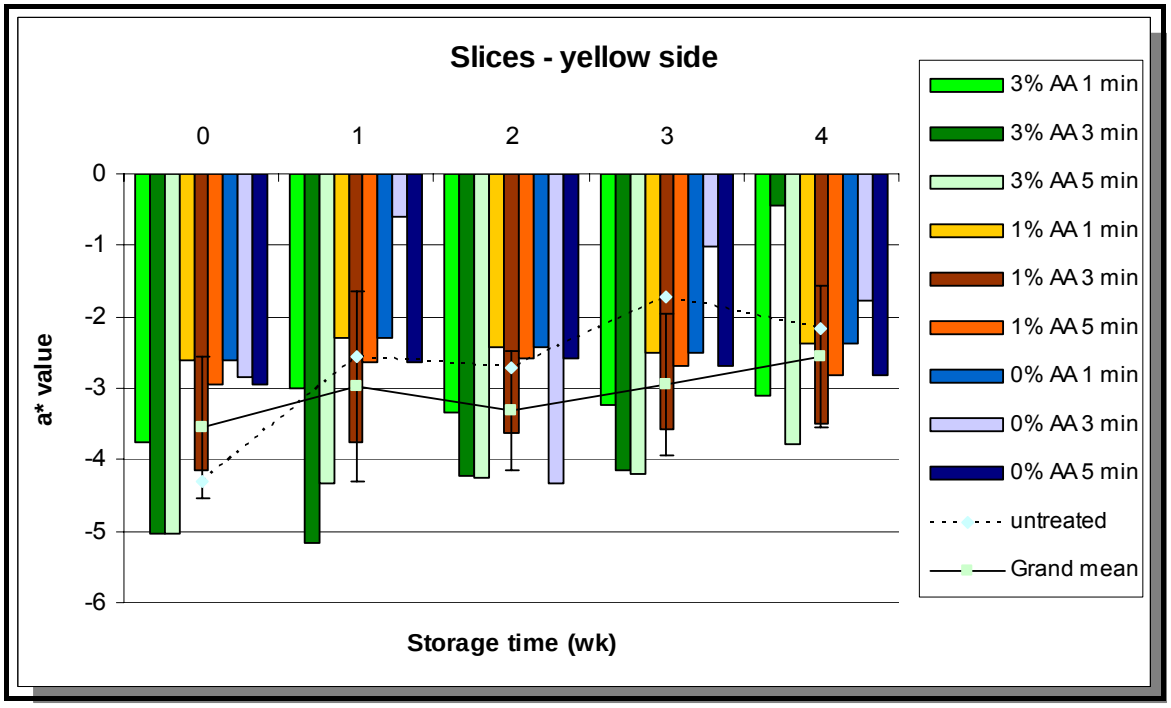

Figure 21: Changes in a* values of the yellow side of avocado slices treated with 0-3% ascorbic acid (AA), vacuum packed in Cryovac B471 bags, treated with HPP at 600MPa for 1 – 5 min and stored at 4°C for 4 weeks. (-a = indicates green : +a = indicates magenta; Grand mean – average of all treatments;Untreated – No HPP and no acidification).

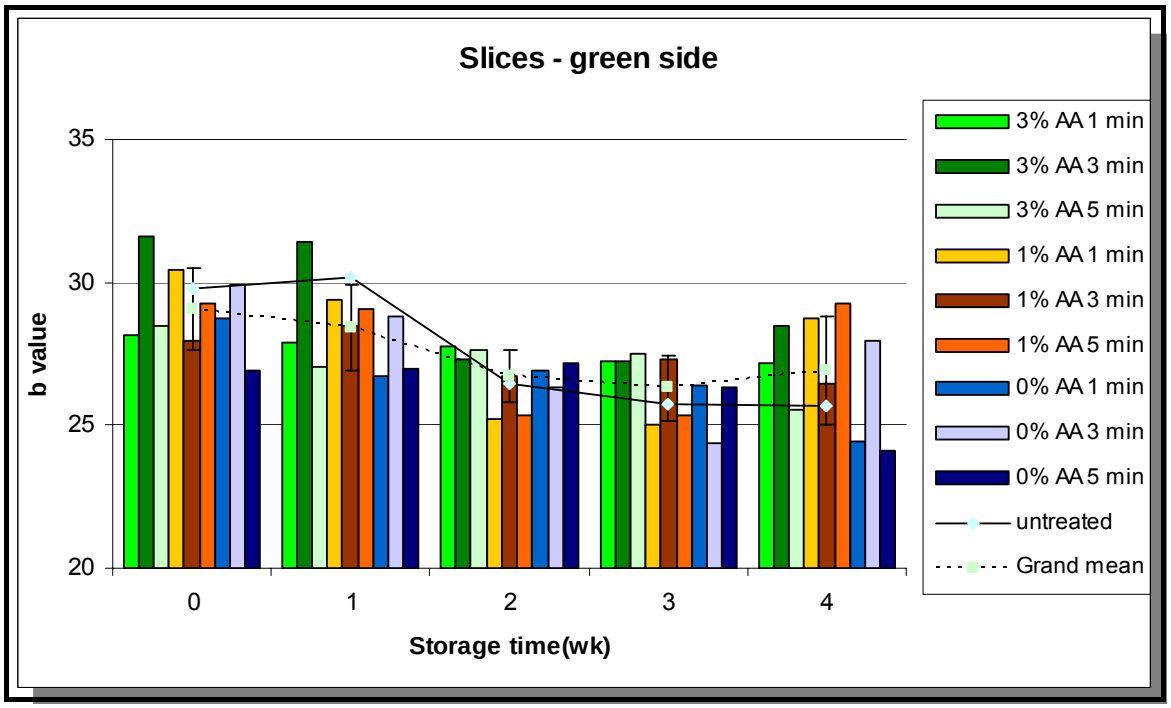

Figure 22: Changes in b* values of the green side of avocado slices treated with 0-3% ascorbic acid (AA), vacuum packed in Cryovac B471 bags, treated with HPP at 600MPa for 1 – 5 min and stored at 4°C for 4 weeks. (-b = indicates blue: +b = indicates yellow; Grand mean – average of all treatments;Untreated – No HPP and no acidification).

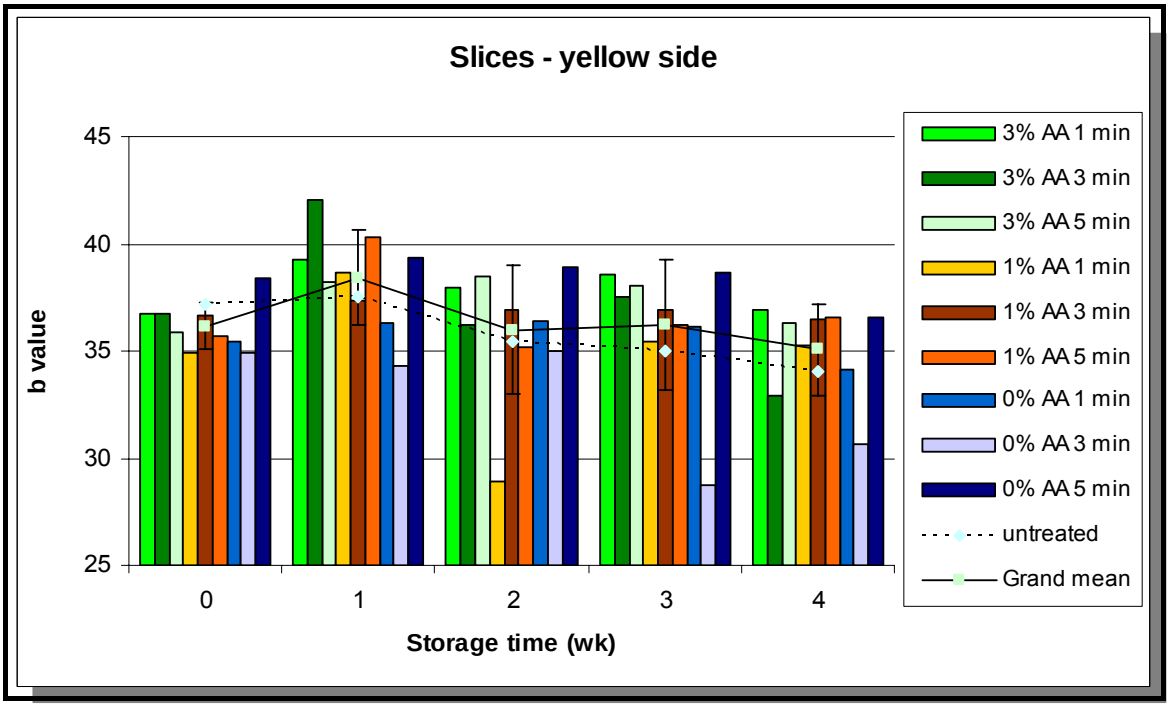

Figure 23: Changes in b* values of the yellow side of avocado slices treated with 0-3% ascorbic acid (AA), vacuum packed in Cryovac B471 bags, treated with HPP at 600MPa for 1 – 5 min and stored at 4°C for 4 weeks. (-b = indicates blue : +b = indicates yellow; Grand mean – average of all treatments;Untreated – No HPP and no acidification).

In Pack Colour Parameters

L value (Lightness): L* values of the treated slices on the green side showed an increase after first week and then a decrease during storage (Figure 18). However, this decrease in untreated samples was much greater than in the treated samples. Trends related to treatments were not observed.

L*values of the yellow side (Figure 19) was much higher than the green side (Figure 18). L* values of yellow side of treated slices also showed an a* value similar to the green side. The untreated samples showed a trend different to the treated samples. Untreated samples showed a continuous decrease in L* values during storage indicating darkening. Treated samples showed a higher and stable L* values during 2 - 4 weeks.

a* values (greenness): The a* values of green side increased from -16 to -11 with storage in all treatments and in the untreated controls (Figure 20). Non acid treated and 1% ascorbic acid treated samples showed a slower increase than the 3% ascorbic acid treated samples. Differences between HPP treatments were not observed.

The a* values of yellow side was higher than the green side (Figure 21). However, a* values of the yellow side were more stable than the a* values of the green side (-5 to -3). On the yellow side a* values of 3% ascorbic treated samples were lower than the 1% acid treated and control samples.

b* value (yellowness): Yellow side showed a higher b* value than green side as expected (Figure 22 and Figure 23). The b* values on the green and yellow sides of the treated samples and the untreated samples showed a similar trend. Differences between acid levels and HPP levels were not very prominent.

Surface pH

The surface pH of the slices showed a differences between the treatments on 0 weeks depending on the acid dip the samples received (Table 38). With storage all slices showed a gradual decrease in pH and reached a value of 5.00 $\pm$ 0.13 (Figure 24) after 4 weeks.

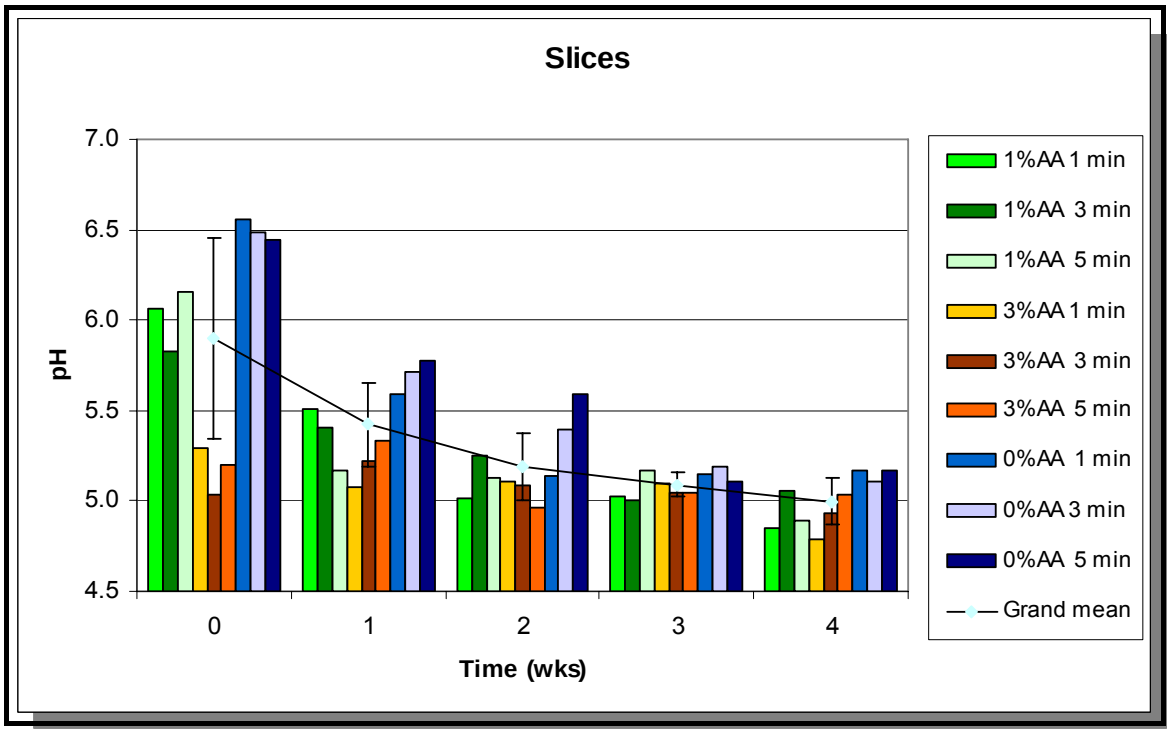

Figure 24: Changes in pH of the avocado slices treated with 0-3% ascorbic acid (AA), vacuum packed in Cryovac B471 bags, treated with HPP at 600MPa for 1 – 5 min and stored at 4°C for 4 weeks (Grand mean – average of all treatments).

Table 38: Statistical significance of main effects and interaction effect on the surface pH values of avocado slices treated with or without ascorbic acid (AA), packed in Cryovac B471 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C on 0 and 4 weeks.

Storage time	Main effect of % AA	Main effect of HPP exposure time	Interaction effect of % AA X HPP exposure time	Probability
pH 0 weeks	Significant 0% AA = 6.52 1% AA = 5.82 2% AA = 5.17 LSD = 0.26	Ns	Ns	P_{pH} = <0.001
pH 4 Weeks	Significant 0% AA = 5.15 1% AA = 4.93 2% AA = 4.92 LSD = 0.16	Ns	Ns	P_{pH} = 0.005

LSD – Least significant difference; Ns – Not significant

The surface pH of acid treated avocado slices significantly decreased with the increase in acid content of the dip used. This was observed on both 0 and 4 weeks. Linear equations were obtained by response surface analysis, to predict the surface pH by using % acid used in the dip (Table 39). Since the pH at 0 weeks and 4 weeks were only influenced by initial pH, response surface curves are not presented in this report.

Table 39: Results of response surface models for surface pH of avocado slices treated with 0-3% ascorbic acid (AA), vacuum packed in Cryovac B471 bags, treated with HPP at 600MPa for 1 – 5 min and stored at 4°C for 4 weeks.

pH after storage	Response surface model
0 weeks	pH_{wk0} = 6.46 -0.428 AA%
4 weeks	pH_{wk4}= 5.12 -0.0754 AA%

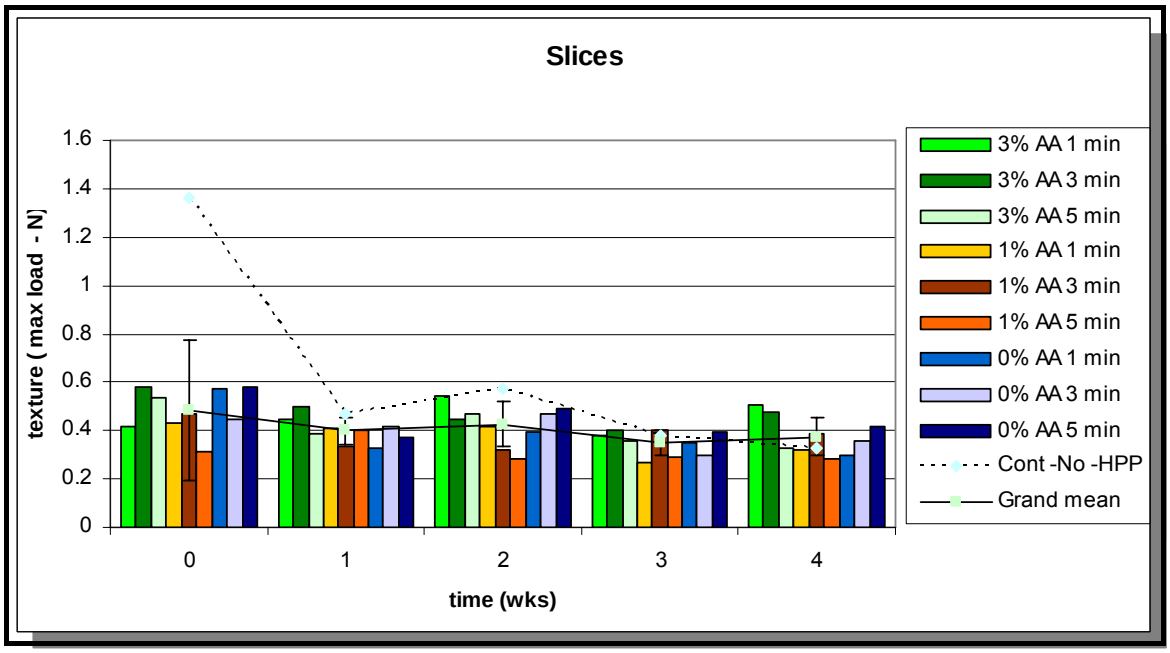

Figure 25: Changes in texture of the avocado slices treated with 0-3% ascorbic acid (AA), vacuum packed in Cryovac B471 bags, treated with HPP at 600MPa for 1 – 5 min and stored at 4°C for 4 weeks(Grand mean – average of all treatments; Cont – No HPP and no acidification).

Texture

Texture of the control-No HPP sample was higher than the all other treatments on day 0 (Figure 25). However, the texture of the control-No HPP sample and all HPP treated samples showed a similar texture after 1 week of storage. During the 4 week storage period the texture values did not show a detectable change. Hence the statistical analysis was not conducted.

Table 40: Statistical significance of main effects and interaction effect on sensory parameters of avocado slices treated with or without ascorbic acid (AA), packed in Cryovac B471 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day.

Parameter	Main effect of % Ascorbic acid in dipping solution	Main effect of HPP exposure time	Interaction effect of pH X HPP exposure time	Probability
Acceptability (A)	Ns	Ns	Ns	
Acidic Taste (AT)	Ns	Ns	Ns	
Out of pack browning (B)	Significant*	Significant*	Significant See table 21	$P_{\%AA}$= 0.002 P_{HPP} < 0.001 $P_{\%AA.HPP}$ = 0.026
Firmness (F)	Significant 0% AA = 4.50 1% AA = 3.53 3% AA = 4.87 LSD = 0.89	Ns	Ns	$P_{\%AA}$ = 0.006

LSD – Least significant difference; Ns – Not significant *- Main effect means are not presented since interaction effect means are presented in Table 21.

Sensory Parameters

Acceptability of slices: According to statistical analysis acceptability of slices was not affected by the level of acid in dipping solution and the HPP treatment (Table 40). The acceptability scores ranged between 6.85 and 4.91 (Table 42).

The response surface analysis was used to obtain a linear relationship to predict acceptability using % ascorbic acid in the acid dip. The relevant equation is given in Table 41.

Table 41: The response surface models for sensory attributes of avocado slices treated with or without ascorbic acid (AA), packed in Cryovac B471 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day.

Sensory attribute	Response surface models
Acceptability	A = 6.42 -0.359 AA %
Acidic taste	AT = 1.96 + 0.550 AA %
Open pack browning	B = 3.09 -0.321 AA %+ 0.270 HPP
Firmness	F = 4.48 -0.882 AA% + 0.331 $(AA\%)^2$

Table 42: Acceptability scores of avocado slices treated with or without ascorbic acid (AA), packed in Cryovac B471 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day of storage at 4°C (pH X HPP - interaction effect).

% AA	HPP exposure time (min)		
	1	3	5
0	6.85	5.24	6.24
1	6.54	6.60	4.94
3	5.48	5.64	4.91

Marginal score = 5

Acidic taste: The statistical analysis did not show significant effects of the % of acid in dipping solution and the HPP treatment on acidic taste (Table 40). Acidic taste ranged from 1.73 for 1% acid dip + 1 min HPP to 4.28 for 3%acid dip + 3 min HPP time (Table 43).

The response surface analysis was used to obtain a linear relationship to predict acidic taste using % ascorbic acid in acid dip. The relevant equation is given in Table 41.

Table 43: Acidic taste scores of avocado slices treated with or without ascorbic acid (AA), packed in Cryovac B471 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day of storage at 4°C (pH X HPP - interaction effect).

% AA	HPP exposure time (min)		
	1	3	5
0	2.31	2.18	1.92
1	1.73	2.23	2.93
3	3.24	4.28	3.84

Out of pack browning: Both statistical analysis and response surface analysis showed that browning of slices was significantly influenced by the interaction effect of % acid in the acid dip and HPP treatment time (Table 40). In general the level of browning was lower than the marginal value of 5. Browning scores were between 1.27 for 3% acid dip + 3 min exposure time and 4.92 for 1% acid dip + 5 min exposure time (Table 44). The lowest browning scores was observed in 3% acid dip +3 min HPP treatment (1.27) and this was significantly different from all other treatments except for 1%acid dip +1 min HPP treatment (2.28).

Out of pack browning values could be predicted using the response surface model given in Table 21. According to Figure 26 browning was lowest at highest acid level and lowest HPP exposure time which is 0.

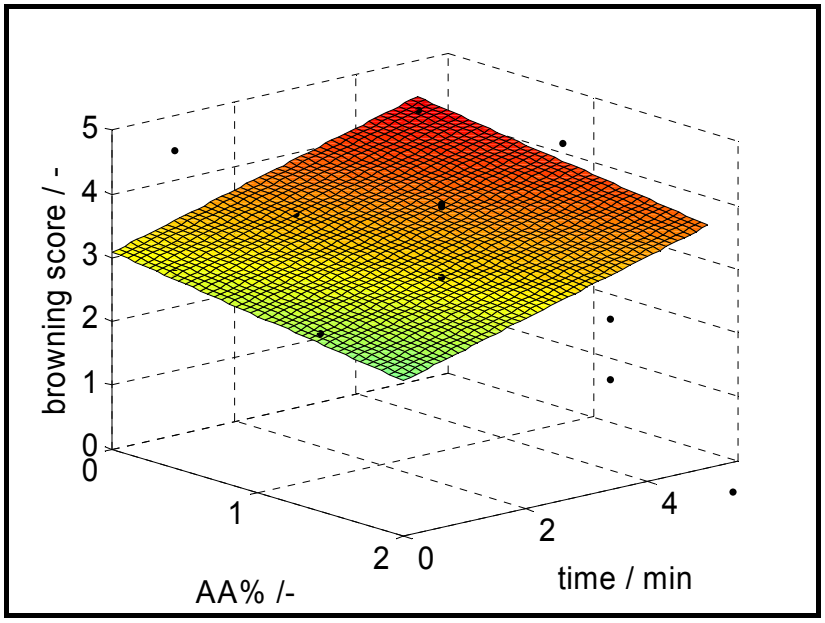

Figure 26: Response surface curve on out of pack browning scores of avocado slices treated with or without ascorbic acid (AA), packed in Cryovac

Table 44: Out of pack browning scores of avocado slices treated with or without ascorbic acid (AA), packed in Cryovac B471 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day of storage at 4°C (pH X HPP - interaction effect).

