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High Hydrostatic Pressure (HHP) for Kiwifruit puree preservation.

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High Hydrostatic Pressure (HHP) for Kiwifruit puree preservation.

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HACEN CONSTAR: que Dña. JACIRA ANTONIA BRASIL ha realizado, bajo su dirección, en el Área de Ciencia y Tecnología de los Alimentos de la Universitat Autònoma de Barcelona, el trabajo titulado “High Hydrostatic Pressure (HHP) for Kiwifruit puree preservation” que presenta para optar al grado de Doctor.

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ABSTRACT

Regular consumption of fruit (including kiwi) in the diet has a beneficial effect on health. It is not only an excellent source of vitamins, fiber and minerals, it also has phytochemicals that contribute to health. On the other hand, more and more consumers demand for fast food with high nutritional quality, sensory and minimum of additives for conservation.

The first impression consumers have from fruit it is the appearance or external aspect, being the most important for acceptance and purchase decision. The shape and size are among the most easily discernible aspects, but generally it is not a decisive quality character. Only if morphological abnormalities or defects could be a cause of reject. During and after kiwi harvest, a certain percentage of the harvest cannot be intended for distribution on not reaching marketing standards.

On the other hand, there is a sector of the population that requires encourage for fruits consumption, specifically kiwis, such as children, the elderly and certain sectors whom for work or practical reasons, is a drawback to wash, peel and cut some fruits.

Based on this approach, in this Thesis we intend to study the application of a non-thermal technology, such as high hydrostatic pressure (HHP), to obtain a kiwi puree safe, healthy (with its nutritional properties) and desirable (without losing organoleptic properties).

In the first phase of this thesis the conditions of pressure, temperature and time of HHP treatment to reduce their endogenous microbial load and to study the evolution of the survivors were determined. Also, different indicators of pathogens were inoculated, obtaining their lethality rates and the resilience of the survivors. In turn, parameters that might influence the survival or growth of microorganisms such as pH, soluble solids, and temperature were monitored.

In a second stage, physicochemical quality parameters (instrumental color, degradation of chlorophylls, rheology), nutritional (vitamin C) and sensory (taste panel), in the conditions that had previously shown a microbiological safety at the beginning and during storage, were studied.

To summarize, we highlight the following results with respect to inactivation of aerobic mesophilic (AM) Total reductions Log 2 and 3 were observed at 300 MPa / 0.1 min and other conditions, respectively. The survivors were sporulated AM (SP), as they match survivors counts. In no case there was growth during storage at 30 days. For inoculated pathogenic microorganisms (S. aureus and E. coli), proved to be much more sensitive as from 500 MPa at

any time and temperature tested, no survivors watch yourself, with reductions of 5 and 7 Log, respectively

The lesser differences in color between pressurized samples versus control (ΔE) were treated at 10 °C, 5 min and 300 <700 <500 MPa. In turn, the content of pigment molecules with HHP treatments tested (300, 500 and 600 MPa and 0.1, 5 and 15 min) was maintained in the range 50-70% for chlorophyll a and 60-80% for chlorophyll b. With the pasteurization heat treatment remained just 0 and 10% for chlorophyll a and b, respectively. Conversely pheohytins formation in samples treated by HHP, hardly changed relative to the control, pasteurized samples doubled its contents.

It was observed that the total content of Vitamin C in the samples treated by HHP, the gap narrowed just about control during storage, only at day 60 compared to control ranged between 50-70% lower.

A level sensory testing, panelists clearly distinguished the samples heat treated and the pressurized control. It should be noted that the treatments tested, samples of 300 and 500 MPa for 15 min, were placed the closest to the control by panelists.

With the results of this study demonstrated that treatment by HHP, are an alternative to increase the life of mashed kiwi, maintaining the microbiological safety and rheological, organoleptic and favorable to consumers nutritional characteristics, at least until the 30th day of storage.

RESUMEN

El consumo habitual de fruta (como el kiwi) en la dieta tiene un efecto muy beneficioso para la salud. No sólo es una excelente fuente de vitaminas, fibra y minerales, sino que además posee compuestos fitoquímicos que contribuyen a la salud. Por otro lado, los consumidores demandan cada día más de alimentos de preparación rápida, con una alta calidad nutritiva, sensorial y el mínimo de aditivos para su conservación.

La primera impresión que los consumidores reciben de las frutas es la apariencia o aspecto externo, siendo la más importante para su aceptación y decisión de compra. La forma y tamaño son unos de los aspectos más fácilmente discernibles, aunque generalmente no es un carácter de calidad decisivo. Solo en caso de malformaciones o defectos morfológicos podría serlo. Durante y después de la cosecha del kiwi, un cierto porcentaje de la recolección no puede ser destinado a su distribución ya que no alcanza los estándares de comercialización.

Por otro lado, existe un sector de población que precisa de facilidades a la hora de incentivar el consumo de frutas, y en concreto de kiwis, como son los niños, los ancianos y ciertos sectores para los cuales, por motivos de trabajo o prácticos, resulta un inconveniente el lavar, pelar y cortar ciertas frutas fuera de casa.

En base a este planteamiento, en la presente Tesis nos hemos propuesto estudiar la aplicación de una tecnología no térmica, como son las Altas Presiones Hidrostáticas (HHP) para la obtención de un puré de kiwi seguro, saludable (con sus propiedades nutritivas) y apetecible (sin perder propiedades organolépticas).

En la primera fase de esta Tesis se determinaron las condiciones de presión, temperatura y tiempos del tratamiento HHP para reducir su carga microbiana endógena y estudiar la evolución de los supervivientes. Además se inocularon diferentes indicadores de patógenos, obteniéndose sus letalidades y capacidad de recuperación de los supervivientes. A su vez, se monitorizaron parámetros que pudieran influenciar en la supervivencia o crecimiento de los microorganismos, como pH, sólidos solubles, temperaturas.

En una segunda fase, se estudiaron los parámetros de calidad fisicoquímica (color instrumental, degradación de las clorofilas, reología), nutricional (vitamina C) y sensoriales (panel de catadores), en aquellas condiciones que previamente habían demostrado una seguridad microbiológica inicialmente y a lo largo de su almacenamiento.

A modo de resumen, destacamos los siguientes resultados obtenidos, respecto a la inactivación de aerobios mesófilos (AM) totales se observaron reducciones de 2 y 3 Log a 300 MPa/0.1 min y resto de condiciones, respectivamente. Los supervivientes en los AM fueron esporulados (SP), ya que coinciden con los recuentos de supervivientes. Pero en ningún caso

hubo proliferación durante su almacenamiento a 30 días. Los microorganismos patógenos inoculados (*S. aureus* y *E. coli*), resultaron ser mucho más sensibles, ya que a partir de 500 MPa a cualquier tiempo y temperatura ensayadas, no se observaros supervivientes, con reducciones de 5 y 7 Log, respectivamente.

Las menores diferencias de color entre las muestras presurizadas frente al control (ΔE) fueron las tratadas a 10 °C, 5 min y 300 < 700 < 500 MPa. A su vez, el contenido de clorofilas *a* y *b*, con los tratamientos de HHP ensayados se mantuvo del orden del 50-70% para clorofila a y del 60-80% para clorofila b. Con el tratamiento térmico de pasteurización aplicado solamente quedó 0 y 10% de clorofila a y b, respectivamente. Por el contrario, la formación de feofitinas (*a* y *b*) en las muestras tratadas por HHP apenas varió respecto al control, mientras que las muestras pasteurizadas doblaron su contenido.

Se pudo observar que el contenido total de Vitamina C en las muestras tratadas por HHP, apenas se redujo respecto a su control, durante su almacenamiento. Sólo al día 60, la diferencia con respecto a su control osciló entre un 50-70% menor.

A nivel de análisis sensorial, los panelistas distinguieron claramente las muestras tratadas térmicamente de las presurizadas y control. Cabe resaltar que de los tratamientos ensayados, las muestras de 300 y 500 MPa a 15 min, fueron las que los panelistas colocaron más cercanas al control.

Con los resultados obtenidos en el presente trabajo se demuestra que los tratamientos por HHP, son una alternativa para aumentar la vida útil del puré de kiwi, manteniendo la seguridad microbiológica y unas características reológicas, organolépticas y nutricionales satisfactorias para los consumidores, como mínimo hasta el día 30 de conservación en refrigeración.

RESUM

El consum habitual de fruita (com el kiwi) en la dieta té un efecte molt beneficiós per a la salut. No només és una excel·lent font de vitamines, fibra i minerals, sinó que a més posseeix compostos fitoquímics que contribueixen a la salut. D'altra banda, cada dia més els consumidors demanen d'aliments de preparació ràpida, amb una alta qualitat nutritiva, sensorial i el mínim d'additius per a la seva conservació.

La primera impressió que els consumidors reben de les fruites és l'aparença o aspecte extern, sent la més important per a la seva acceptació i decisió de compra. La forma i mida és un dels aspectes més fàcilment discernibles, tot i que generalment no és un caràcter de qualitat decisiu. Només en cas de malformacions o defectes morfològics podria ser-ho. Durant i després de la collita del kiwi, un cert percentatge de la collita no pot ser destinat a la seva distribució ja que no arriba als estàndards de comercialització.

D'altra banda, hi ha un sector de població que necessita de facilitats a l'hora d'incentivar el consum de fruites, i en concret de kiwis, com són els nens, els ancians i certs sectors per las quals, per motius de treball o pràctics, és un inconvenient el rentar, pelar i tallar certes fruites fora de casa.

En base a aquest plantejament, a la present Tesi ens hem proposat estudiar l'aplicació d'una tecnologia no tèrmica, com són les Altes Pressions Hidrostàtiques (HHP) per a l'obtenció d'un puré de kiwi segur, saludable (amb les seves propietats nutritives) i desitjable (sense perdre propietats organolèptiques).

En la primera fase d'aquesta Tesi es van determinar les condicions de pressió, temperatura i temps del tractament HHP per reduir la seva càrrega microbiana endògena i estudiar l'evolució dels supervivents. A més es van inocular diferents indicadors de patògens, obtenint letalitats i capacitat de recuperació dels supervivents. A més a més, es van monitoritzar paràmetres que poguessin influir en la supervivència o creixement dels microorganismes, com pH, sòlids solubles, temperatures.

En una segona fase, es van estudiar els paràmetres de qualitat fisicoquímica (color instrumental, degradació de les clorofil·les, reologia), nutritius (Vitamina C) i sensorials (panell de tastadors), en aquelles condicions que prèviament havien demostrat una seguretat microbiològica inicialment i al llarg del seu emmagatzematge.

A mena de resum, destaquem els següents resultats obtinguts, respecte a la inactivació d'aerobis mesòfils (AM) totals es van observar reduccions de 2 i 3 Log a 300 MPa / 0.1 min i resta de condicions, respectivament. Els supervivents en els AM van ser esporulats (SP), ja que van coincidir amb els recomptes de supervivents. Però en cap cas hi va haver proliferació

durant el seu emmagatzematge a 30 dies. Els microorganismes patògens inoculats (*S. aureus* i *E. coli*), van resultar ser molt més sensibles, ja que a partir de 500 MPa a qualsevol temps i temperatura assajades, no es observar supervivents, amb reduccions de 5 i 7 Log, respectivament.

Les menors diferències de color entre les mostres pressuritzades enfront del control (ΔE) van ser les tractades a 10 °C, 5 minuts i $300 < 700 < 500$ MPa. A més a més, el contingut de clorofil·les *a* i *b*, amb els tractaments de HHP assajats es va mantenir de l'ordre del 50-70% per a clorofil·la a i 60-80% per a clorofil·la b. Amb el tractament tèrmic de pasteurització aplicat només va quedat al 0 i 10% de clorofil·la a i b, respectivament. Per contra, la formació de feofitines (*a* i *b*) en les mostres tractades per HHP gairebé no va variar respecte al control, mentre que les mostres pasteuritzades van doblar el seu contingut.

Es va poder observar que el contingut total de Vitamina C en les mostres tractades per HHP, tot just es va reduir respecte al seu control, durant el seu emmagatzematge. Només al dia 60, la diferència respecte al seu control va oscil·lar entre un 50-70% menor.

A nivell d'anàlisi sensorial, els panelistes van distingir clarament les mostres tractades tèrmicament de les pressuritzades i control. Cal destacar que dels tractaments assajats, les mostres de 300 i 500 MPa a 15 min, van ser les que els panelistes van col·locar més pròximes al control

Amb els resultats obtinguts en el present treball es demostra que els tractaments per HHP, són una alternativa per augmentar la vida útil del puré de kiwi, mantenint la seguretat microbiològica i unes característiques reològiques, organolèptiques i nutritives satisfactòries per als consumidors, com a mínim fins al dia 30 de conservació en refrigeració.

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LIST OF ABREVIATIONS

τ	Shear Stress (Pa)
γ	Shear rate (s^{-1})
τo	Initial shear stress (Pa)
“R”	“R” software
*a**	indicates hue on a green (-) to red (+) axis
AA	Ascorbic Acid
AM	Aerobic Mesophilic bacteria
ANOVA	Analisis of Variance
AOAC	Association of Official Analytical Chemists
*b**	indicates hue on a blue (-) to yellow (+) axis
BHT	Butylated hydroxytoluene
BP	Baird Parker agar
CECT	*Spanish Type Culture Collection*
CFU	Coloni Former Units
Chl *a*	Chlorophyll a
Chl *b*	Chlorophyll b
cm	centimeter
Coli ID	Chromogenic medium agar
DHAA	Dehydroascorbic acid
EB	Enterobacteriaceae
EDTA	EthyleneDinitriloTetraAcetic acid
et al.	et alter (and others)
FC	Faecal Coliforms
g	grams

G’	Storage modulus
G’’	Loss modulus
GC	Gas Cromatography
GLM	General Linear Model procedure
h	hours
HHP	High Hydrostatic Pressure
HMF	Hidroxymethylfurfural
HPLC	High Performance Liquid Chromatography
IS	Internal Standard
K	Consistency index (Pa·s)
L	Liter
L*	Lightness (Luminosity)
LAB	Lactic Acid Bacteria
LB	Lactobacillus
LC	Liquid Chromatography
Ln	Neperian Logaritm
Log	Decimal Logaritm
M	Molarity
MB	Mesophilic Bacteria
mg	milligram
min	minute
mL	milliliter
MPa	MegaPascals
MRS	Man-Rogosa-Sharpe agar
MY	Moulds and Yeast
n	Behavior index (dimensionless)

nm	nanometers
PA	Pasteurized
PC	Principal component
PCA	Plate Count Agar
Pheo *a*	Pheophytin a
Pheo *b*	Pheophytin b
PPO	Poliphenoloxidase
PME	Pectinmethylesterase
ppm	Parts per million
$p<0.05$	Significant differences at level of 0.05
$p>0.05$	No Significant differences at level of 0.05
R^2	Coefficient of determination
s	seconds
SD	Standard Deviation
SP	Spores
SS	Soluble Solids
TSB	Triptona Soya Broth
UFP	Ultra-Fresh Profiling
UV	Ultraviolet
Vit C	Total Vitamin C
VRBG	Violet Red Bile Glucose agar
ΔE	Differences of color $\Delta E^*_{ab}= [(\Delta L^{*2}) + (\Delta a^{*2}) + (\Delta b^{*2})]^{0.5}$
µg	microgram
µL	microliter
µM	micromolar

Chapter 1. Literature Review

Kiwifruit (*Actinidia deliciosa* var deliciosa cv. Hayward)

The kiwifruit, often shortened to kiwi, is a berry in an ellipse shaped covered with a skin full of villi. Its size is up to 6 cm and weighs about 80 grams, depending on the variety in question. The skin is brown and is covered with tiny hairs like a hair and the flesh is emerald green, sometimes yellow and brown or white-greenish depending on the variety. The fruit is full of little seeds of black color, arranged in a circle and has a bittersweet flavor and very refreshing (Morton, 2004). There are many commercial cultivars highly appreciated by consumers: Abbot, Bruno, Monty, Hayward, etc.

Actinidia is the genus of this fruit that contains about 70 species. The most commercial are *A. chinesis*, *A. arguta*, *A. kolmikta*, *A. polygama*, and *A. eriantha*. The *A. deliciosa* cv. Hayward eventually became the cultivar of choice because its fruits were larger, had a better appearance, and their flavor was considered by many to be superior. These qualities meant that the fruit of "Hayward" were preferred by consumers in both New Zealand and overseas to that of other kiwifruit cultivars then available. This varietal has a green flesh (flesh), with white core and black seeds (Figure 1-1).

Kiwi tree is a vine plant original from southwestern China. The crop was extended to rest of world after 70s; and in early twentieth century was domesticated in New Zealand. Actually, the kiwifruit is an important commercial crop grown in different parts of the world due to the good results reached in term of productive potential and the fruits quality. Total world production now exceeds a million tonnes per year, more than well-established crops such as raspberries and currants, and is soon likely to exceed production of strawberries or of apricots (Rafols, 2000).

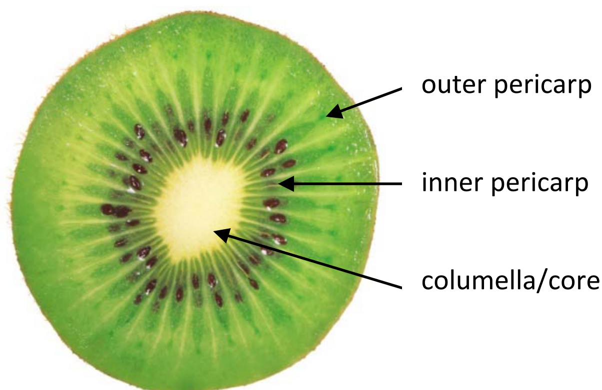

Figure 1-1. Kiwifruit analtomy

World Production of Kiwifruit

World Kiwifruit production is concentrated amongst few countries, with the top ten producing countries contributing over 95 per cent of world production. Table 1 shows the volume and ranking of the major producing countries since 1993. Traditionally, Italy, New Zealand and Chile have been the largest exporters dominating world kiwifruit production. However, there is a diphase in this dates due to a lack of Chinese production data estimates in 2010 480.000 tonnes China thus joined these three countries as a major producer since 2010 (Ward & Courtney, 2013)

Table 1-1. Production of top 10 producers of kiwifruit, 2012. Source: FAO STAT (2014).

Raking	Areas		Production (tonnes).
1	Italy		384,844
2	New Zealand		376,400
3	Chile		240,000
4	Greece		161,400
5	France		65,253
6	Turkey		36,781
7	Iran (Islamic Republic of)		32,000
8	Japan		28,000
9	United States of America		26,853
10	Portugal		25,000
11	Spain		16,200

Actually, data of 2012 of the world production was 1,412,455.00 tonnes. The European Union (EU) is the world's most important kiwifruit growing region accounting for 46.3 % of total world production followed by Oceania (26.9 %), Americas (18.9 %) and Asia (7.9 %). Italy is the largest producer accounting for 59 % of EU production (Figure 1-2). The EU greatly facilitated conversion of crop land to kiwifruit in the 1980s through widespread subsidies. Although most subsidies have reportedly been eliminated, their impact continues (FAO, 2014).

Overview of Kiwifruit in Spain

According FAOSTAT dates Spain is the 11th producer responsible to 16,200.00 tonnes of kiwifruit in 2012 (FAO, 2014), being exceeded by other largest producers including Greece, France, United States of America, Iran, Turkey, Japan and Portugal. Spain produces around 2.5 percent of the EU's kiwis, thereby, positioned this production in 5th place in EU. In 2012, Spain produced around 16,200 tonnes, 31 percent less than from 2011. Approximately 52 percent of production was exported in 2011. Spain turned in the last decades into first import per capita in the EU, consume oscillates in 2 Kg/habitant and year. The variety most consumed is the Hayward even though few years ago since few years came up the "gold" kiwifruit.

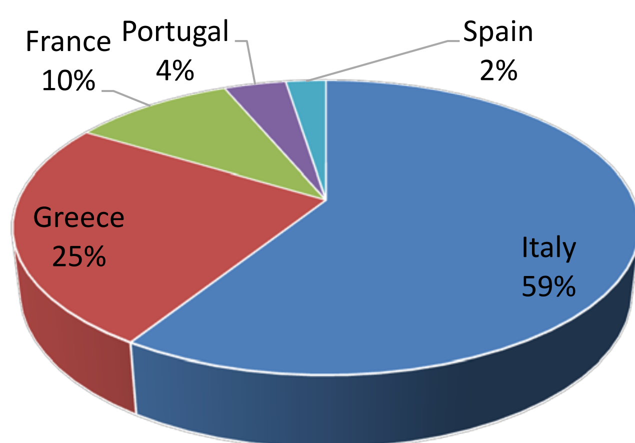

Figure 1-2. Europe kiwifruit crop in 2012. Source: FAO STAT (2014)

The Spanish kiwifruits is grown in the northern areas of Galicia and Asturias since the early 1970's, most of the kiwifruit cultivars used in Spain were originated in New Zealand and the crops are harvested from November to February. The production became profitable, however, only a decade later, when the plant was adapted to the soil and the Spanish market showed high purchase rates and high prices. In 2013, Spain produced around 16,200 tonnes, 31% less than from 2011. At the same time, Spain turned, in the last decades, into the first import per capita in the EU, consume oscillates in 2 Kg/ habitant and year. The variety most consumed is the Hayward even though few years ago since came up the "gold" kiwifruit. As a whole, the sector, which suffered difficulties since its inception, is actually going through a good moment;

it's capable not only of supplying the local market's demand but has also been able to export 52 of its 2011's production to the other European countries. As a matter of fact, Spain produces around 2.5% percent of the EU's kiwifruit, thereby positioned this production in 5° in EU (FAO, 2014).

Survey results on surfaces production area in 2014 shows a total of 954 ha, being Galicia and Comunidad Valencia the bigger producers, with 625 and 282 ha, respectively. These superficies reflect the result of a statistical sampling operation and relate to land cover at the time of field research (summer 2014). They not constitute, therefore, the official figures to spread in the yearbook of agricultural statistic (MAGRAMA, Ministerio de Agricultura, 2014b).

Spanish kiwifruit production peaked around the 2009-2011 seasons, with a decline after that. (Figure 1-3). The beginning of this PhD thesis coincided with the onset of the increased kiwifruit production.

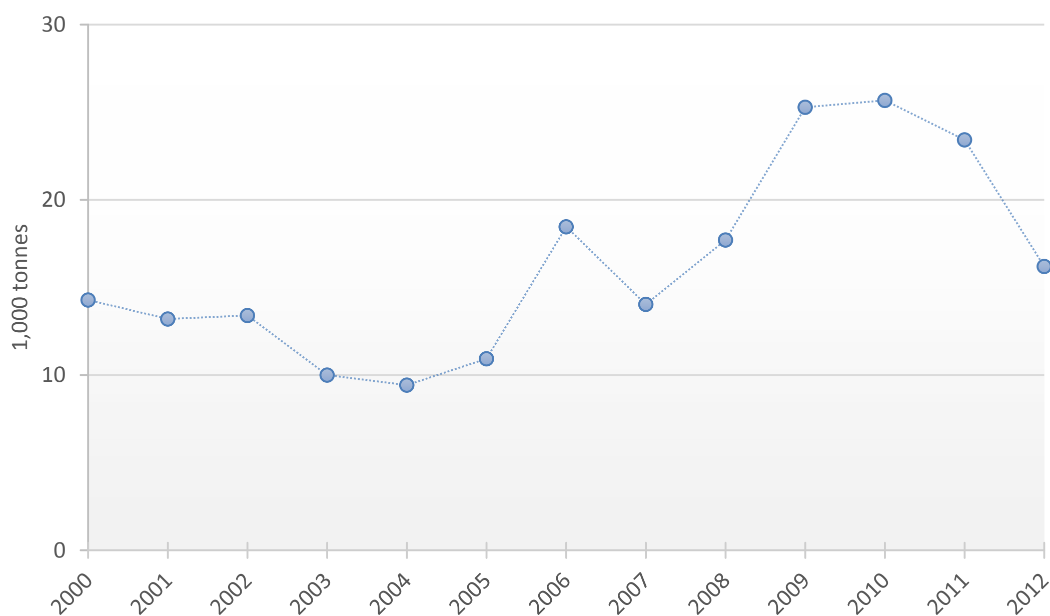

Figure 1-3. Spain kiwifruit production, 2000-2012 (MAGRAMA, Ministerio de Agricultura, 2014a)

Composition and Nutritional Value (Vitamin C) / Health Benefits of Kiwifruit

Kiwifruit has got low caloric value and an excellent source of antioxidants due to presence a wide number of phytonutrients including carotenoids, lutein, phenolics, flavonoids and chlorophyll (Kaya, Aydın, & Kolaylı, 2010; Shastri, Bhatia, Parikh, & Vinaya N., 2012). Chinese pharmacopoeia points out that the fruit aids digestion, reduces irritability, relieves

rheumatism, prevents kidney or urinary tract stones, and cures hemorrhoids, dyspepsia and vomiting (Kiwifruit and health, Hunter, 2010). The fruit is widely available and predominantly used for treatment of hepatitis, edema rheumatoid arthritis, gastric cancer, breast cancer (Shastri et al., 2012). The fruit is related with nutrition benefits such improvement of the immune system and fight the effects of stress and ageing due to its very high vitamin C content. However, kiwifruit also contains others vitamins and minerals that may contribute to possible health benefits, including vitamin K, folate, potassium, magnesium, calcium and phosphorus, as well a dietary fiber and phytochemicals (C. Hunter, Greenwood, Zhang, & A. Skinner, 2011; Cano, 1991) (Table 1-2).