% ascorbic acid	HPP exposure time (min)		
	1	3	5
0	3.25	3.57	4.54
1	2.82	3.50	4.92
3	3.16	1.27	3.51

LSD = 1.61

Firmness: Statistical analysis showed significant main effect of % acid in dipping solution on the firmness of avocado slices (Table 40). Untreated control and 3% AA dip treated samples showed a significantly higher firmness than 1% acid dipped samples.

In general firmness scores ranged between 2.82 (1% AA + 5 min HPP) to 5.33 (3%AA + 1 min HPP) (Table 45).

Table 45: Firmness scores of avocado slices treated with or without ascorbic acid (AA), packed in Cryovac B471 bags, HPP treated at 600 MPa 1-5 min and stored at 4°C for 1 day of storage at 4°C (pH X HPP - interaction effect).

% AA	HPP exposure time (min)		
	1	3	5
0	4.30	4.17	5.04
1	3.72	4.06	2.82
3	5.33	4.72	4.57

According to response surface analysis firmness was only influenced by the acid dip. The equation to predict firmness based on % ascorbic acid in the dip is given in Table 41.

4.3 Experiment 8: Validation Of Results Obtained In Experiment 7 Using Sheppard Variety

This experiment was conducted to validate the observations made in experiment 7 using Sheppard avocado variety. Since acceptance and acidic taste were major limiting factors in some of the more acidic treatments used in experiment 7, 1% malic, 3% ascorbic acid and pH adjustments at 5.0 were used with avocado halves, slices and pulp respectively in experiment 8.

4.3.1 Pulp

Microbiological Counts

Table 46: Standard plate counts of Sheppard avocado pulp, acidified with or without ascorbic acid + malic acid (MA) mix, packed in Amcor bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks.

Treatment	Storage time (week)	
	0	4
pH 6.50 No HPP	2.0	5.3
pH 5.00 No HPP	1.7	4.3
pH 6.50 HPP	1.9	< 1.0
pH 5.00 HPP	2.3	1.0

Minimum detectable level <1.0 $\log_{10}$ cfu/g

Standard plate counts: On day 0 after pressure treatment, unacidified (pH 6.5) and acidified (pH5.5) samples showed no immediate reduction in viable count (Table 46). However, after 4 weeks of storage a 4-5 log unit reduction in viable count of pressure treated samples compared to untreated control samples was observed.

Yeast and mould counts: Yeast and mould counts were minimal throughout storage of pressure treated and untreated samples (Table 47). The highest count (2.6 $\log_{10}$ cfu/g) was observed in unacidified (pH6.5) samples not subjected to HPP.

Table 47: Yeast and mould counts of avocado pulp of Sheppard variety, acidified with or without ascorbic acid + Malic acid (MA) mix, packed in Amcor bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks.

Treatment	Storage time (week)	
	0	4
pH 6.50 No HPP	2.2	2.6
pH 5.00 No HPP	<2.0*	2.3
pH 6.50 HPP	<2.0	2.3
pH 5.00 HPP	<2.0	<2.0*

Minimum detectable level <2.0 $\log_{10}$ cfu/g ; *Counts lower than 2.0 $\log_{10}$ cfu/g were present in one replicate

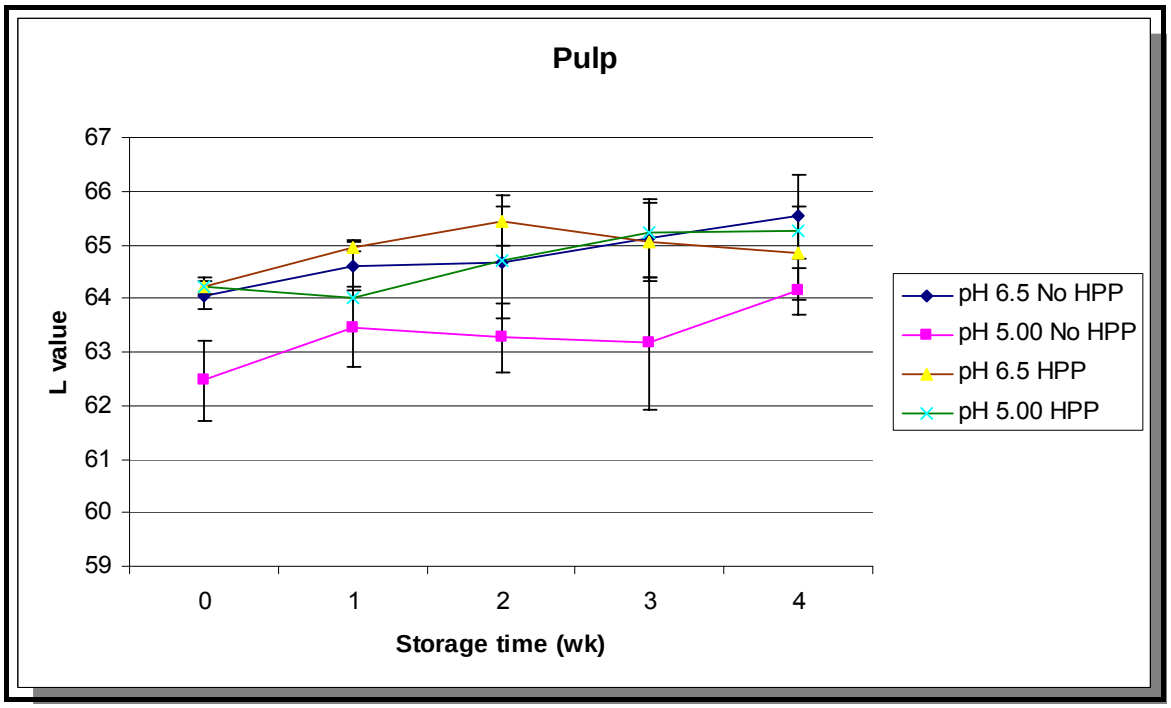

Figure 27: L* values of Sheppard avocado pulp, acidified with or without ascorbic acid + malic acid (MA) mix, packed in Amcor bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks(L*=0 indicates black ; L*=100 indicates white).

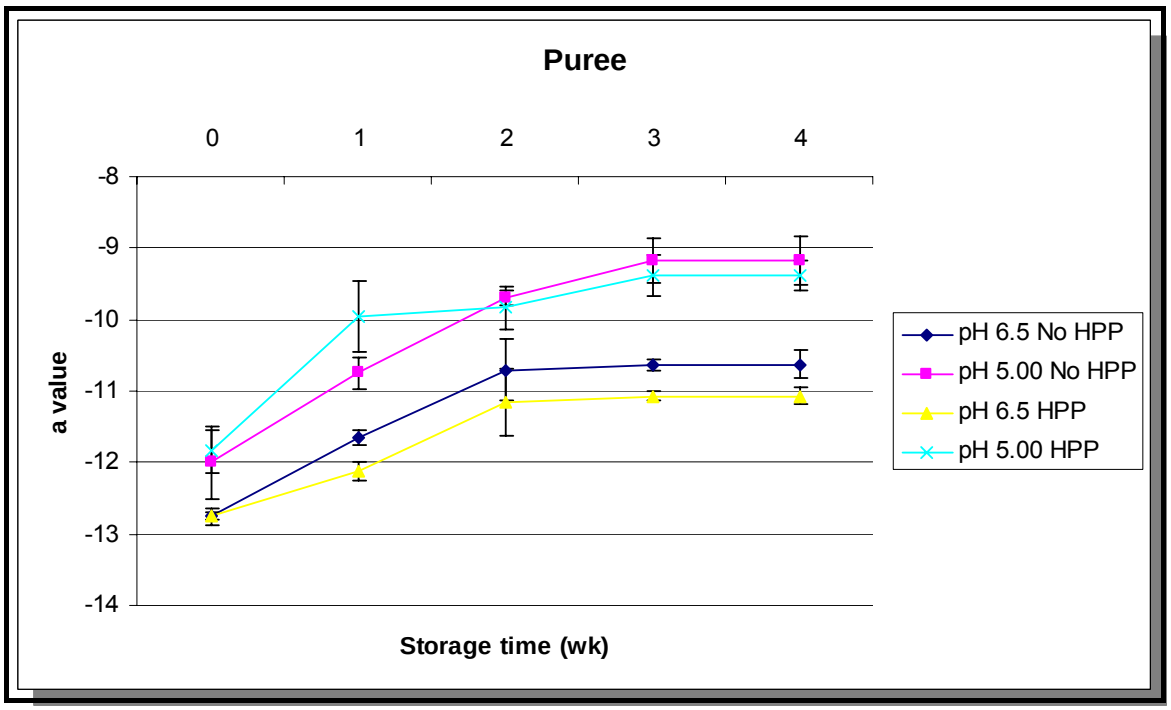

Figure 28: The a* values of Sheppard avocado pulp, acidified with or without ascorbic acid + malic acid (MA) mix, packed in Amcor bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks (-a = indicates green : +a = indicates magenta).

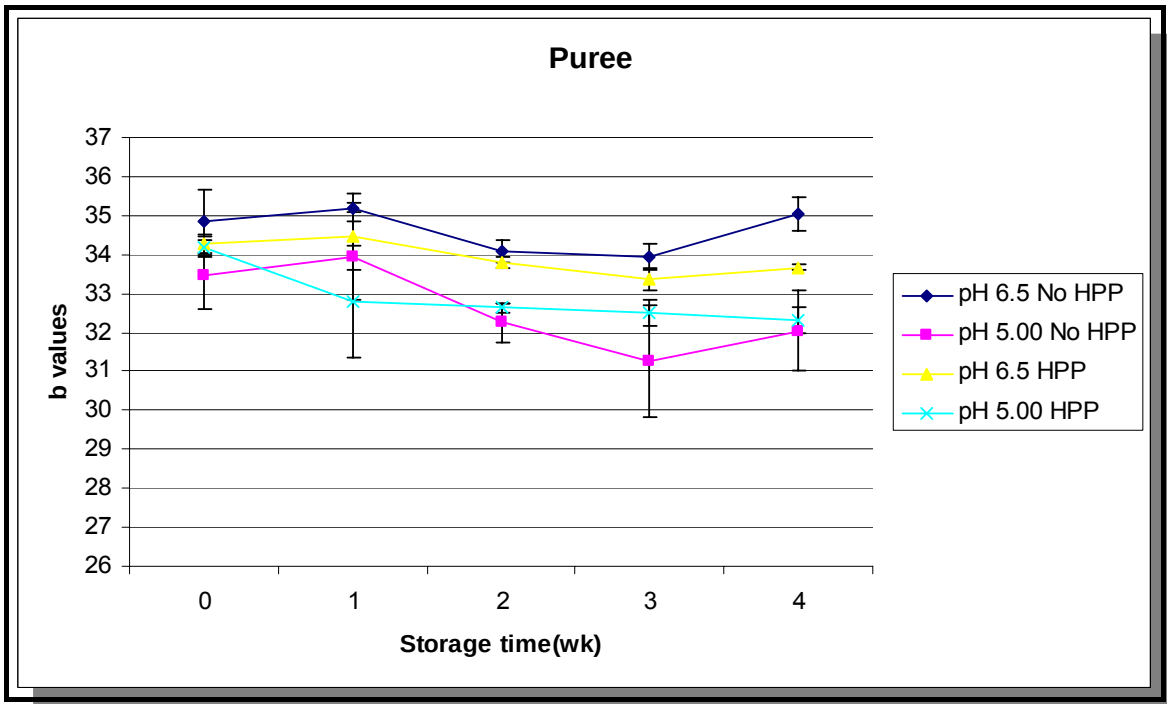

Figure 29: The b* values of avocado pulp of Sheppard variety, acidified with or without ascorbic acid + malic acid (MA) mix, packed in Amcor bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks (-b = indicates blue : +b = indicates yellow).

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Colour

L* value (lightness): The L* values of pulp were slightly increased and showed no difference between treatments over the storage period (Figure 27). Statistical analysis showed pH x HPP effect significant and pH = 5.00 and No HPP treatment showed significantly low L* values.

a* value (greenness): The a* values of pulp showed a gradual increase indicating browning but were stable after 3 weeks (Figure 28). The difference between two pH levels were apparent with a* values. Unacidified samples showed lower a* value than samples acidified to pH5.00. Statistical analysis showed significant effects of pH x HPP and storage time x HPP.

b* value (yellowness): The b* values were stable over the storage period indicating minimal changes in yellowness of the samples (Figure 29). However, the two pH levels showed differences in b* value. Acidified samples showed lower b values. Statistical analysis showed that pH x HPP effect is significant.

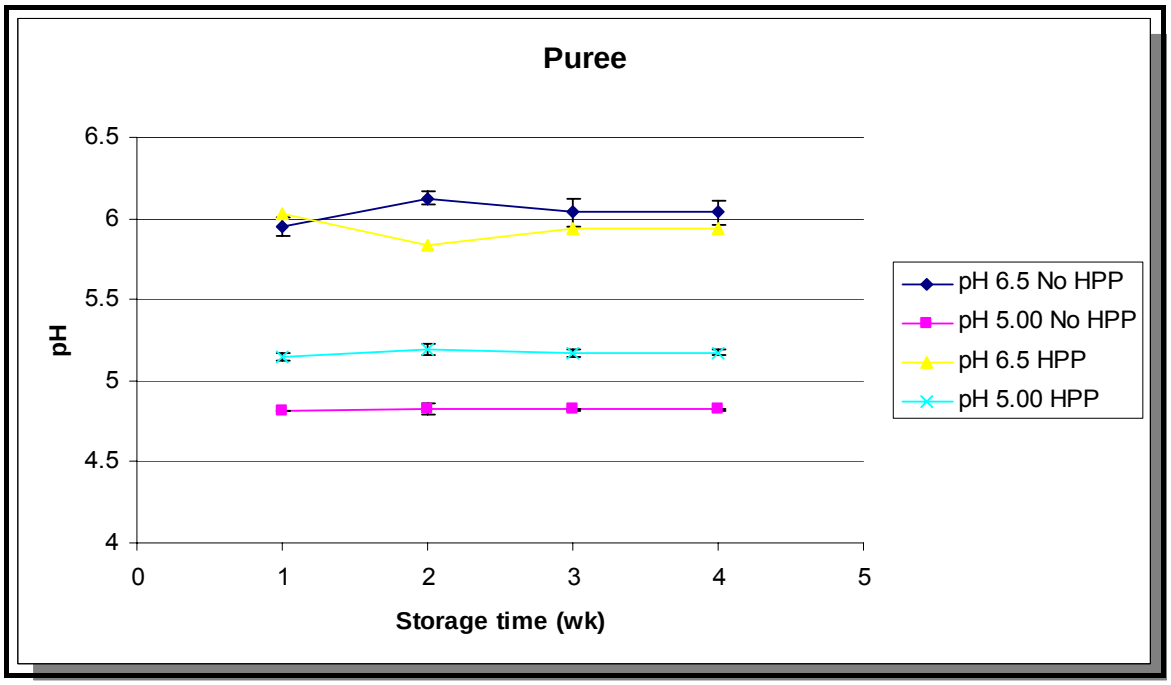

Figure 30: The pH values of Sheppard avocado pulp, acidified with or without ascorbic acid + malic acid (MA) mix, packed in Amcor bags, with or without HPP treatment at 600 MPa 1-5 min and stored at 4°C for 4 weeks.

pH

The avocado pulp showed a stable pH over the 4 week storage period and the pulp pH was dependent on the acidification (Figure 30). Difference between HPP treatment and No HPP was observed only at pH 5.00, where the HPP treatment increased the pH to a level above pH 5.00.

4.3.2 Halves

Microbiological Quality

Standard plate count: Avocado halves treated with acid dip showed no standard plate counts irrespective of the HPP treatment (Table 48). Non acid treated samples showed low plate counts of on the 0 week. After 4 weeks samples that had no HPP treatment showed higher counts at the magnitude of 4 – 5 $\log_{10}$ cfu/g. Samples that received 1% MA dip + HPP treatment showed no standard plate count.

Yeast and mould count Yeast and mould counts were not observed in treatments that had HPP conditions on both 0 and 4 weeks (Table 49). Controls that did not receive an HPP treatment contained 2 – 3 $\log_{10}$ cfu/g magnitude after 4 weeks storage.

Table 48: Standard plate counts of avocado halves of Sheppard variety, acidified with or without 1% malic acid dip (MA), packed in Winpack 7000 bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks.

Treatment	Storage time (week)	
	0	4
No acid dip + No HPP	1.8	5.8
1% MA dip + No HPP	<1.0	4.7
No acid dip + HPP	<1.0*	1.2
1% MA dip + HPP	<1.0	<1.0

Minimum detectable level <1.0 $\log_{10}$ cfu/g ; * Counts at 1.0 $\log_{10}$ cfu/g were observed in replicates.

Table 49: Yeast and mould counts of Sheppard avocado halves, acidified with or without 1% malic acid dip (MA), packed in Winpack 7000 bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks.

Treatment	Storage time (week)	
	0	4
No acid dip + No HPP	2.2	2.2
1% MA dip + No HPP	<2.0*	3.4
No acid dip + HPP	<2.0	<2.0
1% MA dip + HPP	<2.0	<2.0

Minimum detectable level <2.0 $\log_{10}$ cfu/g * counts at 2.0 $\log_{10}$ cfu/g were observed in replicates

Texture

The texture of controls that had no HPP treatments decreased after 1 week of storage (Table 25). HPP treated samples also had a texture that is similar to that were not treated with HPP after one week. Texture remained unchanged during the rest of the storage period up to 4 weeks.

Surface pH: The pH of avocado halves decreased with storage (Figure 32). The highest pH was observed in untreated controls and the lowest pH was observed in 1% MA dip + HPP treatment.

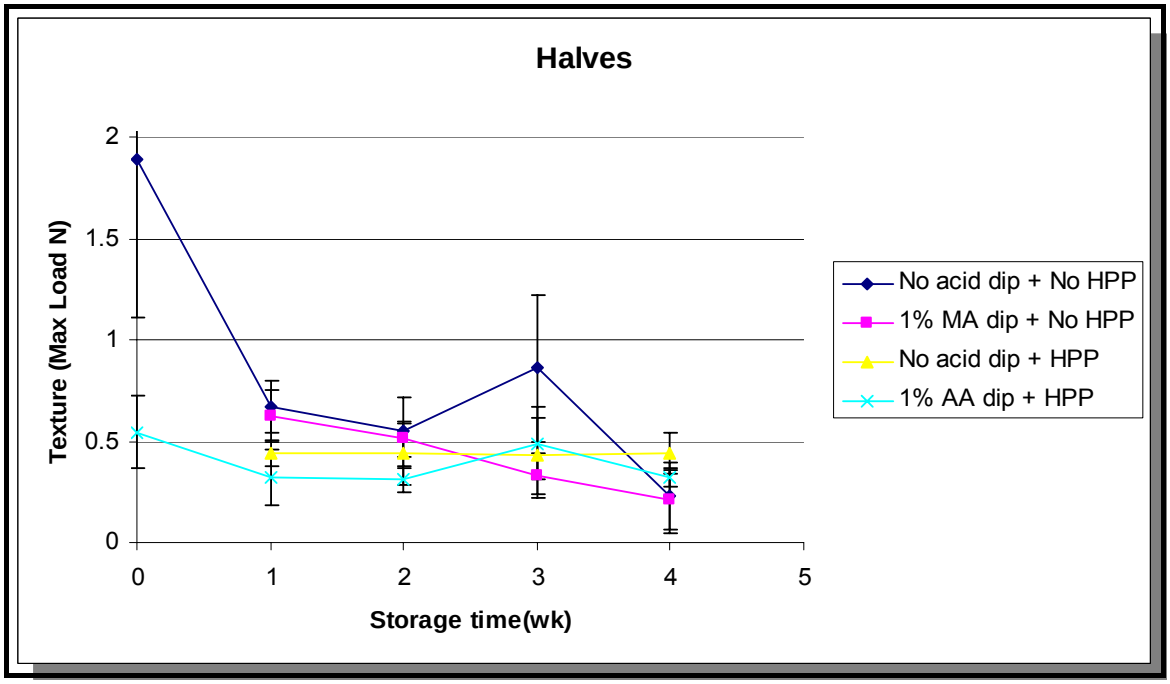

Figure 31: Texture of Sheppard avocado halves, acidified with or without 1% malic acid dip, packed in Winpack 7000 bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks.

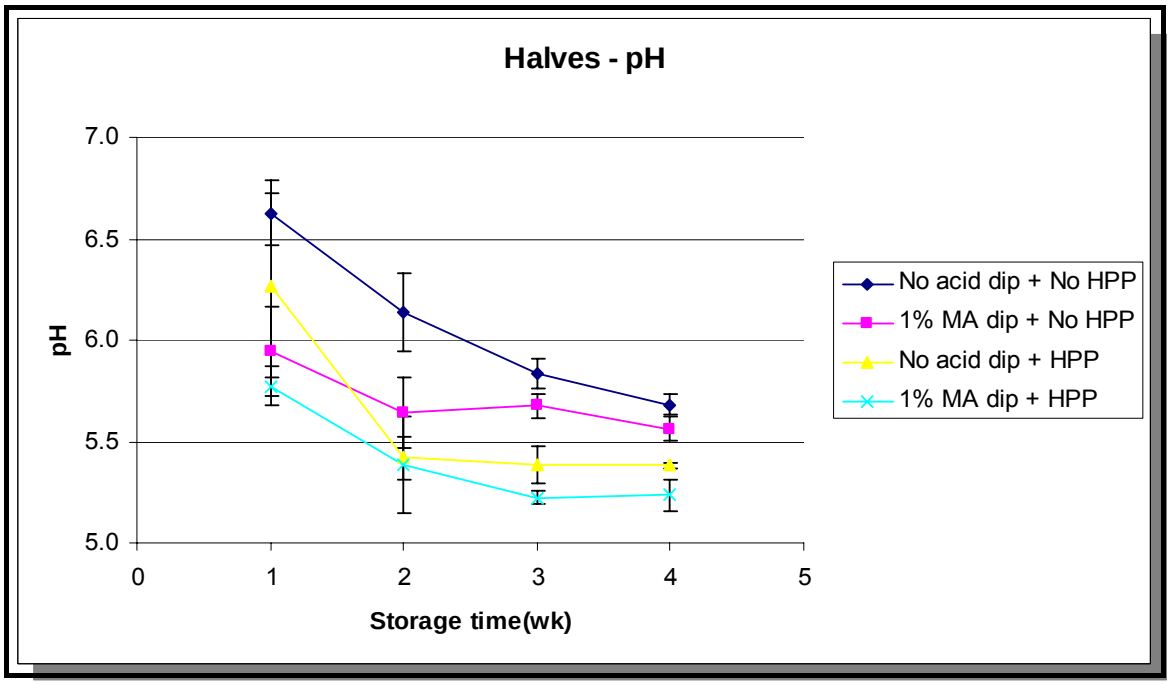

Figure 32: The pH of Sheppard avocado halves, acidified with or without 1% malic acid dip, packed in Winpack 7000 bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks.

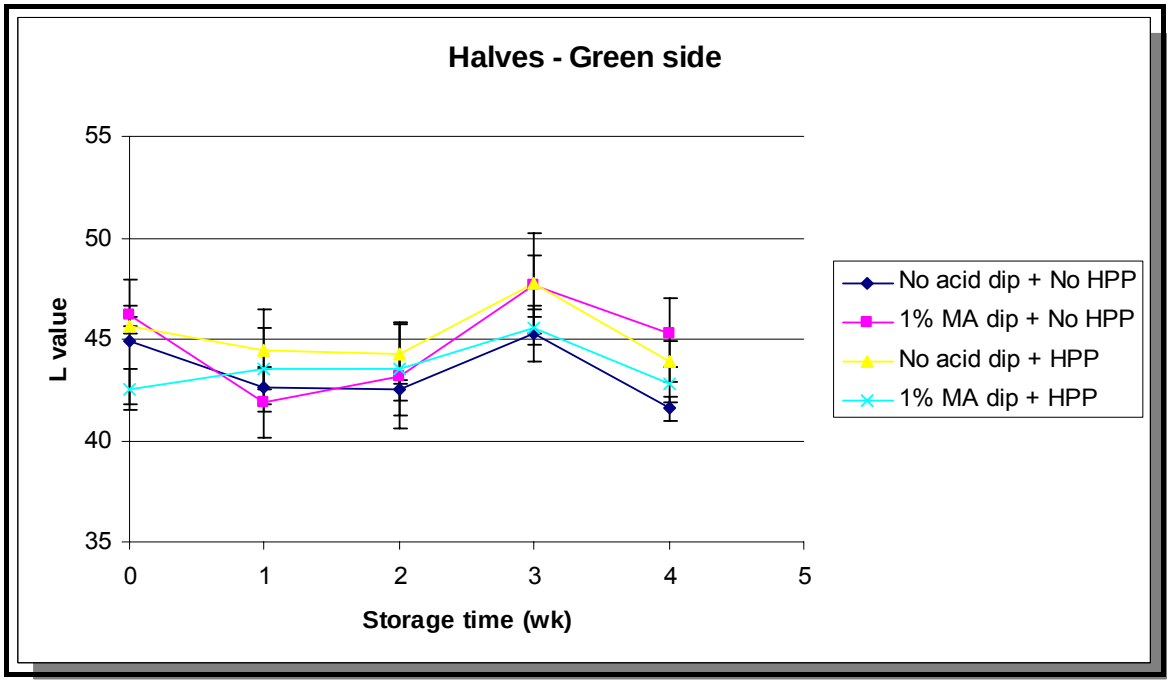

Figure 33: L* values of the green side of avocado halves (Sheppard cv.), acidified with or without 1% malic acid dip, packed in Winpack 7000 bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks (L*=0 indicates black ; L*=100 indicates white).

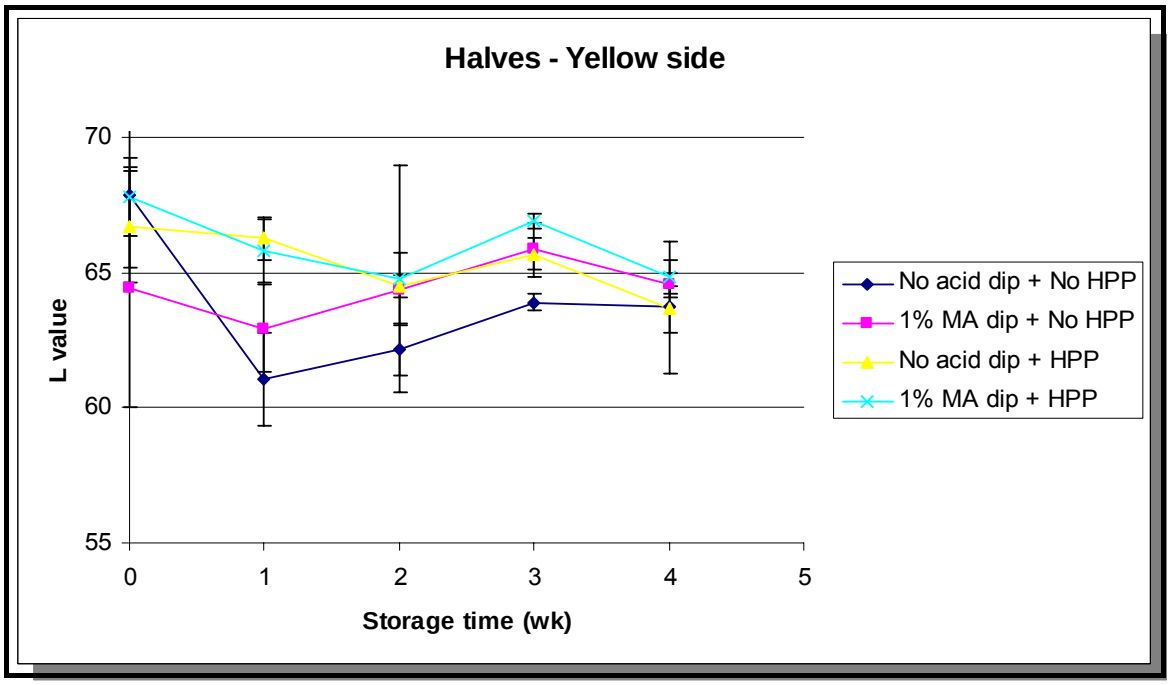

Figure 34: L* values of the yellow side of Sheppard avocado halves, acidified with or without 1% malic acid dip, packed in Winpack 7000 bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks (L*=0 indicates black ; L*=100 indicates white).

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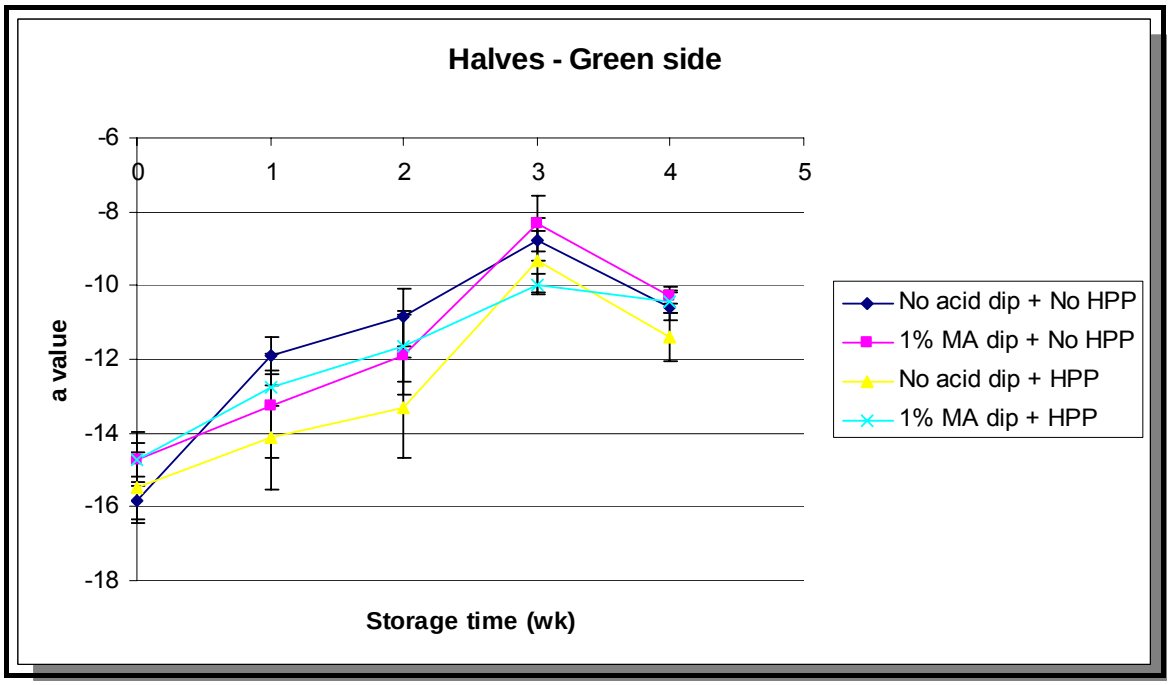

Figure 35: a* values of the green side of Sheppard avocado halves, acidified with or without 1% malic acid dip, packed in Winpack 7000 bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks(-a = indicates green : +a = indicates magenta).

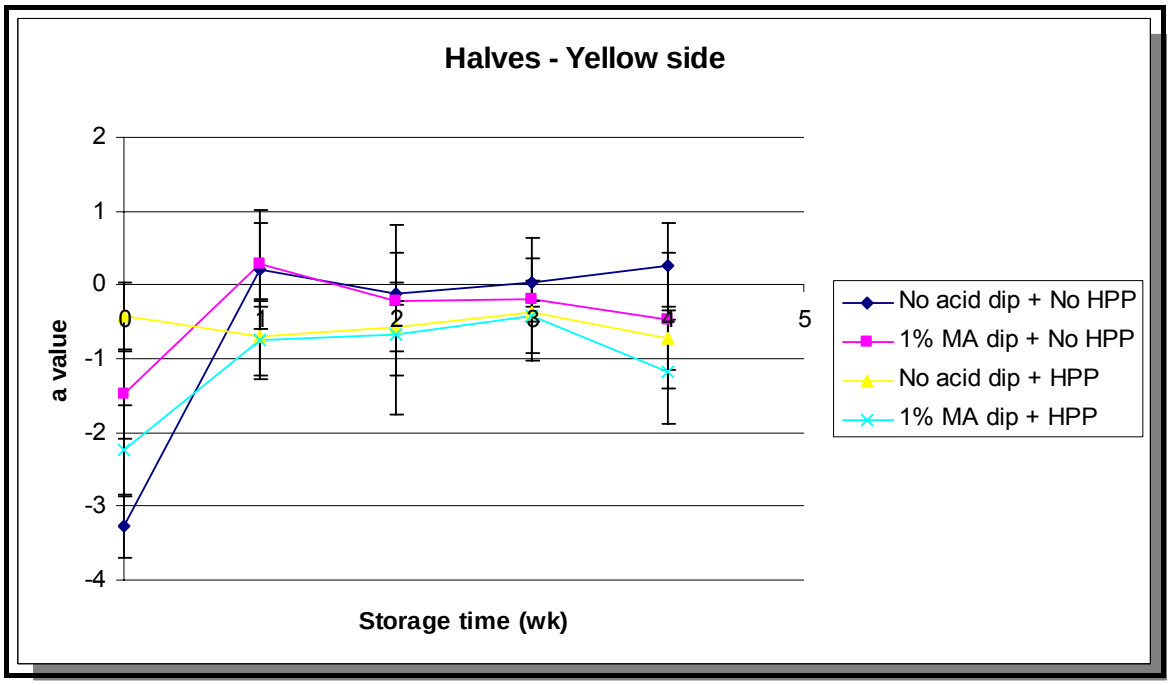

Figure 36: a* values of the yellow side of Sheppard avocado halves, acidified with or without 1% malic acid dip, packed in Winpack 7000 bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks (-a = indicates green : +a = indicates magenta).

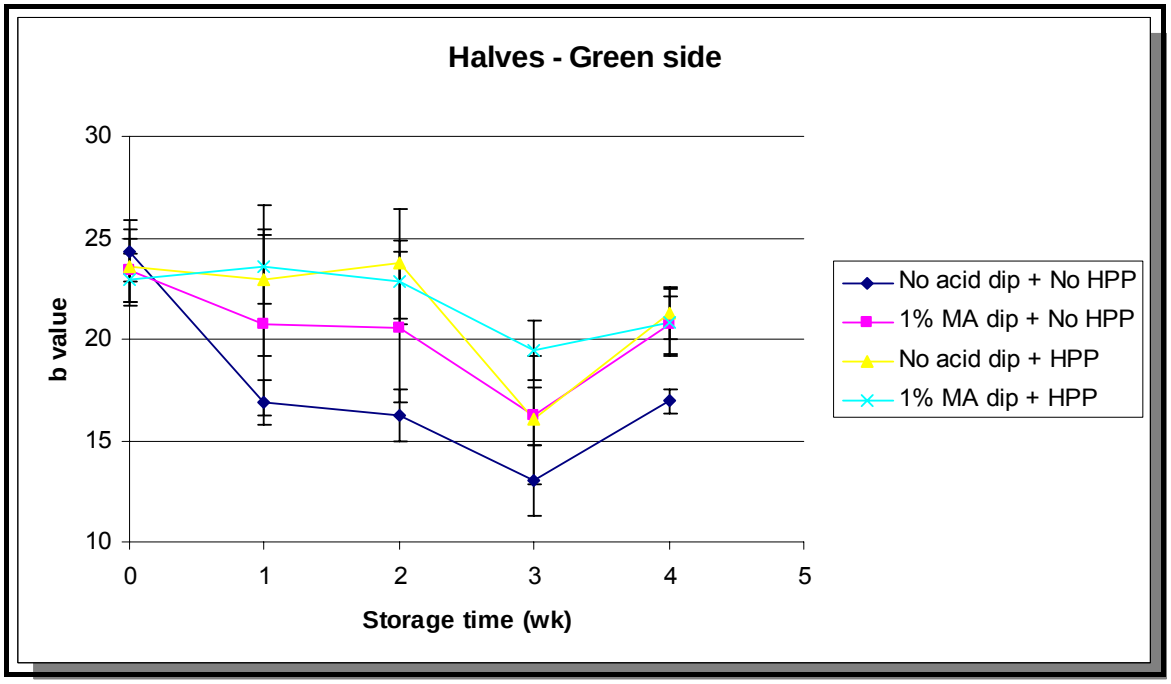

Figure 37: b* values of the green side of Sheppard avocado halves, acidified with or without 1% malic acid dip, packed in Winpack 7000 bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks (-b = indicates blue : +b = indicates yellow).

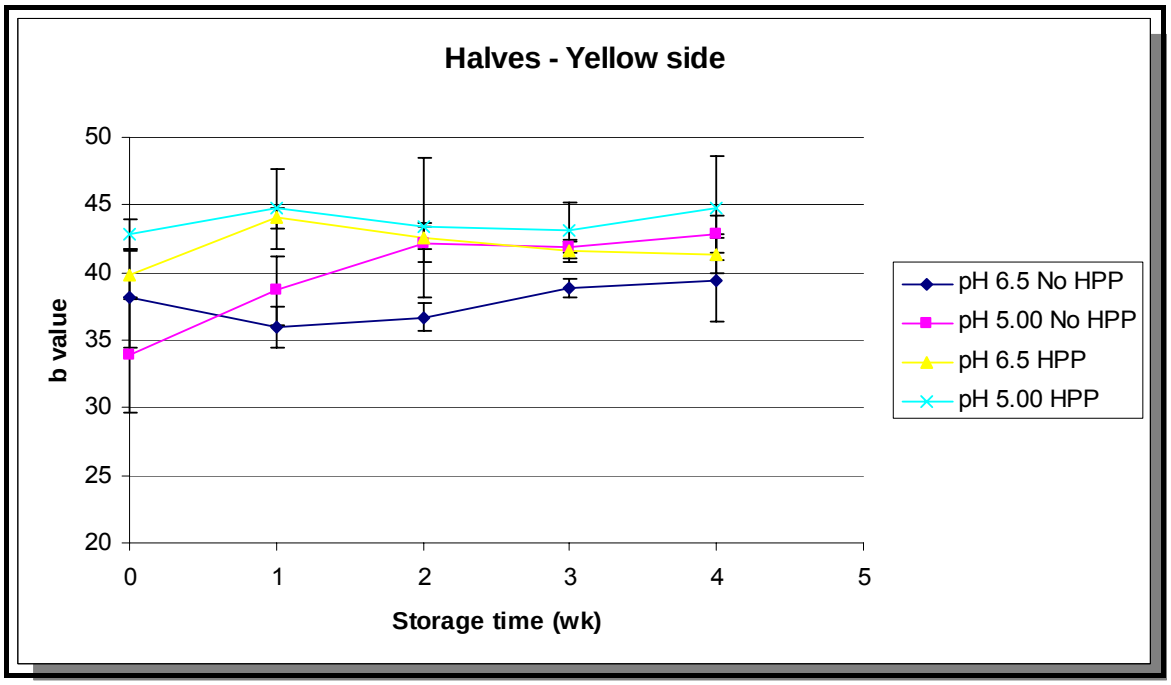

Figure 38: b* values of the yellow side of Sheppard avocado halves, acidified with or without 1% malic acid dip, packed in Winpack 7000 bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks (-b = indicates blue : +b = indicates yellow).

In Pack Colour

L* Value (lightness): The L* value of the green side fluctuated around 45 where as the L* value of the yellow side fluctuated around 65 (Figure 33 and Figure 34). Treatment related differences were not observed in L* values.

a* Value (greenness): a* values showed a slight increase up to week 3 and then decreased on the green side (Figure 35). The a* values of yellow side increased to positive values indicating the increase in reddishness (Figure 36). HPP treated samples showed an increase in a* value but the values remained negative and stable after week 1.

b* Value (yellowness): The b* values of the green side of the avocado halves decreased slightly with storage (Figure 37). The lowest b* values were observed in untreated controls on both green and yellow sides. On the yellow side of avocado halves b* value did not show a decrease and was stable over the storage period (Figure 38).

4.3.3 Slices

Table 50: Standard plate counts of Sheppard avocado slices, acidified with or without 3% ascorbic acid (AA) dip, packed in Cryovac B471 bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks.

Treatment	Storage time (week)	
	0	4
No acid dip + No HPP	<1.0	5.6
3% AA dip + No HPP	<1.0	4.9
No acid dip + HPP	<1.0	<1.0
3% AA dip + HPP	<1.0	<1.0

Minimum detectable level <1.0 $\log_{10}$ cfu/g

Microbiological Parameters Of Slices

Pressure treatment of unacidified and acidified samples resulted in a 4 to 5 log reduction in standard plate count at 4 weeks of storage. (Table 49). Yeast and mould counts were observed

only at low levels (<700cfu/g) in untreated and pressure treated samples after 4 weeks of storage (Table 51).

Table 51: Yeast and mould counts of Sheppard avocado slices, acidified with or without 3% ascorbic acid dip, packed in Cryovac B471 bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks.

Treatment	Storage time (week)	
	0	4
No acid dip + No HPP	<2.0	2.0
3% AA dip + No HPP	<2.0	<2.0
No acid dip + HPP	<2.0	<2.0
3% AA dip + HPP	<2.0	2.8

Minimum detectable level <2.0 $\log_{10}$ cfu/g

Texture

The texture of avocado slices decreased from the initial value to a lower value after one week storage irrespective to the treatments they receive. After the first week texture was maintained for 4 weeks. HPP treated samples showed no difference to the control samples that were not HPP treated (Figure 39).

Surface pH

The initial pH of slices was dependent on the dip treatment. Samples that received acid dip showed a lower pH initially (Figure 40). With storage in all samples pH decreased and reached a value around 5.50.

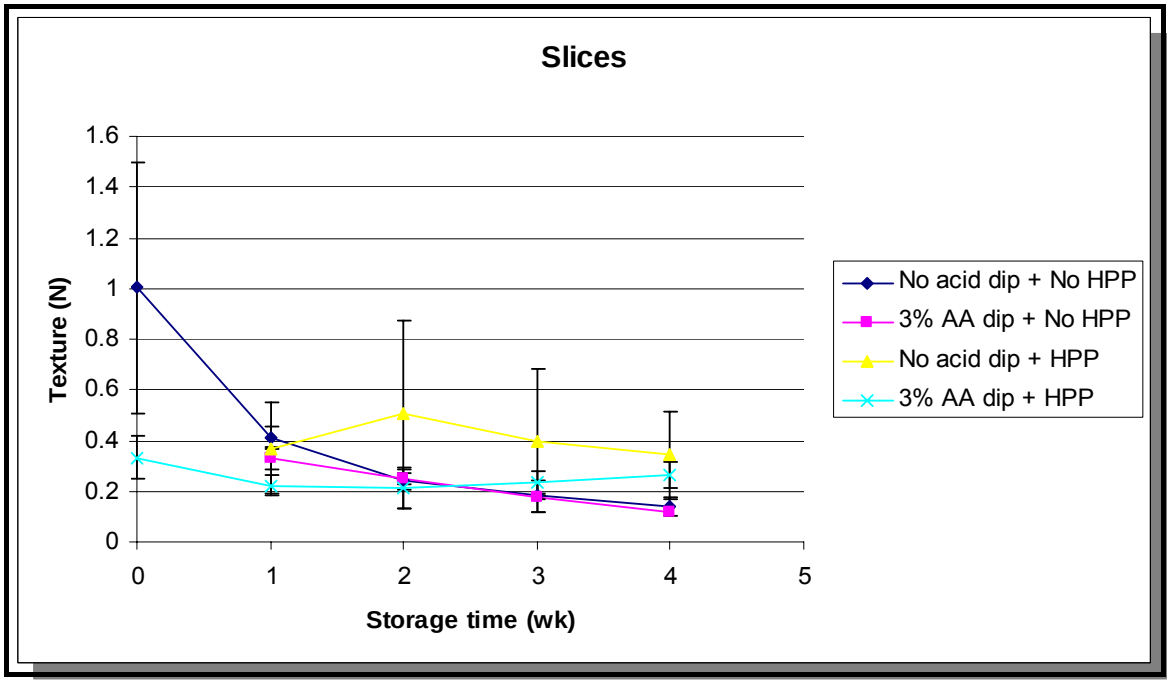

Figure 39: Texture of Sheppard avocado slices, acidified with or without 3% ascorbic acid dip, packed in Cryovac B471 bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks.