Many health benefits have been attributed to ascorbic acid (vitamin C) namely antioxidant, anti-atherogenic and anti-carcinogenic activity, as well as immunomodulation (C. Hunter et al., 2011; Naidu, 2003). Ascorbic acid is one of the most important and essential vitamins for human health. It is necessary for many physiological functions in human biology. Based on available biochemical, clinical and epidemiological studies, the current recommended dietary allowances (RDA) for ascorbic acid is suggested to be 100-120 mg/day to achieve cellular saturation and optimum risk reduction of heart diseases, stroke and cancer in health individuals (Naidu, 2003). The green kiwifruit typically contains 92.7 mg/100g (FW) (Agriculture, 2013) and is also remarkable for the vitamin C concentration in fruits of cv. “Hayward” varies from 37 to 200 mg/100g of weight (Tavarini, Degl’Innocenti, Remorini, Massai, & Guidi, 2008), and as result single fruit can provide 100% of the recommended daily intake.

Table 1-2. Nutrients values of kiwifruit. Source: USDA (2014).

Nutrient	Unit	Value per 100 g
Proximate		
Water	g	83.07
Energy	kcal	61.00
Protein	g	1.14
Total lipid (fat)	g	0.52
Carbohydrate, by difference	g	14.66
Fiber, total dietary	g	3.00
Sugars, total	g	8.99
Minerals		
Calcium, Ca	mg	34.00
Magnesium, Mg	mg	17.00
Phosphorus, P	mg	34.00
Potassium, K	mg	312.00
Vitamins		
Vitamin C, total ascorbic acid	mg	92.70
Folate, DFE	µg	25.00
Vitamin A, IU	IU	87.00
Vitamin E (alpha-tocopherol)	mg	1.46
Vitamin K (phylloquinone)	µg	40.30

An important kiwifruit's characteristic is the significant amounts of pigments, mainly chlorophyll and carotenoids. Kiwifruit is notable for its attractive brightness green flesh because of, mainly the ratio of chlorophyll *a* and *b* (Fuke, Sasago, & Matsuoka, 1985).

Minimally Processed Products

Little Introduction about Fruit Consumption

The consumption of fruit and vegetable is appraised mainly due to the theirs health properties and the contribution for quality life's improvement (Allende, Tomás Barberán, & Gil, 2006). The consumers market's need of convenient, caused by the emergence of chain fast food, leading the consumer foods poor in fiber, vitamins and minerals and rich in salt, fat and sugar. One of the reflections of these alimentary habits became apparent the increasing incidence of obesity and cardiovascular diseases. Mainly due the health conscious consumer and the increasing interest in the role of food for maintaining and improving human well-being, the consumer wants to have access to food at the same time convenient and healthily. With the demand created by the consumer market for fast, nutritious foods, which can be served in individual portions, the fresh cut fruit and vegetable industry started constantly growing and thus the niche for development of new food. (Allende et al., 2006; Ragaert, Verbeke, Devlieghere, & Debevere, 2004)

Kiwifruit pure is one of the products resulting from the creation of this niche. It is directed to children and elders who might find in it an accessible, tasty and easy to consume product as well as to workers who want to consume a kiwi while avoiding the trouble and dirt of peeling it in their offices. Companies might want to use imperfect fruits showing morphology defects related to size to calibre relation.

A Brief Comment on Processed Foods and the Introducing the Minimally Processed.

Food processing is defined as the practices used by food and beverage industries to transform raw plant and animal materials, such as grains, produce, meat and dairy, into consumers product(Johns Hopkins, n.d.).

Consumers demand for foods, which retain their natural flavor, color and texture and contain fewer additives such as preservatives. In response to these needs, one of the most important factors in the food industry has been the development of minimal processing technologies designed to limit the impact of processing on nutritional and sensory quality and to preserve food without the use of synthetic additives. In addition, food manufactures can improve their products in terms of long shelf lives, added dietary nutrients, appealing textures and other features. It possible to create a variety of original food by modifying the flavor, textures, aromas, colors and form of foods and raw ingredients. As a result, food processing can create greater variety in food supply. While food processing can offer many benefits to businesses

and consumers, certain aspects of the industry raise concerns over nutrition, local economies and the environmental issues (Monteiro & Levy, 2010).

The minimally processed vegetables have been named in a several ways: in France and Italy have been denominated "4° Gama" of alimentation, in the Anglo-Saxons countries, "ready to eat" or "ready to use", "minimally processed", "slightly processed" or "partially processed". The most common term in Europe and EE.UU. is "fresh cut". In Spain, there is no specific denomination, although commercially it is usually called "4°Gama" (Artés, 2008).

Minimally Processed Fruit

Minimal processing has been defined as the handling, preparation, packaging and distribution of agricultural commodities in a fresh-like state. Minimal forms of processing of raw fruits and vegetable include washing, peeling/trimming, removing inedible parts, cutting/slicing, chopping/shredding or juicing prior to being packaged for consumption (Alegria et al., 2010; Monteiro & Levy, 2010; O'Connor-Shaw, Roberts, Ford, & Nottingham, 1994). The minimally processed concept signifies that the original products receives a soft treatment that do not produce remarkable changes in their fresh-like properties and nutritional value during its shelf-life (Rico, Martín-Diana, Barat, & Barry-Ryan, 2007). Minimally processed vegetables stay basically prepared to immediate consumption, maintaining almost original product characteristic, and with barely comestible part, and their nutritional value use is optimum (Artés, 2008).

Minimally Processed Kiwifruit

Actinidia is usually eaten fresh, however, the kiwifruit derivatives available on the market are mainly represented by semi-processed products directed to the food industry as ingredients or components for ice cream, yoghurt, cakes, beverages and as flavoring (Gianotti, Sacchetti, Guerzoni, & Rosa, 2001; Shastri et al., 2012). Most of the studies of the kiwifruit processing have got the purpose of obtaining safe and stable products able to retain as much as possible the uniqueness of the fresh fruit, as well as its green color, aroma, nutritional value and structural characteristic (Gianotti et al., 2001).

Studies have been carried out to analyze the several reactions produced in kiwifruit metabolism, after being processed. The green fresh color has been the main problem to maintain the fresh fruit appearance. Other problems hindering the fruit preservation after processing include the microbial contamination, changes in the texture and the loss of nutritional value of the new product. Changes in flavor, texture and appearance occur during

senescence and may be induced or enhanced by minimal processing (O'Connor-Shaw et al., 1994). Minimal processing such as peeling and cutting breaks the cell wall and consequently causes the loss of intracellular material that leads to a dramatic cellular collapse which generates system stimulating ethylene production. Enzymatic activities, softening and ripening of kiwifruit are promoted by ethylene. Therefore, it is difficult to maintain the quality of kiwifruit once it has been processed (Rocculi, Romani, & Rosa, 2005).

Deleterious Processes on Minimal Processing

It is not simple to preserve the natural attributes of vegetables, since processing frequently causes mechanical injuries of tissues (O'Connor-Shaw et al., 1994). Kiwifruit shelf life's is reduced drastically when the fruit is manipulated. Wounding stimulates respiration rate, induces ethylene synthesis, oxidation of phenol, enzymatic activity, lead to an accelerated loss of quality, especially color, firmness and flavor attributes (Allende et al., 2006; Benítez, Achaerandio, Sepulcre, & Pujolà, 2013; Rocha & Morais, 2003). In addition , the formation of exudates, rich in minerals, sugars, vitamins and other nutrients, may support the growth of autochthons microbiota (Oliveira, Maciel de Souza, Morato Bergamini, & De Martinis, 2011). The increase of respiration rate and ethylene production lead to excessive tissue softening and high ethylene sensitivity, which results in short product's life. Studies on kiwifruit showed that one of the changes involved in tissue softening is the solubilization of pectic polymers from cell wall. The loss of pectic galacturonis residues is one of the first changes found in kiwifruit maturation, while solubilization of pectin occurs early in ripening and its depolymerization takes place relatively late in the ripening process (Benítez et al., 2013). Microbial spoilage of fruit takes place through the transfer of skin microflora to fruit flash. The low pH of most fruit is restricted to microbiota to acid tolerant microorganisms such as fungi and lactic acid bacteria (LAB). For assessment of sanitation and manufacturing practices for fruit it is recommended enumeration of yeasts, mold, LAB, Enterobacteriaceae and coliforms, which are part of the microbiota of fruit processing line (O'Connor-Shaw et al., 1994).

Importance of the chlorophyll

The green color of kiwifruit, which is a major attraction to consumer, is mainly due to chlorophyll, which is responsible for the characteristic green color typical of plants (fruit and vegetable). It exists in two structural forms in the terrestrial plants: chlorophyll *a* (dominant, with a green-blue color) and chlorophyll *b* (minor but more stable, with a yellow-green color) (Fuke et al., 1985; Suman, Maria, & Catellani, 2008). The changes in color of ripening and senescent fruits are visible results of chlorophyll degradation. This loss of chlorophyll during

postharvest and senescence of some fruits causes a shift in color from brilliant green to olive brown, a change which is associated with inferior quality (Almela, Fernández-López, & Roca, 2000).

Chlorophylls are highly susceptible to degradation during processing may be the result of cellular structure's disruption and the subsequent liberation of enzymes (which can be inactivated by heating or during extraction with organic solvents), such as lipoxygenase, peroxidase, and polyphenol oxidase (Suman et al., 2008). These degradation processes can be initiated by external factors such as temperature changes, increased levels of ethylene, light, oxygen, water stress, or some other factor or combination thereof, and are mediated by acids and/or enzymes (Almela et al., 2000). Major chemical degradation routes are associated with pheophytinization, epimerization, and pyrolysis, and also with hydroxylation, oxidation or photo-oxidation, if light is implicated (Koca, Karadeniz, & Burdurlu, 2007)

Structure

Chlorophyll is liposoluble pigment anchored in the membrane of thylakoid in the chloroplast of all photosynthesizing plants. The chlorophyll molecule is formed by four pyrrole rings, interconnected by a pair of bonds; this structure is denominated porphyrin. In the center of the rings, interacting with the nitrogen atoms, there is one magnesium atom. In one of the ring there is a linear chain linked covalently by an oxygen atom, formed just by atoms of carbon and hydrogen (polar region). These atoms root the molecule in the membrane of thylakoid letting the rings exposed directly to irradiance. The chlorophylls constitute the group of pigments most widely distributed in nature, in leaves and others green parts of almost all plants. They are essential for photosynthesis, process whereby the energy of light is used for the plants in synthesis of carbohydrates. It occurs in the chloroplasts and is probably associated to proteins and lipids. In the chloroplasts, together with the chlorophylls are found other groups of pigments, the carotenoids. Most of the chlorophyll, which is found in plants include chlorophyll *a* (with a radical methyl – CH_3 group at the C-3 carbon) and chlorophyll *b* (a aldehyde – COH group is bonded to the position). In addition to structural differences between chlorophyll *a* and *b*, their terminal stabilities are different. Chlorophyll *a* was reported to be thermally less stable than chlorophyll *b* (Koca et al., 2007). This pigment is found in the approximate ratio of 3:1 and are obtained easily by extraction with organic solvents. The most indicated between them is the acetone (Koca et al., 2007),

Breakdown Pathway (Catabolites)

The study of the several key chlorophyll catabolites in the last 20 years allowed elucidation of the chlorophyll degradation pathway, which is common to higher plants. The chlorophyll degradation pathway can be divided in two ways: in the first way reactions are occurred with colored pigments ending in the synthesis of "primary" colorless, bluefluorescing breakdown products, termed 'primary' fluorescent Chl catabolite (pFCC); in the second way the pFCC reactions, ending with the isomerization of modified fluorescent Chl catabolites (FCCs) to the respective (nonfluorescent Chl catabolite) NCCs inside the vacuole. (Hörtensteiner & Kräutler, 2011).

Commonly the form of chlorophyll degradation which is described in the literature is accepted for both leaves and fruits as the chlorophyll demetallation to pheophytin, in a process called pheophytinization. The same phenomenon occurs in the dephytylation of chlorophyll pigment to pheophorbide. In fruits, evidence for the dephytylation of chlorophyll *a* by chlrophyllase is first and gives chlrophyllide *a*, followed by demetallation by a magnesium dechelatase to give pheophorbide *a* (Hörtensteiner & Kräutler, 2011)

Chlorophyll is degraded after conversion from chlorophyll *b* to chlorophyll *a*, which involves the enzyme chlorophyll *b* reductase (CBR). Another chlorophyll degradation step is catalyzed by pheophorbide *a* oxygenase (PAO), where the tetrapyrrole ring of pheophorbide *a* is opened by adding two oxygen atoms to yield chlorophyll catabolites (RCC). This step is responsible for the loss of green color associated with chlorophyll and formation of colorless catabolites. In addition, pheophytin pheophorbide hydrolase (PPH) was identified as another enzyme of the chlorophyll degradation pathway. It is responsible for dephytylation of the chlorophyll molecule (Pilkington, Montefiori, Jameson, & Allan, 2012)

A classical scheme for chlorophyll degradation shows that the acidification promotes the loss of the Mg^{+2} of the center of the porphyrin and the alcalinisation leads to the loss of the phytol. From this reaction, which may happen in the same time or not, emerge the pheophytin and chlorophyllides. The loss of phytol from pheophytin and Mg^{+2} from chlorophyllides emerge pheophorbide (Hörtensteiner & Kräutler, 2011; Hortensteiner & Matile, 2004)(Hortensteiner & Matile, 2004)

The conversion of chlorophyll to pheophytin and pheophorbide results in a change from bright green to dull olive-green or olive-yellow, which is ultimately perceived by the consumer as a loss of quality (Koca et al., 2007)

The chlorophylls can be changed chemically by several factors. The phytol can be removed easily by alkalis with formation of the cholophyllide, compound of green color that has got practically the same absorbance of the chlorophylls, although they are more soluble in water. In food processing the most important reaction is undoubtedly the facility by which the magnesium is replaced for protons, by the action of diluted acids, with formation of the pheophytins, compound of olive green color, color that harmed the appearance of foods rich in chlorophylls. *In situ*, even if the plant tissue is acid, the chlorophylls are quite stable, perhaps due to the fact of their association with proteins and lipids, which would have protectress action.

An additional important phenomenon that happens to the chlorophyll is the facility even so the ion of magnesium in these pigments is replaced by divalent metals. The complex is formed with copper has green brilliant color, which makes the foods become more attractive. This complex has the metal firmly bound; only released by the action of concentrated acids that makes it safe to use in food.

Since the green color is one of the major sensory characteristics in determining the final quality of thermally processed green vegetables, it is important to prevent or at least minimize chlorophyll degradation during thermal processing in the food industry (Koca et al., 2007)

Minimal Processing and rates of contamination

Preservation: Minimally Processed by High Hydrostatic Pressure

Selling minimally processed fresh fruit requires a combination of appropriate strategies that extend shelf life while maintaining fruit sensory and organoleptic properties. To extend the shelf life of minimally processed fruit it is necessary overcome the damages caused by the processing. Storage temperature is the single most important factor affecting spoilage of minimally processed fruit and vegetables (Rocha & Morais, 2003). Technologies with potential to increase the quality and safety such as modified atmosphere, ionizing radiation and the use of biopreservatives culture and/or their metabolites (Oliveira et al., 2011). Many other preservation techniques that are currently being used by the fresh-cut industry are antioxidants and chlorines methods (Allende et al., 2006). However, preservation including chemical dipping, edible coating have been used to prolong shelf life and retain the nutritional value of minimally processed kiwifruit (Benítez et al., 2013). Nonetheless, new techniques for maintaining quality and inhibiting undesired microbial growth are necessary in all the steps of

the production and distribution chain, as microorganisms adapt to survive in the presence of previously effective control methods (Allende et al., 2006).

Heat treatment can also be used to preserve minimally processed foods. The partial inhibition of quality-related enzymes such as polyphenoloxidase , polygalacturonase and particularly peroxidase, is one of these treatments. Reduction of respiratory activity is another favorable effects is induced by heat treatments with significant impact over the fresh-like quality and, consequently, extension of its shelf-life (Palou et al., 2000).

High Pressure Processing

High pressure processing is an alternative technology for food safety and preservation. It's capable of inactivating microorganisms and enzymes and effectively pasteurize foods with minimal heating due to the fact that it does not break covalent bonds and that it does not promotes drastic changes in the nutrient content and organoleptic qualities thus it provides an extended shelf life food (Balasubramaniam & Farkas, 2008; R. Earnshaw, 2009; Mor-Mur & Yuste, 2005). High pressure processing is commonly described as high hydrostatic pressure (HHP) processing or ultra-high pressure (UHP) processing (Balasubramaniam & Farkas, 2008; R. Earnshaw, 2009). Heat processing has used to ensure prolonged shelf life and food safety. However, the application of such temperatures is commonly known because promotes damages on processed products. These undesirable changes affect nutritional value as well as organoleptic attributes. Vitamins, color and flavor compounds would harmed. Texture is also negatively affected, frequently vegetable tissues soften and chemical compounds are needed to be add to regain firmness. All these changes result in products that are far from similar to original fresh products (San Martín, Barbosa-Cánovas, & Swanson, 2002). HHP is a nonthermal process, as it only involves minor increases in temperature during pressurization. For a working pressure of 600 MPa, the temperature increment of pure water is only approximately 15 °C (Gutiérrez-López & Barbosa-Cánovas, 2003). High pressure processing (HHP) uses elevates pressures between 100 and 1000 MPa several seconds to minutes (R. Earnshaw, 2009), with or without the additional heat, in order to achieve microbial inactivation or alter the food attributes (Balasubramaniam & Farkas, 2008; Mor-Mur & Yuste, 2005). High hydrostatic pressure has been study for several reasons. This technology has been cited as being of the best innovation in food processing in the last ten years. Using this technology food processors could process foods with cleaner ingredients and fewer additives (Balasubramaniam & Farkas, 2008).

State-of-Art of the Technology

The first reported us of HHP as a method of food preservation was in 1899 in the USA, where experiments were conducted using high pressures to preserve milk, fruit juice, meat and a variety of fruits (R. Earnshaw, 2009). It was demonstrated that pressures of 658 MPa for 10 minutes could destroy microorganisms in these products. In the early years of the twentieth century, other research showed that high pressures could alter the protein structure in egg white. However, these early researchers were constrained by both difficulties in manufacturing high pressure equipment and inadequate packaging materials to contain the foods during processing, and thus research was discontinued. Advances in the design of presses together with rapid advances in packaging materials enable research on HHP to begin again in the 1980s, mainly in Japan (R. Earnshaw, 2009).

The process reached the stage of commercial exploitation in 1990 with a range of high quality pressure processed jams being sold in Japan, including apple, kiwi, strawberry and raspberry. The jams had a shelf life of two months under chilled storage, which is required to prevent enzyme activity. Other companies started production of bulk orange and grapefruit juices and other high acid products such as fruit jellies, sauces fruit yoghurts, purees and salad dressing. These products are suitable for HHP because, owning to their low pH, they are spoiled by microorganisms that are relatively sensitive to HHP, and not by pressure resistant bacterial spores. Similar products later reached the US market followed by pressure treated guacamole, oysters, hummus, chicken strips and fruit "smoothies". HHP orange juice and sliced cooked ham are sold in France and Spain respectively and HHP orange juice in the UK. A survey of consumers in three European countries has found that high pressure processing was acceptable to the majority of people that were interviewed (Butz et al., 2003). There is increasing interest in using HHP to preserve low acid foods, including red meats, poultry, seafoods, foie gras, liquid whole egg and cheese.(R. Earnshaw, 2009; Raso & Barbosa-Cánovas, 2003; Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007).

HHP is effective on a wide variety of foods, such as fruits, juices, vegetables, seafood, sauces, and ready-to-eat meats. During the last decade, the technology has been used by the food industry as an intervention technology for killing *Escherichia coli*, *Salmonella* spp, *Listeria* spp, and *Vibrio* spp pathogens in food products without additional heat processing (Balasubramaniam & Farkas, 2008).

HHP can be used to process both liquid and solid foods in batch equipment, and liquid foods in semi-continuous equipment. Commercial scale high-pressure processing systems cost somewhere between $500,000 and $2.5 million dollars, depending on the equipment capacity and extent of automation. As a new processing technology, with a limited market, pressure-processed products may cost additional 3–10 cents per pound to produce than thermally processed products. With two or more pressure vessels operating under typical food processing conditions, a throughput of approximately 20 million pounds per year is achievable (Balasubramaniam & Farkas, 2008).

Over the last decade, significant progress has been made in high-pressure pasteurization of foods, and a number of commercial products have been introduced into the market. High-pressure pasteurization treatments inactivate pathogenic and spoilage bacteria, yeasts, and molds, but have limited effectiveness against spores and enzymes. Examples of high-pressure pasteurized products available commercially in the United States include: smoothies, guacamole, ready-meal components, oysters, ham, fruit juices, and (Balasubramaniam & Farkas, 2008; Pandrangi & Balasubramaniam, 2005).

The advantages of HHP processing have caused this process be successfully adopted by the food industry, and some products thus produced may be found in the market now (San Martín et al., 2002).

High Hydrostatic Pressure Engineering Principles

As its name suggests, the food (liquid or solid) is subjected to pressures above 100 MPa up to 1000 MPa, with pressures used in commercial systems between 400 and 600 MPa (San Martín et al., 2002). HHP processing begins by packaging untreated products in flexible containers followed by loading them into a high-pressure chamber filled with fluid (usually water) which acts as the pressure transmitting medium. The vessel is sealed and pressure applied isostatically, the process is equally applied in all directions, by forcing additional water into the sealed vessel until the desired processing pressure is reached. The pressure is held for a specific time and then released, and then the processed products are removed and stored. In generally, enzyme inactivation requires using higher pressures than those used for microbial inactivation (Balasubramaniam & Farkas, 2008; Gutiérrez-López & Barbosa-Cánovas, 2003; San Martín et al., 2002). HHP machine may have devices for temperature control (San Martín et al., 2002).

The successful application of HHP technology in the food industry results from the effects that high-pressure treatments cause on microorganisms (spoilage and pathogens) and on some enzymes, which are the most important factors contributing to food safety and stability (San Martín et al., 2002).

Because minimally processed fresh fruit is highly susceptible to deterioration and to lose quality, HHP arise as a alternative in preserve this kind of food. High pressure is recorded by promote microorganism and enzymes inactivation with changes in the organoleptic and nutritional properties, thus it indicates that this technology has potential to be used to preserve kiwifruit.

Chapter 2. Introduction, Objectives and Work Plan

Introduction

The fruit consumption in the daily diet has an effect much beneficial to the health, not only an excellent vitamins, minerals and fiber source, in addition have photochemical components that contribute to the health. The fruit appearance (as in kiwi fruit) is the first impression that the consumer receives and the most important for the acceptance and eventually purchase. Shape is one of the most easily discernible aspects, although generally it is not a decisive character quality, unless the case of deformations or morphological defects.

During and after fruit harvesting a percentage of collected fruit is not destined to the marketing because they do not follow the standard required for the commercialization. The quality concept of official standards are primarily based on external characteristics of the fruit, such as color, size, and free form defects in cortex.

The crop that enters a commercial facility on average of 70 - 80% is packaging into different categories according to size and quality. The rest is considered out of standard. Fruits out of standard can be suitable for the processing industry of juices and canned, because it is fruit of very small sizes or too large.

The destination depends on the classification is made, based on the commercial quality of the fruit, determined by a number of parameters, normally required in all stations, such as size, state of maturity, sugars, pH, color etc. If the fruits are used to industrial use, normal cold, storage in controlled atmosphere, conservation, pre-ripening immediate sale etc., the harvest of these fruits must be made, generating expenses that cannot be covered at the time of sale.

Nowadays, it is perceived that the consumer wants products with increasingly higher degree of processing, leading to different ranges or classifications according to the degree of processing. One way that you can eat is minimally processed; preserved under cold chain. Minimally processed can be obtained through various unit operations of preparation, such selection, peeled, cut, size reduction, washing and packaging, including chemical treatments. Minimally processed can contemplate the possibly to take advantage from fruits that could not be commercialized.

The purpose of minimally processed fruit is to provide the consumer a product a lot like to the fresh, with a long shelf life while ensuring the safety of themselves, keeping nutritional and sensory quality. On the other hand, the conservation of minimally processed products is critical due to physical damage occurring in plant tissues in the process. These damages accelerate the metabolism causing deterioration of desirable sensory characteristics, nutrient loss and growth of microorganisms, leading to a rapid decline in the quality and shortened shelf life.

Find methods to help stop the deterioration of these products is one of the main objectives of the industry sector. In this way, conservation techniques that can extend the life of the product minimizing the modification of sensorial and nutritional characteristics. The technologies traditionally used in the conservation of this type of products are cooling, as a prerequisite both stages of production and distribution, storage and merchandising, and modified atmosphere packaging. Other technologies to extend shelf life are the use of disinfectant solutions, antioxidants, ultraviolet light treatments, addition of color stabilizing agents and texture, application or use of antimicrobial edible coating.

Kiwifruits that do not meet export standards and are not sold in local markets are processed into various products, primarily canned slices in syrup, frozen pulp and slices, juices and wines (Leunda, Guerrero, & Alzamora, 2000) a good alternative to these traditional methods seems to be the application of minimal preservation processes based (Leunda et al., 2000).

An emerging technology that has explored for food preservation and processing is high pressure. High pressures have demonstrated to be efficient in microbial and enzyme inactivation. High pressures lead advantages of being used at low or room temperatures and can avoid the traditional use of heat treatments. High pressures could also preserve the nutritional value and sensory properties of fruits to have limited effect on the covalent bonds of low molecular weight compounds, such as color and flavor compounds.