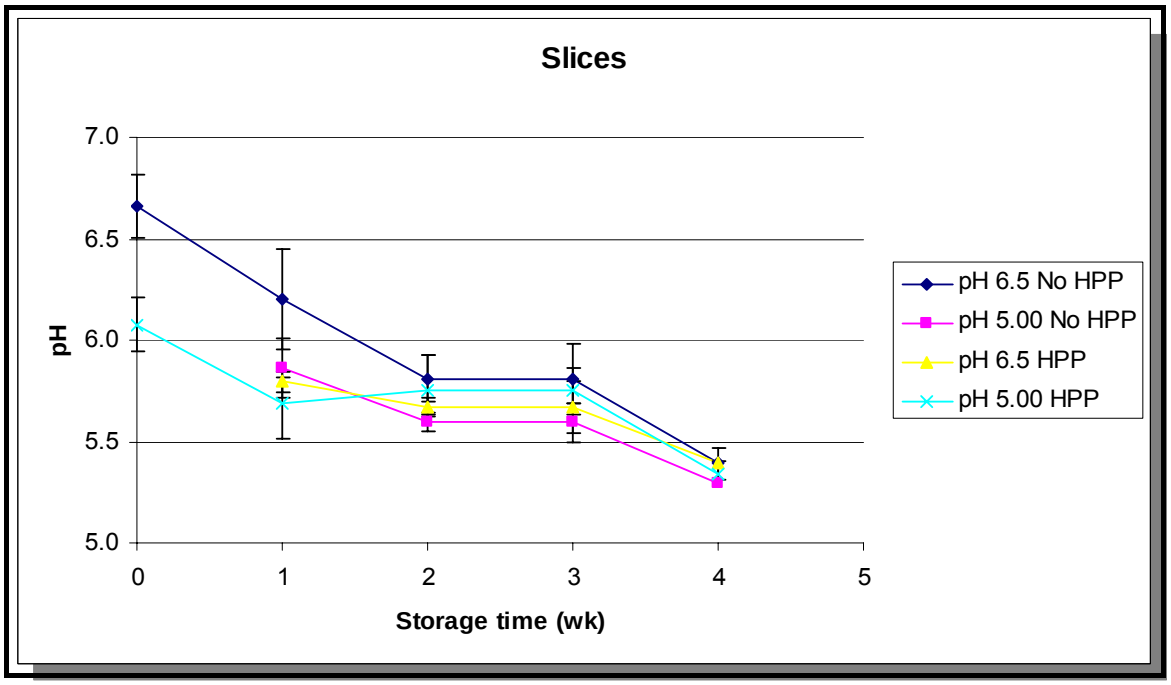

Figure 40: The pH of Sheppard avocado slices, acidified with or without 3% ascorbic acid dip, packed in Cryovac B471 bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks.

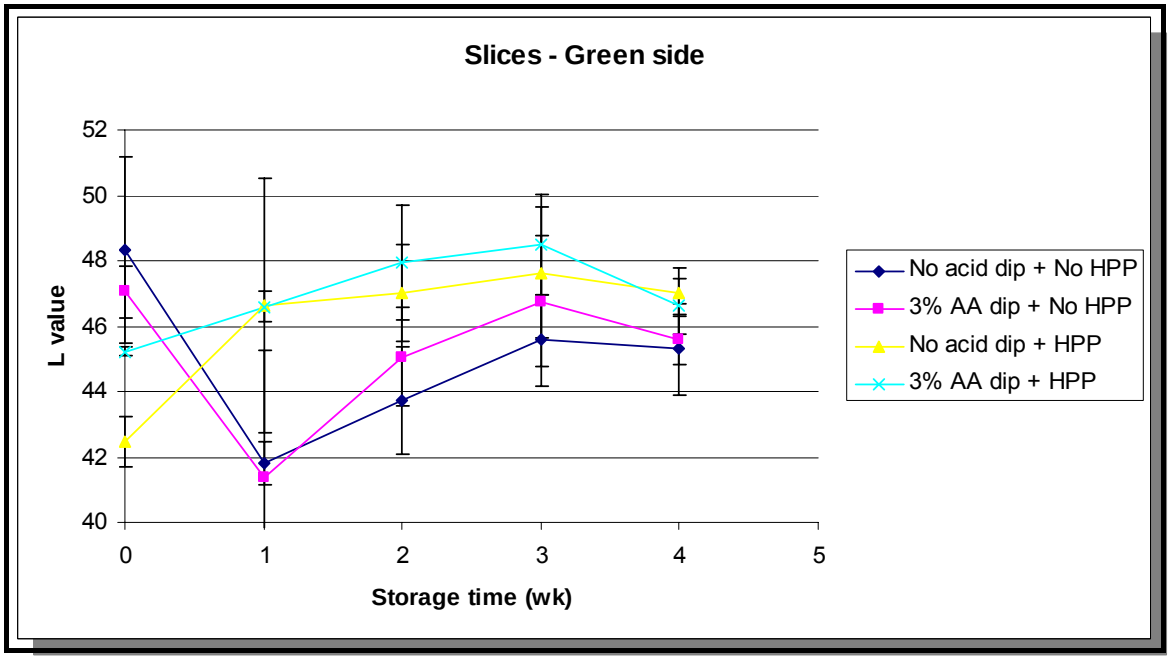

Figure 41: L values of green side of Sheppard avocado slices, acidified with or without 3% ascorbic acid dip, packed in Cryovac B471 bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks (L*=0 indicates black ; L*=100 indicates white).

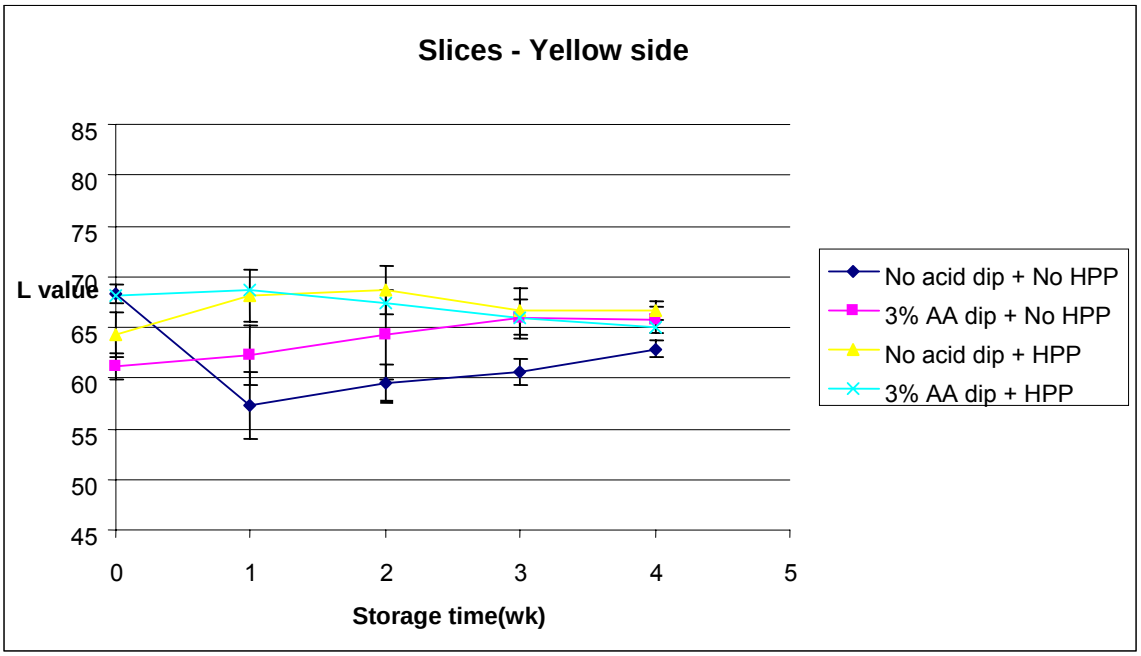

Figure 42: L values of the yellow side of Sheppard avocado slices, acidified with or without 3% ascorbic acid dip, packed in Cryovac B471 bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks(L*=0 indicates black ; L*=100 indicates white).

In Pack Colour

L* value (lightness): On green side L* values ranged between 40 and 50 (Figure 31). The L* values of green side showed a decrease in control samples after 1 week but after storage all samples showed L* values in the same range. The L* values of controls with no HPP treatment were slightly lower than the HPP treated samples. On yellow side which is much lighter, the L* values ranged between 70 -55 (Figure 42). The change in L* values over storage was minimal in HPP treated samples . After 4 weeks all samples showed L* value around 65 on the lighter side. These changes were not very clear to the naked eye.

a* value (yellowness): All sample showed a gradual in crease of a* value on the green side of avocado slices, up to 3 weeks and then the a* value remained stable after 4 weeks (Figure 43). Increase in a* value indicates increase in redness and it signifies development of browning. However, visually detectable level of browning was not observed in any of the samples. Initially the HPP treated samples showed a lower L* value than no HPP samples. On the yellow side (Figure 44) samples with no HPP treatment showed an increase of a* value to the positive range during storage. However the a* values of HPP treated samples remained in minus range indicating no development of browning.

b* value (greenness): On green side b* value ranged between 10- 24 (Figure 45) whereas on the yellow side b* value ranged from 34 to 48 (Figure 46). Initially No HPP controls showed a lower b* value on both sides. The b* values of both sides were stable over the storage period and the differences were minimal between treatments by the 4th week.

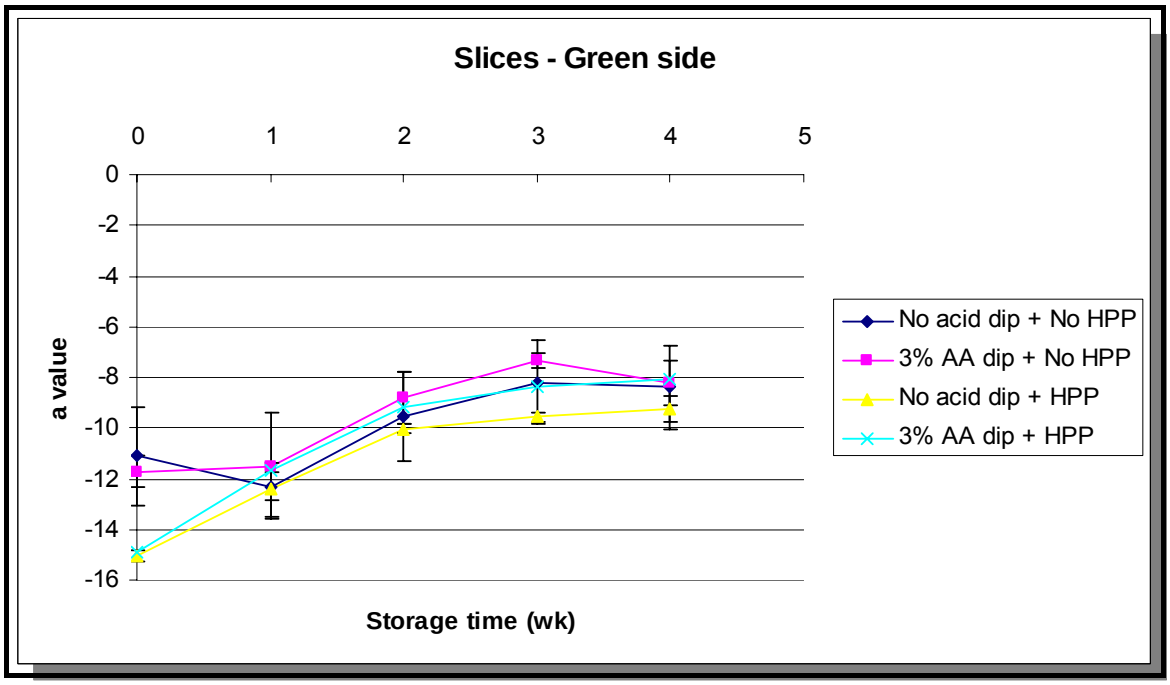

Figure 43: a* values of green side of avocado slices (Sheppard cv.), acidified with or without 3% ascorbic acid dip, packed in Cryovac B471 bags, with or without HPP treatment at 600 Pa 3 min and stored at 4°C for 4 weeks (-a = indicates green : +a = indicates magenta).

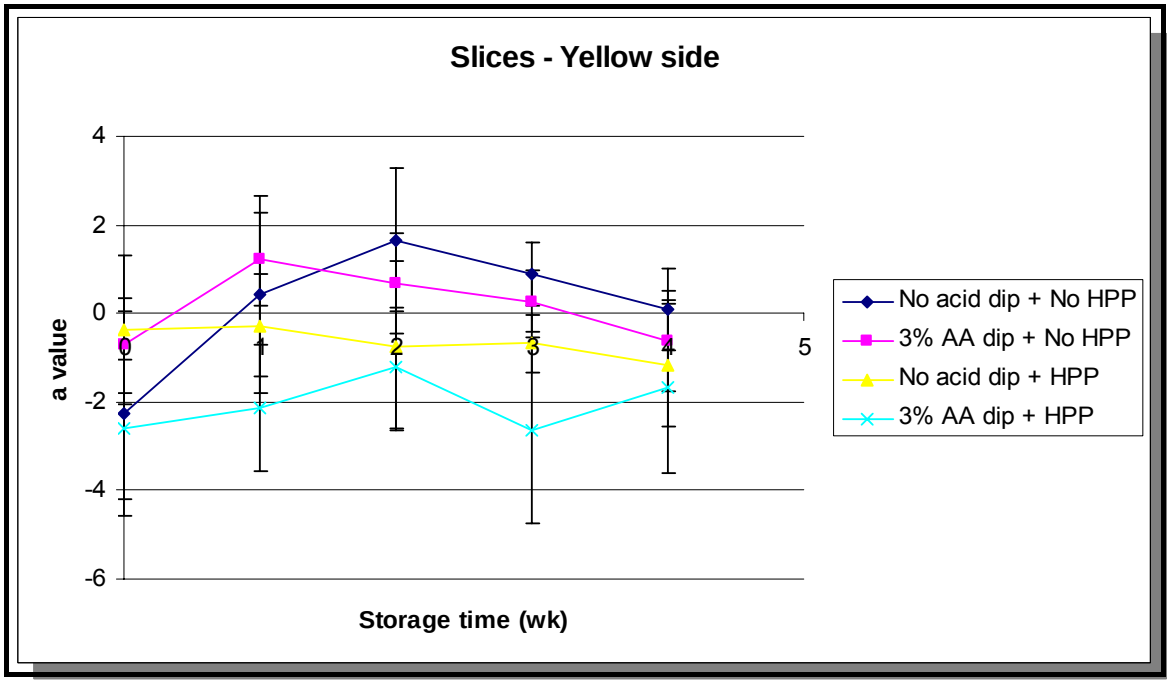

Figure 44: a* values of yellow side of avocado slices (Sheppard cv.), acidified with or without 3% ascorbic acid dip, packed in Cryovac B471 bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks (-a = indicates green : +a = indicates magenta).

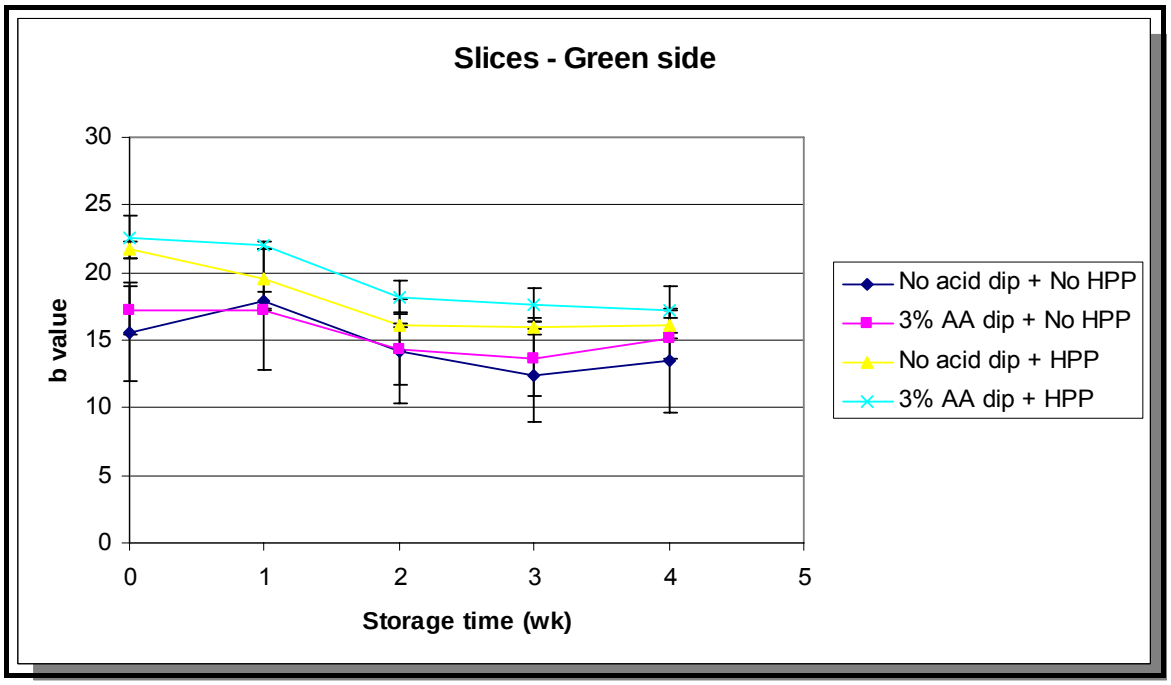

Figure 45: b* values of green side of avocado slices (Sheppard cv.), acidified with or without 3% ascorbic acid dip, packed in Cryovac B471 bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks (-b = indicates blue : +b = indicates yellow).

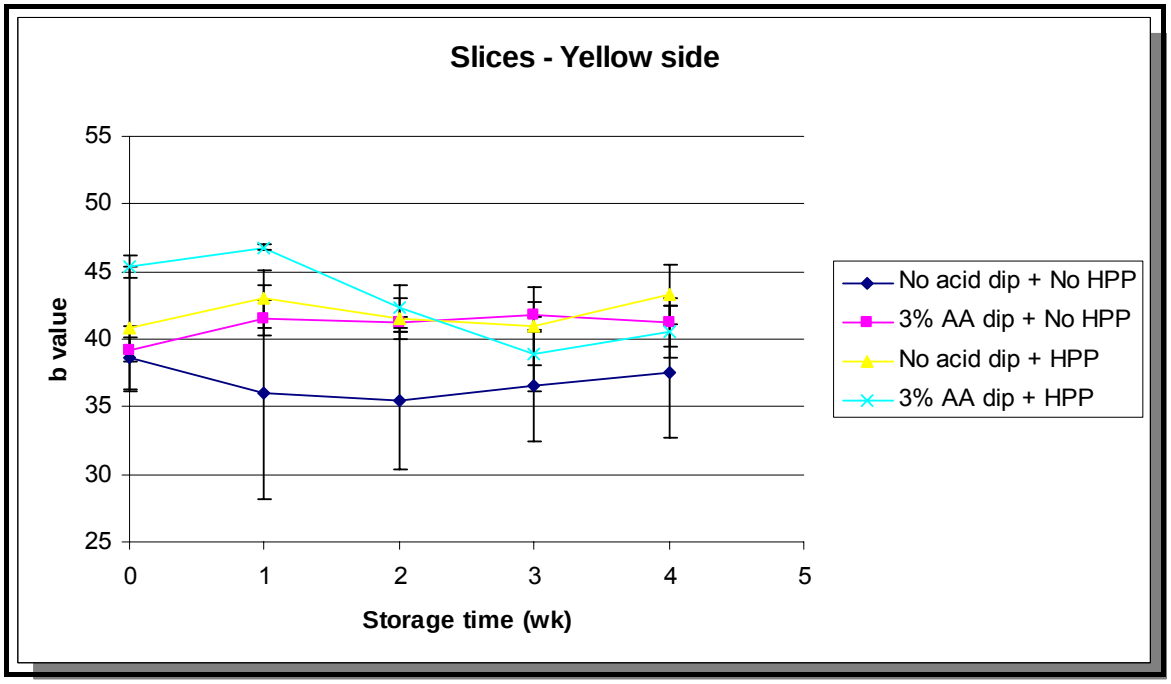

Figure 46: b* values of yellow side of avocado slices (Sheppard cv.), acidified with or without 3% ascorbic acid dip, packed in Cryovac B471 bags, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks (-b = indicates blue : +b = indicates yellow).

5.0 DISCUSSION

Vacuum packaged, high pressure treated avocado products are stored and marketed under refrigerated storage in overseas markets. The technical knowledge on processing details of such products is not readily available in the public domain. This project was initiated by Avocado

Marketing Cooperative to bridge this gap in knowledge to establish a commercial HPP avocado processing facility.

Identification of correct ripeness level in unprocessed avocados, identification of correct packaging material on the evaluation of processing temperature were conducted in experiments 1-5. The materials and methods, results, discussion and conclusions of these experiments are included in Section 10.1 and were submitted in Milestone report 1. The findings of experiments 1-5 were used in conducting the experiments 6-8, which are discussed in this final report.

5.1 Experiment 6: Microbiological Challenge Test

This experiment was conducted to identify the effect of acidification and the application of HPP (600 MPa for 3 min) on *L. monocytogenes* inoculated at a initial microbial load of 7.5 $\log_{10}$ cfu/g.

5.1.1 Listeria Monocytogenes

The challenge of both acidified (pH5) and unacidified (ph 6.5) avocado pulp and quarters with the psychrotrophic pathogen, *Listeria monocytogenes* (log 7.5 cfu/g) revealed that no growth or recovery of viable cells of this organism occurred after treatment with high pressure (600MPa, 3 min). A >6.2 log reduction in viable count of *L monocytogenes* was determined under these processing conditions.

The ability of the product parameters (pH and a_w) and storage conditions (time and temperature) to inhibit the growth of *L. monocytogenes* was evaluated using the publicly available database, ComBase. ComBase enables the combined effects of preservation hurdles on the growth of pathogens to be predicted using models generated from experimental data. While these models are generally regarded to overestimate the ability of pathogens to grow in foods, as data is generated in microbiological growth media (that is designed for supporting optima bacterial growth) the models are useful in evaluating the effects of combined parameters under controlled conditions. Using the parameters from the trial (to achieve a constant growth rate estimation) initial microbial load (log 1.3), acidity (pH 5 and 6.5), water activity (a_w = 1), storage temperature (4°C) and time (4 weeks) ComBase predicts growth of *L monocytogenes* of 3.3 log in the acidified product and 7.1 log in the unacidified product. There is a proposed Food Safety Objective of < 100 cfu/g (<log 2) for *L monocytogenes* in ready-to-eat products at the time of consumption (Szabo et al 2003). Using the data of the potential of the avocado products to support growth combined with the HPP data for determination of inactivation of *L monocytogenes*, the net control over outgrowth of *L monocytogenes* is equivalent to a reduction of 2.9 log units in the acidified products and an increase of 0.9 log units in unacidified products. This would suggest that with the effective implementation of HACCP and process hygiene the proposed FSO for *L monocytogenes* of < log2 could be achieved in both cases but with a much greater degree of certainty for acidified products (equivalent to allowing a 4.9 log initial microbial load in unprocessed avocado) than for unacidified products (equivalent to allowing an initial 1 log microbial load in unprocessed avocado).

5.1.2 Clostridium Botulinum

According to the CCFRA Code of Practice, for long-shelf chilled products (>3°C to 8 °C) of greater than 10 days , the potential for *C. botulinum* outgrowth remains if there is not one or more controlling factors including maintaining pH ≤ 5.0, throughout the product. Other acceptable controlling factors are a heat treatment of no less than 10 min at 90°C or 3.5% salt throughout the water phase or a water activity (a_w) of less than 0.97 (Betts 1996). None of these controlling factors apply to the whole or sliced avocado products investigated, where only the outside was acidified.

Alternatively, where no other controlling factors can be identified the product should be held at temperatures less than 3°C during processing and storage. The pH of the acidified puree does meet this criterion; however the avocado slices and halves, can not be maintained at a pH less than 5.0 throughout the product. HPP treatments as currently used at ambient temperature, are known

not to inactivate spores of *C. botulinum* (Knorr 1995b). Therefore, in this investigation these products were considered permissive for the growth of non-proteolytic *C. botulinum* when stored at temperatures above 3°C. Hence, challenge testing with this organism was deemed unlikely to yield any further information under the conditions used in this study (where pH was >5.0, HPP at ambient temperature and storage at 4°C) and was therefore not undertaken.

5.2 Experiment 7: Effect Of Acidification And HPP Exposure Time At 600 MPa On The Microbiological Quality, pH, Texture And Colour Of Hass Avocado Products During 4 Weeks Storage At 4°C.

This experiment was conducted to identify the effect of acidification and the application of HPP on the microbiological parameters, sensory properties, colour, texture and the pH fluctuations of the product during storage at 4°C. To obtain more information these three types of products i.e. slices, halves and pulp were treated using ascorbic or malic acids or a mixture malic and ascorbic acids (2:1) respectively and were packed in three different types of packaging material. Hass avocado was used in this experiment.

5.2.1 Microbiological Parameter

Pressure treatment effectively controlled microbial outgrowth to very low levels in acidified pulp, halves and slices for 4 weeks (<2.0 $\log_{10}$ cfu/g) and for unacidified slices for 2 weeks (<2.7 $\log_{10}$ cfu/g), held at 4°C. *Enterobactereacea* and lactic acid bacteria growth was not observed for 4 weeks in pulp and halves and in slices for 3 weeks. The initial counts observed in this study were 2.3 - 3.3 $\log_{10}$ cfu/g. No difference was observed between the 1, 3 and 5 min exposure time to HPP. In some instances higher microbial counts were observed in samples treated with HPP for 5 min.

According to Lopez-Malo *et al*. (1999) HPP treatments at 345- 689 MPa, for 10 – 30 min, at pH 3.9 – 4.3 and 1.5% sodium chloride, could reduce initial microbial counts (2.7–3.5 $\log_{10}$ cfu/g) to a level below the limit of detection (<1.0 $\log_{10}$ cfu/g) in avocado puree. Standard plate counts and yeast and mould counts were maintained below the detection level for 35 -110 days at storage temperatures of 5°-25°C. The non HPP treated avocado puree spoiled within the first 5 days at 5°C. In the current study the effect of exposure time was not observed on microbiological parameters.

Although comparable results were observed in our study and the study conducted by Lopez-Malo *et al* (1999) the HPP holding times used in our study (1-5 min) were lower than the holding times (10 -30 min) reported for avocado puree by Lopez-Malo *et al*. (1999). A HPP time of 3 min could be more considerably more commercially viable when compared to 10 – 30 mins.

5.2.2 Sensory Parameter

Sensory analysis was conducted on the request of the HAL program manager, after submission of Milestone Report 1. According to the findings summarised in Table 52 the acidic taste of slices and halves and acceptability of pulp were influenced by the acidification treatments. The HPP treatment time showed an increase in acidic taste of halves.

The acidic taste of halves and slices was dependent on the % acid in dips but the acid dipping did not influence the acceptability of slices and halves. This may be due to the dilution effect that occurs where the acidic outer layer is mixed with the unacidified inner layers of the slices during tasting process.

Acceptability of pulp decreased with the decrease of pulp pH because acidification changed the mild flavour of the unacidified product to an acidic taste. Sensory parameters were evaluated only on day 1 after processing when the pH differences were more prominent between treatments.

During the sensory testing avocado products were exposed to ambient temperature and 21% oxygen level. During preparation and handling of the samples for sensory testing browning was observed in untreated fresh avocado samples whereas very low level of browning was observed in

HPP-treated samples. Open pack browning of the HPP treated avocado halves was not influenced by HPP treatment time whereas the browning of the slices was reduced with the increase in acid % in the dipping solution. Similarly browning of the pulp was lower when the pH was lower than the normal pH of avocado pulp which is 6.5. Avocado slices that received 1-3 min HPP treatment showed less browning than at 5 min exposure time.

Polyphenol oxidase enzyme is responsible for enzymatic browning in avocado. It has been reported that PPO is only partially inactivated by HPP treatment due its extremely high resistance to HPP (Palou *et al*. 1999). However, the partial inactivation in combination with pH reduction, addition of antioxidants and the use of high barrier film in this study has lead to effective control of in-pack browning of the products during refrigerated storage. Esthangi and Knorr (1993), Lopez-Malo *et al* (1999) and Palou *et al* (2000) recommended the use of one or more additional factors such as refrigeration and low pH to control PPO enzyme activity in HPP treated avocado puree and guacamole.

Malic, ascorbic and ascorbic + malic acid mixture was used for acidification of avocado halves, slices and pulp respectively. The positive effects of acidification observed on the prevention of browning in slices and pulp may be due to the use of ascorbic acid as an acidulate. The open pack browning scores of acidified halves, slices and pulp were 2.99 – 3.89, 1.27 – 4.92 and 1.01 - 2.43 respectively. It can be clearly seen that browning scores of slices and pulp were lower than that of the halves which were treated only with malic acid. Therefore it can be concluded that ascorbic acid contributed to the prevention of browning of avocado slices and pulp after opening the packs. Ascorbic acid is widely used for the prevention of enzymatic browning in food industry (Hsu *et al.* 1988; Almeida and Nogueira 1995).

5.2.3 Colour L, a* And b* Values*

In pack instrumental colour measurements showed stable L* values (lightness) and b* values (yellowness) for all three products over 4 weeks storage and no difference was observed between treatments. Lopez-Malo *et al* (1999) reported only about 1% variation in L* value when avocado puree was subjected to 689 MPa for 20 min at 4.1 pH and stored at 5°C. More dramatic changes were observed at 25°C storage temperature. In accordance with our observations, Lopez-Malo *et al* (1999) reported that b* value, the yellow component of the puree colour remained almost constant during storage.

The a* values (greenness) of halves and slices showed no difference between treatments. Higher a* values were observed in pulp with a pH of 4.4 over the 4 week storage. Lopez-Malo *et al*.(1998) performed a sensory evaluation of high hydrostatic pressure treated avocado purees and found a correlation greater than 0.99 between the rank assigned by the judges and the colour *a**values of the avocado purees. They concluded that samples with *a** values below -0.47$\pm$0.3 were well accepted. The a* values observed in experiment 7 were much lower (-7 to -10) than the values observed by Lopez- Malo *et al* (1998) for Hass avocado pulp.

Table 52: Summary of findings for experiment 7 on avocado halves, slices and pulp treated with acid, vacuum packed, treated with HPP at 600MPa for 1 – 5 min and stored at 4°C for 4 weeks..

Attribute	Halves	Slices	Pulp
Acceptability	A	A	Effect of pH was significant. Acceptability decreased with decrease in pH.
Acidic taste	Increased with % acid in dipping solution and increase in HPP time	Increased with % acid in dipping solution.	A

Attribute	Halves	Slices	Pulp
Out of pack browning	A	Significant differences observed between treatments but no trend was observed (Table 21).	Effect of pH was significant. Browning decreased with decrease in pH.
Firmness / consistency	Only HPP exposure time was significant. Interaction effect was not significant.	Only dipping treatment effect was significant. The interaction effect was not significant.	A (consistency.)
L value	B	B (But untreated showed darkening on yellow side) .	B (But untreated samples showed darkening).
a* value	B (Low a* values in acid dipped samples).	B (Low values in 3% acid dipped samples on yellow side).	At pH 4.4 higher a* value than other treatments and control.
b* value	B (Low in acid dipped samples).	B	B
Texture	A	A	Not assessed.
pH	Decrease with storage in unacidified and increase in acid dipped.	Decrease with storage in unacidified and less decrease in acid dipped.	Decrease with storage in unacidified and increase in acid treated

A - No difference between treatments and unacidified control (with no HPP)

B - No difference between treatments, unacidified control (with no HPP) and untreated (no HPP and no acidulation) on both green and yellow side.

5.2.4 Texture

Sensory, firmness scores of the halves and slices were not showing any trend with acidification, HPP exposure time or the interaction effect. Instrumental texture analysis confirmed the results observed with firmness. However the variability of texture in a batch of fruit was difficult to control under our conditions. Texture of the treatments was not different between the treatment combinations evaluated in this study. The texture remained unchanged over the 4 week storage period. The firmness and the texture readings of the product were dependent on the initial texture of the raw material as shown in experiments 1-5.

The texture of the HPP treated and untreated avocado halves and slices were in the same range after 1 week of storage and retained the texture at the same level over a period of 4 weeks. This indicates that the reduction in texture that occurred due to HPP treatment was equal to the texture reduction that occurred during storage of controls that had no HPP treatment.

Tabilo-Munizaga *et al* (2005) studied the rheological properties of avocado pulp treated with HPP. Avocado puree showed a shear thinning behaviour and time dependency as a result of the HPP treatment. In the current study the rheological properties of avocado puree were not assessed.

5.2.5 Packaging Material Evaluation

The material used for packaging avocado halves (Winpack 7000) and pulp had (Amcor retort pouches) very good barrier properties when compared to the Cryovac B 471 bags in which the slices were packed. Some delamination in Cryovac B471 bags were observed after high pressure treatment at 600MPa for 1-3 min. As mentioned before the use of ascorbic acid in the dipping solution contributed to the prevention of browning and maintaining the colour of avocado slices.

The in pack colour of avocado pulp (L values increased) was improved by the application of HPP. As described above the partial inactivation of avocado PPO by HPP may be responsible for the good colour retention of HPP treated samples during storage at 4°C. Yen and Lin (1996) reported partial inactivation of PPO in guava pulp treated at 400 – 600 MPa for 15 min at 25°C. The lightness and greenness of pulp was reported to decrease with storage continuously but the pulp retained acceptable colour for 20 – 40 days at 4°C. In our study the colour of HPP treated avocado products did not deteriorate during storage. Since PPO is only partially inactivated by HPP, the retention of high barrier properties of the packaging material after application of HPP is very important for the retention of colour in HPP treated browning susceptible products, during storage.

Table 53: Summary of findings from experiment 8 on Sheppard avocado halves ,slices and pulp with or without acidification, packed under vacuum, with or without HPP treatment at 600 MPa 3 min and stored at 4°C for 4 weeks.

Attribute	Halves	Slices	Pulp
L value	B	B	B (Lowest values observed at pH5.00+No HPP).
a* value	No difference between treatments on green side. Positive a* values on yellow side for treatments with No HPP	Slight increase over storage on the green side. On yellow side values increased to positive values in No HPP samples.	Lower a values with pH 6.5 +No HPP
b* value	Untreated controls had lowest b values. Values were stable over storage.	B	B
Texture	A	A	Not assessed.
pH	Decrease with storage in all treatments.	Decrease with storage in unacidified and acidified.	A (Stable pH over 4 weeks)

A - No difference between treatments and unacidified control (with no HPP)

B - No difference between treatments, unacidified control (with no HPP) and untreated (no HPP and no acidulation) on both green and yellow side.

5.3 Experiment 8: Validation Of Results Obtained In Experiment 7 Using Sheppard Variety

This experiment was conducted to validate the observations made in experiment 7 using Sheppard avocado variety. Since acceptance and acidic taste were major limiting factors in some of the more acidic treatments used in experiment 7, 1% malic, 3% ascorbic acid and pH adjustments at 5.0 were used with avocado halves, slices and pulp respectively in experiment 8.

5.3.1 Microbiological Parameters

Microbial outgrowth in pressure treated acidified and unacidified avocado samples was maintained at < 2.8 log $_{10}$ cfu/g in samples stored at 4°C for 4 weeks compared with non HPP treated samples that developed total viable counts in the order of 4 - 5 log $_{10}$ cfu/g. As discussed above similar effects of HPP on initial microbial loads have been reported by others on avocado products (Lopez-Malo *et al*.1999).

5.3.2 Colour Measurements L, a* And b* Values*

As observed in experiment 7, the L*, a* and b* values of the acidified and HPP treated avocado halves, slices and pulp of the experiment 8 were similar to the acidified + No HPP and untreated controls over the 4 week storage period (Table 53). These results indicates that the acidification and HPP treatments have not caused any undesirable changes in the instrumental colour parameters of the three avocado products made with Sheppard variety.

The two varieties Has and Sheppard used in experiment 7 and 8, respectively, showed a similar changes in colour parameters in treated and untreated controls. However, only in untreated and acidified controls (with no HPP), of Sheppard avocado halves, the a* value of yellow side, increased to positive values. These values were higher than - 0.46 which was mentioned as the limit of sensory acceptance of colour by Lopez-Malo *et al* (1998). HPP treatment helped to retain the a* value at minus range over the 4 week storage period. All HPP treated samples had acceptable visual colour during storage. Lopez- Malo *et al* (1998) suggested that the time needed to reach an a* value close to zero could be defined as the storage period to observe an unacceptable colour change and thus define an acceptability storage time (AST). The AST for HPP treated avocado slices in experiment 8 was more than 4 weeks while it was less than 1week for untreated controls.

5.3.3 Texture

The observations made on texture in experiment 8 confirmed the observations made in experiment 7. As mentioned before the texture values of all treatments decreased during the first week of storage. Both Hass and Sheppard varieties followed a similar trend in textural changes.

5.3.4 pH

The pH of treated and untreated halves and slices decreased gradually and reached values (pH 5.5) that were in the same range. Treatment related effects were not observed after 4 weeks storage in halves and slices. The pH of pulp samples showed differences with acidification and showed minimal changes over the storage period. With halves and slices the reduction of surface pH may be related to the buffering capacity of the Avocado pulp. Similar observation was observed with Hass variety in experiment 7.

The observations made in this study indicate that the combined effect of acidification, presence of antioxidants (ascorbic acid), vacuum packaging in low OTR material and HPP treatment at 600 MPa for 1-5 min is essential to retain the colour and other sensory properties and to reduce the microbial counts of avocado products to safe levels.

6.0 CONCLUSIONS

6.1 Experiment 6 : Microbiological Challenge Test

Results indicated an initial log reduction of greater than 6.2 $\log_{10}$ cfu/g with an initial inoculum level of 7.5 $\log_{10}$ cfu/g and a limit of detection of 1.3 $\log_{10}$ cfu/g. No recovery or growth of *L. monocytogenes* cells following seven days of storage was observed.

6.2 Experiment 7: Effect Of Acidification And HPP Exposure Time At 600 MPa On The Microbiological Quality, pH, Texture And Colour Of Hass Avocado Products During 4 Weeks Storage At 4°C.

Acidification and application of HPP helped in reducing the initial total aerobic mesophile load of the avocado products. Application of acidification treatment influenced the acceptability of pulp acidified with ascorbic + malic acid mixture (2:1). With acidification, the acidic taste of halves treated with malic acid and slices treated with ascorbic acid, increased but the acceptability was not affected. Acidification with ascorbic acid helped in prevention of browning in slices and pulp when compared to acidification of halves with malic acid. Acidification and HPP treatment did not cause any undesirable changes in the instrumental colour and texture measurements of the avocado products.

Amcor retort packs and Winpack 7000 packs were found to be effective in maintaining low oxygen conditions in avocado products packed under vacuum and treated with HPP.

6.3 Experiment 8: Validation Of Results Obtained In Experiment 7 Using Sheppard Variety

Results of experiment 8 on Sheppard variety confirmed the observations made in experiment 7 with Hass variety. Acidification and HPP treatments did not case undesirable effects on the colour parameters L, a*, b* and texture as in experiment 7. Initial pH adjustments of pulp and the acid dips of slices and halves influenced the final pH of the products after HPP and after storage. However, a gradual reduction of pH was observed during storage of all treatments in experiment 8 where Sheppard avocados were used.

6.4 Overall Conclusions

Use of avocado at correct ripeness level (≥7.0 N with peel) was found to be important for maintaining a desirable texture after processing and material handling during processing (Experiments 1- 5).

Studies conducted on packaging material selection and the two storage studies revealed the need of high oxygen barrier films for vacuum packaging the avocado products (<5 cc/m^2/24 hr). The selected packaging material were able to retain their barrier properties during and after high pressure treatment. Winpack 7000 packs could be used effectively to provide the barrier properties required for HPP treated avocado halves and slices. Avocado pulp could be packed in either Winpack 7000 or Amcor retort pouches.

Acidification to a pH 4.4 and HPP treatment at 600 MPA for 3 min was essential to obtain lower microbial count. Results of the challenge test indicated that *L. monocytogenes* could be effectively controlled by acidification and HPP treatment at 600 MPa for 3 min. Once the microbial counts were reduced by the acidification and HPP treatment the counts remained at low level during the storage at 4°C for 4 weeks in this study. The acidification to a pH 4.4 however, reduced the acceptability of avocado pulp. The acidified pulp could be used for production of guacamole. Acidification of the pulp at pH 5.0 could minimise the effects on acceptance and acidic flavour and this acidic pH still provides a factor to control outgrowth of *C. botulinum* at the storage temperatures of 4°C, used in these trials.

Ascorbic acid or a mixture of ascorbic acid and malic acid (2:1) could be used effectively for acidification of HPP treated avocado products. In contrast, halves and slices exposed to acid dips at the 1% level to adjust the surface pH did not result in internal acidification of the avocado tissue and so do not provide a controlling factor for outgrowth of *C. botulinum.*

As pressure treatment alone will not inactivate bacterial spores additional processing hurdles including effective acidification pH ≤ 5 combined with storage temperature of 4°C or storage temperature control below 3°C would be required for controlling the risk of *C. botulinum* outgrowth.

The avocado varieties Hass and Sheppard, both showed similar trends in microbiological parameters, colour and texture after HPP treatment and during storage.

7.0 TECHNOLOGY TRANSFER

During the life of this project, meetings were held between technical personnel from Avure technologies Inc. and Food Science Australia team to plan and discuss the findings of the research conducted. This in formation was conveyed to Mr Brian Prosser of Avocado Marketing Cooperation.

One milestone report was submitted to Mr Brian Prosser and Horticulture Australia in July 2006. A presentation of the findings will be made to Mr Brian Prosser after submission of this report.

We wish to seek permission from Mr Brian Prosser and Horticulture Australia to publish the results of this project in peer reviewed scientific journals after the agreed confidentiality period.

7.1 RECOMMENDATIONS

High pressure processing could be used to extend the shelf life of avocado halves, slices and pulp products effectively by controlling the microbial growth while maintaining sensory properties.

The success of this process depends on the selection of raw material at the correct maturity and the selection of packaging material that can withstand the HPP treatment. The avocados should be at the correct ripeness level (≥7.0 N with peel) to facilitate material handling during processing and to maintain a desirable texture after processing.

The packaging material should be able to retain a low oxygen transmission rate (<5 cc/m^2/24 hr) after HPP treatment and possess adequate flexibility to allow folding over the surface of avocado halves. It should also result in a minimum head space after application of vacuum at -1.0 bar for 5 seconds.

It is recommended that refrigerated (4°C) extended shelf-life avocado products be formulated to an acidic level of pH 5 throughout the product and pressure treated at 600MPa for 3 min to achieve optimal microbiological stability and quality.

Storage of non-acidified products at <3°C is essential for the safe extension of shelf life. Acidified pulp (pH 5.0) products could be stored at 4°C for up to 4 weeks. Time temperature combinations for unacidified products, involving storage temperatures above 3°C would require specific risk assessments and evaluation of the cool chain and process hygiene requirements under commercial conditions.

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10.0 APPENDICES

10.1 Methods, Results, Discussion And Conclusions Of Experiments 1-5

10.1.1 Methods – Experiments 1- 5.

Experiment 1: Effect Of Packaging Material On In Pack Browning Of Avocado Halves During Storage.

Avocado halves were peeled and packed in 3 different types of bags; Holmes, Cryovac B471 and Amcor retort pouches using a Webomatic vacuum packaging machine with a vacuum of -1 bar for 5 sec. Samples were treated with HPP at 600MPa for 3 min at 22°C.

Experiment 2: Effect Of Avocado Firmness On Quality Of Stored Halves.

Avocados of Hass variety treated with ethylene for 24 hrs were used in this experiment. Based on the observations made in experiment 1, Amcor retort pouches and Cryovac B471 pouches were used for packaging avocado pulp and halves respectively. A Webomatic vacuum packaging machine was used to heat seal the packages after subjecting packages to a vacuum of -1 bar for 5 seconds.

After discussions with the supplier on how to obtain different firmness levels the avocado batches were left at room temperature and in cold room storage as described below.

Table 54: Conditions used for the ripening of avocado in experiment 2

Firmness level	Firmness @ processing (Kg with 8 mm probe)	Days at cold room at 4°C prior to ripening	Days at room temperature	Days at cold room at 4°C after ripening
T1	0.7 ± 0.5	0	5	5
T2	0.6 ± 0.2	2	4	4
T3	< 0.5	3	4	3

Fruits at each firmness level were divided into two subgroups. One sub group was processed at 5°C and the other was processed at 22°C before applying pressure. After peeling and packaging, the HPP treatment was applied at 4°C and 22°C respectively. For 5°C the pressure plant was filled with water at 5°C and the vessel temperature was set at 5°C

After HPP processing all samples were stored at 4°C and changes in colour were observed objectively and subjectively. Objective colour measurements were taken from three replicate samples per treatment while the avocado halves were in the bags. Same samples were used repeatedly for colour measurement during storage.

The three untrained panellists who performed informal sensory testing of the samples during storage assigned subjective colour, taste and texture scores for each treatment. A sample of the questionnaire used for the informal sensory analysis is in Section 10.1.

Avocado halves were homogenized into a pulp and the pH of the samples was recorded at each evaluation as described above.

Experiment 3: Effect Of pH Adjustment On The Quality Of Avocado Pulp.

Avocado fruit at eating ripeness was used in this experiment (<0.5 Kg with penetrometer). Fruit pulp was separated and homogenized with a Robot-Coupe blender. The pulp sample was divided into two subgroups with one subgroup acidified with food grade citric acid crystals (Cargill, USA) at 5g citric acid/Kg of pulp, ratio and the pH was reduced to 4.5. Acidified and unacidified pulp was packed in Amcor retort pouches and heat sealed using a Webomatic vacuum packaging machine operated to a vacuum of -1 bar for 5 seconds. Each subgroup was divided into two and HP processed (600 MPa for 3 min) at 4°C and 22°C (sample, HPP vessel and water temperature). After processing all samples were stored at 4°C for 28 days.