It is necessary studies relating to how high pressure affect the color, flavor and texture of this fruit based food and elucidation of possible mechanisms behind the changes. In addition attention to the impacts of high pressure on vitamin c and microorganism in the food preservation. High pressure has been studied in haul of foods, but few data have been gotten on puree fruit based. The knowledge how high pressure effects kiwi puree could make easier the disseminating both technology as the product produced with this technology.

Objectives

General Objectives

The aim of the present work was to investigate the potential of high hydrostatic pressure as a processing technology to keep the kiwi fruit quality and extend its shelf life without addition of preservatives.

The study was performed on kiwi fruit puree in order to have a more uniform matrix and sustain more robust conclusions.

To asses if the objective was achieved, some safety and quality characteristics were assessed, and their study are the specific objectives.

Specifics Objectives

- To determine the optimal conditions (pressure, time and temperature) of HHP treatment, enable to reduce the pathogen and endogenous microbiota, equal or greater than a pasteurization process.
- To identify changes in physical-chemical properties (such as, pH, ºBrix, etc.) and their relationship with the damage produced by the HHP processing and development of microbial survival.
- To evaluate changes in color and degradation or formation of chlorophylls and pheophytins (derivatives), partly responsible for color change after processing and during the shelf-life.
- To evaluate the effect of HHP on the rheological properties (viscosity) of kiwifruit puree after processing and during the shelf-life.
- To evaluate the content of ascorbic acid, dehydroascorbic acid, and total vitamin C after processing and during the storage study
- To evaluate the consumer preference among kiwifruit puree fresh, treated by HHP and a commercial treatment (pasteurization).

Working Plan

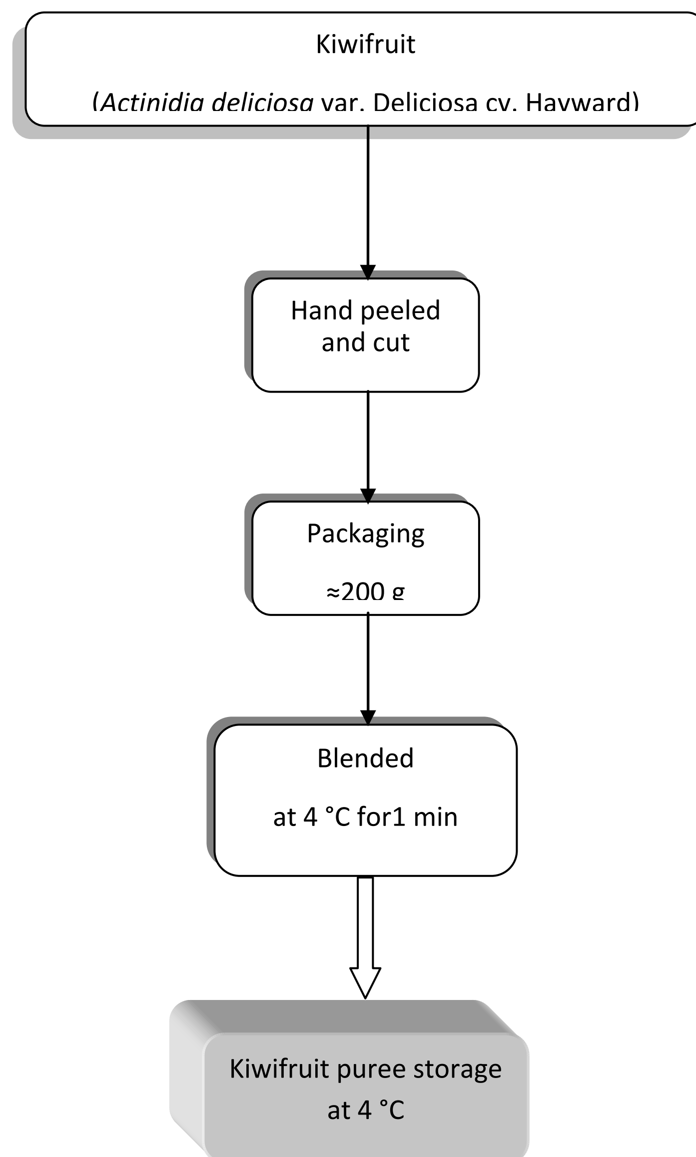

Figure 2-1. Work plan corresponding to the Kiwifruit puree production

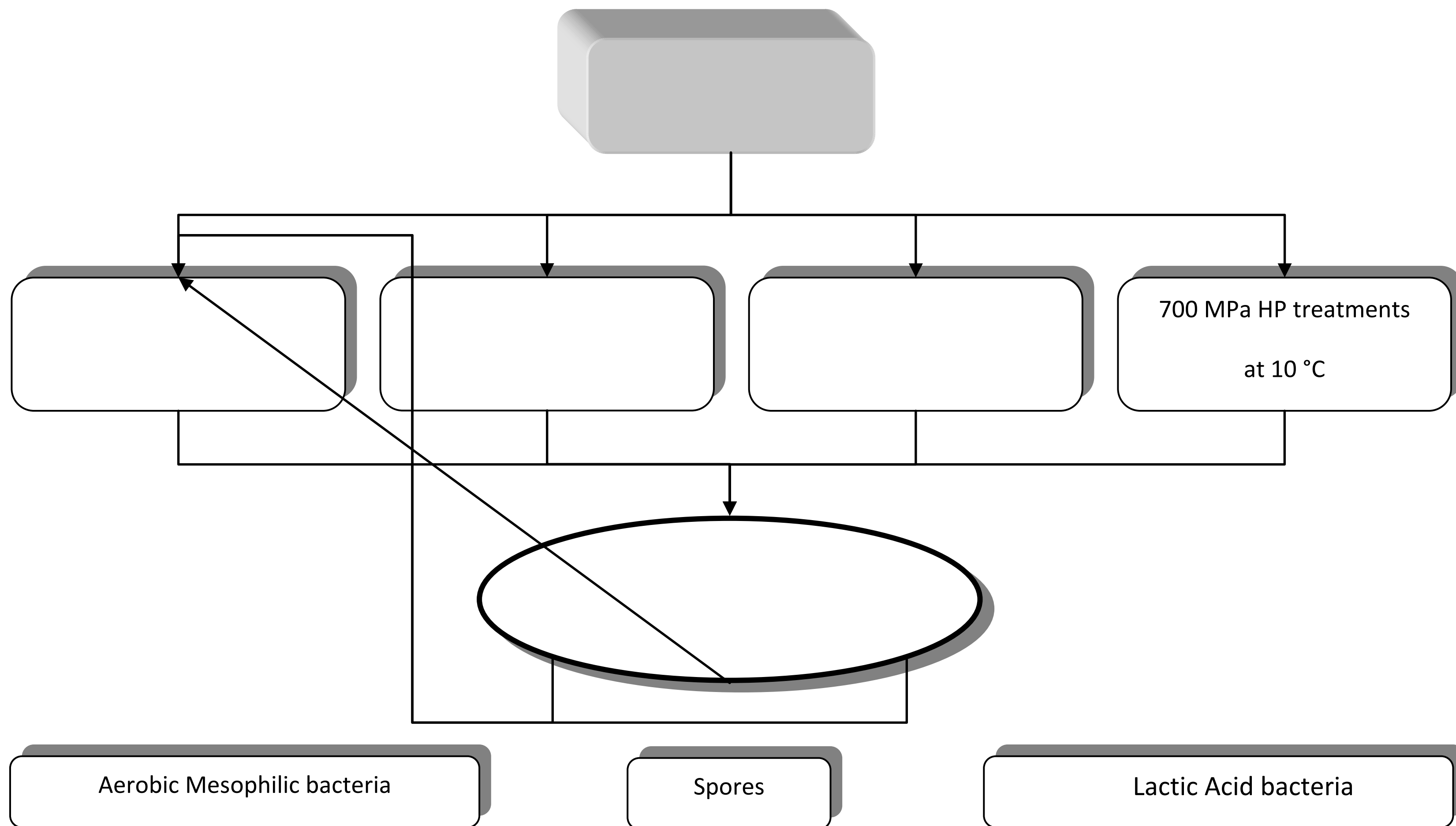

Figure 2-2. Workplan for the evaluation of he effect of HHP treatments on kiwifruit puree microbiota

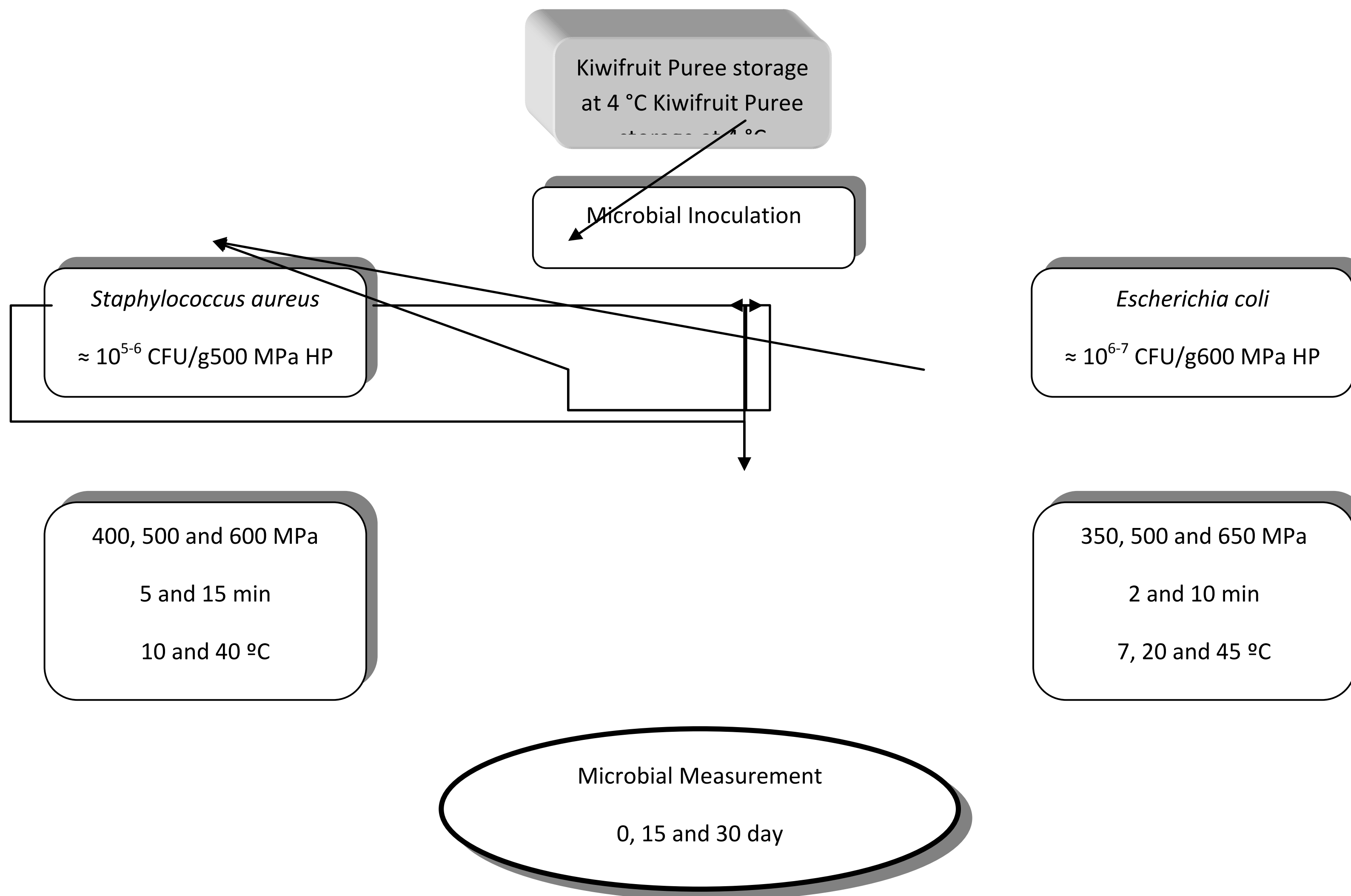

Figure 2-3. Workplan for the innoculation experiment

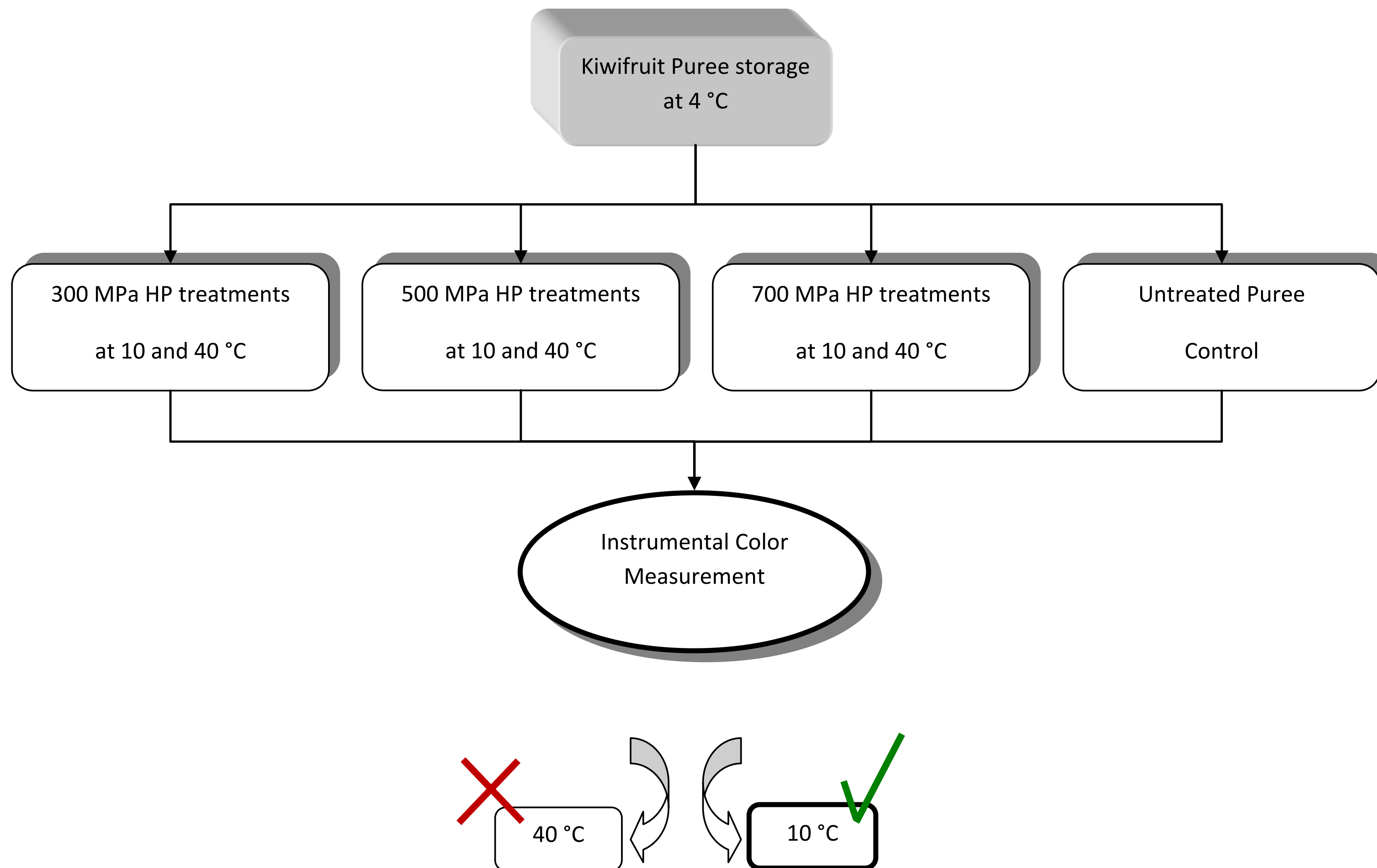

Figure 2-4. Work plan corresponding to the preliminary analysis of kiwifruit

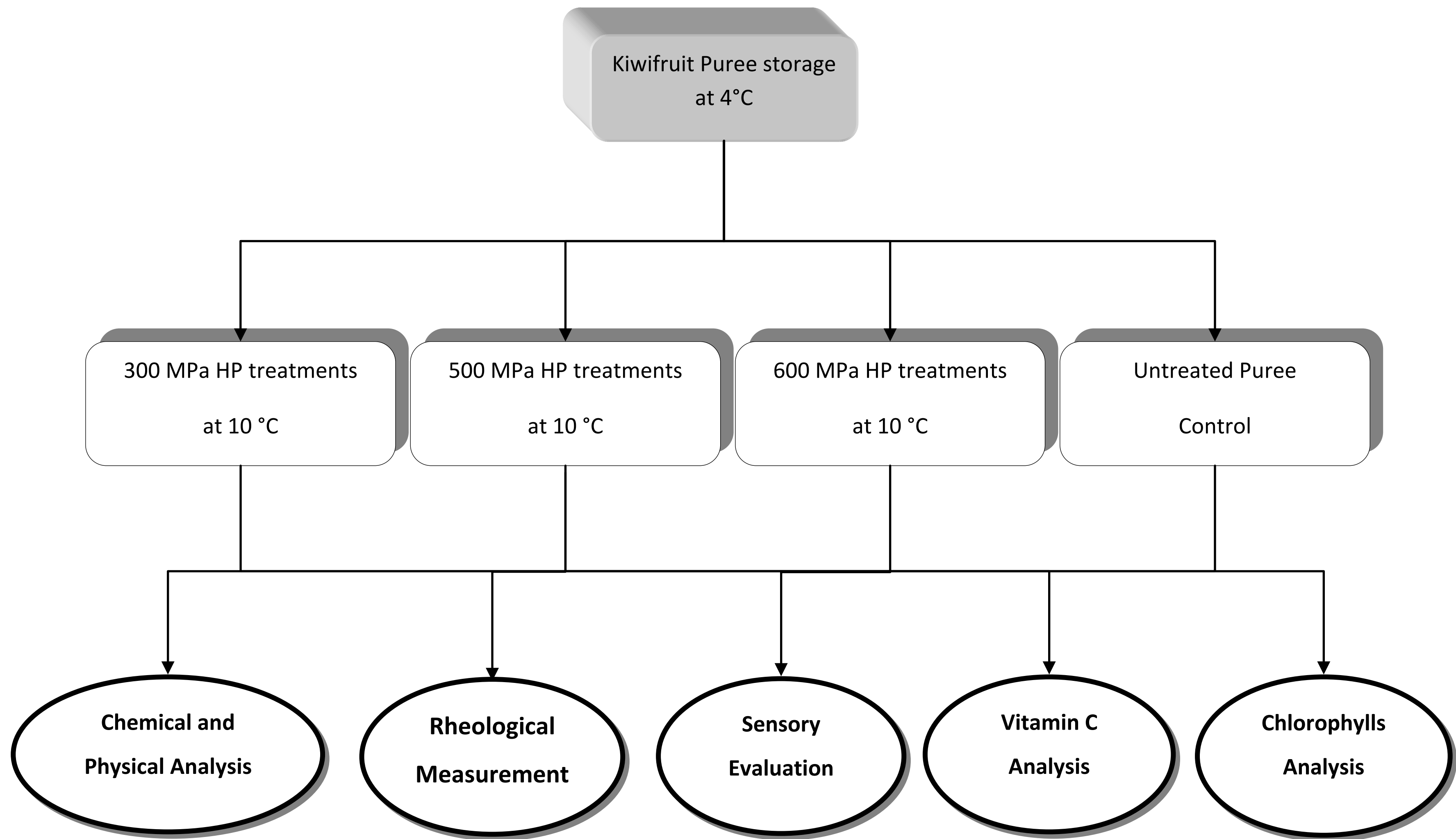

Figure 2-5. Work plan corresponding to the studies of kiwifruit

Chapter 3. Materials and Methods

Raw Material

Kiwifruit (***Actinidia deliciosa*** var. Deliciosa cv. Hayward) was purchased from a local retailer. The fruits were screened for damage and ripeness, and were stored at 4 °C until processing. This kind of kiwi was chosen because in the Spanish plantations the varieties used is denominated "***Actinidia deliciosa***" that proceed from the cultivatr Hayward and its availability at a regular quality throughout the whole year.

Kiwifruit Puree Elaboration

Hand peeled and cut kiwifruit were blended for 1 min at 4 °C 12 rpm using a homogenizer (Universal Machine Stephan® UMC 5, Stephan Food Service Equipment GmbH, Hameln, Germany) and packaging in a bag made of high-strength plastic material, approximately around 200 g. The puree was conserved at 4 °C during the packaging processing. The kiwifruit puree bags were overwrapped in an outer polyethylene bag and exposed to high hydrostatic pressure treatments.

Kiwifruit Puree Treatment: High Hydrostatic Pressure (HHP) and Storage

HHP processing was performed on high pressure system (Mark/Model ISOLAB-FPG11500, Stansted Fluid Power Ltd., Essex, UK) with vessel of 110 mm internal diameter x 550 mm internal height and maximum work pressure of 800 MPa. Samples were placed in the high pressure vessel and subjected to a pressure of 300, 500 and 600 MPa for 0.1, 5 and 15 min. with at 10 and 40 °C, a mixture of water and propylene glycol was used as drive fluid.

After treatments, kiwi puree products were stored at 4 °C. The purees were analyzed at 0, 15, 30 and 60 days after storage.

Thermal treatment was carried out in 19.5 x 24 cm plastic bag with 150 g of sample. Puree was exposed to temperature of 80 °C in water bath (± 1 °C) in bath water by 3 min. Immediately after sample was heated to the experimental temperature for the prescribed time, it was cooled in a cool-water in order to stop the heat accumulation.

Microbial Analysis and Sample Inoculation

Microbial analysis was carried out the day after experiment to avoid the stress effect post-treatment in the microorganisms. Decimal dilution in peptone water solution was used for

microbial enumeration in different microbiological culture media. Aerobic mesophilic bacteria (AM) counts were enumerated in plate count agar (PCA, Oxoid Ltd, UK), incubated at 30 °C for 48 h. For to count total spores, kiwi puree was heated at 85 ˚C for 5 min, cooled in ice and plated on PCA, incubated at 30 °C for 48 h . Enterobacteriaceae (EB) counts were enumerated on violet red bile glucose agar (VRBGA, Oxoid), incubated at 37 °C for 24 h. Lactic acid bacteria (LAB) were enumerated on non acidified Man-Rogosa-Sharpe agar (MRS, Oxoid), incubated at 30 °C for 48 h. Molds and yeasts (MY) were detected on Rose Bengal agar chloramphenicol medium (Oxoid), incubated at 25 °C for 5 days. Faecal coliforms (FC) were enumerated on Coli ID selective chromogenic medium agar (Coli ID, bioMérieux S.A., Madrid, Spain), incubated at 37 °C for 24 h.

KiwiFruit Puree Inoculation with *Escherichia coli* and *Staphylococcus aureus*

In order to show the effectiveness of high pressure treatment an inoculation experiment was carried out with pathogenic bacteria indicator: ***Escherichia coli*** (***E. coli***) and ***Staphylococcus aureus*** (***S. aureus***). The bacterial strains were obtained from Spanish Type Culture Collection (CECT, Valencia, Spain): ***Escherichia coli*** (CECT 5947) and ***Staphylococcus aureus*** (CECT 534)

Inoculum Preparation

Bacterial colonies were obtained from 24 h cultures on Triptone Soy Broth (TSB, Oxoid) at 37 °C. One milliliter of the first suspensions was transferred to a new sterile tube with TSB, this suspension was incubated at 37 °C for 24 h and it was considered as the initial inoculum. After dilutions in TSB, a concentration of 10^8 CFU/mL were confirmed by viable cell counts on Plate Count Agar (PCA, Oxoid Ltda, UK) and Coli ID Selective Chromogenic Medium Agar (Coli ID, bioMérieux S.A., Madrid, Spain) for ***S. aureus*** and ***E. coli***, respectively. The cultivars were storage at 4˚C around one day until they were inoculated in kiwifruit samples.

In a Pyrex® 1000 mL bottle, approximately 500 g of kiwi puree was sterilized in a rotating autoclave at 121 °C for 15 min. Ten mL of inoculum was inserted in the bottle. The sample was thoroughly shaken and divided in appropriate sterile bags, immediately samples were treated by high pressure.

Chemical and Physical Analysis

The pH of kiwi puree was measured with a pH meter (model GLP 21 + Crison, Spain). Dry matter was analyzed by reference AOAC method (AOAC, 2000). Soluble solids (SS) were measured with a Spectronic Instrument refractometer (Rochester, N.Y., USA) at 20 °C, results are reported as °Brix.

Instrumental Color Measurement of Kiwi Fruit Puree

The color change of kiwifruit puree was calculated from 15 measurements with MiniscanTM XE (Hunter Associates Laboratory, Reston, VA, EE.UU.), the equipment was set up for illuminate D_{65} (10° observer angle) and the calibrated using a standard white and black references tiles. Readings were obtained applying the standard CIE $L^{*}a^{*}b^{*}$ color system, where L^{*} is the lightness, a^{*} indicates hue on a green (-) to red (+) axis, and $b*$ indicates hue on a blue (-) to yellow (+) axis. For measuring, puree was poured into a cylindrical sample cup and measured at day 0, 15, 30 and 60. The color degradation can be also expressed as a single numerical value ΔE. This value defines the magnitude of the color difference. The ΔE is expressed by $\Delta E^{*}_{ab} = [(\Delta L^{*2}) + (\Delta a^{*2}) + (\Delta b^{*2})]^{0.5}$, color differences were calculated by subtracting mean control reading of the raw puree ($L_0^{*}a_0^{*}b_0^{*}$) from individual $L^{*}a^{*}b^{*}$ reading after treatment.