Colour changes in samples were assessed objectively and subjectively. Three panellists evaluated the samples during storage for subjective assessment of colour, taste and texture. The pH of the samples was recorded at each evaluation.

Experiment 4: Evaluation Of Suitable Texture For Vacuum Packaging And HPP Treatment.

Avocado fruits were received after subjecting to ethylene ripening treatment at Melbourne Markets for 24 hrs.

On arrival the majority of the ethylene treated fruits were ripened and the peel colour was brown. The green (unripe) and brown (ripe) fruits were separated immediately. From the two groups of fruits, half the fruits were stored at 22°C and the remainder at 4°C. The softening pattern was observed using an Instron Texture measuring device as described above.

Avocado fruits that had not undergone ethylene ripening treatment were also obtained. Half of the fruits were stored at 22°C and the remainder 4°C. Fruits stored at 22°C started to ripen within 6 to 9 days from picking. The texture of ripening avocados was measured with and without peel at the initiation of ripening using an Instron Texture measuring device. At this stage fruits were warmer and the peel colour was beginning to change. Fruits at different textures were evaluated on the ease of peeling and vacuum packaged at -1 bar for 5 seconds in B471 and Amcor retort pouches.

Experiment 5: Evaluation Of Suitable Acid Types For HPP Treated Avocado Products.

Experiment 5A: Food grade ascorbic acid (BASF, Denmark), citric acid (Cargill Foods, USA), fumaric acid, lactic acid, malic acid and tartaric acid (Winery Products Pty Ltd, Australia) were used in making 1 and 25 solutions of above acids using potable tap water. The pH of the solutions was recorded.

Hass avocado slices were dipped in 2% acid solutions of ascorbic acid, citric acid, fumaric acid, lactic acid, malic acid and tartaric acid. Some slices were packed in Amcor retort pouches under vacuum (-1.0 bar). The remaining slices were pureed and pH was measured. The packaged slices were treated with high pressure at 600 MPA for 3 min at 22°C. After HPP slices were pureed and the pH of the puree was measured.

Experiment 5b: Selection Of Suitable Acid Types For Acidification Of Avocado Pulp

Hass avocado pulp was made as described in section 2.3. The pulp was divided in to 7 sub portions. The pH of six sub portions were adjusted using ascorbic acid, citric acid, fumaric acid, lactic acid, malic acid and tartaric acid

10.1.2 Results

Experiment 1

Table 55: Effect of high pressure processing on packaging materials.

Type/ Name	Effect of HPP on packaging	Supplier
Holmes*	Creasing observed	Cryovac
B471 (Cheese bags)	Delamination in some bags	Cryovac
Retort pouches **	No effect at or below 22°C	Amcor

* at 23ºC/75% RH

** post retort at 23°C/50% RH

The packaging material used in this experiment showed different after effects due to the application of HPP (Table 55). The Holmes bags had many creases while delamination was observed in most B 471 bags in areas above the sealing line of the package. Delamination was observed in some bags below the sealing line where the avocado samples remained.

Table 56: Development of brown colour in HPP (600 MPa, 3 min, at 22°C) treated Sheppard avocado halves packed in three different package types during storage at 4°C.

Package type	Storage time			
	3 days	6 days	12 days	27 days
Holmes	No browning	Slight browning	Browning very obvious	Browning very obvious
B471 (Cheese bags)	No browning	No browning	Very light browning in juice	Browning obvious with juice
Amcor retort pouches	No browning	No browning	No browning	No browning

Browning was observed in the avocado pieces when packed in the Holmes bags after 12 days of storage (Table 54). Browning was observed in the juice that is located in a thin layer inside the bag when halves or slices were packed in B471 bags. This browning was very prominent only after 21 days.

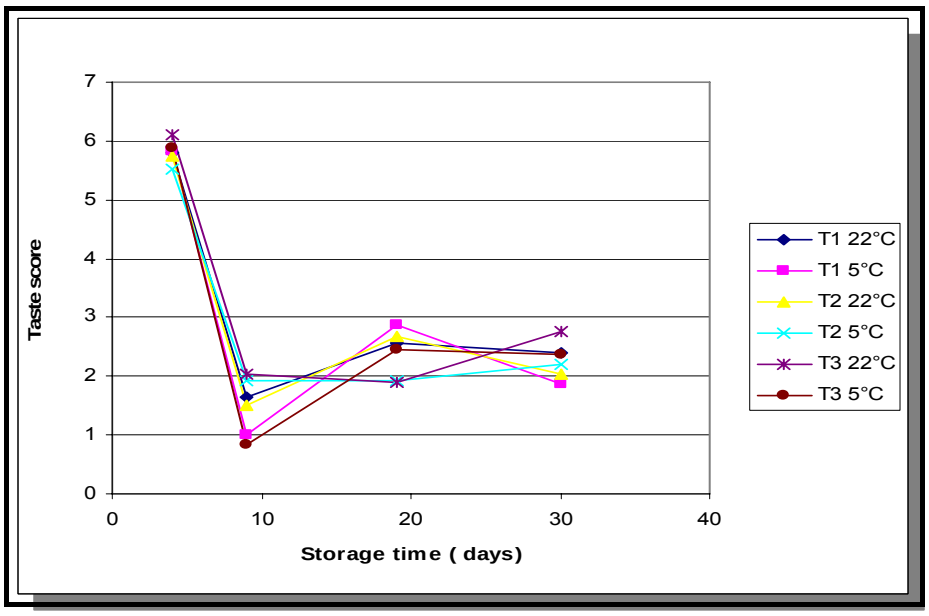
The stiffness of Amcor retort pouches prevented their use for halves and slices. During vacuum packaging the shape of the halves and slices were deformed when packed in Amcor retort pouches. Based on the observations made in this experiment, B471 bags were selected for further testing for avocado halves and slices, and Amcor bags were selected for further testing for pulp.

Experiment 2

As shown in Table 54 the fruits used in this experiment had very close texture readings.

The ripening method suggested by the grower did not result in fruits with different texture levels as expected. Fruits with a texture measurement between 0.7-<0.5 kg were not able to withstand the peeling, vacuum packaging and HPP processing. After processing the shape of the fruit halves remained intact but the halves were too soft to handle.

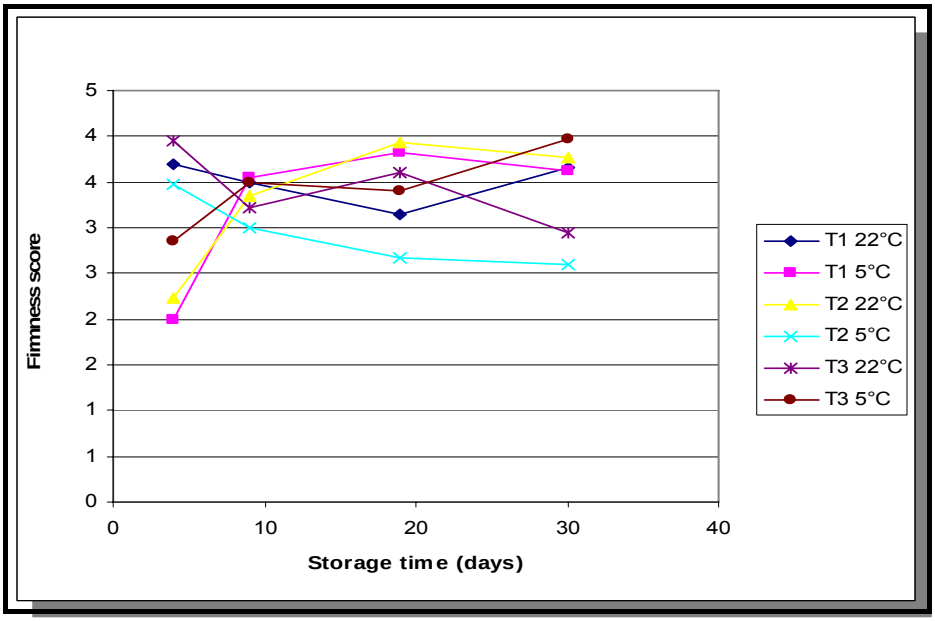
Sensory attributes of the avocado halves are shown in Figure 47 to Figure 49.



Taste score: 0 – Low avocado taste; 10 – Fresh like avocado taste

Figure 47 - Changes in average taste scores (n=3) of avocado halves of three initial texture levels (T1, T2, T3), packed in Cryovac B471 bags, processed at 5°C and 22°C with HPP (600 MPa for 3 min) and stored at 4°C.

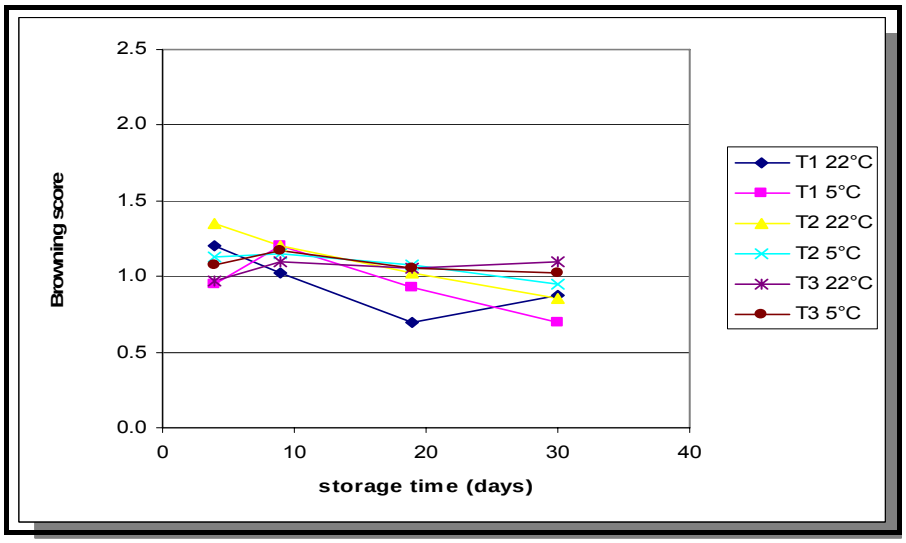
Taste attributes of avocado halves decreased rapidly from day 4 to day 9 (score 6 to 1.5). After further storage the taste scores varied only slightly and ranged between 2.9-2.0. Effects of texture level or processing temperature were not obvious with reference to taste.



Firmness score: 0- Softer; 10 - Firmer

Figure 48 - Changes in average firmness scores (n=3) of avocado halves of three initial texture levels (T1, T2, T3), packed in Cryovac B471 bags, processed at 5°C and 22°C with HPP (600 MPa for 3 min) and stored at 4°C.

Firmness scores were lower than 5 which was the mid point in the scale. No difference in firmness was observed between the three texture levels used. Firmness did not show a change with storage time, texture levels or processing temperature.

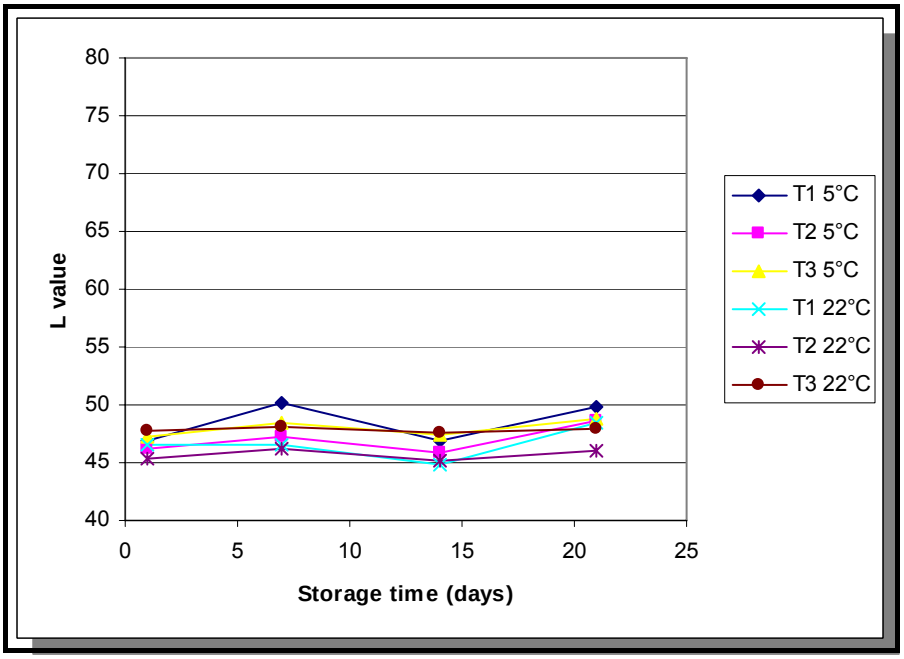


Browning score: 0 – No browning; 10 – 100% brown on surface

Figure 49 - Changes in average browning scores (n=3) of avocado halves of three initial texture levels (T1, T2, T3), packed in Cryovac B471 bags, processed at 5°C and 22°C with HPP (600 MPa for 3 min) and stored at 4°C.

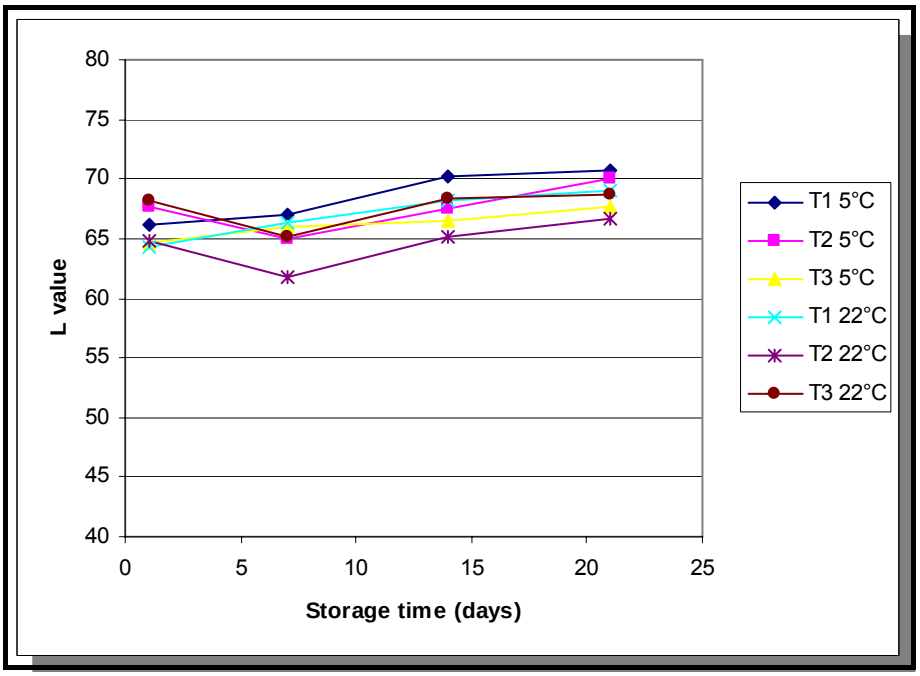
Browning levels of the samples were very low and ranged between 0.6 and 1.2. Samples did not show any visually detectable browning during the storage period of 28 days.

The L*, a* and b* values were measured on the peel side and on the cut surface near the stem end and blossom end of the avocado, and inside the seed cavity. Peel side measurements were different from the measurements observed on the cut surface and the seed cavity. Hence graphs on peel side and seed cavity are presented in this report.



L=0 yields black and L*=100 indicates white*

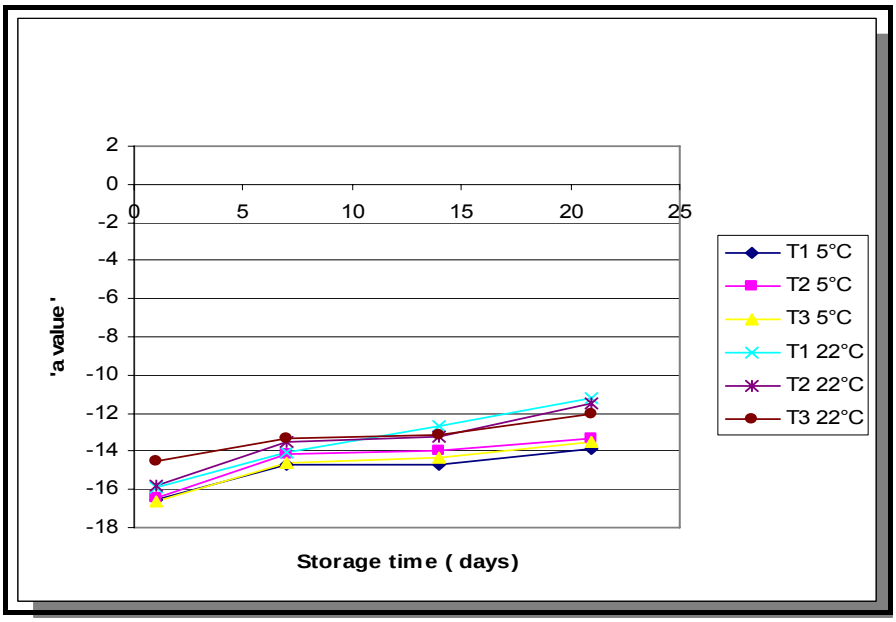
Figure 50 – Changes in average "L values" (n=3) of the green side of avocado halves of three initial texture levels (T1, T2, T3), packed in Cryovac B471 bags, processed at 5°C and 22°C with HPP (600 MPa for 3 min) and stored at 4°C.



L=0 yields black and L*=100 indicates white*

Figure 51 - Changes in average "L values" (n=3) of the seed cavity of avocado halves of three initial texture levels (T1, T2, T3), packed in Cryovac B471 bags, processed at 5°C and 22°C with HPP (600 MPa for 3 min) and stored at 4°C.

L* values of the green side were lower than that of the cut surfaces and the seed cavity (Figure 50 and Figure 51). This is expected due to the lightness of the cut surface and of the seed cavity compared to that of the green side. L* values for both the green side and the seed cavity remained unchanged during the storage period indicating that the lightness of the halves remained unchanged during this period. There was no difference in L* values between the 3 texture levels used and the two processing temperatures used. The trend observed with L* value was comparable to the observations made on the overall browning of the samples.

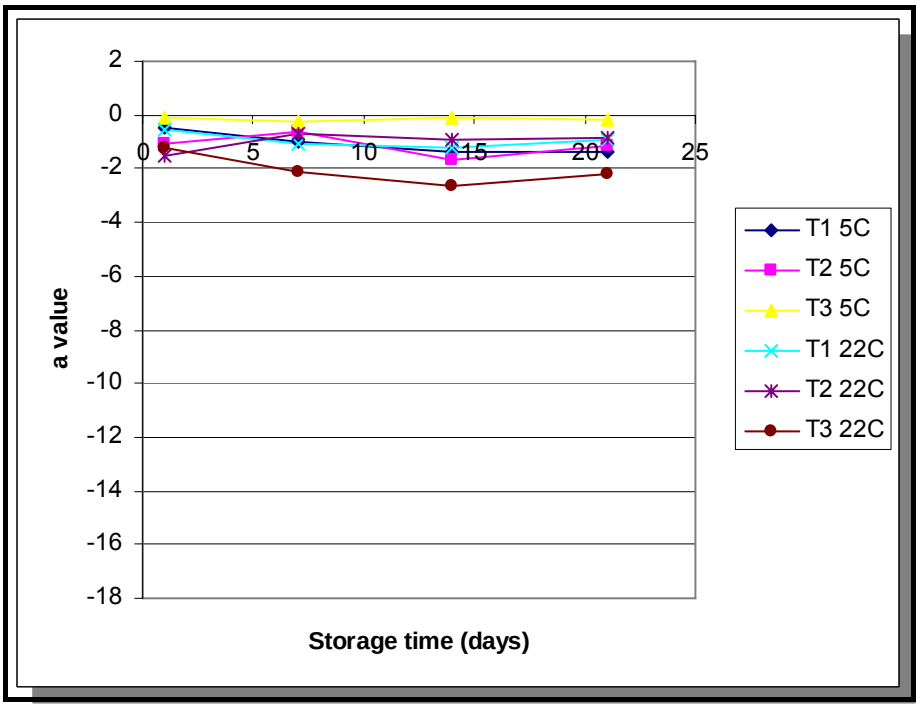


a- negative values indicate green while positive values indicate magenta*

Figure 52 – Changes in average "a values" (n=3) of the green side of avocado halves of three initial texture levels (T1, T2, T3), packed in Cryovac B471 bags, processed at 5°C and 22°C with HPP (600 MPa for 3 min) and stored at 4°C.

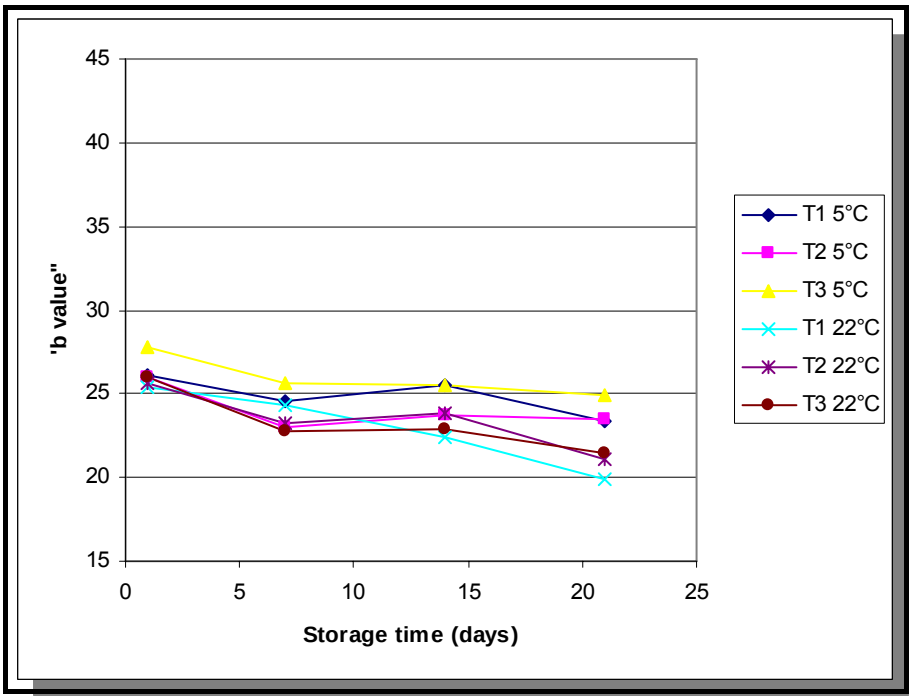
a* values of the green side were lower than that of the seed cavity and cut surfaces (Table 46 and Table 47). a* values of the green side showed a slight increase in a* values when compared to the values observed in the seed cavity. This indicated a slight reduction in the green colour on the green side of the avocado halves during storage.

b* values of the green side were lower than the values for the seed cavity indicating the prominent yellow colour in the seed cavity (Table 48 and Table 49). As with a* values, the b* values of the green side slightly decreased during storage. The b* values seed cavity remained fairly unchanged during storage.