Rheological Measurements

Flow curves were obtained using a Rheological measurements were performed on HAAKE RheoStress 1 Rheometer (Thermo Fisher Scientific, Inc., Karlsruhe, Germany) driven by an electrically commutated motor, supported by axial and radial air bearings, the dates were collected and analyzed through RheoWin Pro Job Manager (software associated to rheometer). The instrument was fitted with cone and plate geometry (60 mm; 1°, titan). Samples were placed onto the base plate and the gap was set to 4 mm. Samples were allowed to rest for 240 s to achieve a constant test temperature of 20 °C and for relaxation of residual stresses. Frequency sweep was performed with constant strain of 0.1% at frequency between 0.5 Hz – 15.0 Hz to determine the linear viscoelastic region. The dates were reported as G’ (storage modulus) and G’’ (loss modulus). Thereafter, flow curves were obtained applying increasing shear rate from 1.0 s^{-1} to 20.0 s^{-1} in linear ramp over a period of 60 s. Analyses were repeated nine times providing the means of nine determinations for each data point. The experimental values of shear stress and shear rate were fitted to the Herschel-Bulkley model $\tau = \tau_0 + \mathrm{K}(\gamma)^{\mathrm{n}}$, where:

τ = Shear Stress (Pa)

τo =Initial shear stress (Pa)

γ = Shear rate (s-1)

K = Consistency index (Pa.s)

n = Behavior index (dimensionless).

Sensory Evaluation

To compare sensory characteristics of kiwi puree processed by different HHP conditions, “napping” test (Morand & Pagès, 2006) was used. Napping® denomination provide from French term “nappe” that mean tablecloth. This technical seeks establish sensorial distance by similarities perceived. The panelist makes a positioning of the sample in a rectangular white tablecloth approximately (40 x 60 cm), in accordance with similarities that the panelists perceive between the samples and from the coordinates of each sample place is constructed the sensorial space.

Protocol: the kiwi puree samples were given to panelist, approximately 20 mg in a plastic transparent cup. The coach instructed the panelist to locate each sample simultaneously on the tablecloth, in a way that the samples perceived as similar were together (close) and the different were far away. Then after had located all samples, the panelist wrote on the tablecloth the identification of the sample.

Later, it used a grid to determine with exactness the coordinates of each ticked point on the tablecloth, after that there were two dimensions for each point. This coordinates allowed calculate the sensorial distance. Thus, each panelist provided a special visualization of the product. Analyses of all tablecloths provided an average visualization. In this experiment, it was applied Napping® plus other descriptive method, the Ultra-fresh profiling (UFP) in order to complement the analysis. In the UFP, after the panelist had situated the samples on the tablecloth it asked for to write on point ticked descriptive words to describe the samples characteristics. The dates were recorded and processed by using SensorMiner (Le & Husson, 2008) routine in “R” statistical software, applying the Procrustes Multiple Factor Analysis test (Husson, Le, Cadoret, & Husson, 2015). All results came from triplicate analysis.

Data became from a total 8 different kiwifruit samples, 6 pressurized, one pasteurized and one sample with no treatment (control). The sensorial test had participated of 20 panelists.

It was made a descriptive test also; the panelist should evaluate the intensity of sample sensation of sweetness, green color, smell and texture in a scale no structured. The data were analyzed by analysis of variance (ANOVA) GLM procedure was conducted using Statistica 7.0 (Statsof, Inc., OK, USA) and significance limit was set at $p<0.05$. Newman–Keuls test was performed for comparison to data.

Vitamin C Analysis

The concentration of ascorbic acid and total vitamin C (ascorbic plus dehydroascorbic acid) were measured, following the method used by Suárez-Jacobo et al. (2011) with a little

modification, briefly, to determine the ascorbic acid content, two grams of kiwi puree was homogenized with 20 mL of extraction solution, 3% metaphosphoric acid 8% plus acetic acid. The mixture was centrifuged 13000 rpm for 10 min and filtered through a 0.45 μm membrane filter and 20 μL of filtrate was directly injected into a high performance liquid chromatographic (HPLC) system. The procedure for determining total vitamin C was the same as that used to determine ascorbic acid, with the difference that DL-dithiothreitol (reducing reagent) was added to the kiwi puree and the mixture was left to react for 2 h at room temperature and in darkness in order to reduce dehydroascorbic acid to ascorbic acid just after the samples was filtered and 20 μL injected. Ascorbic acid was resolved by reserved-phase HPLC of H_2SO_4 (0.01% v/v) in an isocratic gradient. Dionex P680 HPLC pump; a TCC-100 Thermostatted column compartment and a Dionex UVD1700U detector at a wavelength of 245 nm were used of analysis. Separation of ascorbic acid was performed by using a reversed phase C18 Hypersil ODS (5 μm) stainless steel column (150 x 4.0 id mm) (Teknokroma, Barcelona, Spain). The flow rate was fixed at 1.0 mL min^{-1}. Calibration curves were made with a minimum of 7 concentrations of ascorbic acid standard to confirm linearity range. Chromatographic data were collected and recorded by Chromaleon software (Dionex Corporation, Sunnyvale, USA). The dates was analyzed by analysis of variance (ANOVA) GLM procedure was conducted using Statistical 7.0 (Statsof, Inc., OK, USA) and significance limit was set at $p<0.05$. Newman–Keuls test was performed for comparison to data**.**

Identification and Quantification of Chlorophylls and Catabolites

Chlorophylls and derivatives were determined by high-performance liquid chromatography (HPLC).For the extraction, a total of 2 g of kiwi puree were homogenized with 5 mL of 0.01% BHT acetone, 50 μL of EDTA 100 mM and 3 mg of Na_2CO_3 in a homogenizer (Heidolph DIAX-900; Heidolph Instruments GmbH & Co.KG, Schwabach, Germany), after that the puree was centrifuged 10000 g at 5 ºC for 5 min. The supernatant was recovered and reserved; the pellet was through a new extraction. The extraction was repeated until the pulp had lost the green color. In the end of the extraction the spindle used to extract was cleaned with 10 mL of NaCl saturated solution and more 5 mL 0.01% BHT acetone, all volume supernatant plus NaCl were centrifuged one more time. The total supernatant was evaporated to reduce the volume then it was made a second extraction with 0.001% BHT hexane. Sample was centrifuge in the same way described before. The supernatant was recovered, evaporated and stored at -32 ºC until analyze. Sample was reconstituted to 1 mL with 0.01% BHT acetone and analyzed. Results were expressed in mg of compound in Kg of kiwi puree.

Analysis was carried out with a Dionex UVD170U detector, a Dionex P680 HPLC pump, a TCC – 100 Thermostatted column compartment. A C-30 column (250 x 4.6 cm, s - 5 μm, YMC - Pack), a guard column was employed for separations. Data was analyzed by Chromeleon software (version 6.7) (Dionex Corporation, Sunnyvale, USA).

Mobile phases were %A – Tert-buthyl-ether; %B – Methanol and %C – Water. The multi-step gradient was 0 – 15 min: 90 %B, 4 %C, 15 – 30 min: 66 %B, 4 %C, 30 – 45 min: 4 %B, 0 %C, 45 – 54 min: 90 %B, 4 %C. Column temperature was 30 ºC with a flow rate of 1 mL min^{-1}. Samples were injected in 20 μL aliquots. Monitored wavelengths were 432 nm for chlorophyll a and pheophytin b; 466 nm for chlorophyll b and 408 nm for pheophytin and pheophorbid a.

To control de recovery compounds it was used the internal standard (IS) zinc phthalocyanine, it was added to sample before extraction and monitored at 340 nm.

Chapter 4. Microbiologial evaluation

Results of counts of mesophilic bacteria (MB), spores (SP) and lactic acid bacteria (LAB)

The effect of high pressure at diverse conditions are compared to raw kiwi puree in Figure 4-1 and Table 4-1. In the raw puree, mostly mesophilic bacteria were present, about 3.7 Log. The raw puree tendency was to be stable during storage time with a slightly decline 0.2-0.4 Log. Pressurization up to 300 MPa caused only a minor inactivation comparing to the others pressures, gets to survive until 1.7 Log. Treatments from 500 MPa presented did not differences between them, but in 700 MPa it is possible to observe a decay in the presence of mesophilic bacteria from day 0 until day 60, that survivors was less than 0.5 Log (0.1 min). No differences were found in holding time when applied pressures of 500, 600 and 700 MPa, except 300 MPa. The study of storage shows a trend to preservation the initial survivors' number. During storage treatment of 300 MPa/ 0.1 min decrease approximately 1 Log to the day 30 and the treatment of 700 MPa/ 0.1 min which in day 30 did not observed no survivors (no detected).

Table 4-1. Effect of HHP on microbial populations (Log cfu/g) of fresh kiwi puree after HHP processing at 10 °C

Treatments		Microbial Group											
MPa	min	Aerobic mesophilic				Spores				Lactic acid bacteria			
0	0	3.7*	±	0.4*	a**	0.4	±	0.2	a	1.4	±	0.7	a
300	0.1	1.7	±	0.5	bc	0.6	±	0.1	a	1.2	±	0.5	bc
300	5	0.7	±	0.3	c	0.3	±	0.2	a	0.3	±	0.3	c
300	15	0.6	±	0.1	c	0.4	±	0.2	a	0.3	±	0.0	c
500	0.1	0.4	±	0.5	c	0.4	±	0.3	a	0.3	±	0.0	c
500	5	0.6	±	0.6	c	0.7	±	0.4	a	0.0	±	0.0	c
500	15	0.2	±	0.1	c	0.2	±	0.2	a	0.0	±	0.0	c
600	0.1	0.8	±	0.3	c	0.7	±	0.2	a	0.0	±	0.0	c
600	5	0.5	±	0.2	c	0.1	±	0.1	a	0.0	±	0.0	c
600	15	0.6	±	0.3	c	0.3	±	0.2	a	0.0	±	0.0	c
700	0.1	0.4	±	0.2	c	0.6	±	0.2	a	0.0	±	0.0	c
700	5	0.0	±	0.0	c	0.0	±	0.0	a	0.0	±	0.0	c
700	15	0.0	±	0.0	c	0.0	±	0.0	a	0.0	±	0.0	c

* Values are means (Log CFU+1/g) ± standard deviations of duplicate analysis from three different productions.

** Values in the same column with different letter differ significantly ($p<0.05$)

In Figure 4-2 and Table 4-1 spores recounts are exposed. After processing (day 0) results show that, no differences are found between treatments include raw sample, samples had counts <1 Log. During storage time, no differences were found in the

spores count values in 300, 500 and 600 MPa. In purees pressurized at 700 MPa for 5 and 15 min no spores was detected during storage time. In a general overview the samples did not changed during time of thirty days.

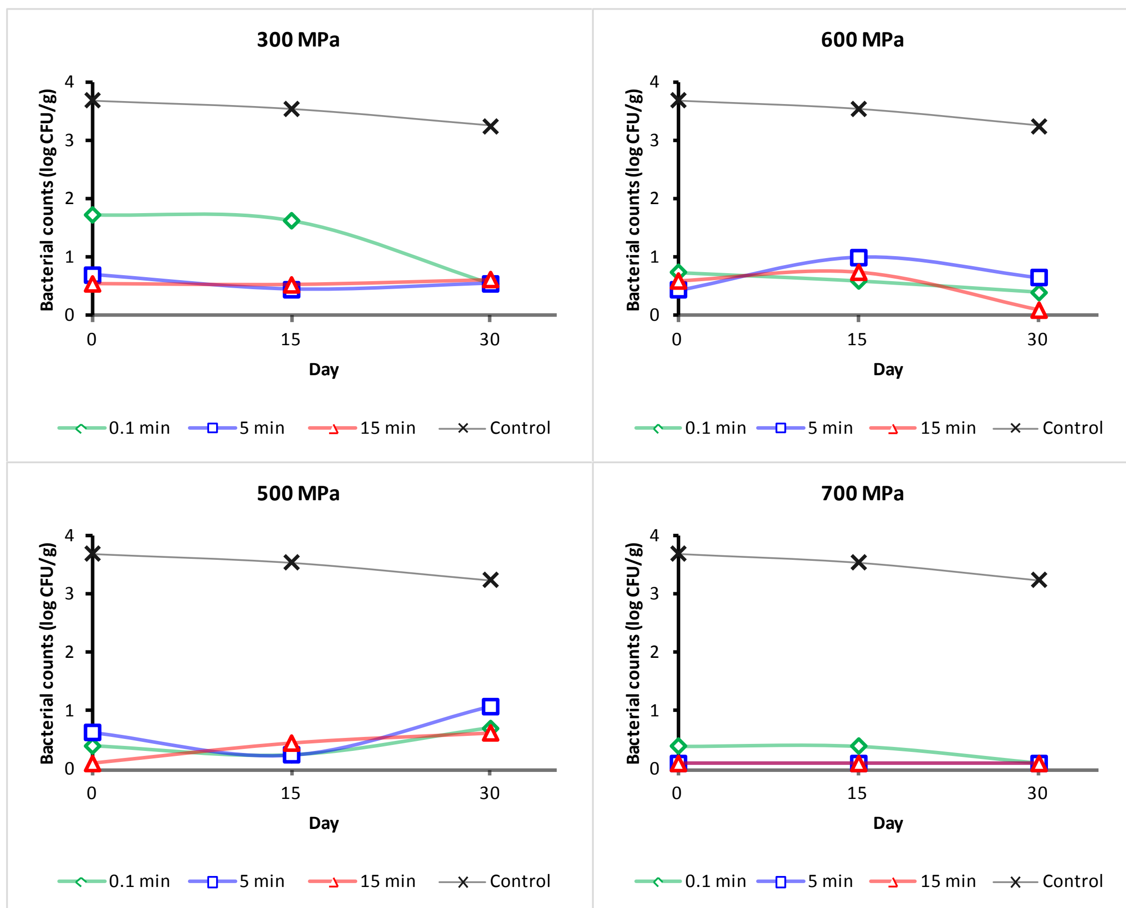

Figure 1-1. Inactivation and evolution curve of total aerobic mesophilic in untreated and HHP treated kiwi puree during storage at 8°C for 30 days. Raw sample (X), 0.1 min (◊), 5 min (□), 15 min (△).

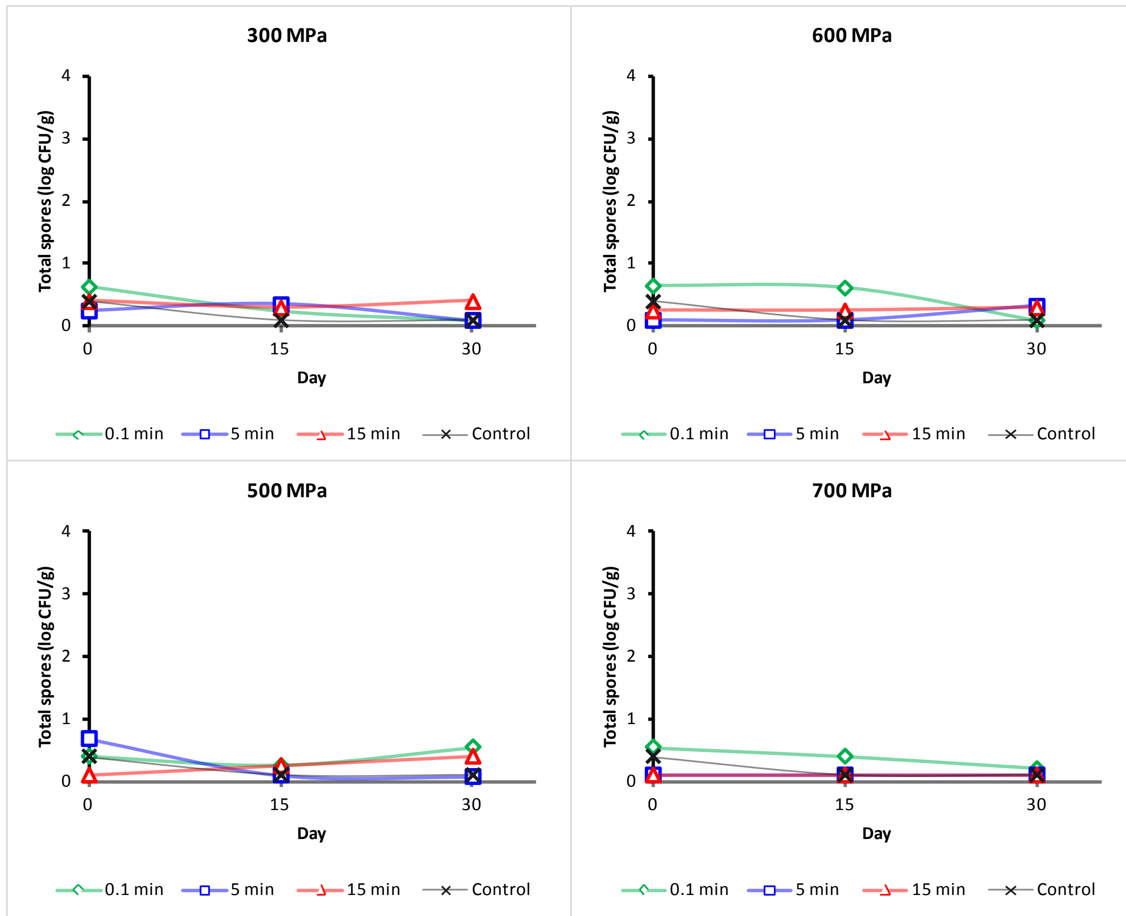

Figure 1-2. Inactivation and evolution curve of total spores in untreated and HHP treated kiwi puree during storage at 8°C for 30 days. Raw sample (X), 0.1 min (◊), 5 min (□), 15 min (△).

Results of lactic acid bacteria reported (Figure 1-3) that raw sample had the higher counts in day 0 with tendency to linear increasing during storage; its count grow up from 1.4 Log (day 0) to 3.4 Log (day 30). When pressure of 300 MPa for 0.1 min was applied, a small effect was observed. With the exception of treatment of 500 MPa at 0.1 min it was not observed survivors in day 0 for treatments of 500, 600 and 700 MPa. At 300 MPa only holding time of 5 and 15 were capable to inactivate approximately 1 Log. Dealing with holding time, it had no influence with pressure applied or during storage, except 300 MPa, mainly for the total destruction at 600 and 700 MPa. During the storage period, samples maintained the initial counts until day 30. Treatment at 300 MPa/ 0.1 min obtained less lethality, but with trend to decrease until day 30.

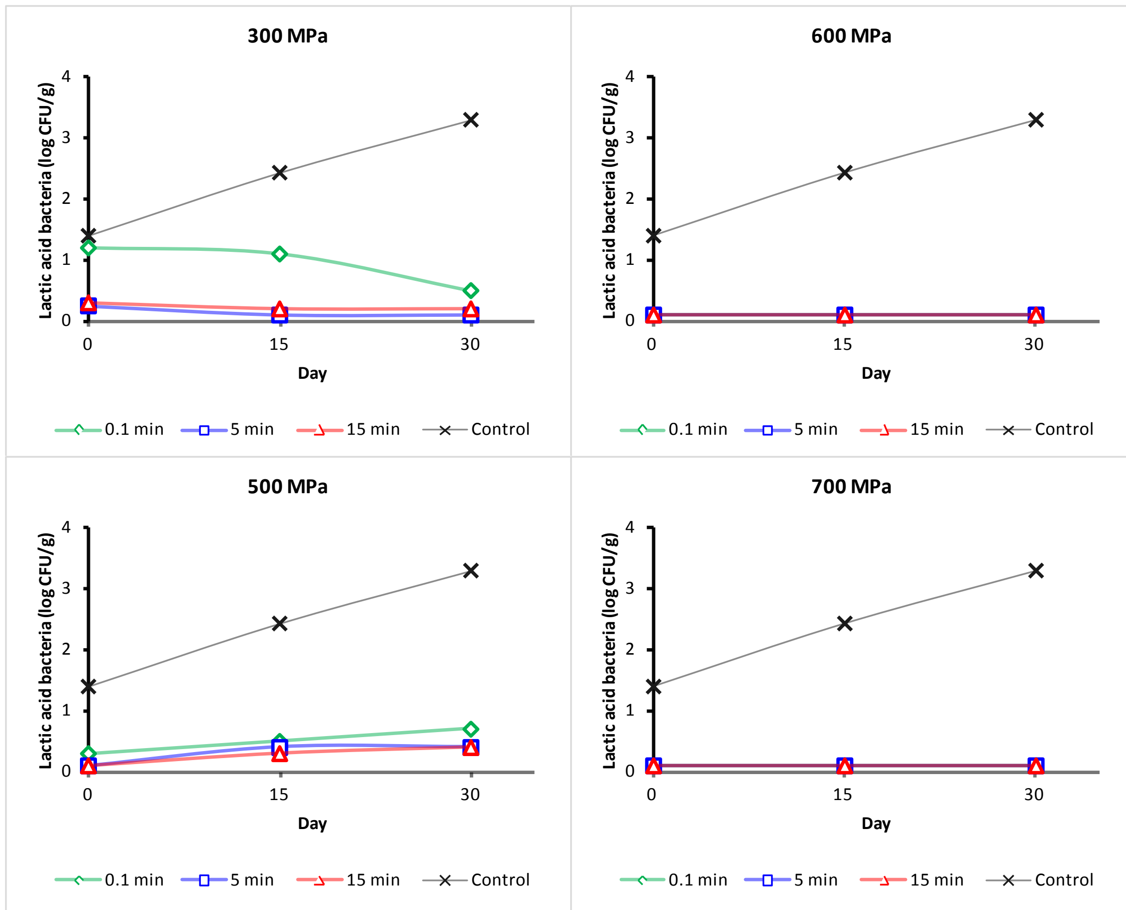

Figure 1-3. Inactivation and evolution curve of lactic acid bacteria in untreated and HHP treated kiwi puree during storage at 8°C for 30 days. Raw sample (X), 0.1 min (◊), 5 min (□), 15 min (△).

Staphylococcus aureus and *Escherichia coli* inactivation

In order to assess the effect of high pressure on selected microorganisms when present on kiwifruit puree two test bacteria (*Staphylococcus aureus* and *Escherichia coli*) were inoculated to the puree with the aim to obtain results that will allow modeling the pressure inactivation.

The product inoculated with *S. aureus* was subjected to HHP treatment at different temperatures (10, 40 °C) and pressures (400, 500, 600 MPa) for two different holding time (5, 15 min). The kiwifruit puree was plated on non-selective and on selective media for each of the microorganisms studied and temperature of treatment for all 6 condition plus a control sample. The evolution of the surviving microorganisms was followed for up to 30 days on refrigerated storage (sampling times at 0, 3, 5 and 30 days).

S. aureus found difficult to survive in kiwifruit puree and the initial counts decreased on the day 3 count (Table 1-2). At day 5, and until the end of the study at day 30, the presence of *S. aureus* was not detected.

Pressure treatments cause a dramatic reduction on microbial counts. *S. aureus* was only detected on PCA on samples treated at 400 MPa for any of the treatment temperatures (Table 1-2). As in control samples, the presence of *S. aureus* was not detected from day 5, and until the end of the study at day 30. For all the treatments at 500 and 600 MPa no surviving *S. aureus* was detected. There was no growth on BP agar for any pressure-processed sample.

It seems that most survivors of 400 MPa treatments have some kind of injury, because there were detected survivors in a generic medium (PCA) while in the selective medium (BP) there were no microorganisms capable to growth. BP medium components acts avoiding the general bacterial growth and allows *S. aureus* growth. Once *S. aureus* is injured, it is not capable to grow in a hostile environment. No selective effect of the medium was detected for control samples, were no injuring effect appeared.

Kiwi has very low pH (3.3), what could influence in the microorganism inactivation together with its organics acids content. The inhibitory effect during storage is most probably due to some mechanism similar to HHP processing. The matrix of the puree could promote some cell injury, as protein and enzyme unfolding,

including partial or complete denaturation; cell membranes undergoing a phase transition and change of fluidity; disintegration of ribosomes in their subunits; and intracellular pH changes related to the inactivation of enzymes and membrane damage (Georget et al., 2014). According to Buzrul et al., (2008), who studied kiwi and pineapple juices, during storage time the most probably cause of the inhibitory effect is the interaction of the HHP treatment impact and low pH values.

Table 1-2. Effect of HHP on survival and evolution of S. aureus *inoculated in kiwi puree. For refrigerated storage at day 5 and longer, counts were below detection level for all the conditions.*

Storage	Temp	Pressure	time	PCA *			BP *		
(days)	(°C)	(MPa)	(min)	Mean		SD	Mean		SD
0		Control		5.4 **	±	0.3 a ***	5.7	±	0.1 a
	10	400	5	0.5	±	0.7 b		n.d	
		400	15	0.5	±	0.7 b		n.d	
		500	5		n.d			n.d	
		500	15		n.d			n.d	
		600	5		n.d			n.d	
		600	15		n.d			n.d	
	40	400	5	0.5	±	0.3 b		n.d	
		400	15	0.5	±	0.4 b		n.d	
		500	5		n.d			n.d	
		500	15		n.d			n.d	
		600	5		n.d			n.d	
		600	15		n.d			n.d	
3		Control		2.8	±	1.4 a	2.7	±	1.5 a
	10	400	5	1.6	±	0.8 b		n.d	
		400	15	0.4	±	0.3 cd		n.d	
		500	5		n.d			n.d	
		500	15		n.d			n.d	
		600	5		n.d			n.d	
		600	15		n.d			n.d	
	40	400	5	2.0	±	1.4 ab		n.d	
		400	15	1.5	±	0.7 bc		n.d	
		500	5		n.d			n.d	
		500	15		n.d			n.d	
		600	5		n.d			n.d	
		600	15		n.d			n.d	

* Plate agar count (PCA) and Baird-Parker agar (BP).

** Values are means (Log CFU+1/g) ± standard deviations of duplicate analysis from three different productions.

*** Values in the same column and day with different letter differ significantly ($p<0.05$).

The kiwifruit puree inoculated with *E. coli* was pressure treated at 350, 500, 650 MPa for 2 or 10 min. The treatment temperatures selected were 7, 20 and 45 °C. On the 45 ºC treatment no growth was observed for any of the experimental conditions, in any of the 3 repetitions of the experiment.

All the control samples suffered a trend to reduce the number of *E. coli* during the storage, declining from 7 to 5 and 1 Log approximately in the days 0, 3 and 30 respectively, in both generic (PCA) and selective medium (Coli ID) (Table 1-3). Except treatments at 350 MPa, the others treatments did not presented survivors in the day 0. Those treatments also no differ ($p>0.05$) when analyzed the different times used.

Holding times presented difference only in treatments at 350 MPa, but in general no differences ($p>0.05$) were found. As in *S. aureus*, *E. coli* inactivation resulted in no detected counts (0 Log), giving us none differences statistic. The temperatures employed also did not resulted to effect in the inactivation ($p>0.05$), although that temperature of 45 °C seems to promote more lethality.

During of samples evolution it was observed that the treatments with survivors (350 MPa) the counts remained and in some samples decreased below detection level.

It seems that most *E. coli* survivors have injuries, even though not as marked as in *S. aureus*. Perhaps it is because Coli ID is a medium less stressful for *E. coli* than BP is for *S. aureus* or due to the inactivation mechanism of HHP treatments are different for these two microorganisms.

Table 1-3. Effect of HHP on survival and evolution E. coli inoculated in kiwi puree.

Storage (days)	Temp (°C)	Pressure (MPa)	time (min)	PCA * Mean		SD	Coli ID * Mean		SD
0		Control		7.2**	±	0.3 a***	7.3	±	0.3 a
	7	350	2	1.1	±	0.2 b	1.2	±	0.2 b
		350	10		n.d.			n.d.	
		500	2		n.d.			n.d.	
		500	10		n.d.			n.d.	
		650	2		n.d.			n.d.	
		650	10		n.d.			n.d.	
	20	350	2	0.5	±	0.3 bc	0.1	±	0.1 b
		350	10	0.1	±	0.1 c	0.0	±	0.0 b
		500	2		n.d.			n.d.	
		500	10		n.d.			n.d.	
		650	2		n.d.			n.d.	
		650	10		n.d.			n.d.	
3				5.4	±	0.3 a	5.7	±	0.1 a
		350	2	1.1	±	0.4 b	0.9	±	0.2 b
		350	10		n.d.			n.d.	
		500	2		n.d.			n.d.	
		500	10		n.d.			n.d.	
		650	2		n.d.			n.d.	
		650	10		n.d.			n.d.	
		350	2	0.5	±	0.3 bc	0.2	±	0.1 bc
		350	10	0.5	±	0.4 bc	0.2	±	0.2 bc
		500	2		n.d.			n.d.	
		500	10		n.d.			n.d.	
		650	2		n.d.			n.d.	
		650	10		n.d.			n.d.	
30				0.9	±	0.2 a	1.1	±	0.3 a
		350	2		n.d.			n.d.	
		350	10		n.d.			n.d.	
		500	2		n.d.			n.d.	
		500	10		n.d.			n.d.	
		650	2		n.d.			n.d.	
		650	10		n.d.			n.d.	
		350	2	0.5	±	0.0 bc		n.d.	
		350	10	0.5	±	0.0 bc		n.d.	
		500	2		n.d.			n.d.	
		500	10		n.d.			n.d.	
		650	2		n.d.			n.d.	
		650	10		n.d.			n.d.	

* Plate agar count (PCA) and *Escherichia coli* selective medium (Coli ID).

** Values are means (Log CFU+1/g) ± standard deviations of duplicate analysis from three different productions.

*** Values in the same column and day with different letter differ significantly ($p<0.05$).

Chapter 5. Results and Discursion. Color, Chlorophyll

Table 5-1. Effect of HHP treatment on color parameters in kiwifruit puree day 0.

MPa	min	° C	L*			Chroma*			Hue*			ΔE*		
Control			42,44	0,94	a	22,91	0,46	de	102,27	0,16	d			
300	5	10	40,07	1,26	b	22,10	0,68	ef	103,94	0,49	c	2,71	1,17	fg
300	15	10	39,46	0,71	bcde	21,05	0,97	gh	104,63	0,41	a	3,70	0,94	def
500	5	10	39,58	0,93	bcd	21,53	1,09	fg	104,19	0,65	bc	3,33	1,29	ef
500	15	10	38,46	1,09	e	20,08	0,96	h	104,49	0,41	ab	4,98	1,36	bc
700	5	10	36,42	0,42	f	25,11	0,51	bc	101,62	0,20	e	1,00	0,44	h
700	15	10	37,11	0,86	f	26,04	0,43	ab	102,59	0,34	d	1,87	0,72	gh
300	5	40	39,98	0,43	bc	26,22	1,55	a	96,38	0,17	h	4,96	1,12	bc
300	15	40	38,60	0,67	de	23,70	0,78	d	95,65	0,16	i	4,84	0,44	bc
500	5	40	39,31	0,59	bcde	24,95	0,78	c	95,37	0,25	i	4,78	0,57	bcd
500	15	40	39,04	0,83	cde	22,86	0,79	de	97,04	0,39	g	4,08	0,74	cde
700	5	40	39,61	0,91	bcd	22,49	0,88	ef	98,16	0,24	f	5,18	0,88	b
700	15	40	38,77	0,84	de	17,83	0,42	i	95,32	0,38	i	8,40	0,49	a

Values are means ± Standard deviation (n=45).

Values in the same column with different superscripts differ significantly

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Effects of HHP on instrumental color parameters of kiwifruit puree

Instrumental Color Measurement (L*, Hue*, Chroma* and ΔE*) Day 0

The color behavior of the different samples of kiwifruit puree was, however, very much masked by the large variation within and between the samples, partly due to the different parts of the kiwifruit puree which was compound by the columella or core (white), the inner pericarp with the seeds and the outer pericarp. Each part differs from the others in composition and texture.

The luminous intensity of a color, its degree of lightness is measured in the CIEL*a*b* scale with L* values that reflects as light or dark a color can be. L* values close to 100 represents light colors and values close to 0 means it is darker color. Analysis of kiwifruit puree showed a variation of lightness between 36.42 – 42.44 (Figure 1-1).

All samples treated by HHP presented L* values lower than control, in special treatment of 700 MPa at 10 °C. Did not observed differences in treatments at 10 °C of 300 MPa for 5 or 15 min and 500 MPa for 5 min. When samples were pressurized at 40 °C, pressure of 300 MPa for 15 min and 500, 700 MPa for 5 o 15 min did not presented differences. Comparing temperatures used in the experiment, except samples treated at 700 MPa for 5 or 15 min at 10 °C and samples treated at 40 °C presented L* values lower.

Maskan (2001) studying color change of kiwifruit during hot drying obtained formation of dark compounds that reduced luminosity, this behavior was possibly due to the presence of heat sensitive reactions in the process involving the degradation of thermolabile pigments, which in turn result in formation of dark compounds that reduced luminosity. As a whole, the development of discoloration of samples during drying may be related to pigment destruction, ascorbic acid browning and non-enzymatic Maillard browning. HHP treatment (at low and moderate temperatures) has a limited effect on pigments (e.g. chlorophyll, carotenoids, anthocyanins, etc.) responsible for the color of fruits and vegetables. The color compounds of HHP processed fruits and vegetables can, however, change during storage due to incomplete inactivation of enzymes and microorganisms, which can result in undesired chemical reactions (both enzymatic and non-enzymatic) in the food matrix (Oey, Van der Plancken, Van Loey, & Hendrickx, 2008).

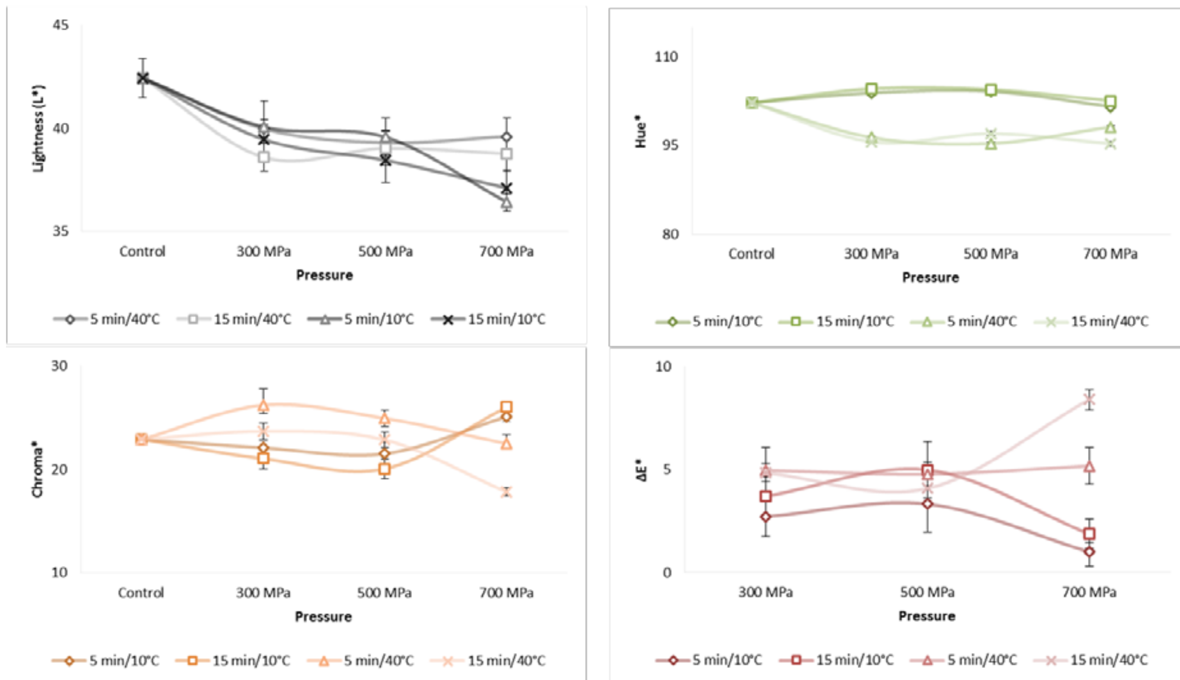


Figure 1-1. Change in the L values of HHP processed kiwifruit puree treated at 10 °C (a) and 40 °C (b).*

To identify the color of a sample, it will most likely speak first of its hue. Quite simply, hue° is how it is perceive a sample color. Hue° denotes hue angle, an angular measurement. In the CIEL*a*b* scale the green colors are around the 180°. Hue which value close to 90° means that the color became yellowish color.

After HHP treatment samples treated with different temperatures presented decrease when heat was applied. Hue° values from heated pressurized samples presented differences being that pressure of 700 MPa for 5 min closer to control. Except pressures of 700 MPa for 5 or 15 min the rest of samples treated at 10 °C produced samples whose Hue° values were even higher than the control. Pressurized samples at 300 and 500 MPa for 15 min showed to be greener (Table 5-1. Effect of HHP treatment on color parameters in kiwifruit puree day 0.Table 5-1).

Color saturation represented by chroma* showed values between 17.83-26.22 (Table 5-1). Pressurized purees by 700 MPa at 10 °C showed more saturated than control as 300 MPa 15 min and 500 MPa for 5 o 15 min had saturation lower than control. For 300 and 500 MPa samples did not differ when applied the same holding time process.

Pressurized samples at 40 °C showed a behavior opposite to treatment at 10 °C. While saturation increased when treated at 700 MPa at 10 °C decreased when treated at

40 °C similar behavior happened with the others samples, but it was possible observe no correlation. Treatment by 300 MPa for 5 min showed more saturation, even in the heated treatment it showed tend of less saturation when holding time was longer.

Delta E* is defined as the difference between two colors in an L*a*b* color space.

Pressurized samples at 10 °C treated for 15 min presented values higher than samples treated for 5 min presenting tendency to increase the color difference when pressurized for long time, nevertheless samples treated more hard (700 MPa / 5 and 15 min) showed to be the samples alike to the control. It is important to record that the color difference is calculated taking into account the control. Results showed that when purees were pressurized with heat 700 MPa / 15 min produced the biggest difference. The purees treated by 300 MPa / 5 and 15 min and 500 and 700 MPa for 5 min did not differ between them (Table 5-1).

Except samples treated at 700 MPa for 5 and 15 min at 40 °C the others treatment did not presented differences between them although these samples presented more difference when compared to the control. Samples treated more severely presented more difference in relation to control.

For to verify the situation of a color in relation to reference, delta E* was used which is difference or the distance between two colors using the space L*a*b* to define the values of each colors. So as closer to the 0 value, in other words, as smaller the difference, closer to the selected standard.

Figure 1-2. **Evolution of Lightness (L*) during storage at 4 ºC of kiwifruit puree treated by high hydrostatic pressure**

Observing color evolution for treatment at 10 °C, samples presented faint tendency to reduce luminosity during storage. This was not true for control and 300 MPa / 15 min which not presented L* value higher in the day sixtieth.

When was applied pressure at 40 °C samples showed a slightly decrease in luminosity mainly in the day 60. Control and sample treated at 300 and 500 MPa (15 min) presented behavior different from the others samples, which presented stability until day 30 but decrease during the following thirty days (Figure 1-2).

Comparing temperatures used, in the day 0, samples did not vary their L* value, just samples of 700 MPa presented increase at 40 °C, as well as. During the storage the samples luminosity come to presenting increasing until day 60. Luminosity appeared to increase when treated at 40 °C, mainly during in analysis of day 15 and 30.

Samples started green shades and became yellowish in the end of experiment. This results means that the product is useful in the market just 15 days. So it is not interesting because is needed products that have a large shelf-life.

As a vegetable product perishable kiwifruit puree keeps the senescence and damages initiated first by the fruit manipulation and kept it later during 60 days promoted a complete degradation of the system. The major visual changes in kiwifruit are caused by both enzymatic and/or no enzymatic development of brown pigmented substances and by the decoloration due to chlorophyll degradation (Leunda et al., 2000). Natural coloring pigments like chlorophylls may be lost by various mechanisms resulting in a decrease of color intensity. During processing and storage, there is a release of intercellular acids and enzymes which can then come into intimate contact with chlorophyll-protein complexes. This contact as well as the physical damage to the tissue would be the initiating step in chlorophyll degradation. This process results in the formation of five groups of intermediate compounds (pheophytin, Chlorophyllide, Pheophorbide, flurescent compound and rusty pigment). The acid removal of the ion Mg^{+2} in the chlorophyll molecule occurs to form pheophytin. Cleavage of the phytol chain by the enzyme chlrophyylase leads chlorophyll to chlorophyllide. This last is

converted to pheophorbide through the loss of its magnesium ion and pheophytin is subsequently converted to pheophorbide through the loss of its phytol chain. Pheophorbide is then transformed to colorless compounds (Leunda et al., 2000).

Results for Hue° values showed accentuated decrease for pressurized samples at 10 °C. Samples started at day 0 more green and all values higher than 100. Differently of samples pressurized at 40 °C which also presented decrease in Hue* values, but these samples started in the day 0 less green. When comparing temperatures of treatments, it was clearly the loss of green color in samples treated at 40 °C in the first day of analysis. During the day 15 samples showed a little increase when applied heat in the pressurization, except for sample 300 MPa / 15 min and 500 MPa / 5 min. From the day thirty, except sample 300 MPa / 15 min (day 30), 300 and 700 MPa for 5 min (day 60) the samples did not showed big variation in Hue* values.

Saturation of kiwifruit puree treated at 10 °C presented decrease in the day 15 and soon increase in the day 30 keeping values until day 60, in general the treatments presented slightly tender to saturate color in special treatment of 700 MPa / 15 min. When heat was applied, except 300 MPa / 5 min and 500 MPa / 15 min the others samples presented variation of up and down, decreasing in the day 30 and increasing in the day 60. Comparing temperatures it was possible to observe decrease in the saturation when treated at 40 °C in the day 0, except for sample 500 MPa / 5 min and 700 MPa / 5 and 15 min, in the day 15 happened similar things, Chroma* value increase, except 500 / 700 MPa (15 min) in the other way in day 60 almost samples presented decrease in saturation maintained stable until final of the study.

The color difference of kiwifruit puree processed at 10 °C presented tendency to increase during storage. In the day sixtieth all samples presented the maximum delta E* (ΔE*) values, but during the storage period the color difference presented variation. The first fifteen days were necessary to produce decrease in the ΔE* values of 300 MPa / 15 min, and 500 MPa / 5 and 15 min which means that samples turn more similar to the control. The same thing happened with 300 MPa / 5 min and 700 MPa / 15 min in the thirtieth day of storage. In the treatment at 40 °C samples also presented

more color difference in the day 60, except sample 500 MPa / 5 min which did not change ΔE^* values during storage. Analysis of those samples showed ΔE^* values started higher than when treated at 10 °C, except sample treated by 300 and 500 MPa / 15 min, but in the day 60 presented ΔE^* values lower or equal to treatment at 10 °C.

The color of green vegetables is mainly determined by the chlorophyll pigments present in plant material to catch the energy from sun light. The chlorophyll pigments degrade during heat treatments, with the consequence that the green color changes. The change in green color due to heat treatments consists initially of an increase in color followed by a decrease. This initial increase in green color has been reported in literature, but not modelled or expressed quantitatively. Most studies on changes in green color due to time and temperature treatments only mention a decrease of green color. Only a few authors mention an initial increase in green color upon heating. MacKinney and Weast (1940) attribute the initial increase of the green color in green vegetables to air removal around the fine hairs on the surface of the plant and to the expulsion of air between the cells.

Table 1-2. Effects of HHP on the chlorophylls a and b and pheophityn a and b contain of kiwifruit puree. Day 0

MPa	min	Chl *a(mg/100g)*	Chl *b(mg/100g)*	Pheo *a(mg/100g)*	Pheo *b(mg/100g)*
Control		1.08 ± 0.06 a	1.24 ± 0.01 a	1.60 ± 0.03 bc	0.02 ± 0.00 c
Heated sample		0.01 ± 0.02 e	0.08 ± 0.03 e	3.17 ± 0.62 a	0.13 ± 0.02 a
300	0.1	0.42 ± 0.04 d	0.69 ± 0.07 d	1.70 ± 0.08 b	0.04 ± 0.00 b
	5	0.62 ± 0.02 bc	0.73 ± 0.02 cd	1.15 ± 0.00 c	0.02 ± 0.00 c
	15	0.60 ± 0.01 bc	0.79 ± 0.03 bcd	1.26 ± 0.04 bc	0.02 ± 0.00 bc
500	0.1	0.59 ± 0.02 c	0.81 ± 0.08 bc	1.41 ± 0.22 bc	0.02 ± 0.00 bc
	5	0.60 ± 0.04 bc	0.79 ± 0.03 bcd	1.50 ± 0.04 bc	0.02 ± 0.00 bc
	15	0.62 ± 0.01 bc	0.76 ± 0.02 bcd	1.54 ± 0.04 bc	0.03 ± 0.00 bc
600	0.1	0.69 ± 0.08 bc	0.86 ± 0.08 b	1.52 ± 0.18 bc	0.02 ± 0.00 bc
	5	0.61 ± 0.00 bc	0.82 ± 0.03 bc	1.52 ± 0.01 bc	0.03 ± 0.00 bc
	15	0.56 ± 0.06 c	0.73 ± 0.10 cd	1.51 ± 0.08 bc	0.03 ± 0.00 bc

* All concentrations in mg/100g
** Mean values with different letters in the same column are significantly different ($p<0.05$)

Effect of High Hydrostatic Pressure on the chlorophyll *a* and *b* and pheophytin *a* and *b* of kiwifruit puree.

The major visual changes in kiwifruit are caused by both enzymatic and/or nonenzymatic development of brown pigmented substances and by the decoloration due to chlorophyll degradation (Leunda et al., 2000). Natural coloring pigments like chlorophyll may be lost by various mechanisms resulting in a decrease of color intensity (Leunda et al., 2000).

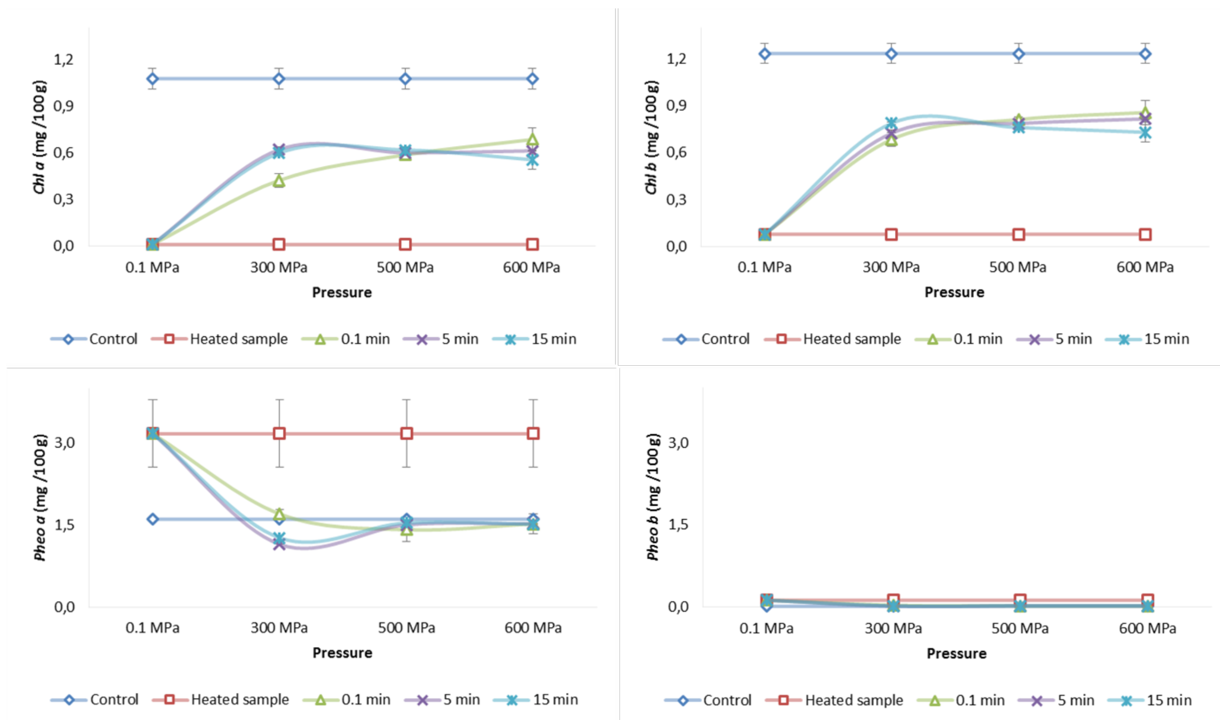
During processing and storage, there is a release of intercellular acids and enzymes, which can then come into intimate contact with chlorophyll-protein complexes. This contact as well as the physical damage to the tissue would be the initiating step in chlorophyll degradation (Leunda et al., 2000).

This process results in the formation of five groups of intermediate compounds (pheophytin, chlorophyllide, pheophorbide, fluorescent compound and rusty pigment). The acidic removal of the ion Mg^{+2} in the chlorophyll molecule occurs to form pheophytin. Cleavage of the phytol chain by the enzyme chlorophyllase leads chlorophyll to chlorophyllide. This last is then converted to pheophorbide through the loss of its magnesium ion, and pheophytin is subsequently converted to pheophorbide through the loss of its phytol chain. Pheophorbide is then transformed to colorless compounds (Leunda et al., 2000)

Possible strategies proposed by various authors for controlling chlorophyll degradation include (Heaton & Marangoni, 1996): inactivation of chlorophyllase with minimal conversion of chlorophyll to pheophytin; addition of Mg^{+2} salts to prevent the loss of magnesium from chlorophyll or chlorophyllide; addition of Cu^{+2} or Zn^{+2} salts, which form a new bright green complex chlorophyll ion metal; control of the pH, temperature and ionic strength of food products to minimize chlorophyllase and dioxygenase activities in tissues: addition of antioxidants to prevent the induction of the oxidation of chlorophyll by light; and peroxidase-hydrogen peroxide and/or lipoxygenase control (Leunda et al., 2000).

Chlorophyll a

Figure 1-3. Pigments on pressurized samples were treated at 10 °C. Day 0



Chlorophyll (Chl) *a* presented value higher in the control sample. All pressurized sample presented Chl *a* contain less than control, but higher than pasteurized sample. When kiwifruit puree was heat the loss of Chl *a* was almost 100 %. Pressurized samples by 300 MPa / 0.1 min showed to be the most affected by pressure while the others pressurized samples did not differ between them. The chlorophyll *b* contain as chlorophyll *a* was higher in the control. All the pressurized samples presented Chl *b* contain were higher than pasteurized sample. Samples treated by 300 MPa for different hold time did not differ between them, the same thing happened with treatment by 500 MPa. Samples treated by 600 MPa presented higher value when hold time was 0.1 min. Observing the sample set just 300 and 600 MPa for 0.1 presented differences.

Factors likely to have a significant effect on the rate of chlorophyll degradation include pH, temperature, water activity, and time (Cano, Marin, Ancos, & Hayward, 1993). Others mechanisms of chlorophyll degradation could have been taking place, probably as a consequence of enzymatic reactions that produced an evident bleaching of the fruit tissue (Cano et al., 1993).

Several authors have reported the regeneration of peroxidase, in some frozen fruit tissues during prolonged storage periods. Studies on the behavior of POD (polyphenol oxydase) in frozen kiwi fruits showed that the POD activity strongly increased form 200 days frozen storage in Abbot and Montly kiwifruit slices, whereas this regeneration was slight in Bruno and Hayward samples (Cano et al., 1993).
The color this difference continuously increased during frozen storage, and was the most evident change that took place in Montly kiwifruit slices (Cano et al., 1993). The total color differences relating to the corresponding fresh product; this could related to the observed loss of chlorophylls (27%)

In conclusion, the most suitable cultivar of kiwifruit for freezing preservation in terms of color deterioration could be cultivar Hayward and Bruno (Cano et al., 1993).
The change color parameters such as hue during storage could explain the losses or breakdown of certain classes of pigments (Cano et al., 1993).

It was observed that the degradation rate of chlorophyll a and chlorophyll b accelerated as pH decreased (Koca et al., 2007).

The rate constants of green color loss and chlorophyll degradation decreased with increasing pH, indicating that the green color was retained at higher pH conditions. It was found that chlorophyll *a* degraded faster than chlorophyll *b* at all pH values for each temperature applied. The results revealed that chlorophyll *a* was more susceptible to thermal degradation than chlorophyll *b* in acidic conditions study blanched green peas (Koca et al., 2007)

The natural color of fruit and vegetables is mostly unaffected by HHP, with no significant losses of chlorophyll and color of processed fruit in jams being retained (Richard Earnshaw, 1996).

Pheophytin (Pheo) *a* presented higher value in the heated sample. No differences presented the pressurized samples when compared with the control, but between the samples pressurized by 300 MPa for 0.1 min presented more Pheo *a* contain. *P*heohytin *b* results presented very low values between 0.015-0.128. The higher values were from the heated puree. Sample pressurized by 300 MPa differ when treated for 0.1 and 5 min.

In a study of pigment and color stability of frozen kiwifruit slices during prolonged storage Cano et al. (1993) pheophytin *b* was the chlorophyll derivate found in these samples prolonged storage periods. This compounds was not observed in chromatograms of fresh and just-frozen kiwifruit extracts (Cano et al., 1993)

The change in other objective color parameters such as Hue* during storage could explain the losses or breakdown of certain classes of pigments (Cano et al., 1993) correlation studies between this parameters (HUE*) and the two more important pigment (chlorophyll and xanthophyll) classes in this fruit are shown in correlation coefficients of -0.7726 was obtained for total chlorophyll/Hue parameter, this results confirm that in the changes in kiwifruit appearance, samples tend to be more yellow and luminous, and other chemical compounds produced by other bio-chemical mechanism must be taken into account (Cano et al., 1993). However, the correlation values indicated that the most important factor responsible for color changes is pigment breakdown. (Cano et al., 1993). Previous reports on chlorophyll stability during processing and storage showed that the analyses of degradation products indicates that oxidation during storage was not a dominant factor in chlorophyll conversion and loss color (Cano et al., 1993). Not only chlorophyll breakdown was implicated in the color changes (Cano et al., 1993).

However, the results from the frozen kiwifruit slices agreed with the premise that changes in visual color were noticeable only after a considerable degradation of

chlorophyll compounds had taken place. (Cano et al., 1993). The higher correlation coefficients between the most important chemical class of pigments in this fruit and the Hunter Lab color parameters were obtained with the total ratio of chlorophyll/hue*. Chlorophyll and xanthophyll breakdown was observed during storage but causative mechanism of color deterioration of kiwifruit slices at prolonged frozen storage periods.

Figure 1-4. Chlorophylls behavior during storage time in pressurized kiwifruit puree

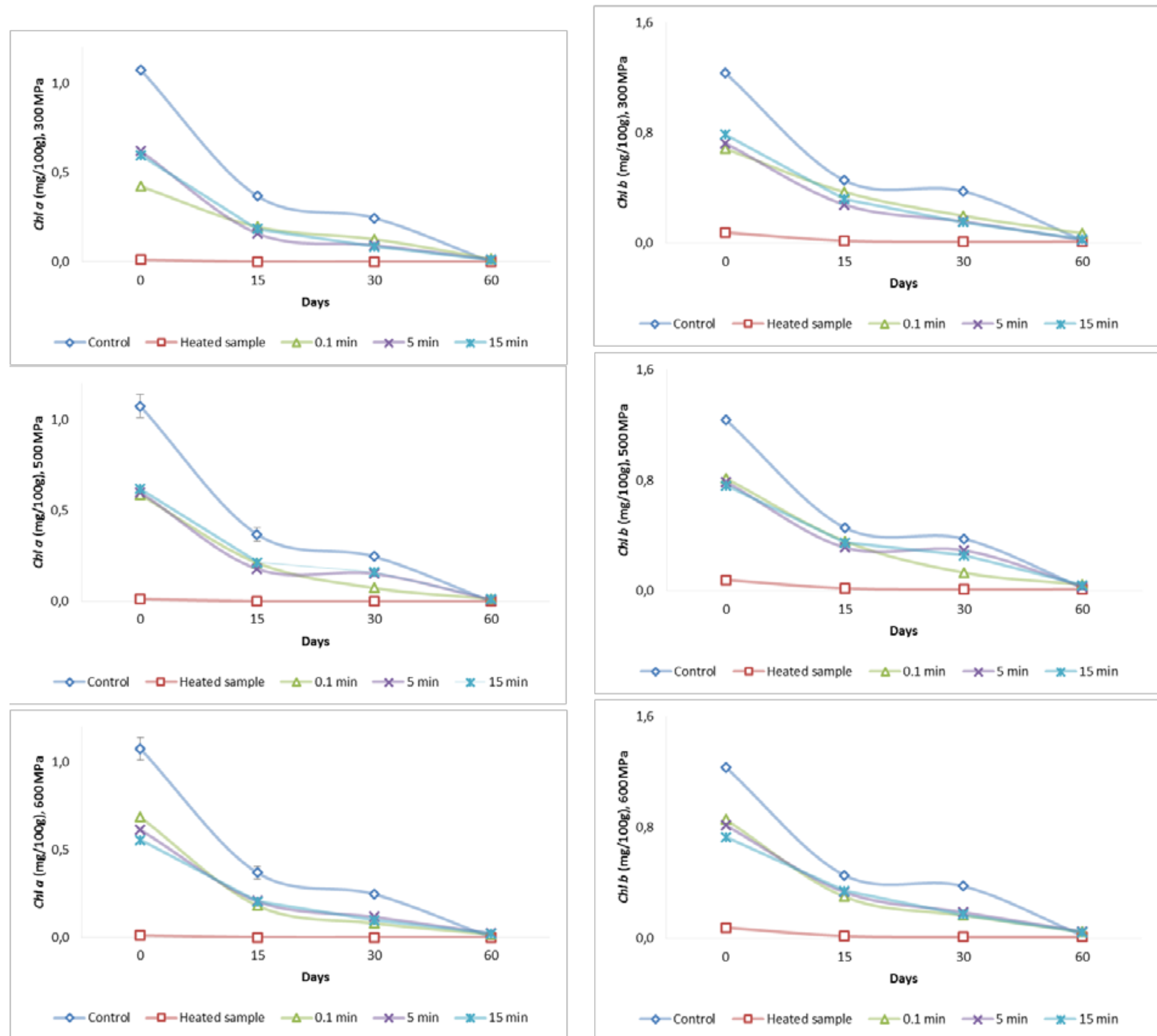
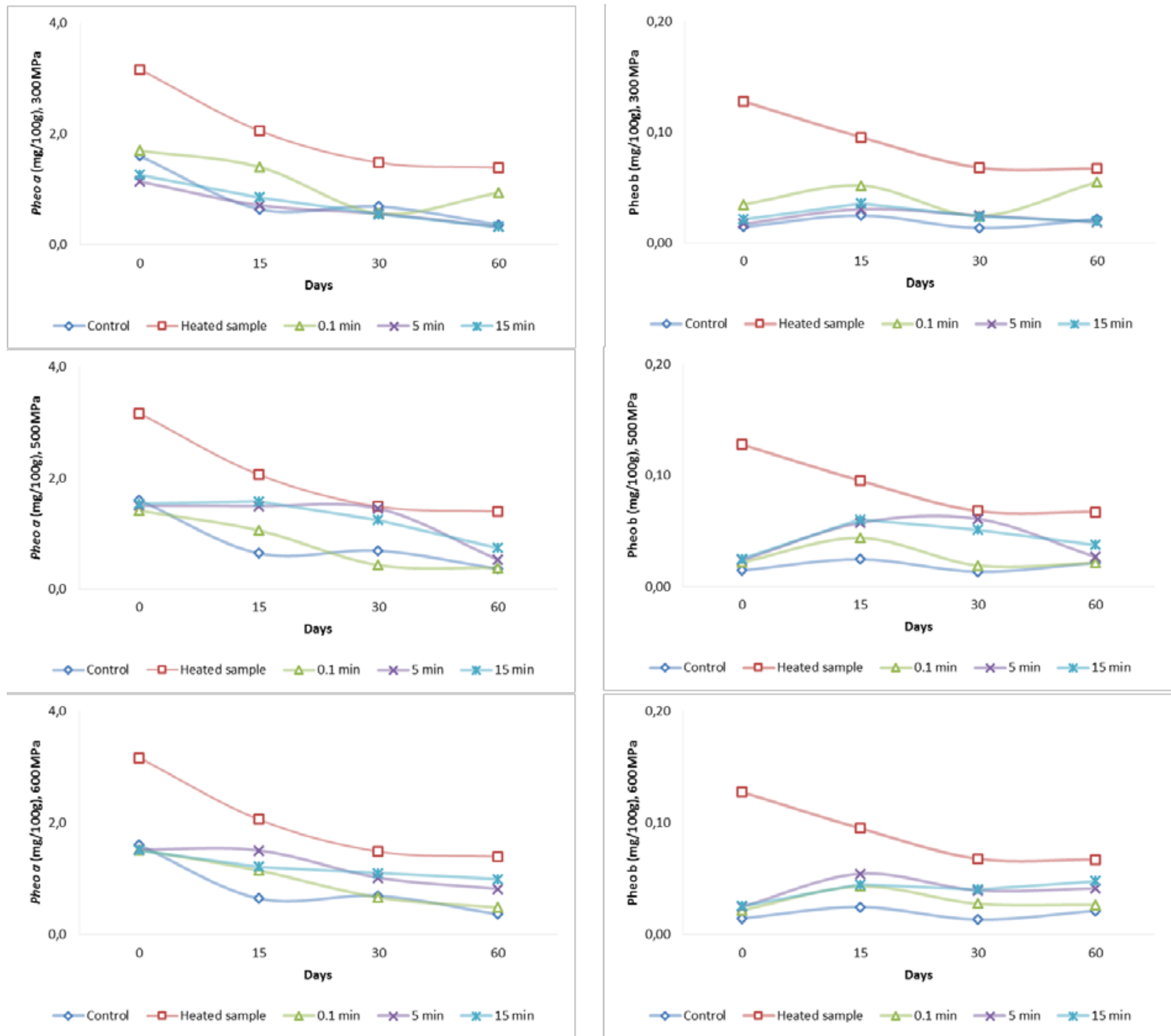


Figure 1-5. Pheophytin behavior during storage time in pressurized kiwifruit puree



Chapter 6. Results and Discursion. Rheological Properties

Rheological properties were obtained by curve fitting using Otswald Waele (Power Law). The parameters, values of consistency coefficient, K, and the flow behaviour index, *n*, are shown in Table 6-1. The model presented good adjustment of the data showing values for the coefficient of determination R^2>0.98.

Several factors affect the rheological behaviour of fruit pulp, including temperature and concentration of soluble solids. The consistency index decrease as decline the soluble solid concentrate (Puntes, Rubio, & Cabeza, 2012). The consistency index of kiwifruit puree did not show decreasing when applied pressure of 300, 500 or 600 MPa. Although soluble solid presented, lower than control sample in all pressurized puree.

A shear-thinning behaviour can be observed, because values of flow behaviour (*n*) were less than one. This behaviour has been reported in others studies as rheological behaviour of blueberry (Antonio, Faria, Takeiti, & Park, 2009), mango puree and papaya (Properties, Mango, & Nectar, 2005).

Otswald Waele predicts that viscosity would decrease, when *n* is smaller than one, together with increasing shear rate. Fluids with this characteristics, are denominates pseudoplastics fluids. Non-Newtonian and pseudoplastic is common in pulp, puree and fruit juice (Properties et al., 2005). In a great number of rheological characterization of food material also is finding this behaviour as raspberry, strawberry, prune, and peach purees (Maceiras, Álvarez, & Cancela, 2007); and blueberry in a study of.(Nindo, Tang, Powers, & Takhar, 2007).

According to Figure 6-1, pressurized puree at 300 MPa during 5 min presented higher values of shear stress then others purees different from treatment of 500 and 600 MPa, which control sample presented flow curves above of the others flow curves.

It is noted when applied pressure of 500 and 600 MPa samples reaches plateau next 10 s^{-1}. Pressure of 300 MPa presented this effect just for sample with holding time of 15 min. The cessation of ascendant curve means that no more stress is required to carry on the flow. Possibility the change in the flow is due to a breakdown, arrangement or entanglement of micro or nanostructure of material. Resembles pressure is capable of rearranges the internal structure considering that higher pressure or holding time applied in the puree.

Because the viscosity is ratio between shear stress and shear rate, apparent viscosity is also related to the reason, which affects the shear stress values. With an increase in shear rate occurs a decrease in apparent viscosity of kiwifruit puree. Apparent viscosity at shear of 10 and 20 s^{-1} are shown in Table 6-2. After HHP treatment no differences were found in viscosity at 10 s^{-1} when comparing with control. Considering pressure parameter sample that was treated at 5 and 15 min at 300 MPa differ between them. Viscosity at 20 s^{-1} did not differ from de control, but was lower than viscosity measured at 10 s^{-1}. This fact can be seen in the Figure 6-1 where it is possible to see the curve pendent change with the increasing shear rate.

The decreasing in viscosity in fruit purees could be explained by the arrangement breakdown of the molecules due to the shear rate applied and promoting the alinement of the constituent molecules (Antonio et al., 2009).

The flow behaviour and consistency index values for the kiwi puree storage for 30 days given in Table 6-1. Consistency index values on day 15 shows no difference between the samples treated analyzed, but the samples pressurized at 500 MPa/0.1 and 5 min were bigger than 300 MPa/0.1 and 5 min from day 0 at 500 MPa for 0.1 and 5 min. Results from day 30 shows that pressure and holding time did not influence K values from samples analyzed. Neither storage time promoted any changes in these parameters.

Purees had n values on day 15 ranged between 0.7 to .14, while n for day 30 ranged between 0.10 to 0.13. These results indicated that purees behaved as a non-Newtonian fluid during the storage time (pseudoplastic fluid). In day 15 samples treated at 300 MPa for 5 min and 600 MPa for 5 and 15 min demonstrated significant increase compared to the control from day 0. Observing results from day 30, control obtain lower value than treated samples and no differences were found in between treated purees.

The viscosity during storage time did not change in the 10 s^{-1} until day 15. With exception of sample 500 MPa/0.1 and 5 min the viscosity measured at point 20 s^{-1} did not differ from control in day 0. The high values for viscosity were observed in day 30, purees treated at 600 MPa for 5 and 15 min. These same samples also presented one of the highest consistency index.

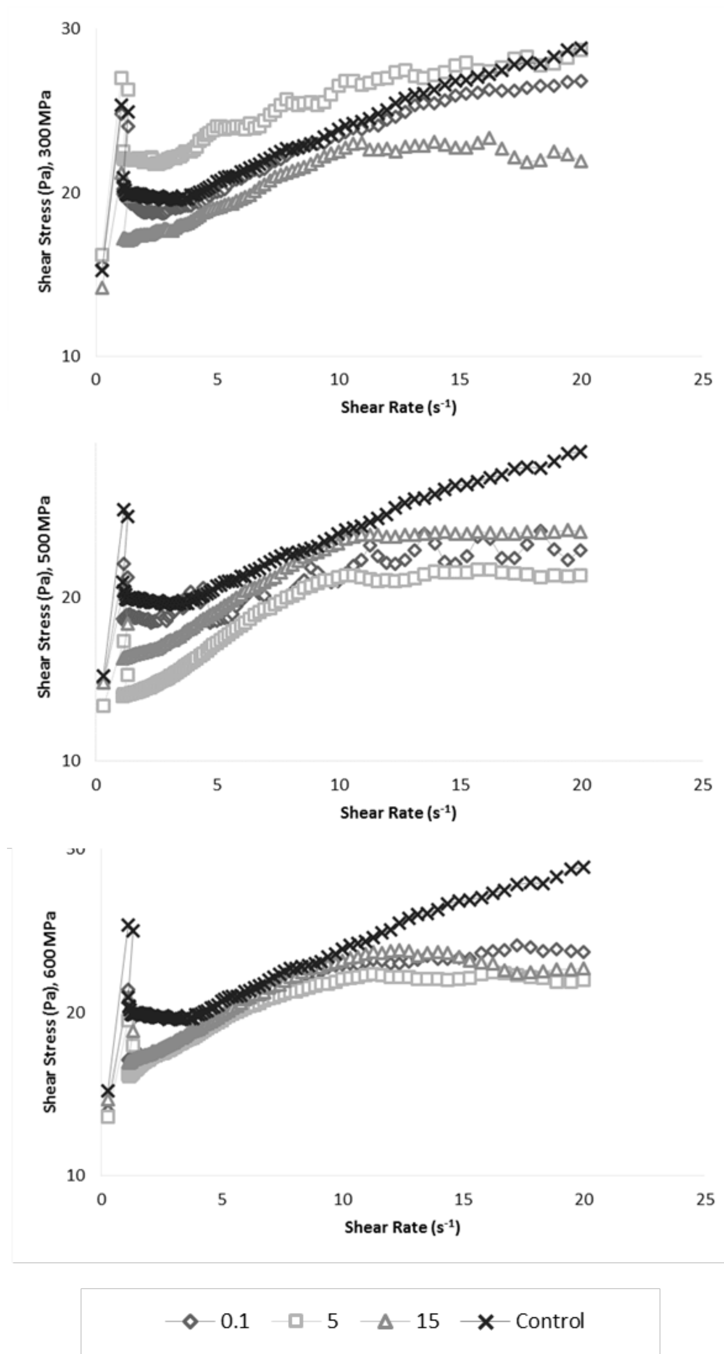


Figure 6-1. Shear stress versus shear rate for kiwi puree pressurized by 300, 500 and 600 MPa

Table 6-1. Rheological coefficients of Ostwald Waele model (Power Law) for high pressure treated kiwi puree, as a function of holding time at three aging time

MPa	min	consistency index K Day 0	consistency index K Day 15	consistency index K Day 30	flow behaviour n Day 0	flow behaviour n Day 15	flow behaviour n Day 30
Control		16.7 ± 3.7 [abc]	16.2 ± 2.8 [abc]	15.8 ± 5.4 [abc]	0.05 ± 0.1 [de]	0.07 ± 0.0 [abcde]	0.02 ± 0.1 [e]
300	0.1	18.5 ± 5.7 [ab]	13.9 ± 1.7 [bc]	14.8 ± 3.3 [abc]	0.09 ± 0.1 [abcd]	0.07 ± 0.0 [abcde]	0.11 ± 0.1 [abcd]
	5	17.2 ± 2.9 [ab]	15.1 ± 5.6 [bc]	17.5 ± 2.6 [abc]	0.09 ± 0.0 [abcde]	0.14 ± 0.0 [ab]	0.10 ± 0.1 [abcde]
	15	15.2 ± 4.8 [abc]	15.9 ± 5.1 [abc]	16.4 ± 4.7 [abc]	0.10 ± 0.1 [abcd]	0.12 ± 0.1 [abcd]	0.11 ± 0.0 [abcd]
500	0.1	14.1 ± 1.1 [abc]	12.7 ± 2.2 [c]	16.8 ± 2.2 [abc]	0.08 ± 0.0 [abcde]	0.12 ± 0.1 [abc]	0.10 ± 0.1 [abcde]
	5	13.9 ± 1.6 [bc]	12.9 ± 2.3 [c]	16.1 ± 5.3 [abc]	0.11 ± 0.0 [abcd]	0.10 ± 0.0 [abcd]	0.11 ± 0.1 [abcd]
	15	15.6 ± 3.8 [abc]	17.9 ± 2.7 [abc]	14.5 ± 2.0 [abc]	0.10 ± 0.1 [abcd]	0.11 ± 0.0 [abcd]	0.11 ± 0.1 [abcd]
600	0.1	15.3 ± 2.6 [abc]	16.5 ± 4.8 [abc]	13.5 ± 3.5 [abc]	0.09 ± 0.1 [abcd]	0.10 ± 0.0 [abcd]	0.11 ± 0.1 [abcd]
	5	13.9 ± 0.8 [bc]	17.4 ± 3.9 [abc]	18.6 ± 1.9 [ab]	0.13 ± 0.0 [abc]	0.14 ± 0.0 [abc]	0.12 ± 0.0 [abcd]
	15	14.9 ± 2.3 [abc]	15.8 ± 5.1 [abc]	19.7 ± 5.0 [a]	0.10 ± 0.0 [abcd]	0.14 ± 0.0 [a]	0.13 ± 0.1 [abc]

Different superscripts in the same row and column indicate significant difference. Compare row and columns of consistency index separated of flow behaviour. ($p<0.05$) ($n=3$)

Table 6-2. Apparent viscosity of kiwi fruit treated by HHP storage for 30 days. Specifically two shear rate points (10 s^{-1} and 20 s^{-1})

Day	MPa	min	Apparent viscosity 10 s^{-1}	Apparent viscosity 20 s^{-1}
0	Control		2.39 ± 0.73 ^{cdefghi}	1.44 ± 0.49 ^{k}
	300	0.1	2.35 ± 0.60 ^{cdefghi}	1.34 ± 0.35 ^{klm}
		5	2.65 ± 0.56 ^{abcde}	1.43 ± 0.34 ^{k}
		15	2.26 ± 0.62 ^{fghij}	1.10 ± 0.29 ^{klm}
	500	0.1	2.10 ± 0.42 ^{ghij}	1.15 ± 0.23 ^{klm}
		5	2.12 ± 0.25 ^{ghij}	1.07 ± 0.19 ^{klm}
		15	2.33 ± 0.33 ^{cdefghij}	1.20 ± 0.20 ^{klm}
	600	0.1	2.29 ± 0.45 ^{defghij}	1.18 ± 0.28 ^{klm}
		5	2.20 ± 0.26 ^{ghij}	1.10 ± 0.20 ^{klm}
		15	2.31 ± 0.40 ^{defghij}	1.13 ± 0.22 ^{klm}
15	Control		2.32 ± 0.50 ^{defghij}	1.29 ± 0.35 ^{klm}
	300	0.1	2.15 ± 0.34 ^{ghij}	1.15 ± 0.20 ^{klm}
		5	2.39 ± 0.65 ^{cdefghi}	1.23 ± 0.41 ^{klm}
		15	2.46 ± 0.62 ^{abcdefg}	1.19 ± 0.31 ^{klm}
	500	0.1	2.01 ± 0.27 ^{ij}	1.04 ± 0.14 ^{lm}
		5	2.07 ± 0.49 ^{hij}	1.00 ± 0.22 ^{m}
		15	2.43 ± 0.44 ^{bcdefgh}	1.09 ± 0.22 ^{klm}
	600	0.1	2.38 ± 0.85 ^{cdefghi}	1.16 ± 0.40 ^{klm}
		5	2.59 ± 0.49 ^{abcdef}	1.28 ± 0.25 ^{klm}
		15	2.45 ± 0.68 ^{bcdefg}	1.21 ± 0.34 ^{klm}
30	Control		1.94 ± 0.51 ^{j}	1.12 ± 0.37 ^{klm}
	300	0.1	2.24 ± 0.51 ^{efghij}	1.20 ± 0.33 ^{klm}
		5	2.77 ± 0.56 ^{abc}	1.43 ± 0.27 ^{kl}
		15	2.37 ± 0.68 ^{bcdefghij}	1.17 ± 0.44 ^{klm}
	500	0.1	2.72 ± 0.45 ^{abcd}	1.43 ± 0.32 ^{kl}
		5	2.15 ± 0.58 ^{ghij}	1.09 ± 0.35 ^{klm}
		15	2.20 ± 0.27 ^{fghij}	1.07 ± 0.20 ^{klm}
	600	0.1	2.08 ± 0.60 ^{ghij}	1.04 ± 0.21 ^{klm}
		5	2.83 ± 0.33 ^{ab}	1.30 ± 0.18 ^{klm}
		15	2.89 ± 0.72 ^{a}	1.30 ± 0.30 ^{klm}

Different superscripts in the same row indicate significant difference ($p<0.05$). Data average ± SD (n=3)

Chapter 7. Results and Discussion. Vitamin C

Effect of high hydrostatic pressure on vitamin C from kiwifruit puree

Effect of pressurization on concentration of ascorbic acid (AA), dehydroascorbic acid (DHAA) and total vitamin C (Vit C) is summarized in Table 7-1. Analysis carried out after pressure treatment resulted in purees with value of AA (88.3-92.7 mg/100 g), equivalent to the content in kiwifruit, that is 92.7 mg/100 g (Tavarini et al., 2008). As is to be expected, values for total Vit C content were higher than AA content, as the total Vit C is the result of AA and DHAA. DHAA shown higher variation in its content, but neither AA nor Vit C presented significant differences (*p>0.05*) right after pressure treatment for kiwifruit puree samples. In spite the fact the differences were not significant (*p>0.05*), the highest of the measured values for AA, DHAA and Vit C were found in the non-pressurized sample (Table 7-1)

Several authors have found slightest or none losses in the initial Vit C content in apple puree (Landl, Abadias, Sárraga, Viñas, & Picouet, 2010), and in kiwifruit puree treated at 500 MPa for 3 min. (Butz et al., 2003; Fernández-Sestelo et al., 2013). found that HHP treatment did not induce loss of Vit C in vegetable and fruit products in measurements directly after treatment. (Yen & Lin, 1996) studying guava puree also found no pressure influence in AA decline in the first day of analysis, but report that puree began to decline after 10 and 20 days.

Storage time

The study of kiwifruit puree storage is presented in (Table 7-1). During storage, a slight, but constant decline of AA content was observed. After the first fifteen days, the AA decay occurred in all samples. When compared to themselves at day 0, pressurized purees lost around 25% of AA. These losses were significant (*p<0.05*), being bigger to treatment of 300 MPa at 5 and 15 min and 600 MPa at 15 min. Holding time did not influence in results for day 15, and samples of this day did not differ significantly (*p>0.05*) between them. But again, the highest values for AA were found on non-pressurized samples.

Samples got to the day 30 with little additional losses (7%) on AA. The decrease approached to 29% comparing to day 0 and 32% comparing to control from day 0. The

non-pressurized purees reached the lower AA contain. Differences were found between control (day 30) and treatments of 500 and 600 MPa for 5 min.

Ascorbic acid reached at day 60 with total losses around 79% since first day of storage, and 80% regarding fresh puree. The losses in relation to the others days were around 71% (day's 15 and 30). Samples showed more retention for control and sample treated at 300 MPa for 0.1 min and less for 500 MPa/0.1 min.

Dehydroascorbic acid results from day 0 did not present difference for treated samples and control. The biggest loss occurred for samples pressurized at 300 MPa/ 0.1 min. In general, losses were around 20% after pressurization. As in AA contain DHAA showed trend to retain more compound at 300 and 500 MPa when applied larger holding time.

In analysis of day 15 it is observed average losses of 56%, the control and pressurized purees samples were that lost more. The DHAA decline increase for 65% if compared to control from day 0. Differences only were observed between samples of 300 MPa at 5 min and 600 MPa at 0.1 and 5 min.

Observed results for DHAA in days 30 losses got to 66% in relation to the day 15 and 85% to day 0. No influence of pressure of time were found in this day. In the final of study (day 60) no DHAA was detected.

The Vit C start to have significant losses in day 15 (33%) and followed this trend until day 60 with losses around 85%. HHP processing do not affect negatively the Vit C contents at day 15, on the other hand do not affect in the preservation. The deterioration seems be due to the storage time as non-pressurized sample also had a reduction on Vit C because of losses of AA and DHAA. The Vit C losses in minimally processed fruit is related for various authors and the loss is extremely variable among different fruits and vegetables (Tavarini et al., 2008).This degradation could be explained by the oxidation of AA in DHAA, which is irreversible (Landl et al., 2010)

Table 7-1. Effect of different HHP treatments at 10 °C on the ascorbic acid, dehydroascorbic acid and total vitamin C of kiwifruit puree storage for 60 days at 8 °C.

Day	MPa	min	Ascorbic acid (mg/100 g)	Dehydroascorbic acid (mg/100 g)	Total Vit C (mg/100 g)
0	Control		94.4 ± 5.4 ^{a}	33.4 ± 3.2 ^{a}	127.9 ± 8.5 ^{a}
	300	0	87.6 ± 2.0 ^{a}	25.0 ± 3.2 ^{abc}	112.6 ± 5.3 ^{a}
		5	87.4 ± 5.1 ^{a}	31.3 ± 1.9 ^{a}	118.7 ± 7.0 ^{a}
		15	90.2 ± 3.5 ^{a}	32.0 ± 3.8 ^{a}	122.2 ± 7.3 ^{a}
	500	0	91.1 ± 3.8 ^{a}	25.9 ± 3.3 ^{abc}	117.0 ± 7.0 ^{a}
		5	89.6 ± 3.1 ^{a}	28.9 ± 3.7 ^{a}	118.4 ± 6.8 ^{a}
		15	90.9 ± 0.5 ^{a}	31.1 ± 6.4 ^{a}	122.0 ± 6.9 ^{a}
	600	0	87.5 ± 0.6 ^{a}	25.2 ± 2.1 ^{abc}	112.7 ± 2.7 ^{a}
		5	92.7 ± 0.0 ^{a}	26.8 ± 0.4 ^{abc}	119.5 ± 0.4 ^{a}
		15	88.3 ± 0.2 ^{a}	25.7 ± 5.5 ^{abc}	114.0 ± 5.7 ^{a}
15	0	0	71.5 ± 2.2 ^{b}	9.7 ± 0.9 ^{defghi}	81.2 ± 1.3 ^{bcd}
	300	0	66.8 ± 0.0 ^{bc}	12.0 ± 1.4 ^{defg}	78.8 ± 1.4 ^{bcdef}
		5	65.8 ± 2.6 ^{bcd}	6.5 ± 3.3 ^{fghij}	72.3 ± 5.9 ^{bcdefghi}
		15	66.0 ± 0.6 ^{bcd}	10.6 ± 1.7 ^{defgh}	76.6 ± 2.4 ^{bcdefg}
	500	0	66.9 ± 1.7 ^{bc}	11.2 ± 2.5 ^{defgh}	78.2 ± 4.2 ^{bcdef}
		5	69.7 ± 2.7 ^{bcde}	13.1 ± 1.0 ^{def}	79.7 ± 3.0 ^{bcde}
		15	68.5 ± 1.0 ^{bc}	11.5 ± 2.3 ^{defgh}	80.0 ± 1.3 ^{bcde}
	600	0	69.1 ± 0.9 ^{bc}	17.5 ± 2.8 ^{bcd}	86.6 ± 3.8 ^{b}
		5	67.3 ± 0.6 ^{bc}	16.6 ± 2.4 ^{cde}	83.9 ± 3.0 ^{bc}
		15	65.8 ± 2.5 ^{bcd}	13.3 ± 0.1 ^{def}	79.1 ± 2.6 ^{bcdef}
30	Control		55.9 ± 0.2 ^{ef}	3.3 ± 0.0 ^{ghij}	59.2 ± 0.2 ^{hij}
	300	0	65.3 ± 1.5 ^{bcde}	4.8 ± 0.6 ^{fghij}	70.1 ± 2.1 ^{cdefghi}
		5	60.4 ± 3.5 ^{cdef}	1.2 ± 1.1 ^{ij}	61.6 ± 4.6 ^{ghij}
		15	61.3 ± 3.8 ^{cdef}	2.5 ± 0.3 ^{hij}	63.8 ± 3.4 ^{fghij}
	500	0	61.2 ± 1.5 ^{cdef}	3.2 ± 0.1 ^{ghij}	64.4 ± 1.5 ^{efghij}
		5	64.0 ± 1.1 ^{bc}	10.0 ± 0.4 ^{defghi}	77.1 ± 2.1 ^{bcdefg}
		15	64.7 ± 3.1 ^{bcde}	3.1 ± 1.8 ^{ghij}	67.7 ± 1.3 ^{defghij}
	600	0	64.7 ± 0.8 ^{bcde}	7.7 ± 3.1 ^{efghij}	72.4 ± 2.3 ^{bcdefghi}
		5	66.6 ± 4.3 ^{bc}	6.7 ± 2.1 ^{fghij}	73.3 ± 2.3 ^{bcdefgh}
		15	63.3 ± 3.2 ^{bcdef}	4.5 ± 0.2 ^{fghij}	67.8 ± 3.4 ^{defghij}
60	Control		56.8 ± 2.1 ^{def}	0.0 ± 0.0 ^{j}	56.8 ± 2.1 ^{ij}
	300	0	54.2 ± 0.9 ^{f}	0.0 ± 0.0 ^{j}	54.2 ± 0.9 ^{j}
		5	21.5 ± 0.8 ^{ghi}	0.0 ± 0.0 ^{j}	21.5 ± 0.8 ^{k}
		15	15.5 ± 1.0 ^{hij}	0.0 ± 0.0 ^{j}	15.5 ± 1.0 ^{k}
	500	0	11.2 ± 0.8 ^{j}	0.0 ± 0.0 ^{j}	11.2 ± 0.8 ^{k}
		5	13.8 ± 0.4 ^{ij}	0.0 ± 0.0 ^{j}	13.8 ± 0.4 ^{k}
		15	15.6 ± 0.7 ^{hij}	0.0 ± 0.0 ^{j}	15.6 ± 0.7 ^{k}
	600	0	25.6 ± 1.2 ^{g}	0.0 ± 0.0 ^{j}	25.6 ± 1.2 ^{k}
		5	14.8 ± 0.6 ^{hij}	0.0 ± 0.0 ^{j}	14.8 ± 0.6 ^{k}
		15	24.5 ± 2.4 ^{ghi}	0.0 ± 0.0 ^{j}	24.5 ± 2.4 ^{k}

Values are means ± standard deviation (n=6)

Values in the same column with different letter differ significantly ($p<0.05$) of the storage period and for the same storage day

Chapter 8. Results and Discussion. Sensory analyis

Sensory analysis of kiwifruit puree treated by high hydrostatic pressure, and tested by multiple factor analysis (MFA).

Sensory analysis in the first day after HHP treatment

Napping is a holistic sensory method to get the sensory distances between products. This was the test selected to determine similarities and differences on the treated samples. Panelists have to arrange all the products on a blank paper sheet according to their own criteria. The closer products are, the more alike the panelist perceives them. To complete Napping test the panelists were asked to describe the appearance, aroma, taste and texture of each sample or group. Data analysis was performed a MFA with coordinates for each panelist as group, as suggested by Pagès (2005). In this way, all the judges have the same weight in the analysis. The data of each group are not scaled because we needed the distances between products.

The averaged judgment of sensory properties of kiwifruit puree based on the position of the samples in the tablecloth by the panelists is presented in

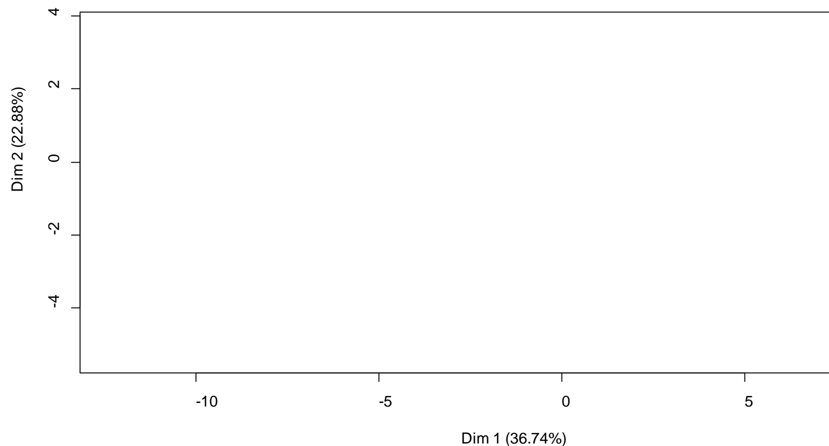


Figure 8-1. On the assessment of the samples, pasteurized puree was perceived as clearly different to control and pressurized samples, as their representation lay apart in the first axis which has an eigenvalue of 14.7. This first axis accounted for 36.74% of the total variance. On the left side in the first axis is the heated sample, apart from the freshly aspect of control and pressurized ones. The second axis, with an eigenvalue of 9.2 and accounting for 22.88% of the total variance, opposes almost all the pressurized sample to the control and pasteurized samples. The relatively high eigenvalue for the third axis of 5.4 suggest to consider it to fully describe the sensory sample characterization. This axis accounted for 13.52% of the total variance on the sensory evaluation. Control and pasteurized samples lay close, along with sample pressurized at 500 MPa/ 15 min. These group was differentiated from the rest of pressurized samples (300 MPa/ 5 min and 600 MPa/ 5 min; 300 MPa/ 15 min, 500 MPa/ 5 min and 600 MPa/ 15 min) that are gathered together.

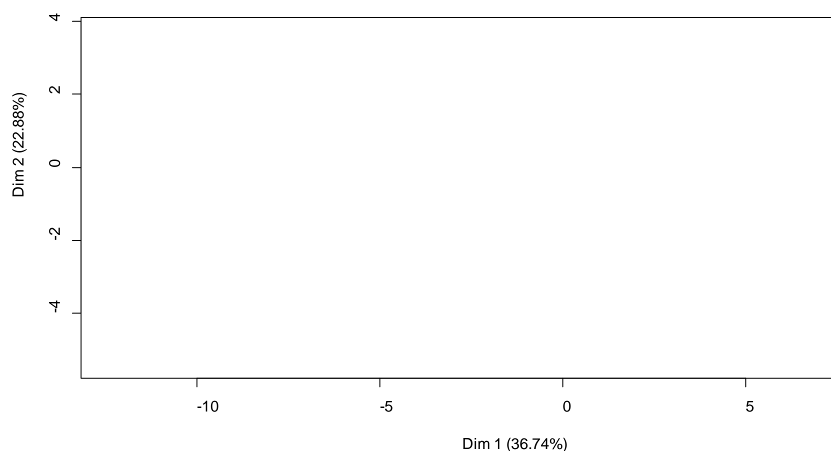


Figure 8-1 Individual factor map obtained from MFA of kiwi puree: (C) Control, (P) Pasteurized, (1) 300 MPa/5 min, (2) 300 MPa/15 min, (3) 500 MPa/5 min, (4) 500 MPa/15 min, (5) 600 MPa/5 min, (6) 600 MPa/15 min.

The hierarchical clustering of puree samples according to the two first axes in MFA analysis is shown in Figure 8-2. Panelists clearly distinguished the thermal effect on the kiwifruit puree. Among the non-thermally treated samples the one closest to the fresh, untreated control sample is one processed at a non extreme pressure intensity (500 MPa for 15 min).

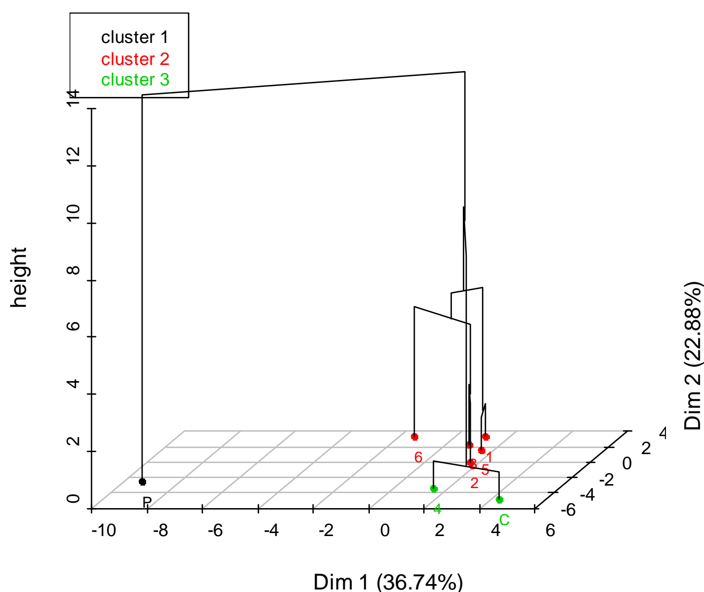


Figure 8-2. Clustering of the kiwifruit pure according to the sensory analysis. Axes correspond to MFA analysis. Code: (C) Control, (P) Pasteurized, (1) 300 MPa/5 min, (2) 300 MPa/15 min, (3) 500 MPa/5 min, (4) 500 MPa/15 min, (5) 600 MPa/5 min, (6) 600 MPa/15 min.

Descriptors assessment

The words associated with each sample can be used to analyze the relations between the samples. Results for descriptor analysis reveal that the first three principal components account for 83.91% of the total variance (Dim1: 44.37%. Dim2: 24.14%. Dim3: 15.40%). Axis 1 has an eigenvalue of 5.32, 2.89 for the second axis and 1.84 for the third axis.

The first component is strongly correlated with characteristics such as strong odor, homogeneity and freshness, and negatively correlates with bad taste, and yellowness (Figure 8-3, correlation circle). Color appearance is better represented in the second axis, with positive scores for green and negative for yellow. This second component shows correlation between the yellow and fluid descriptors and opposite

to green and viscous descriptors. The opposition between sweet and sour descriptors can be seen in dimension 2 and 3 (Figure 8-4, correlation circle).

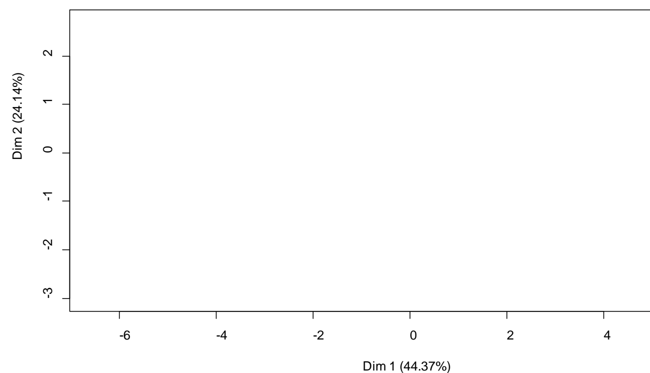


Figure 8-3. Factor map for the two first dimensions obtained from MFA analysis of descriptors of kiwifruit puree. Code: (C) Control, (P) Pasteurized, (1) 300 MPa/5 min, (2) 300 MPa/15 min, (3) 500 MPa/5 min, (4) 500 MPa/15 min, (5) 600 MPa/5 min, (6) 600 MPa/15 min.

Figure 8-4. Factor map for the dimensions 2nd and 3rd obtained from MFA analysis of descriptors of kiwifruit puree. Code: (C) Control, (P) Pasteurized, (1) 300 MPa/5 min, (2) 300 MPa/15 min, (3) 500 MPa/5 min, (4) 500 MPa/15 min, (5) 600 MPa/5 min, (6) 600 MPa/15 min.

Pasteurized kiwifruit puree was classified apart from all the other samples when paying attention to descriptors (Figure 8-3). Negative scores, that characterize the thermal treated sample, are associated to yellow color, weak odor, bad taste and also sweetness, while the positive scores, that characterize the control sample, are linked to homogenous, strong odor and fresh characteristics. Dimension 2 separates the pressure-treated samples and having high scores at viscous, green and sour descriptors (Figure 8-3). The descriptors that most contribute to the Dimension 3 are sweet and sour. These descriptors were scored opposite in the biplot presentation of Dimension 2 and 3 (Figure 8-4).

The clustering analysis (Figure 8-5) confirms the existence of three groups. The most distant group has only the pasteurized kiwifruit puree. The other two groups, more similar among them, include control and pressure-treated samples. Control was perceived as more similar to puree treated for 15 min at 300 MPa or 500 MPa. Puree treated at 600 MPa (5 or 15 min) and the samples treated for 5 min at 300 MPa or 500 MPa were perceived as similar.

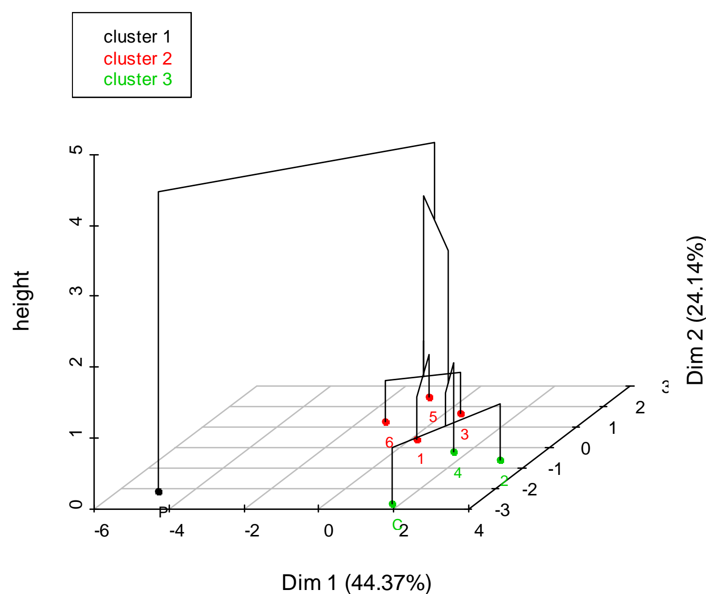


Figure 8-5. Hierarchical clustering of the factor map obtained from MFA analysis of descriptors of kiwifruit puree. Code: (C) Control, (P) Pasteurized, (1) 300 MPa/5 min, (2) 300 MPa/15 min, (3) 500 MPa/5 min, (4) 500 MPa/15 min, (5) 600 MPa/5 min, (6) 600 MPa/15 min.

Sensorial analysis of kiwifruit puree storage

Day 15

On the sensory analysis after 15 days of refrigerated storage there were still relevant differences between samples. The MFA of the perceived differences accounts for 77% of the total variance with the 3 first components (Table 2). The first dimension alone explains more than 50% of the variance. The scores of the samples on this dimension 1 are clearly different for control, pasteurized and pressure-treated samples. While unprocessed samples have large positive scores and thermal-treated have large negative all pressure-treated samples lay clustered with intermediate scores in the first dimension (*Figure 8-6*).

Table 8-1. Descriptive characteristics of the MFA analysis of perceived differences

	Eigenvalue	*percentage of variance*	*cumulative percentage of variance*
Dim 1	16.85	52.66	52.66
Dim 2	4.09	12.78	65.44
Dim 3	3.84	12.00	77.44

Attending to the information contained on the two first dimensions of MFA the pressurized samples belong to a tight group (Figure 8-6 and Figure 8-7). It is noteworthy that control sample was prepared freshly for the sensory analysis and was not preserved 15 days as their counterparts. Untreated kiwifruit puree cannot withstand so long time, even under refrigeration. As pressurized samples have evolved a little bit during these 15 days, the difference with the unprocessed puree is larges that the distance at day 0.

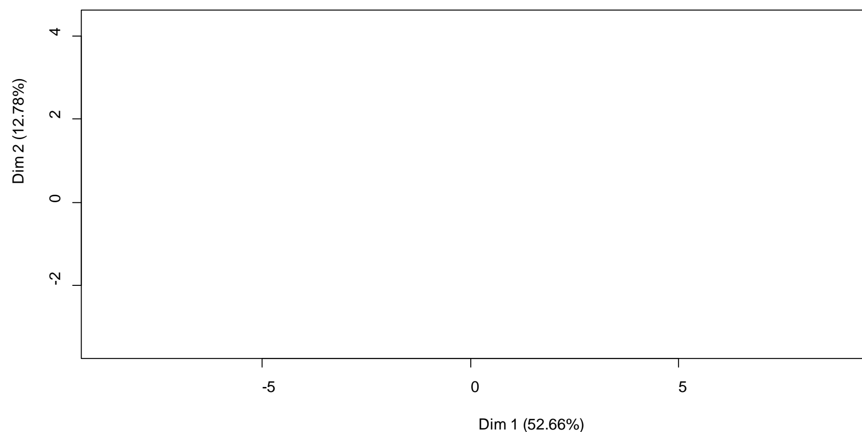


Figure 8-6. Factor map obtained from MFA analysis of the perceived differences on kiwifruit puree. Code: (C) Control, (P) Pasteurized, (1) 300 MPa/5 min, (2) 300 MPa/15 min, (3) 500 MPa/5 min, (4) 500 MPa/15 min, (5) 600 MPa/5 min, (6) 600 MPa/15 min

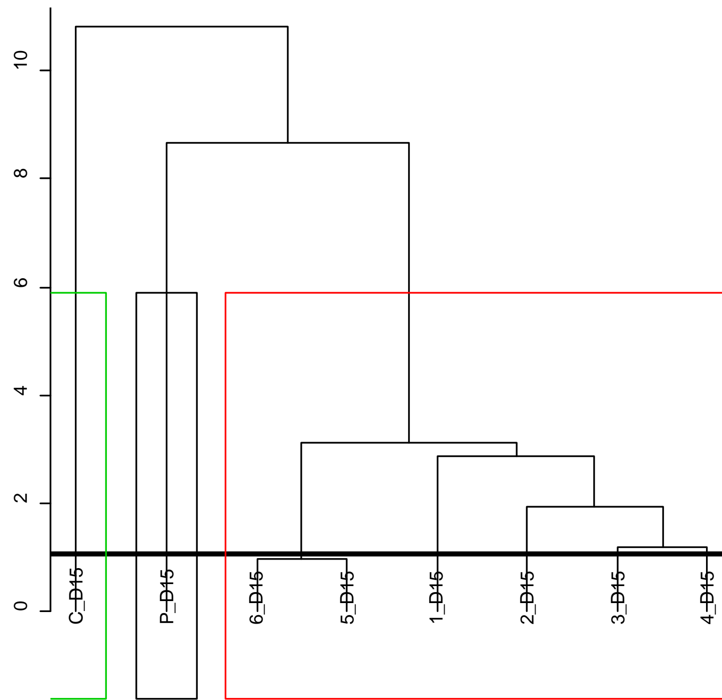


Figure 8-7. Hierarchical analysis of the clusters obtained from MFA analysis of the perceived differences of kiwi puree. Code: (C) Control, (P) Pasteurized, (1) 300 MPa/5 min, (2) 300 MPa/15 min, (3) 500 MPa/5 min, (4) 500 MPa/15 min, (5) 600 MPa/5 min, (6) 600 MPa/15 min.

Third dimension contains information related with the intensity of pressure treatments. Lower pressure and shorter time treatments lay on the lower part and the more intense treatments receive higher scores.

Words day 15

The MFA analysis of the words used by the panelist to describe the samples stored for 15 days also presents high loadings on the first dimension. The 3 first dimensions accounts for 87% of the variance, being the first one responsible of 59% of the total variance (Table 3).

Table 8-2. Descriptive characteristics of the MFA analysis of de descriptors used for the kiwifruit puree

	Eigenvalue	*percentage of variance*	*cumulative percentage of variance*
Dim 1	5.92	59.19	59.19
Dim 2	1.72	17.20	76.39
Dim 3	1.12	11.24	87.63

Regarding the vocabulary used in samples of day 15, four knew words were employed: bitter, dark, cooked and fermented. These descriptors were associated to

thermally-treated and pressure-treated samples, all withstanding two weeks of refrigerated storage. Whereas control was related with descriptors as sweet, fresh, green, firm and yellow. Second axis allows to distinguish the samples pasteurized and treated at 300 MPa for 5 min from all the others. Its profile was described as cooked and fermented (Figure 8-8).

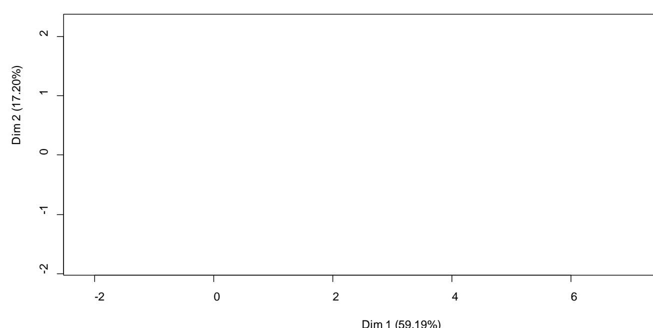


Figure 8-8. Factor map obtained from MFA analysis of descriptors of kiwfruiti puree. Code: (C) Control, (P) Pasteurized, (1) 300 MPa/5 min, (2) 300 MPa/15 min, (3) 500 MPa/5 min, (4) 500 MPa/15 min, (5) 600 MPa/5 min, (6) 600 MPa/15 min.

Day 30

When samples preserved for 30 days under refrigerated conditions were compared to a freshly prepared-unprocessed kiwifruit puree, the distances perceived by the panelists could be described with 3 principal dimensions under MFA analysis. These 3 dimensions accounted for 76% of total variance.

Table 8-3. Descriptive characteristics of the MFA analysis of perceived diferences

	Eigenvalue	*percentage of variance*	*cumulative percentage of variance*
Dim 1	17.10	47.51	47.51
Dim 2	5.94	16.51	64.02
Dim 3	4.55	12.65	76.67

The appearance of purees in the day 30 is exposed in Figure 8-9. As well as happened with the purees in day 15, again panelists grouping pressurized samples separated from pasteurized and control in axis 1 (Figure 8-10). In axis 2 it is perceived an approach between pasteurized and pressurized purees which get closer to the axis left side. The third axis discriminate sample treated 300 MPa for 5 min and 500 MPa for 15 min opposing against to the others pressurized samples.

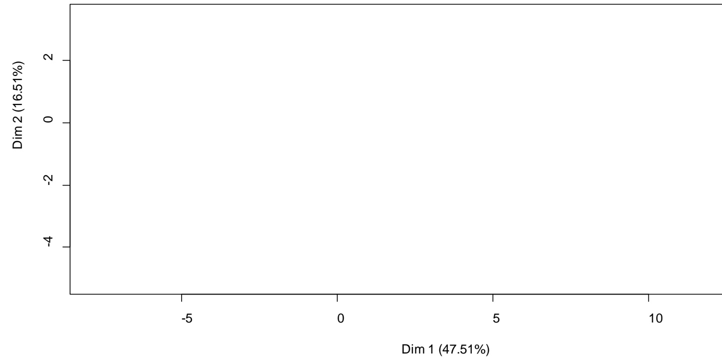


Figure 8-9. Factor map obtained from MFA analysis of the perceived differences on kiwifruit puree. Code: (C) Control, (P) Pasteurized, (1) 300 MPa/5 min, (2) 300 MPa/15 min, (3) 500 MPa/5 min, (4) 500 MPa/15 min, (5) 600 MPa/5 min, (6) 600 MPa/15 min

Figure 8-10. Hierarchical analysis of the clusters obtained from MFA analysis of the perceived differences of kiwifruit puree. Code: (C) Control, (P) Pasteurized, (1) 300 MPa/5 min, (2) 300 MPa/15 min, (3) 500 MPa/5 min, (4) 500 MPa/15 min, (5) 600 MPa/5 min, (6) 600 MPa/15 min.

Words day 30

Table 8-4. Descriptive characteristics of the MFA analysis of the vocabulary used to describe the kiwifruit puree

	Eigenvalue	*percentage of variance*	*cumulative percentage of variance*
Dim 1	6.28	52.31	52.31
Dim 2	2.98	24.82	77.13
Dim 3	1.41	11.72	88.86

Map projected from the terms used by panelists in analysis at day 30 are represented in Figure 8-11. Like panelist arrange samples on the tablecloth, also the descriptors were mentioned in a way that the control opposed the others samples. Correlating descriptors to the individual map, it is possible to observe that all samples, which were storage, had characteristics like off flavor fermented, fluid, cooked, dark, bitter and yellow, with distance between pressurized and pasteurized samples.

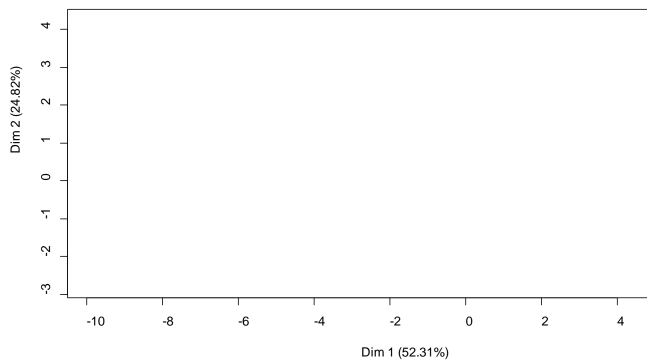


Figure 8-11. Factor map obtained from MFA analysis of descriptors of kiwifruit puree. Code: (C) Control, (P) Pasteurized, (1) 300 MPa/5 min, (2) 300 MPa/15 min, (3) 500 MPa/5 min, (4) 500 MPa/15 min, (5) 600 MPa/5 min, (6) 600 MPa/15 min.

Chapter 9. References

Agriculture, U. S. D. of. (2013). USDA National Nutrient Database. Retrieved from http://ndb.nal.usda.gov/

Alegria, C., Pinheiro, J., Gonçalves, E. M., Fernandes, I., Moldão, M., & Abreu, M. (2010). Evaluation of a pre-cut heat treatment as an alternative to chlorine in minimally processed shredded carrot. *Innovative Food Science & Emerging Technologies*, *11*(1), 155–161. http://doi.org/10.1016/j.ifset.2009.10.008

Allende, A., Tomás Barberán, F., & Gil, M. (2006). Minimal processing for healthy traditional foods. *Trends in Food Science & Technology*, *17*(9), 513–519. http://doi.org/10.1016/j.tifs.2006.04.005

Almela, L., Fernández-López, J. a, & Roca, M. J. (2000). High-performance liquid chromatographic screening of chlorophyll derivatives produced during fruit storage. *Journal of Chromatography. A*, *870*(1-2), 483–9.

Antonio, G. C., Faria, F. R., Takeiti, C. Y., & Park, K. J. (2009). Rheological behavior of blueberry. *Ciência E Tecnologia de Alimentos*. http://doi.org/10.1590/S0101-20612009000400006

Artés, F. (2008). PROCESO GENERAL DE ELABORACIÓN DE LOS PRODUCTOS VEGETALES MÍNIMAMENTE PROCESADOS : HORTALIZAS. In *II Curso Internacional Tecnología PostCosecha y Procesado Mínimo* (pp. 1–25). Cartagena - España.

Balasubramaniam, V. M., & Farkas, D. (2008). High-pressure Food Processing. *Food Science and Technology International*, *14*(5), 413–418. http://doi.org/10.1177/1082013208098812

Benítez, S., Achaerandio, I., Sepulcre, F., & Pujolà, M. (2013). Aloe vera based edible coatings improve the quality of minimally processed "Hayward" kiwifruit. *Postharvest Biology and Technology*, *81*, 29–36. http://doi.org/10.1016/j.postharvbio.2013.02.009

Butz, P., Fern, A., Lindauer, R., Dieterich, S., Bogn, A., & Tauscher, B. (2003). Influence of ultra high pressure processing on fruit and vegetable products, *56*, 233–236.

Buzrul, S., Alpas, H., Largeteau, A., & Demazeau, G. (2008). Inactivation of Escherichia coli and Listeria innocua in kiwifruit and pineapple juices by high hydrostatic pressure. *International Journal of Food Microbiology*, *124*(3), 275–278. http://doi.org/10.1016/j.ijfoodmicro.2008.03.015

C. Hunter, D., Greenwood, J., Zhang, J., & A. Skinner, M. (2011). Antioxidant and "Natural Protective" Properties of Kiwifruit. *Current Topics in Medicinal Chemistry*, *11*(14), 1811–1820. http://doi.org/10.2174/156802611796235134

Cano, M. P. (1991). HPLC Separation of Chlorophyll and Carotenoid Pigments of Four Kiwi Fruit Cultivars. *J. Agric. Food Chem.*, *39*, 1786–1791.

Cano, M. P., Marin, M. A., Ancos, B. De, & Hayward, D. S. (1993). Original paper Pigment and colour stability of frozen kiwi-fruit slices during prolonged storage, 346–352.

Earnshaw, R. (1996). High pressure food processing, *96*(2), 8–11. http://doi.org/10.1108/00346659610108966

Earnshaw, R. (2009). High pressure food processing. In *Nutrition & Food Science* (Vol. 96, pp. 8–11). MCB UP Ltd. http://doi.org/10.1108/00346659610108966

FAO, F. and A. O. of the U. N. (2014). FAOStat. Retrieved August 8, 2014, from http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor

Fernández-Sestelo, A., de Saá, R. S., Pérez-Lamela, C., Torrado-Agrasar, A., Rúa, M. L., & Pastrana-Castro, L. (2013). Overall quality properties in pressurized kiwi purée: Microbial, physicochemical, nutritive and sensory tests during refrigerated storage. *Innovative Food Science and Emerging Technologies*. http://doi.org/10.1016/j.ifset.2013.06.009

Fuke, Y., Sasago, K., & Matsuoka, H. (1985). Determination of Chlorophylls in Kiwi Fruit and Their Changes during Ripening. *Journal of Food Science*, *50*(5), 1220–1223. http://doi.org/10.1111/j.1365-2621.1985.tb10446.x

Georget, E., Kapoor, S., Winter, R., Reineke, K., Song, Y., Callanan, M., … Mathys, A. (2014). In situ investigation of Geobacillus stearothermophilus spore germination and inactivation mechanisms under moderate high pressure. *Food Microbiology*, *41*, 8–18. Retrieved from http://www.sciencedirect.com/science/article/pii/S0740002014000100

Gianotti, A., Sacchetti, G., Guerzoni, M. E., & Rosa, M. D. (2001). Microbial aspects on short-time osmotic treatment of kiwifruit, *49*.

Gutiérrez-López, G. F., & Barbosa-Cánovas, G. V. (2003). *Food Science and Food Biotechnology*. CRC Press LLC.

Heaton, J. W., & Marangoni, A. G. (1996). Chlorophyll degradation in processed foods and senescent plant tissues. *Trends in Food Science & Technology*, *7*(1), 8–15. http://doi.org/10.1016/0924-2244(96)81352-5

Hörtensteiner, S., & Kräutler, B. (2011). Chlorophyll breakdown in higher plants. *Biochimica et Biophysica Acta*, *1807*(8), 977–88. http://doi.org/10.1016/j.bbabio.2010.12.007

Hortensteiner, S., & Matile, P. (2004). How Leaves Turn Yellow : Catabolism of Chlorophyll, 189–202.

Husson, A. F., Le, S., Cadoret, M., & Husson, M. F. (2015). Package " SensoMineR ."

Johns Hopkins. (n.d.). Food Processing Background Reading. In *Teaching the Food System: A Project of the Johns Hopkins Center for a Livable Future* (pp. 1–9).

Kaya, A., Aydın, O., & Kolaylı, S. (2010). Effect of different drying conditions on the vitamin C (ascorbic acid) content of Hayward kiwifruits (Actinidia deliciosa Planch). *Food and Bioproducts Processing*, *88*(2-3), 165–173. http://doi.org/10.1016/j.fbp.2008.12.001

Koca, N., Karadeniz, F., & Burdurlu, H. S. (2007). Effect of pH on chlorophyll degradation and colour loss in blanched green peas. *Food Chemistry*, *100*(2), 609–615. http://doi.org/10.1016/j.foodchem.2005.09.079

Landl, a., Abadias, M., Sárraga, C., Viñas, I., & Picouet, P. a. (2010). Effect of high pressure processing on the quality of acidified Granny Smith apple purée product. *Innovative Food Science and Emerging Technologies*. http://doi.org/10.1016/j.ifset.2010.09.001

Le, S., & Husson, F. (2008). SENSOMINER: a package for sensory data analysis. *Journal of Sensory Studies*, *23*(1), 14–25. http://doi.org/10.1111/j.1745-459X.2007.00137.x

Leunda, M. A., Guerrero, S. N., & Alzamora, S. M. (2000). Color and chlorophyll content changes of minimally processed kiwifruit. *Journal of Food Processing and Preservation*, *24*(1), 17–38. http://doi.org/10.1111/j.1745-4549.2000.tb00403.x

Maceiras, R., Álvarez, E., & Cancela, M. a. (2007). Rheological properties of fruit purees: Effect of cooking. *Journal of Food Engineering*, *80*(3), 763–769. http://doi.org/10.1016/j.jfoodeng.2006.06.028

Mackinney, G., & Weast, C. A. (1940). Color Changes in Green Vegetables. *Industrial & Engineering Chemistry*, *32*(3), 392–395. Retrieved from http://dx.doi.org/10.1021/ie50363a025

MAGRAMA, Ministerio de Agricultura, A. y M. A. (2014a). Anuario de Estadística. Retrieved from http://www.magrama.gob.es/es/estadistica/temas/publicaciones/anuario-de-estadistica/

MAGRAMA, Ministerio de Agricultura, A. y M. A. (2014b). Encuesta sobre Superficies y Rendimientos Cultivos (ESYRCE): mapas de los principales grupos de cultivos. Retrieved from http://www.magrama.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/esyrce/mapas-de-los-principales-grupos-de-cultivos/

Maskan, M. (2001). Kinetics of colour change of kiwifruits during hot air and microwave drying. *Journal of Food Engineering*, *48*(2), 169–175. http://doi.org/10.1016/S0260-8774(00)00154-0

Monteiro, C. A., & Levy, R. B. (2010). A new classification of foods based on the extent and purpose of their processing. *Cadernos de Saúde Pública*, *26*(11), 2039–2049.

Morand, E., & Pagès, J. (2006). Procrustes multiple factor analysis to analyse the overall perception of food products. *Food Quality and Preference*, *17*(1-2), 36–42. Retrieved from http://www.sciencedirect.com/science/article/pii/S0950329305001527

Mor-Mur, M., & Yuste, J. (2005). Microbial Aspects of High- pressure Processing. In *Emerging Technologies for Food Processing* (pp. 0–12).

Morton, J. F. (2004). Fruits of Warm Climates. In *Fruits of Warm Climates* (pp. 293–300). 20534 SW 92 Ct. Miami, FL. 33189: Julia F. Morton.

Naidu, K. A. (2003). Vitamin C in human health and disease is still a mystery? An overview. *Nutrition Journal*, *2*(1), 7. http://doi.org/10.1186/1475-2891-2-7

Nindo, C. I., Tang, J., Powers, J. R., & Takhar, P. S. (2007). Rheological properties of blueberry puree for processing applications. *LWT - Food Science and Technology*, *40*(2), 292–299. http://doi.org/10.1016/j.lwt.2005.10.003

O'Connor-Shaw, R. E., Roberts, R., Ford, A. L., & Nottingham, S. M. (1994). Shelf life of minimally processed honeydew, kiwifruit, papaya, pineaple and cantaloup. *Journal of Food Science*, *59*(6), 1202–1215.

Oey, I., Van der Plancken, I., Van Loey, A., & Hendrickx, M. (2008). Does high pressure processing influence nutritional aspects of plant based food systems? *Trends in Food Science & Technology*, *19*(6), 300–308. http://doi.org/10.1016/j.tifs.2007.09.002

Oliveira, M. A. de, Maciel de Souza, V., Morato Bergamini, A. M., & De Martinis, E. C. P. (2011). Microbiological quality of ready-to-eat minimally processed vegetables consumed in Brazil. *Food Control*, *22*(8), 1400–1403. http://doi.org/10.1016/j.foodcont.2011.02.020

Pagès, J. (2005). Collection and analysis of perceived product inter-distances using multiple factor analysis: Application to the study of 10 white wines from the Loire Valley. *Food Quality and Preference*, *16*(7), 642–649. Retrieved from http://www.sciencedirect.com/science/article/pii/S0950329305000224

Palou, E., Hernández-Salgado, C., López-Malo, a., Barbosa-Cánovas, G. V., Swanson, B. G., & Welti-Chanes, J. (2000). High pressure-processed guacamole. *Innovative Food Science & Emerging Technologies*, *1*(1), 69–75. http://doi.org/10.1016/S1466-8564(99)00002-8

Pandrangi, S., & Balasubramaniam, V. (2005). *Emerging Technologies for Food Processing*. *Emerging Technologies for Food Processing*. Elsevier. http://doi.org/10.1016/B978-012676757-5/50004-9

Pilkington, S. M., Montefiori, M., Jameson, P. E., & Allan, A. C. (2012). The control of chlorophyll levels in maturing kiwifruit. *Planta*, *236*(5), 1615–28. http://doi.org/10.1007/s00425-012-1723-x

Properties, R., Mango, O. F., & Nectar, P. (2005). RHEOLOGICAL PROPERTIES OF MANGO AND PAPAYA NECTAR, *43*(2), 665–685.

Puntes, E. M., Rubio, L. a, & Cabeza, M. S. (2012). Scientia Agropecuaria Comportamiento de flujo y caracterización fisicoquímica de pulpas de durazno Flow behavior and physicochemical characterization of peach pulps.

Rafols, M. (2000). *Guía completa del cultivo del kiwi*. (E. De Vecchi, Ed.).

Ragaert, P., Verbeke, W., Devlieghere, F., & Debevere, J. (2004). Consumer perception and choice of minimally processed vegetables and packaged fruits. *Food Quality and Preference*, *15*(3), 259–270. Retrieved from http://www.sciencedirect.com/science/article/pii/S0950329303000661

Raso, J., & Barbosa-Cánovas, G. V. (2003). Nonthermal Preservation of Foods Using Combined Processing Techniques. *Critical Reviews in Food Science and Nutrition*, *43*(3), 265–285. Retrieved from http://www.tandfonline.com/doi/abs/10.1080/10408690390826527#.Vgm4vRLtmko

Rastogi, N. K., Raghavarao, K. S. M. S., Balasubramaniam, V. M., Niranjan, K., & Knorr, D. (2007). Opportunities and challenges in high pressure processing of foods. *Critical*

Reviews in Food Science and Nutrition, *47*(1), 69–112. http://doi.org/10.1080/10408390600626420

Rico, D., Martín-Diana, a. B., Barat, J. M., & Barry-Ryan, C. (2007). Extending and measuring the quality of fresh-cut fruit and vegetables: a review. *Trends in Food Science & Technology*, *18*(7), 373–386. http://doi.org/10.1016/j.tifs.2007.03.011

Rocculi, P., Romani, S., & Rosa, M. D. (2005). Effect of MAP with argon and nitrous oxide on quality maintenance of minimally processed kiwifruit. *Postharvest Biology and Technology*, *35*(3), 319–328. http://doi.org/10.1016/j.postharvbio.2004.09.003

Rocha, a. M. C. ., & Morais, a. M. M. . (2003). Shelf life of minimally processed apple (cv. Jonagored) determined by colour changes. *Food Control*, *14*(1), 13–20. http://doi.org/10.1016/S0956-7135(02)00046-4

San Martín, M. F., Barbosa-Cánovas, G. V, & Swanson, B. G. (2002). Food processing by high hydrostatic pressure. *Critical Reviews in Food Science and Nutrition*, *42*(6), 627–45. http://doi.org/10.1080/20024091054274

Shastri, K. V., Bhatia, V., Parikh, P. R., & Vinaya N., C. (2012). Actinidia deliciosa: a review. *International Journal of Pharmaceutical Sciences and Research*, *3*(10), 3543–3549.

Suárez-Jacobo, Á., Rüfer, C. E., Gervilla, R., Guamis, B., Roig-Sagués, A. X., & Saldo, J. (2011). Influence of ultra-high pressure homogenisation on antioxidant capacity, polyphenol and vitamin content of clear apple juice. *Food Chemistry*, *127*(2), 447–454. http://doi.org/10.1016/j.foodchem.2010.12.152

Suman, M., Maria, R. De, & Catellani, D. (2008). Chromatographic evaluation of chlorophyll derivatives in pasta-based food products : effects of pasteurization treatments and correlation with sensory profiles, *478*(August 2007), 471–478. http://doi.org/10.1002/jsfa

Tavarini, S., Degl'Innocenti, E., Remorini, D., Massai, R., & Guidi, L. (2008). Antioxidant capacity, ascorbic acid, total phenols and carotenoids changes during harvest and after storage of Hayward kiwifruit. *Food Chemistry*, *107*(1), 282–288. http://doi.org/10.1016/j.foodchem.2007.08.015

Ward, C., & Courtney, D. (2013). Kiwifruit: taking its place in the global fruit bowl. *Advances in Food and Nutrition Research*. Retrieved from https://books.google.es/books?hl=ca&lr=&id=FtkmUL1yCjsC&oi=fnd&pg=PA1&dq=kiwi+ward+2013+carol&ots=wc6CwFb-xT&sig=FCEm1nFaGVyeh8XQRS4KSemCWww

Yen, G.-C., & Lin, H.-T. (1996). Comparison of high pressure treatment and thermal pasteurization effects on the quality and shelf life of guava puree. *International Journal of Food Science and Technology*, *31*(2), 205–213. http://doi.org/10.1111/j.1365-2621.1996.331-32.x

Chapter 10. Conclusions

1. The microbiological evaluation revealed that pressures applied in this study were able to inactivate the natural microbiota of kiwifruit puree. Spores were the only survivors and were not affected by the pressure. During storage time the survivors were not able to evolve in the conditions studied.
2. *Staphylococcus aureu*s were diminished by treatments at 500 and 600 MPa with holding time of 5 and 15 min and maintained below detection limit during storage time. HHP treatments at 350 MPa for 10 min and 500 and 650 MPa for 2 and 10 min were sufficient to reduce the initial *Escherichia coli* counts.
3. Treatments between 300 MPa for 5 min at 10 and 40 °C and 700 MPa for 15 min at 10 and 40°C caused the pressurized kiwifruit puree to present less luminosity. Based on the results of HHP treatments at 10°C, the kiwifruit was less affected by green colour loss. The process resulted in fruits that maintained and even improved the initial green colour of kiwifruit puree. However, during the storage time the HHP treatment was not able to maintain the green colour of the fruit and the result was a puree that resembled the control and the samples pressurized at 40 °C.
4. The chlorophylls were not preserved after high pressure treatments, while the opposite fact was found to pheophytins. The HHP treatment was not capable of maintaining the chlorophylls contents until the end of the storage study. Nevertheless, do not detected an increase of pheophytin. Further studies are necessaries in order to determine the HHP effect on the chlorophylls degradations in kiwifruit puree.
5. The study indicated that the kiwifruit puree is a non-Newtonian fluid with plastic behaviour. The rheological parameters k and n were affected in a minimal form by the HHP treatments. During the storage time the flow behaviour has shown almost no changes. The viscosity of kiwifruit puree was not affected in a pertinent way by the analysis.
6. Vitamin C is not affected immediately by the HHP treatment. Nonetheless, 85% of the vitamin was lost during the study. The lost was, thus, caused by storage time and not by the HHP treatment.
7. Napping test has been effective in order to discriminate pressurized and heated puree and fresh ones. The descriptors allowed understanding the napping configuration and creating the possible to distinguish a correlation between the components and product characteristics perceived by the panellist.
8. Finally, the HHP did not affect in a negative way the kiwifruit puree (with the notable exception of the chlorophylls). However, it is difficult to determine the exact levels of treatment due to the irregular results of each pressure on the different analysed aspect of the puree. Most of the harmful effects were caused by storage time.