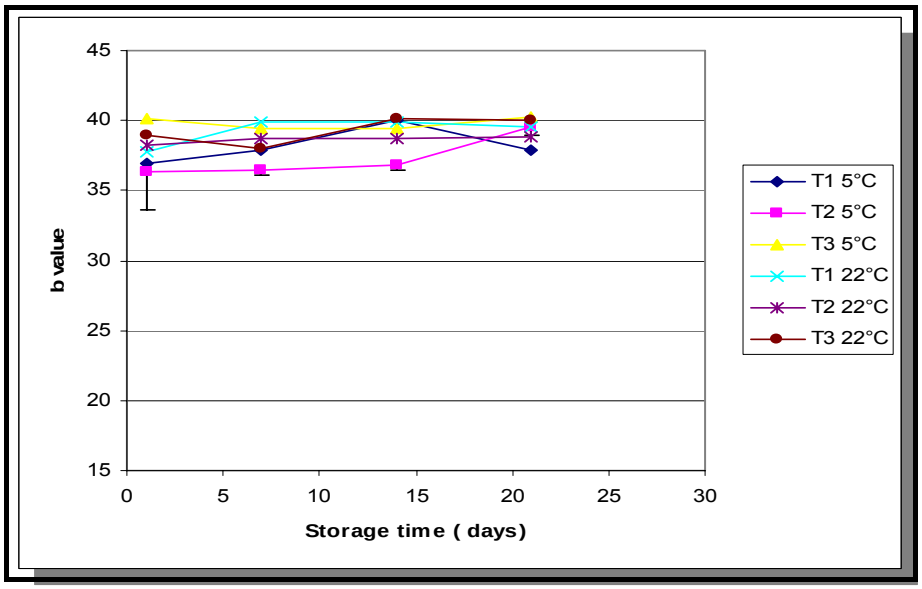
a- negative values indicate green while positive values indicate magenta*

Figure 53 – Changes in average "a values" (n=3) of the seed cavity of avocado halves of three initial texture levels (T1, T2, T3), packed in Cryovac B471 bags, processed at 5°C and 22°C with HPP (600 MPa for 3 min) and stored at 4°C.



b - negative values indicate blue and positive values indicate yellow*

Figure 54 – Changes in average "b values" (n=3) of the green side of avocado halves of three initial texture levels (T1, T2, T3), packed in Cryovac B471 bags, processed at 5°C and 22°C with HPP (600 MPa for 3 min) and stored at 4°C.



b- negative values indicate blue and positive values indicate yellow*

Figure 55 – Changes in average "b values" (n=3) of the seed cavity of avocado halves of three initial texture levels (T1, T2, T3), packed in Cryovac B471 bags, processed at 5°C and 22°C with HPP (600 MPa for 3 min) and stored at 4°C.

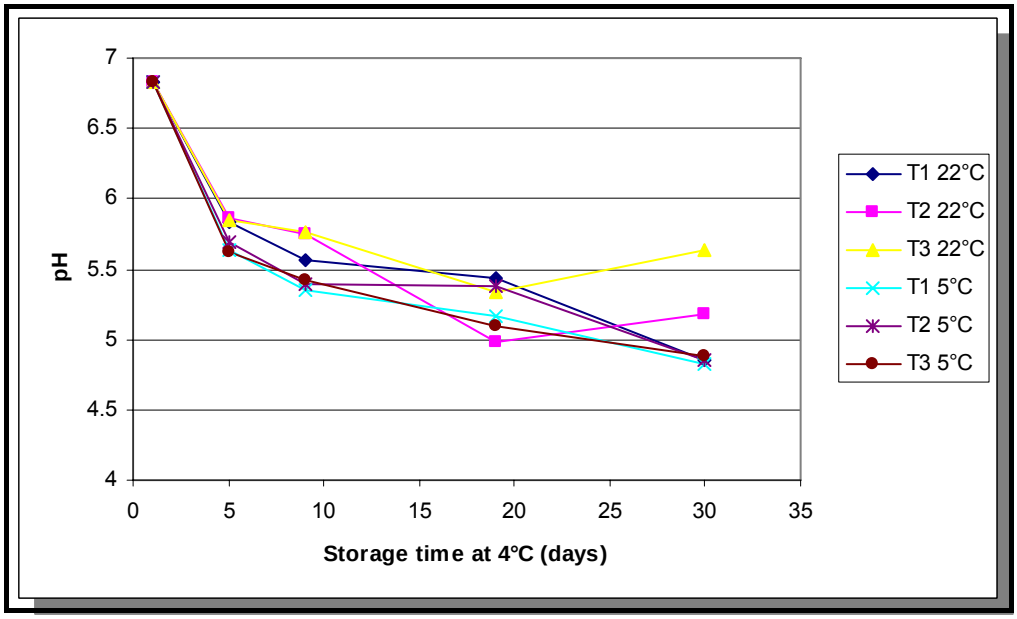
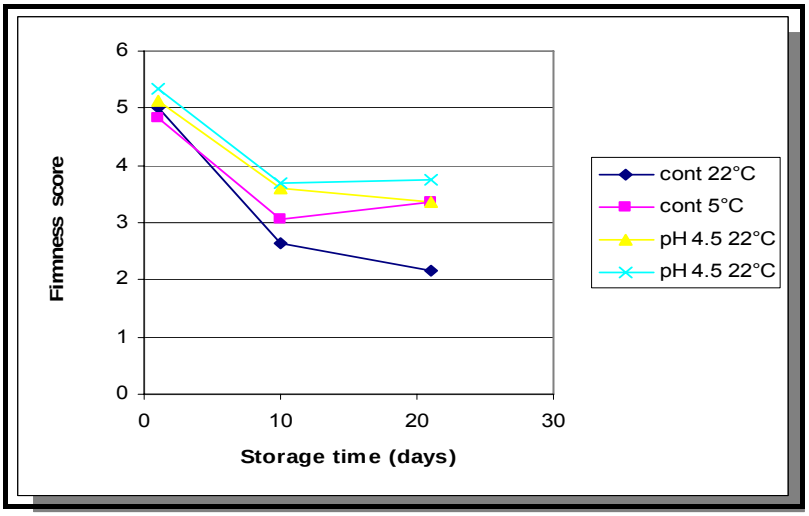


Figure 56 – Changes in average pH value (n=3) of avocado halves of three initial texture levels (T1, T2, T3), packed in Cryovac B471 bags, processed at 5°C and 22°C with HPP (600 MPa for 3 min) and stored at 4°C.

The pH of avocado halves decreased gradually from pH 7 to 5. This decrease was observed in all treatment combinations (Table 50).

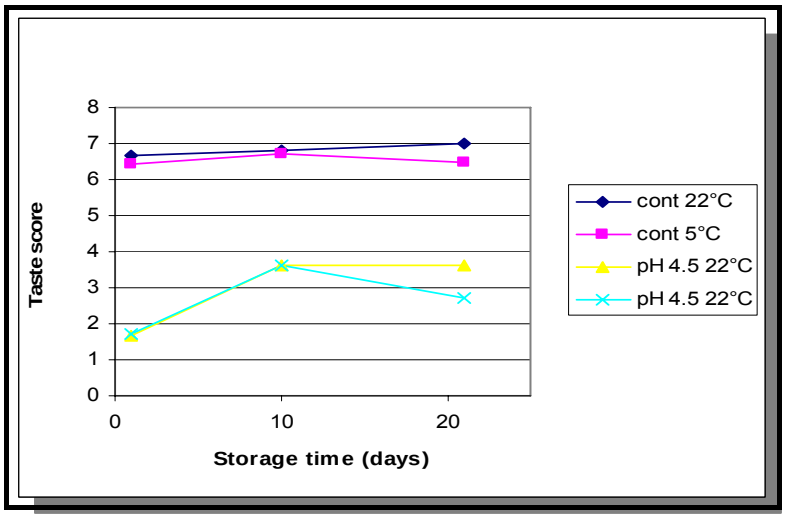
Experiment 3



Firmness score: 0- Softer; 10 - Firmer

Figure 57 – Changes in average firmness scores (n=3) of avocado pulp packed in Amcor retort pouches, processed at 5°C and 22°C with HPP (600 MPa for 3 min) and stored at 4°C.

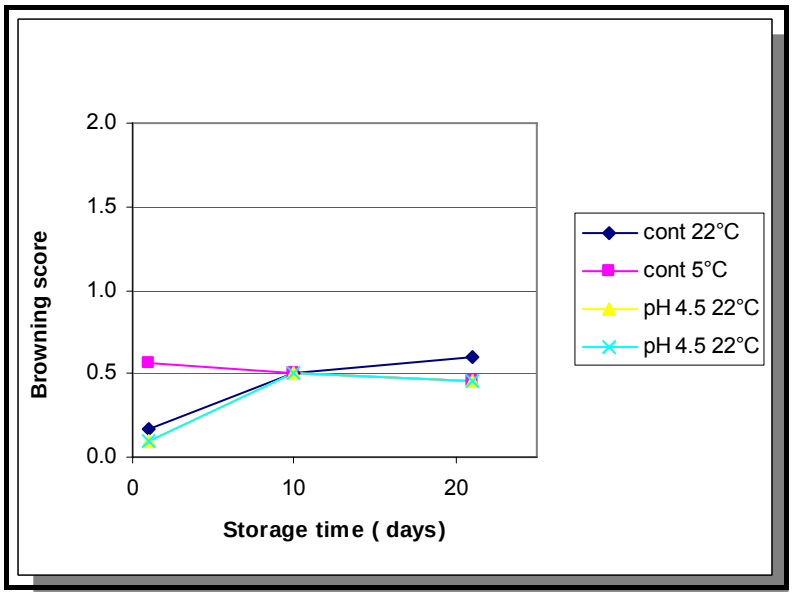
A decrease in firmness scores was observed from day 1 to day 9 however the scores remained stable during the remainder of the storage period (Figure 57).



Taste score: 0 – Low avocado taste; 10 – Fresh like avocado taste

Figure 58 – Changes in average taste scores (n=3) of avocado pulp packed in Amcor retort pouches, processed at 5°C and 22°C with HPP (600 MPa for 3 min) and stored at 4°C.

Taste scores were higher for the control samples compared to the acidified samples (Figure 58). Taste scores of the control samples were constant during storage. The taste scores of acidified samples improved after the first 9 days of storage.



Browning score: 0 – No browning; 10 – 100% brown on surface

Figure 59 – Changes in average browning scores (n=3) of avocado pulp packed in Amcor retort pouches, processed at 5°C and 22°C with HPP (600 MPa for 3 min) and stored at 4°C.

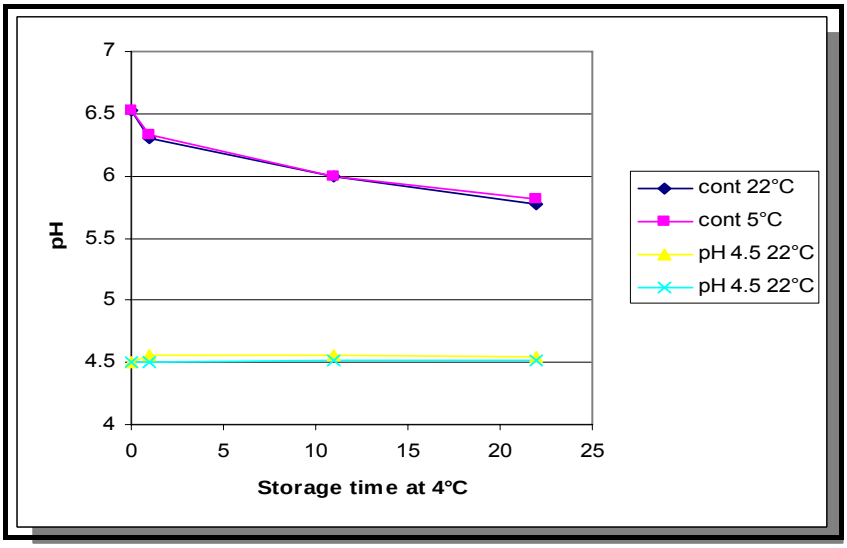
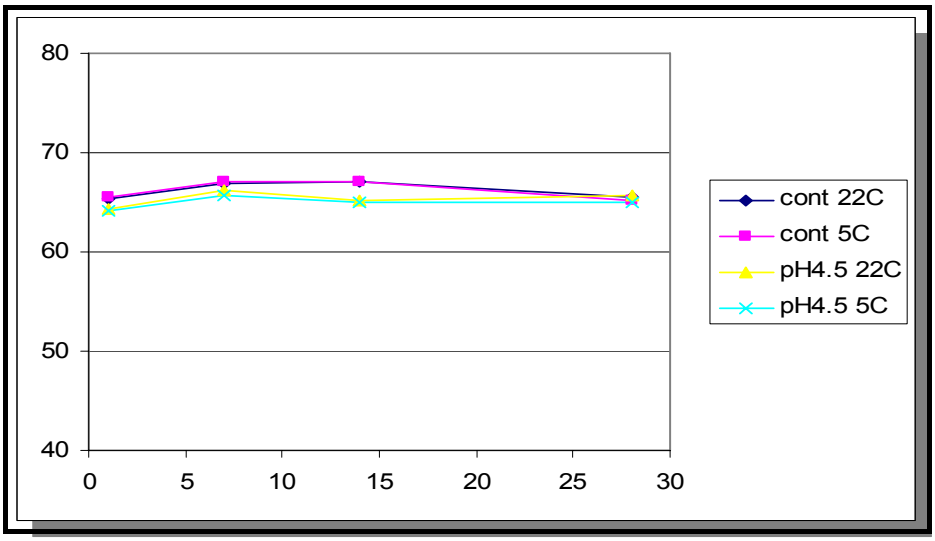


Figure 60 – Changes in average pH (n=3) of avocado pulp packed in Amcor retort pouches, processed at 5°C and 22°C with HPP (600 MPa for 3 min) and stored at 4°C.

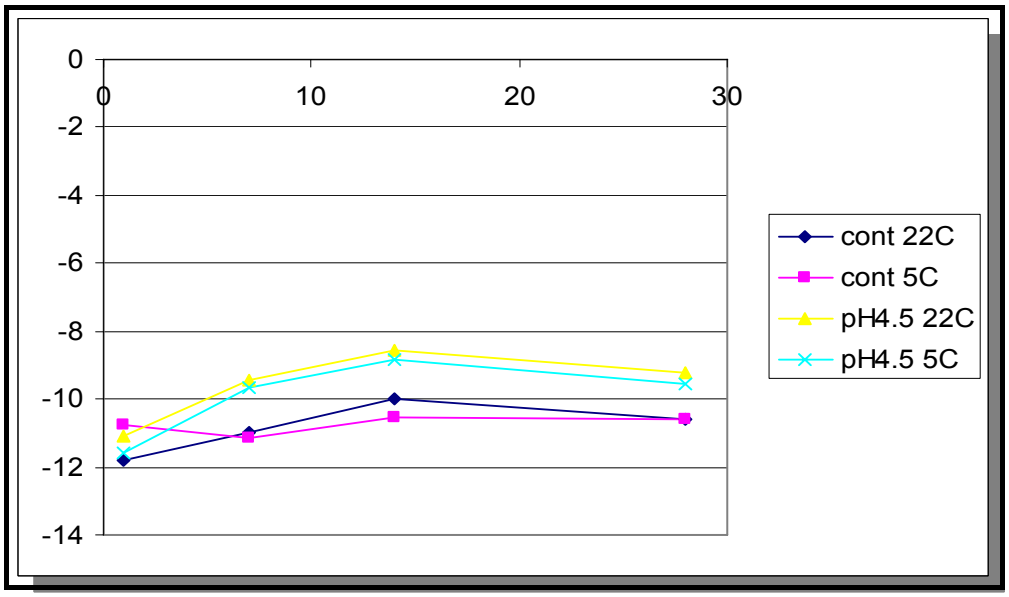
In all samples browning scores were lower than 1 during storage (Figure 59). The differences between treatments were not very distinct.

The pH values of the control samples were higher than the acidified samples as expected. The pH of acidified samples remained very stable whereas the controlled samples showed a slight decrease in pH over the storage period.



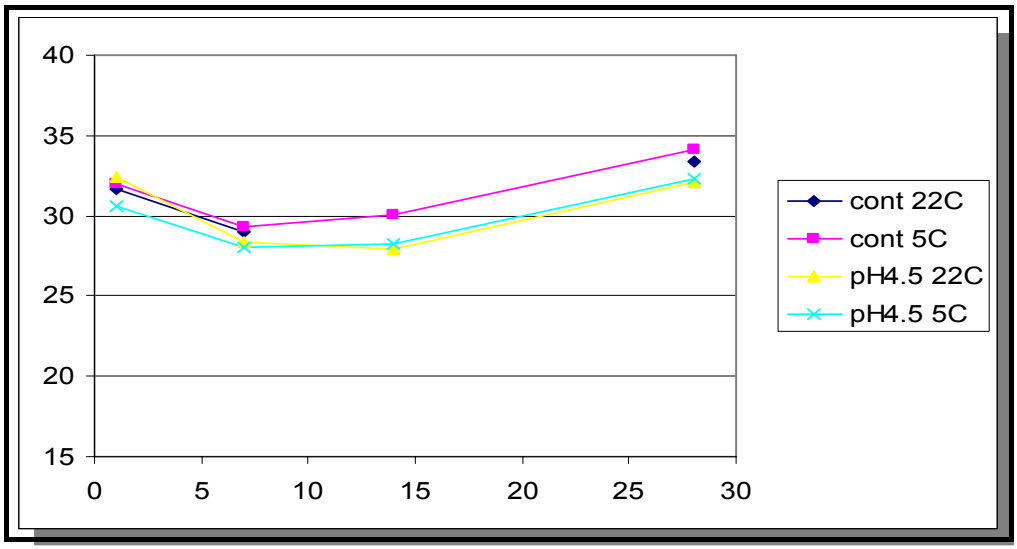
L=0 yields black and L*=100 indicates white*

Figure 61 – Changes in average L* values (n=3) of avocado pulp packed in Amcor retort pouches, processed at 5°C and 22°C with HPP (600 MPa for 3 min) and stored at 4°C.



a- negative values indicate green while positive values indicate magenta*

Figure 62 – Changes in average L* values (n=3) of avocado pulp packed in Amcor retort pouches, processed at 5°C and 22°C with HPP (600 MPa for 3 min) and stored at 4°C.



b - negative values indicate blue and positive values indicate yellow*

Figure 63 – Changes in average b* values of avocado pulp packed in Amcor retort pouches, processed at 5°C and 22°C with HPP (600 MPa for 3 min) and stored at 4°C.

L* values of the pulp remained unchanged and a difference in L* value between the treatments was not observed (Figure 61). Both a* values and b* values of the control samples were different from pH adjusted samples (Figure 62 and Figure 63). a* values in all samples remained unchanged during storage while b* values in all samples showed a slight increase. This indicated an increase in yellowness of the samples during storage.

Experiment 4

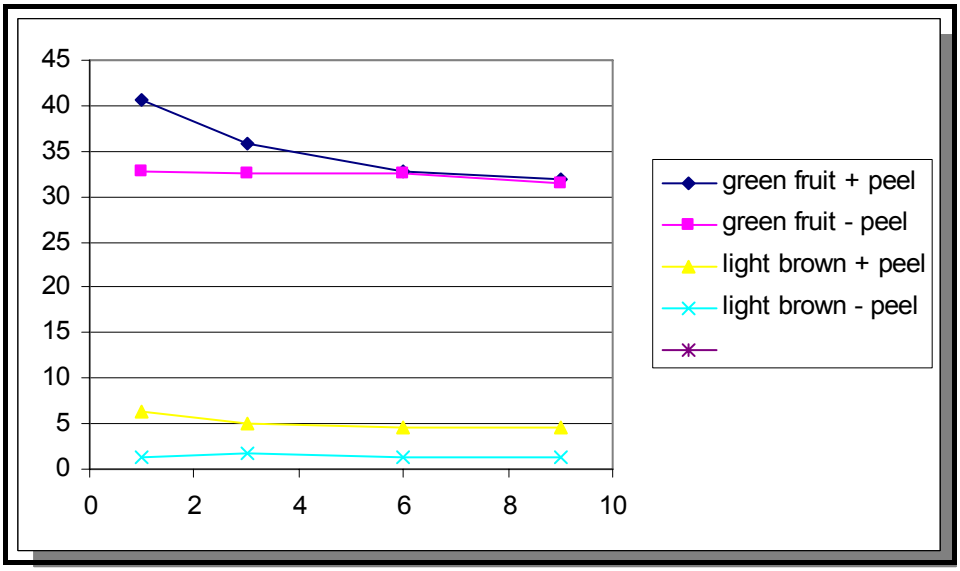


Figure 64 – Firmness pattern of ethylene treated (100 ppm 24 hrs), green-unripe and ripening-brown avocados stored at 4°C during 10 days of storage.

Avocado fruit firmness changed slowly when stored at the 4 ºC for a short period of 7 days (Figure 64). Brown fruits which were undergoing ripening showed a lower firmness reading when measured without peel than with peel. At this texture level (< 2.0 N) the fruits were too soft for handling and processing. At <2.0N texture level peeling of the halved fruits caused damage to the soft pulp and resulted in poor appearance of the halves or slices.

Green unripe fruits stored at room temperature showed a rapid decrease in texture. The texture of both green and brown fruit types reached the same level of textural softness within 6 days after arrival at the laboratory (Figure 65).

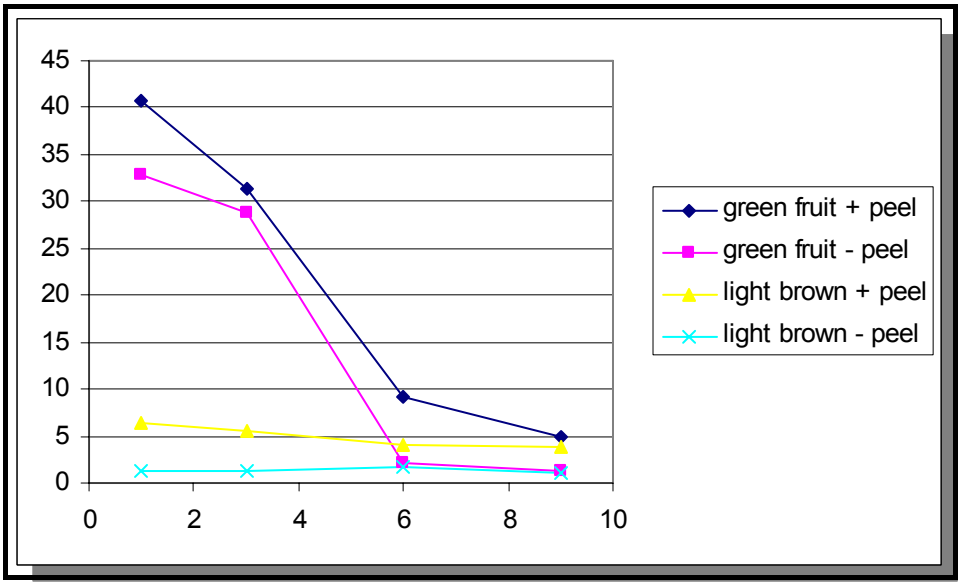


Figure 65 – Firmness pattern of ethylene treated (100 ppm 24 hrs), green-unripe and ripening-brown avocados stored at room temperature at 22°C during 10 days of storage.

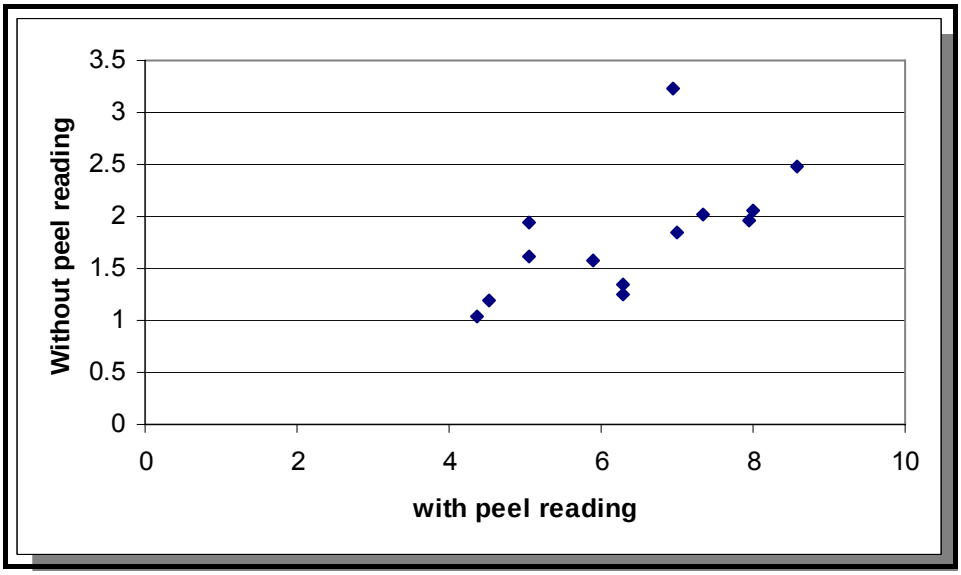


Figure 66 – Scatter diagram of firmness with and without peel of untreated green avocados 6 days after harvesting and storage at 22°C.

This study evaluated the firmness of untreated fruits stored at room temperature at the initiation of texture change. Fruits with a peel texture measurement above 4.0 N were measured with and without peel as shown in Figure 66 after 6 days from harvesting. There was no strong correlation between the two measurements (r^2= 0.6). However, fruits with a peel reading above 6.0 N had a good texture for peeling and handling during the packing stage. The effect of HPP treatment at this stage of ripening could not be detected due to the unavailability of the HPP unit. Similar observations were also made with fruits tested 9 days after harvesting (Figure 67).

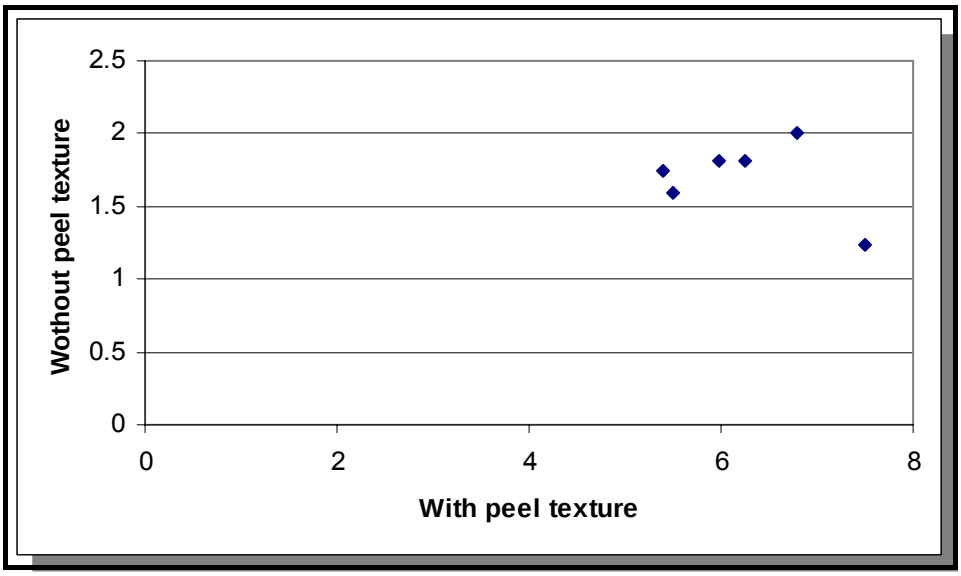


Figure 67 – Scatter diagram of firmness with and without peel of untreated green avocados 9 days after harvesting and storage at 22°C.

Experiment 5

Experiment 5 A- Evaluation Of Suitable Acid Types For HPP Treated Avocado Products.

Table 57: The pH of 1 and 2% (w/w) acid solutions made with different acid types

Acid type	pH of the 1% solutions	pH of the 2% solutions
Ascorbic	2.73	2.65
Citric	2.36	2.27
Fumaric	2.39 (< 1% saturated)	
Lactic	2.33	2.36
Malic	2.39	2.28
Tartaric	2.31	2.19

Table 58: The pH of the pureed avocado slices before and after HPP treatment with 2% (w/w) acid solutions made of different acid types.

Acid type	pH before HPP	pH after HPP
Ascorbic	6.93	6.47
Citric	5.76	5.71
Fumaric	6.59	6.18
Lactic	6.15	5.85
Malic	5.8	5.94
Tartaric	6.13	6.05
Untreated with acid	6.56	6.21

As expected the pH of the acid solutions decreased with the increase of acid percentage from1% to 2% (Table 57).

The pH of the pureed slices did not show a higher variation in pH values before and after HPP treatment (Table 58).

Table 59: Acidity and acceptability scores of HPP treated (600 MPa for 3 min) avocado pieces treated with different acid types

Acid type	Acidic flavour	Acidic flavour Rank	Acceptability	Acceptability Rank
Ascorbic	3.00 ± 2.39	4	5.86 ± 1.25	7
Citric	5.14 ± 2.47	7	6.14 ± 3.55	6
Fumaric	2.00 ± 1.20	1	8.57 ± 1.27	1
Lactic	3.57 ± 2.26	5	6.71 ± 2.82	4
Malic	2.57 ± 2.32	3	7.43 ± 0.76	3
Tartaric	4.00 + 3.55	6	6.71 ± 2.82	5
Untreated with acid	2.29 ± 1.67	2	7.86 ± 1.01	2

Differences of the acidity acceptability scores were not statistically significant due to the high variability in results (Table 59). Therefore, based on previous experience and knowledge available on the acids, malic acid and ascorbic acid were selected for further experimentation. Malic acid was observed to have better anti-microbial properties when used with avocado products (personal communication with Avure Technologies). Ascorbic acid was selected based on antioxidant properties.

Experiment 5B - Selection Of Suitable Acid Types For Acidification Of Avocado Pulp

Table 60: Acidic flavour and acceptability scores of HPP treated (600 MPa for 3 min) avocado pulp treated with different acid types.

Acid type	Acidic flavour	Acidity rank	Acceptability	Acceptability Rank
Ascorbic	3.0 ± 2.6	4	5.9 ± 2.8	2
Citric	5.1± 2.7	7	5.3 ± 2.9	1
Fumaric	2.0 ± 1.3	1	8.6 ± 0.8	7
Lactic	3.6 ± 2.4	5	6.7 ± 2.3	5
Malic	2.6 ± 2.5	3	6.7 ± 2.7	4
Tartaric	4.0 ±3.8	6	6.4 ± 3.2	3
Untreated with acid	2.3 ±1.8	2	7.9 ± 2.0	6

The lowest acidic flavour and highest acceptability rank was observed for fumaric acid (Table 60). However, based on the above results and our previous experience 2% ascorbic acid, 2% malic acid and 1% malic acid + 1% ascorbic acid were recommended for microbiological challenge test and storage trials.

10.1.3 Discussion:

Experiments 1-4

The aim of these initial experiments was to provide information regarding the raw material quality needed for the production of high quality avocado products. Another objective was to collect the basic information required to develop a sequence of unit operations to process avocado halves, slices and pulp by HPP processing. The preliminary experiments conducted during this project concentrated on:

- The optimum texture requirements of raw material for HPP treated avocado halves and slices;
- Packaging material requirements with reference to the OTR, ability of the packaging material to withstand HPP treatments and the flexibility of the packaging material to produce a neatly moulded final pack with minimum head space;

- The effects of processing temperature on the final quality and storage stability of the product.

Discussions were held with Mr. Brian Prosser (Proprietor of SunFresh Avocados Pty. Ltd.) regarding the ripening process of the avocados to be used for the trials. It was decided to send untreated avocados from Queensland to the Melbourne wholesale markets and treat the avocados with ethylene using a commercial scale ethylene treatment facility. After 24 hrs of exposure to 100ppm ethylene the avocados were returned to ambient conditions and sent to Food Science Australia by the Melbourne agent for SunFresh Avocados Pty. Ltd.

On most occasions the avocados exhibited a firm texture upon arrival at Werribee but were warm and in the ripening stage. The texture of the avocado fruits changed very rapidly and it proved difficult to retain the texture at a desirable state. In the first two experiments the texture was measured using a hand -held penetrometer where the texture was recorded as 0.6Kg using an 8mm probe. It was decided that it was also necessary to use the Instron texture measuring device to monitor the texture with and without peel to achieve greater precision in the results.

In the first experiment three different types of packaging materials (Amcor retort pouch, Cryovac B471 cheese bag, and Cryovac Holmes bags) were evaluated for packaging HPP treated halves and slices. Maximum vacuum was applied for 5 seconds to remove headspace air. This was essential to prevent development of browning on the cut surfaces and the seed cavity of the avocados during storage. The Amcor retort pouches showed very good barrier properties and prevented browning in avocado pulp during storage. This was demonstrated using both objective and subjective measurements of colour.

This packaging material could not be used for the avocado halves due to the high rigidity of the pouch. When packed in the Amcor retortable pouches with -1bar vacuum for 5 seconds, the avocado halves and slices were significantly deformed in shape. This was probably due to the high vacuum and softness of the avocados used and the low flexibility of the packaging material. Lower vacuum levels and shorter exposure times were trialled but both conditions led to the development of detectable levels of browning in the product after 24 hrs storage at 4°C (data not presented).

Cryovac B471 bags were also used for the packaging avocado halves and the samples retained colour satisfactorily over a period of 28 days. The L*,a* and b* values and the informal subjective browning scores also indicated minimum changes to product colour occurred during storage at 4°C. This packaging material was very flexible and was able to mould to the shape of the avocado halves, resulting in a very small headspace after vacuum packaging. However, the avocado juice that was smeared in between the two layers of packaging material showed detectable levels of browning after 21 days of storage at 4°C. This may be due to the higher OTR and lower thickness exhibited by the B471 bags compared to the Amcor retort pouches.

Based on observations made on the browning of avocado halves, slices and pulp in experiment 1, the B471 bags were selected for avocado halves and slices, and Amcor retort pouches were selected for avocado pulp for experiments 2 and 3. It is important to note that the selection of suitable packaging material was essential for the success of this project.

According to the results of the informal sensory evaluations made by 3 panellists in experiment 2, the taste scores of avocado halves decreased markedly after eight days of storage at 4°C. The sensory scores remained constant at around 2-3 for the remainder of the storage period. The initial loss in taste may be due to the loss of some flavour notes during storage. This loss may be related to the barrier properties of the B471 packaging material and/or the drop in pH.

In experiment 3 acidification of avocado pulp reduced the taste scores considerably compared to the unacidified control samples. This may be due to the increased acidity masking the subtle mild flavour of avocado. However, acidification of the pulp would reduce the risk from microbial contamination thus making it suitable for use in manufacturing guacamole or salsa products. Unacidified pulp was acceptable in taste up to 21 days of storage. The retention of taste during the storage period may be related to the good barrier properties of the Amcor retort pouches.

However, sensory testing of the final products would need to be conducted by a formally trained panel to confirm these findings.

The pH values of the avocado halves decreased gradually during storage from 7 to 5. This followed a trend similar to that recorded for the taste scores. The decrease in pH may be due to either or both microbial activity or breakdown of compounds in the avocado tissues. A similar reduction in pH was observed in unacidified avocado pulp packed in Amcor retort pouches but at a lower magnitude. In acidified avocado pulp the pH was also stable during storage.

The texture difference observed in the raw avocado material was not detectable in the HP processed product. It was therefore decided to collect more data on the texture of fruits during ripening to determine a suitable texture. It was demonstrated that selection of optimum firmness of raw material was important for maintaining a good avocado flavour and for reducing damage during peeling and packaging of high pressure treated avocado halves and slices. Very soft halves and slices resulted in poor quality products after peeling and packaging.

There was no detectable difference observed between the samples processed at 5°C and those processed at 22°C in any of the parameters tested in this study. However, processing at a lower temperature could minimize the acceleration of potential microbial growth during the processing phase.

Experiment 4 demonstrated that storing of unprocessed whole avocado fruits at a lower temperature could maintain the firmness without significant change. However, prolonged storage of fruits at 4°C resulted in chilling injury as expected. Ethylene treated fruits stored at room temperature rapidly decreased in texture value but once they reached a low texture value the texture changes were then minimal. For ethylene treated fruits it was difficult to monitor texture change between 10-5N using peel texture measurements. Hence non-ethylene treated fruits were obtained and their texture was monitored with and without peel in these measurements. Fruits exhibiting a firmness of $\geq$ 7.0N with the peel intact recorded a flesh firmness of $\geq$ 1.5 N after removal of the peel. At this firmness level fruits possessed a satisfactory taste and were able to withstand the handling required during peeling and packaging and probably HPP processing.

Experiments 5

Six different types were evaluated for their suitability for avocado product acidification. The obtained results showed a wide variation. Fumaric acid showed the lowest acidic flavour and highest acceptability scores. Based on the experience of the team members ascorbic acid and malic acid were selected to for experimentation with avocado products.

10.1.4 Conclusions

Experiments 1-4

These studies have shown that it will be important to obtain a packaging material which is superior to Cryovac B471 in OTR and thickness characteristics. The browning problem associated with B471 packaging perhaps could be minimized by using smaller bags (short and narrow) to reduce the total package surface area and/or adding a small volume of ascorbic acid solution into the seed cavity. However, it would be preferable that the packaging materials should possess barrier properties comparable to those exhibited by the Amcor retort pouches (OTR < 3.0 cc/ m^2/24 hrs@ atm.,23°C, 50% RH pressure, but with a flexibility similar to the B471 bags and which can withstand high pressure treatments.

Processing at 5°C and 22°C showed no effect on the sensory parameters of HPP treated avocado products.

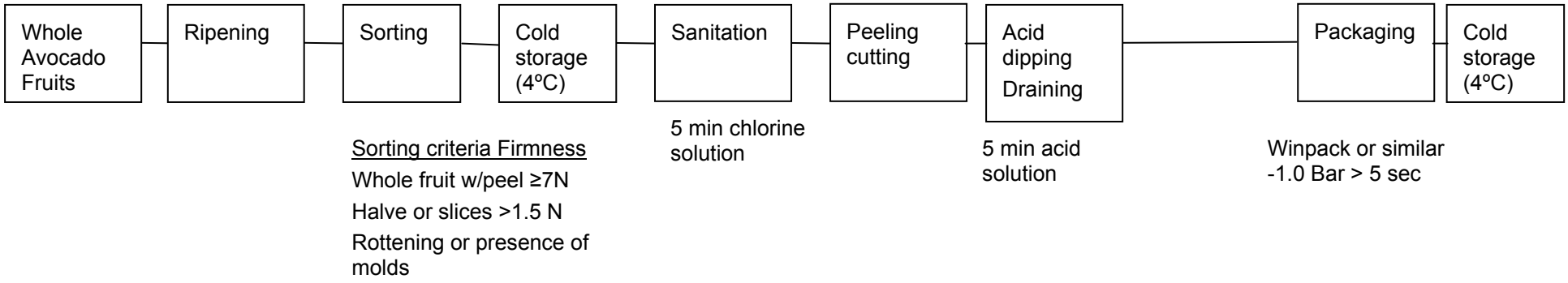
It is believed that avocado fruits with a $\geq$7.0 N with peel texture readings would be able to withstand the peeling, halving and packaging stages required for these products.

Experiment 5

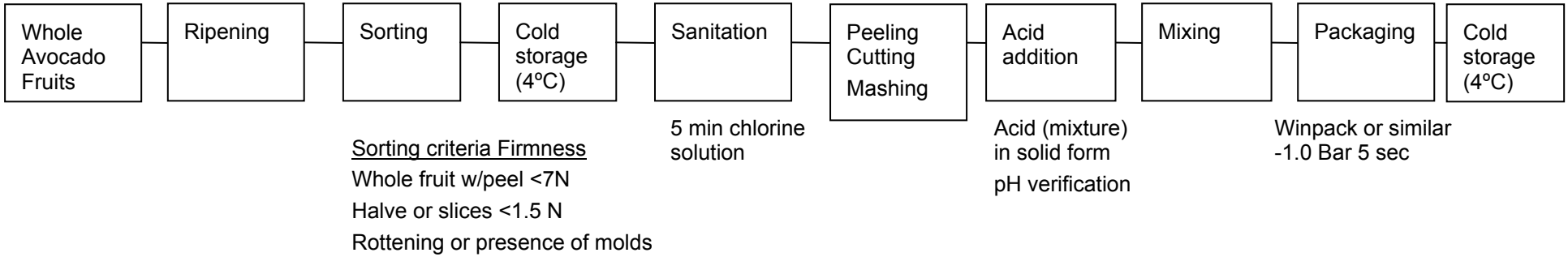
Ascorbic acid was selected for further experimentation with avocado products based on it's reported anti browning properties. Malic acid was selected based on anti-microbial properties that were observed under HPP conditions.

10.2 Flow Diagram Of The Suggested Processing Steps For HPP Avocado Products

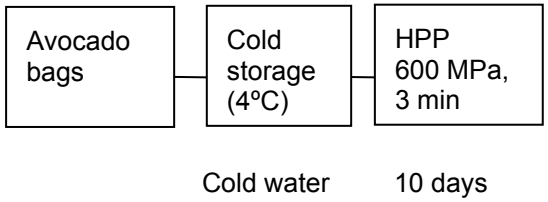
10.2.1 Preparation Of Avocado Halves And Slices



10.2.2 Preparation Of Avocado Puree



10.2.3 High Pressure Process



10.2.4 Description

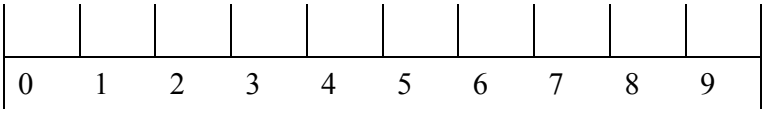
Avocados need to be selected based on their texture or firmness. According to the findings of this study the preferred texture of fruit with peel ≥7.0 N (1.5 N texture reading with peeled slices or halves). After ripening to correct maturity, cold storage of fruit helped maintain the desirable texture and microbiological quality of the unpeeled fruit. Prior to processing the fruit needs to be washed with sanitizing solution such as Tsunami®/Chlorine solution. In these trials, the fruits were immersed in the sanitizing solution for 5 minutes before washing. Processing of the fruit should be conducted at low temperature. In this study there was no difference in quality observed when processed at 4c or 22c. However to maintain microbial contamination and growth under control, it is recommended processing be done at 4c. Once they are peeled and cut the fruit should be placed in acid solution. In the trials halves and slices were kept in acid solution for 5 minutes to imitate commercial conditions where the product is transported from processing step 1 to processing step 2 to be packaged. The slices and halves should be drained from the solution and placed in Winpack 7000 bags or a bag with a similar OTR and flexibility. Bags should be vacuum packed with -1.0 bar for 5 seconds to completely evacuate air from the seed cavity area of the halves. Packaged products should be stored at 4°C pending HPP processing. HPP processing should be conducted with chilled water or water at room temperature. However, it is recommended chilled water be used to maintain a high microbiological standard of the product. HPP processing at 600 MPa for 3 min. Processing for 3 min is recommended in order to achieve a higher kill rate of the initial microbial load of the raw material. According to the Australian Standards on refrigerated products, storage of product at 4°C is recommended for up to 10 days.

10.3 Sensory Evaluation Forms

10.3.1 Sensory Evaluation Of Avocado Pulp

1. Sample No. –

Taste



Very low acidity High acidity

Acceptable / not acceptable (Select one option)

Comment –

2. Sample No. –

Taste

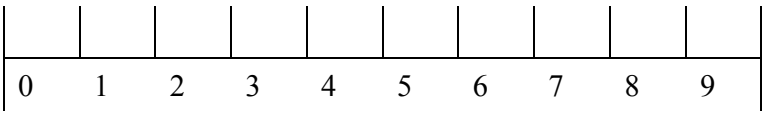
Very low acidity High acidity

Acceptable / not acceptable (Select one option)

Comment –

3. Sample No. –

Taste



Very low acidity High acidity

Acceptable / not acceptable (Select one option)

Comment –

10.3.2 Sensory Evaluation Of Avocado Paste

Name

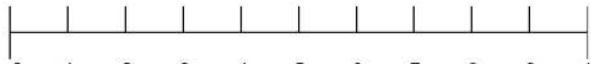
Sensory evaluation of avocado paste **SAMPLE No ____**

Please taste the samples and provide relevant information.

Acceptability

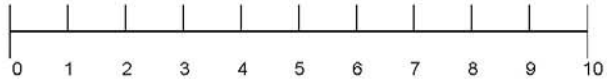
Extremely dislike Extremely like

Acidic taste



Very low acidity Very acidic

%Browning



0% 50% 100%

Consistency (mouthfeel)

Thin Thick

Note: 5 is the consistency you would expect in an avocado paste

Comments:

10.3.3 Sensory Evaluation of Avocado Pieces

Sensory evaluation of avocado pieces **SAMPLE No ____**

Please taste the samples and provide relevant information.

Acceptability

Extremely dislike Extremely like

Acidic taste

Very low acidity Very acidic

%Browning

0% (green) 50% 100% (browned)

Firmness

Very soft Very firm

Note: 5 is the firmness you would expect in a fresh avocado piece

Comments: