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Improving marinade absorption and shelf life of vacuum packed marinated pork chops through the application of high pressure processing as a hurdle

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ABSTRACT

The objective of this study was to determine the efficacy of HPP to accelerate marinade (piri-piri) absorption in pork chops and to study the effects on the physicochemical, sensory and microbiological characteristics during storage. HPP (300 MPa, 400 MPa or 500 MPa) and organic acids Inbac™ (0.3%) were used as hurdles to extend the shelf life. The results showed that HPP ≥ 400 MPa increased ($P < 0.05$) the marinade absorption which enhanced the flavour acceptability of the marinated pork chops. The piri-piri marinade masked the discolouration caused by HPP and increased ($P < 0.05$) the tenderness of the pork chops over storage. From the microbiological point of view, HPP at 300, 400 or 500 MPa and Inbac™ (0.3%) extended ($P < 0.05$) the shelf life by 16, 22 and 29 days, respectively. The results highlighted the potential of combined effects of HPP and antimicrobial Inbac™ to accelerate marinade absorption and extend the shelf life of marinated pork chops.

1. Introduction

Marinade technology has been used in the meat industry for several decades. The role and perception of marinades has evolved from flavouring and tenderising to enhancing yield and quality of meats. Marinades are also applied to meat products for preservation and to improve colour (Yusop, O'sullivan, & Kerry, 2011). Based on their functionality marinade ingredients are classified into two categories: 1) Ingredients that affect the water-binding or textural properties, and condition the meat to bind water via ionic strength and pH such as water, salt, phosphates, organic acids, hydrocolloids, protein isolates, curing aids and enzymes and 2) ingredients which affect the consumer appeal and the eating quality of marinated meat products such as herbs and spices, flavour extracts and sweeteners (Toledo, 2007).

The demand for value added meat products continues to increase in the marketplace and an increase in the range of commercially available marinade products was reported (Hall, Marks, Campos, & Booren, 2008; Yusop et al., 2011) and flavour components such as barbeque and piri-piri marinade are in high consumer demand (Nachay, 2011). Marinades can increase the sensory acceptability of meat products by enhancing flavour (Yusop et al., 2011). Kim et al. (2010) found that pork marinated with garlic and onion juice had significantly higher ($P < 0.05$) flavour attributes than control samples which were unmarinated. Previous studies have also reported the tenderising effect of acidic marinades (i.e. organic acids) on beef and chicken (Aktaş, Aksu, & Kaya, 2003; Berge et al., 2001; Burke & Monahan, 2003; Lewis & Purslow, 1991; Oreskovich, Bechtel, Mckeith, Novakofski, & Basgall, 1992; Bowker, Callahan, & Solomon, 2010; Birk et al., 2010).

HPP is an alternative method for food preservation which subjects liquid and solid foods, with or without packaging, to pressures between 100 and 800 MPa (Bermúdez-Aguirre & Barbosa-Cánovas, 2011). HPP technology has the advantage of inactivating microorganisms and enzymes at ambient or low temperatures without affecting the nutritional properties of food (Indrawati, Van Loey, Smout, & Hendrickx, 2003); however, pressure levels > 300 MPa can negatively affect some other important product qualities, such as tenderness, colour and lipid oxidation (Cheftel & Culioli, 1997). Synergetic effects on microbial inactivation of HPP when used in combination with organic acids, antimicrobial peptides, the lactoperoxidase system, and phenolic compounds have been reported in the literature (Rodriguez-Calleja, Cruz-Romero, O'sullivan, Garcia-Lopez, & Kerry, 2012; Cheftel & Culioli, 1997; Manas & Pagán, 2005; Raso & Barbosa-Cánovas, 2003).

Several authors have reported that HPP primarily affects the physicochemical properties of raw/uncooked meat products and has minimum effects on cooked products (Considine, Kelly, Fitzgerald, Hill, & Sleator, 2008; Neto et al., 2015; Bansal, Siddiqui, & Rahman, 2015). While many studies report the ability of HPP to increase and safety and shelf life of meat products (Kruk et al., 2011; Garriga, Grebol,

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Aymerich, Monfort, & Hugas, 2004; Wang, Yao, & Gänzle, 2015; Karłowski et al., 2002), many authors also report the negative impact of HPP on the colour (Rodriguez-Calleja et al., 2012; Karłowski et al., 2002; Bajovic, Bolumar, & Heinz, 2012), texture (Sun & Holley, 2010; McArdle, Marcos, Kerry, & Mullen, 2011) and lipid oxidation (Kruk et al., 2011; Medina-Meza, Barnaba, & Barbosa-Cánovas, 2014; He et al., 2012). Such altered physicochemical characteristics may have a negative effect on the sensory acceptability of HPP meat; however, marinades may be able not only to mask the physicochemical changes such as colour and improve the tenderness but also increase sensory acceptability by enhancing flavour of marinated meat products.

Kruk et al. (2011) used HPP at 300–600 MPa for 5 min to extend the shelf life of raw chicken breast fillets and found that HPP at 600 MPa for 5 min inactivated all microorganisms below delectable levels and improved shelf life for 7–14 days; however lipid oxidation, lightness and shear force were significantly increased. Rodriguez-Calleja et al. (2012) demonstrated the strongly potential synergetic interaction of HPP (300 MPa for 5 min) and a mix of organic acids as hurdles extending the shelf life of skinless chicken breast fillets up to 4 weeks and concluded that the combined effect of the antimicrobial edible coating Articoat™ and HPP was more effective than either treatment alone. Wang et al. (2015) examined the effects of HPP (350–600 MPa for 3 min) on the quality and shelf life of honey garlic marinated pork chops and concluded that the marinade partially masked meat discolouration due to HPP, the pH of high pressure processed marinated pork chops was higher ($P < 0.05$) than the control pork chops and a HPP level of 450 MPa or higher for 3 min can extend the shelf life from 10 days to 31 days with minimal effects on meat quality. Kingsley et al. (2015) found that a combination of Sriracha® hot sauce flavouring and HPP at 600 MPa for 5 min yielded a raw oyster with improved sensory quality in regards to flavour and also lower bacterial counts (4 log) over 10 days of refrigerated storage.

In the present study, an industrial scale HPP unit and commercially available organic acids were used to treat marinated pork chops which have the advantage of easily scaling up. HPP and cooking of products in their final packaging also result in an extremely convenient product for consumer use. Other technologies such as hydrodynamic pressure (HDP) have been used to increase marinade absorption. Bowker et al. (2010) applied HDP to turkey breasts before marination via tumbling in a brine consisting of water, salt, and phosphate and found that HDP enhanced the marinade absorption, increased processing yield, which resulted in improved tenderness; however, to the best of our knowledge, there are no studies which have been carried out examining the ability of HPP to accelerate the marinade (e.g. piri-piri sauce) absorption/yield and flavour acceptability of pork chops, and their subsequent physicochemical quality during chilled storage. There are also few studies regarding the ability of various marinades to mask the discolouration caused by HPP. Hence, the objectives of this research was to determine the efficacy of HPP to accelerate the marinade (e.g. piri-piri marinade) absorption in pork chops and investigate the effects of a combination of HPP and a commercially available mix of organic acids on the physicochemical, sensory and microbiological quality of marinated pork chops during chilled storage at 4 °C.

2. Materials & methods

2.1. Materials

Twelve pork loins were obtained on two separate occasions from a local meat processor (Ballyburden, Ballincollig, Cork). Piri-Piri marinade (Rapeseed oil 60%, Spices and flavourings 36% (chilli, garlic, jalapeno, black pepper, onion, paprika, lovage root, fenugreek seed, bird clover, onion leek, coriander, turmeric, ginger, cumin seed, fennel, sugar, grapefruit, passion fruit, papaya, mango, palm fat) and Salt 4%) was obtained from Oliver Carty (Athlone, Co. Roscommon, Ireland). A commercial antimicrobial mix of organic acids Inbac™ (a mix of Sodium acetate 43%, Malic acid 7%, emulsifier-mono and diglycerides of fatty acids and technological coadjuvants; anticaking agents, calcium phosphate, magnesium carbonate and silicon dioxide ˜50%) was obtained from Chemital (Chemital Ltd, Barcelona, Spain).

2.2. Methods

2.2.1. Marination of pork chops

The pork loins were cut into 3 cm chops including the fat ring, weighed and placed in a combivac vacuum pouch (20 polyamide/70 polyethylene bags (Alcom, Campogalliano, Italy) and piri-piri marinade which contained Inbac™ (0.3%) at a weight ratio 80:20 (Pork chop:-marinade) was added and then vacuum packed using a Webomatic vacuum packaging system (Werner Bonk, type D463, Bochum, German) and then randomly assigned to each treatment (0.1 MPa, 300 MPa, 400 MPa, 500 MPa). Marinated control samples were then stored in a chill room at 4 °C. For samples requiring HPP (300 MPa, 400 MPa or 500 MPa), marinated pork chop samples were high pressure processed (as outlined in section 2.2.2) before storage in a chill room at 4 °C for the duration of the shelf life.

2.2.2. High pressure processing

After 24 h, vacuum-packed marinated pork chops were high pressure processed using an industrial Hiperbaric 420 L unit (Burgos, Spain) at the HPP Tolling facilities (HPP tolling, St. Margaret's, Dublin) using water as the pressure transmitting medium. The speed of pressurisation was 130 MPa per minute, the speed of depressurisation was instantaneous (˜ 1 s) and the holding time was 3 min. The temperature of the pressure transmitting medium (water) was 10 °C. The initial temperature of the raw marinated pork chops before HPP was 3.4 °C and the final temperature measured after HPP was 6.5 °C.

2.2.3. Marinade absorption

The initial weight of raw unmarinated pork chops was recorded. Samples were then marinated as described in Section 2.2.1 and after 24 h storage at 4 °C untreated and high pressure processed samples were placed on an elevated stainless steel wire rack for 5 min to allow dripping of the excess marinade and then re-weighed. Calculation for marinade absorption was as follows;

% marinade absorption = (weight after 24 h marination – initial unmarinated weight) / (initial unmarinated weight) * 100.

2.2.4. Cooking

Vacuum-packed marinated pork chops were cooked at full steam (100 °C) in a Zanussi oven (Zanussi Professional, Italy) and temperature monitored using a thermocouple data logger (Omega Engineering Ltd., Manchester, UK) inserted into the coldest point of the marinated pork chops until an internal temperature of 74 °C was reached. The samples were then cooled down at room temperature before analysis was carried out.

2.2.5. Cook loss

The cook loss of both untreated control and high pressure processed marinated pork chops was determined on day 1. Briefly, the initial weight of the raw marinated pork chops was recorded after samples had been placed on an elevated stainless steel wire rack for 5 min. After cooking the samples were re-weighed and cook loss calculated as follows:

% cook loss = (cooked weight – initial raw weight) / (initial raw weight) * 100

2.2.6. Compositional analysis

To obtain a representative sample for proximal composition analysis marinated pork chops, the outer layer of fat was removed after cooking and then homogenised for 1 min in a Buchi™ mixer B-400 (Büchi Labortechnik, Switzerland). Compositional analysis (fat, moisture, protein and ash) of cooked marinated pork chops was determined on day 1 using the methods previously described by O' Neill, Cruz-Romero, Duffy, and Kerry (2018)).

2.2.7. Microbiological analysis

Microbiological analysis of the raw marinated pork chops was carried out throughout storage at 4 °C. Briefly, 10 g of the surface of the raw marinated pork chop was weighed aseptically into a stomacher bag in a vertical laminar-flow cabinet and a primary 10-fold dilution was performed by the addition of 90 ml of sterile maximum recovery diluent (MRD) (Oxoid, Basingstoke, U.K.), stomached (Steward Stomacher 400 Lab Blender, London, UK) for 3 min and homogenates were 10-fold serially diluted using MRD solution. For the enumeration of total viable counts (TVC) 1 ml of each appropriate dilution was inoculated on duplicated plates in the centre of compact dry-total count plates (20 cm^2) (Nissui Pharmaceutical, Co. Ltd., Japan) following incubation at 37 °C for 48 h. Lactic acid bacteria (LAB) was determined on overlaid de Man Rogosa Sharpe medium (Oxoid); 1 ml of each appropriate dilution was inoculated on duplicated plates and incubated at 30 °C for 48 h. *Escherichia coli* (*E. Coli*) and total coliforms were determined using Compact Dry EC plates (Nissui Pharmaceutical, Japan) to which 1 ml of each appropriate dilution was inoculated on duplicated plates (20 cm^2) and incubated at 37 °C for 24 h. At the start and the end of the shelf life, marinated pork chops were tested also for the presence or absence of *Salmonella* spp. in Compact dry SL plates (Nissui Pharmaceutical, Co. Ltd., Japan). For this, pre-enrichment process was carried out by weighing 25 g of sample into a sterile filter stomacher bag and then 225 ml of Buffered Peptone water (Oxoid) was added and homogenised with a stomacher for 1 min and incubated at 37 °C for 24 h. The bag was taken from the incubator and 0.1 ml of enriched specimen was then dropped gently on the sheet 1 cm from the edge of the plate. After inoculation of the enriched culture, 1 ml of sterilized water was dropped at the opposite point where the specimen was dropped. The sterilised water diffused automatically and the sheet was wetted uniformly. The inoculated compact dry SL plates were incubated at 42 °C for 24 hs. All results (except *Salmonella*) were expressed as $\log_{10}$ colony-forming units (CFU/g).

As the shelf life of the products varied, it was impossible to have a similar sampling interval for all samples. For example, the shelf life of the control untreated marinated pork chops was short; therefore, the microbiological and physicochemical analyses had to be carried out more frequently at shorter intervals in order to determine the shelf life of these products.

2.2.8. pH

The pH of raw and cooked untreated and high pressure processed marinated pork chops was measured using a digital pH metre (Mettler-Toledo GmbH, Schwerzenbach, Switzerland) by inserting the glass probe directly into the sample. The pH was measured throughout the shelf life.

2.2.9. Warner-Bratzler shear force

Warner-Bratzler Shear force (WBSF) was measured according to the method outlined by Shackelford et al. (1991). Briefly, the 3 cm thick marinated pork chops were cooked as described in Section 2.2.3 to an internal temperature of 74 °C and then cooled at room temperature (20 °C). Four cylinders of a 1.27 cm diameter were obtained from each pork chop parallel to the muscle fibre direction using a corer. The pork steak cylinders were sheared using a Texture Analyser TA-XT2 (Stable Micro Systems, Surrey, UK) attached with a Warner Bratzler V-shaped shearing device at a crosshead speed of 4 mm/s. The WBSF of the untreated and high pressure processed marinated pork chops were determined throughout the shelf life.

2.2.10. Colour

The colour of the surface of the raw and cooked marinated pork chop was measured as described by O' Neill et al. (2018). CIE L-, a- and b values (Lightness, redness and yellowness, respectively) during chilled storage at 4 °C are reported.

2.2.11. Sensory evaluation

Sensory acceptance testing was carried out using a 25 member taste panel to evaluate the cooked untreated and high pressure processed marinated pork chops over two sessions using a 9-point hedonic scale. The panellists were recruited from staff and postgraduate students at the School of Food and Nutritional Sciences, University College Cork and chosen based on their experience in the sensory analysis of meat products, their liking of marinated pork chops and on their availability. The same 25 panellists partook in all sensory evaluation sessions.

Vacuum pouches containing the raw marinated pork chops were labelled with a three digit random number and panellists evaluated the appearance of the vacuum packed untreated and high pressure processed raw marinated pork chops before sensory evaluation of the cooked samples. Samples for cooked sensory analysis were then labelled and cooked as described in Section 2.2.3 before being removed from the packaging and served warm (~60 °C) in duplicate on labelled polystyrene plates. The tested attributes were: Liking of Appearance (raw), Liking of Appearance (cooked), Liking of Texture, Liking of Flavour, Juiciness, Tenderness, and Overall sensory acceptability (OSA).

To ensure that all samples were safe for consumption, microbiological analysis was carried out before each sensory test. Sensory analysis was carried out at day 1 and when samples reached Log 5 CFU/g because this is directly below the microbiological limit for TVC ($< 5 \times 10^6$ CFU/g of raw meat product) and indicates samples are coming close to the end of shelf life but have not reached it yet. For control samples (0.1 MPa), sensory analysis was carried out on day 8 while that for samples high pressure processed at 300, 400 or 500 MPa was carried out on day 13, 29 or 34, respectively.

2.2.12. Lipid oxidation

Lipid oxidation of the raw marinated pork chops was measured using the 2-thiobarbituric acid (TBA) assay (Siu & Draper, 1978). The malondialdehyde (MDA) content was calculated using an extinction coefficient of $1.56 \times 105\,l\,mol^{-1}\,cm^{-1}$. The lipid oxidation was measured throughout the shelf life and results were expressed as 2-thiobarbituric acid-reactive substances (TBARS) in mg MDA/kg sample.

2.2.13. Statistical analysis

Cook loss, marinade absorption and compositional analysis were tested using one way ANOVA. A two way ANOVA test was carried out on parameters which were measured over storage time (colour, texture, pH, sensory) in order to determine significant differences among treatments. Significant interactions were found between the level of HPP applied and storage time for every analysed parameter ($P < 0.05$), so comparison of data was done by means of one-way ANOVA carried out separately on treatment and storage time. Significance was assessed using Tukey's test at 5% significance level using SPSS software package (SPSS for Windows, version 21 IBM Corp., Armonk, NY, USA.

Principle component analysis (PCA) was carried out using the Unscrambler software package version 10.3 (CAMO ASA, Trondheim, Norway).

3. Results and discussion

3.1. Physicochemical analysis

3.1.1. Marinade absorption & Cook loss

The results showed that marinade absorption/yield of the pork chops high pressure processed at 300 MPa did not increase significantly compared to control untreated samples; however, an increased

Table 1
Physicochemical changes of marinated pork chops.*.

HPP (MPa)	Marinade absorption (%)	Cook Loss (%)	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
0.1	1.95 ± 0.22 [a]	19.00 ± 1.04 [a]	63.37 ± 1.03 [a]	28.15 ± 0.49 [a]	4.42 ± 1.35 [a]	1.54 ± 0.11 [a]
300	2.16 ± 0.19 [ab]	19.10 ± 1.56 [a]	63.50 ± 2.04 [a]	28.31 ± 0.82 [a]	4.78 ± 1.47 [a]	1.59 ± 0.04 [a]
400	2.63 ± 0.31 [bc]	17.75 ± 1.87 [a]	62.85 ± 2.19 [a]	28.51 ± 1.22 [a]	4.9 ± 1.82 [a]	1.56 ± 0.04 [a]
500	2.9 ± 0.48 [c]	17.38 ± 2.09 [a]	63.29 ± 1.04 [a]	29.11 ± 0.38 [a]	4.02 ± 1.92 [a]	1.58 ± 0.34 [a]

* Values are Mean ± standard deviation. [a,b,c] Different superscripts in the same column indicate significant difference ($P < 0.05$) between treatments.

($P < 0.05$) marinade absorption was noticed when the marinated pork chops were high pressure processed at pressures ≥400 MPa (Table 1). It was reported that marinades diffuse from the meat surface into the interior of the meat due to the gradient formed from the higher concentration of marinade to the lower concentration of fluid in the interior of the meat (Yusop et al., 2011). In this study, the higher HPP levels may have accelerated this diffusion process. While previous studies reported that injection of a marinade consisting of salt, tripolyphosphate and bicarbonate can increase the yield and tenderness of pork loin (Sheard & Tali, 2004); however; to the best of our knowledge, there are no studies on the ability of HPP to increase the marinade (e.g. piri-piri) absorption of flavour components via immersion.

3.1.2. Compositional analysis & Cook loss

The results for compositional analysis showed that HPP did not significantly affect the moisture, protein, fat or ash content of the cooked marinated pork chops (Table 1) which correlated with the cook loss results which were not significantly different. Conversely, Kruk et al. (2011) reported HPP increased significantly the moisture content of cooked chicken breast fillets when chicken breast fillets were high pressure processed at 300, 450 or 600 MPa for 5 min and this moisture increase was attributed to the significant cook loss differences.

3.1.3. Colour

At day 1, high pressure processed raw marinated pork chops had significantly ($P < 0.05$) higher CIE L-, and b-values (lightness and yellowness, respectively) and the lowest a-values (redness) compared to control untreated raw marinated pork chops (0.1 MPa) (Table 2). Untreated control samples were the darkest ($P < 0.05$) with lightness increasing ($P < 0.05$) proportionally as the pressure level applied increased with raw marinated pork chops high pressure processed at 500 MPa showing the highest ($P < 0.05$) lightness. Colour changes in muscle food products after HPP have been reported that may be related to the denaturation of the myofibrillar and sarcoplasmic proteins (Ma & Ledward, 2013; Zhou, Xu, & Liu, 2010). Similar results have been reported by Carlez, Veciana-Nogues, and Cheftel (1995)) who suggested that fresh meat discolouration after HPP at 200–350 MPa is due to a "whitening" effect (increase in L* values) caused by globin denaturation, haem release or displacement or by oxidation of ferrous myoglobin to ferric metmyoglobin when fresh meat is high pressure processed at pressures ≥400 MPa. Goutefongea, Rampon, Nicolas, and Dumont (1995)) also suggested discolouration due to HPP occurs as a results of protein coagulation which would affect sample structure and surface properties. Kruk et al. (2011) high pressure processed raw chicken breast fillets at 300, 450 or 600 MPa for 5 min and found that the lightness and yellowness increased significantly and this increase was proportional to the pressure level applied which is in agreement with our findings that the increased lightness depended on the pressure level applied.

During storage, the lightness of both raw untreated control and raw high pressure processed marinated pork chops decreased ($P < 0.05$) significantly and these significant changes were noticed on day 11 for raw untreated control marinated pork chops or on day 16, 23 or 30 for samples that were high pressure processed at 300, 400 or 500 MPa, respectively. The decreased lightness may be due to the presence of oxidised products of meat pigments which have a brown and darker colour (Wettasinghe & Shahidi, 1997). It was also reported that the enzymatic systems can also be affected by HPP and this could explain the progressive accumulation of metmyoglobin content during storage (Jung, Ghoul, & de Lamballerie-Anton, 2003). Regarding the redness and yellowness, these colour parameters did not change significantly during storage in untreated control or high pressure processed samples and this may be due to presence on the surface of the meat of the piri-piri marinade which is highly pigmented with L-, a and b-values of 26.86, 17.68 and, 54.28, respectively. Throughout storage, the colour differences observed on day 1 among raw untreated control samples and high pressure processed samples (higher lightness, higher yellowness and decreased redness in high pressure processed samples) were also observed during chilled storage.

After cooking, untreated and high pressure processed marinated pork chops became significantly ($P < 0.05$) darker, less red and less yellow (Table 3). The decrease in lightness may be due to the denaturation of myoglobin as the cooked pigment is denatured metmyoglobin which is darker in colour (Boles & Pegg, 1999). Decreased redness and yellowness may be due to loss of the red and yellow marinade pigments due to cook loss. Compared to cooked untreated control samples, greater colour changes were noticed on cooked high pressure processed marinated pork chops which may be due to the fact that when marinated pork chops were high pressure processed it may have caused denaturation of proteins before cooking and then the cooking process may have caused further protein denaturation and apparently resulting in higher degree colour change.

On day 1, independent of the pressure level applied cooked high pressure processed marinated pork chops were lighter ($P < 0.05$) than untreated control samples; however, no significant differences between samples were observed in regards to redness and yellowness, and this may be due to the presence of the marinade on the surface of all marinated cooked pork chops. During storage, the lightness of both cooked untreated marinated pork chops and high pressure processed cooked marinated pork chops decreased ($P < 0.05$) significantly and these significant changes were noticed on day 11 for cooked untreated marinated pork chops or on day 23, 16 or 23 for samples that were high pressure processed at 300, 400 or 500 MPa, respectively. This decrease in lightness over storage time also may be due to oxidised products of meat pigments which have a brown and darker colour (Wettasinghe & Shahidi, 1997). Similar to raw marinated pork chops, the redness and yellowness did not change over storage time in both untreated or high pressure processed cooked marinated pork chops and this may be due to presence on the surface of the meat of the piri-piri marinade which is highly pigmented. Throughout storage, the colour differences observed on day 1 among cooked untreated control samples and high pressure processed samples (higher lightness in high pressure processed samples) was also observed during storage.

3.1.4. Texture

Results on day 1 showed that as the pressure level increased, the WBSF of the cooked marinated pork chops increased ($P < 0.05$). Untreated control marinated pork chops had the lowest WBSF and the marinated pork chops that were high pressure processed at 500 MPa had the highest WBSF indicating that these samples were the toughest

Table 2
Changes in colour and pH during chilled storage of raw untreated and HPP marinated pork chops.*.

	Treatment (MPa)	Day 1	Day 7	Day 9	Day 11	Day 16	Day 23	Day 30	Day 37	Day 44
Lightness	0.1	55.06 ± 2.68 ^{aA}	54.77 ± 1.40 ^{aA}	54.03 ± 1.42 ^{aAB}	51.97 ± 1.21 ^{aBC}	51.29 ± 2.34 ^{aC}	/	/	/	/
	300	63.64 ± 3.05 ^{bA}	63.20 ± 2.25 ^{bAB}	63.01 ± 1.48 ^{bAB}	62.89 ± 2.85 ^{bAB}	61.93 ± 1.81 ^{bBC}	58.11 ± 2.41 ^{aC}	58.01 ± 2.31 ^{aC}	/	/
	400	66.79 ± 1.83 ^{cA}	65.75 ± 3.27 ^{bcA}	64.95 ± 2.55 ^{bcA}	63.69 ± 1.45 ^{bcAB}	63.59 ± 3.85 ^{bcAB}	60.02 ± 3.29 ^{aBC}	58.69 ± 2.74 ^{aC}	58.62 ± 2.70 ^{aC}	/
	500	68.18 ± 2.39 ^{cA}	67.11 ± 1.62 ^{cA}	66.95 ± 2.06 ^{cAB}	66.54 ± 2.81 ^{cAB}	65.51 ± 2.99 ^{cAB}	64.02 ± 2.53 ^{bAB}	62.53 ± 2.15 ^{bB}	62.51 ± 1.16 ^{bB}	59.92 ± 1.77 ^{B}
Redness	0.1	14.12 ± 1.95 ^{aA}	13.71 ± 3.40 ^{aA}	14.36 ± 2.96 ^{aA}	13.23 ± 2.79 ^{aA}	13.03 ± 1.30 ^{aA}	/	/	/	/
	300	11.58 ± 1.01 ^{bA}	10.94 ± 1.58 ^{bA}	11.45 ± 1.86 ^{bA}	10.23 ± 2.72 ^{bA}	10.37 ± 1.34 ^{bA}	10.34 ± 1.09 ^{aA}		/	/
	400	11.30 ± 1.45 ^{bA}	10.98 ± 1.14 ^{bA}	11.46 ± 1.73 ^{bA}	10.37 ± 0.92 ^{bA}	10.81 ± 1.38 ^{bA}	10.54 ± 1.44 ^{aA}	11.19 ± 1.75 ^{aA}	10.87 ± 2.05 ^{aA}	/
	500	10.65 ± 1.37 ^{bA}	11.04 ± 2.01 ^{bA}	10.87 ± 1.81 ^{bA}	10.64 ± 2.14 ^{bA}	10.57 ± 1.20 ^{bA}	10.48 ± 0.88 ^{aA}	10.69 ± 1.73 ^{aA}	10.22 ± 2.26 ^{aA}	10.17 ± 1.69 ^{A}
Yellowness	0.1	26.74 ± 2.67 ^{aA}	24.41 ± 3.86 ^{aA}	25.1 ± 2.41 ^{aA}	27.09 ± 4.24 ^{aA}	25.95 ± 3.67 ^{aA}	/	/	/	/
	300	36.54 ± 2.40 ^{bA}	34.21 ± 3.51 ^{bA}	37.54 ± 2.47 ^{bA}	38.31 ± 3.95 ^{bA}	38.44 ± 2.89 ^{bA}	38.08 ± 4.03 ^{aA}	38.39 ± 2.47 ^{aA}	/	/
	400	34.21 ± 4.47 ^{bA}	32.59 ± 4.74 ^{bA}	33.58 ± 3.57 ^{bA}	34.57 ± 4.01 ^{bA}	37.63 ± 2.94 ^{bA}	38.13 ± 4.92 ^{aA}	37.76 ± 3.93 ^{aA}	37.44 ± 2.84 ^{aA}	/
	500	37.36 ± 3.35 ^{bA}	35.00 ± 2.77 ^{bA}	37.3 ± 4.84 ^{bA}	37.94 ± 3.68 ^{bA}	37.08 ± 3.12 ^{bA}	37.09 ± 4.09 ^{aA}	37.76 ± 3.12 ^{aA}	35.14 ± 3.47 ^{aA}	37.85 ± 2.97 ^{A}
pH	0.1	5.59 ± 0.02 ^{aA}	5.48 ± 0.04 ^{aB}	5.46 ± 0.05 ^{aB}	5.40 ± 0.08 ^{aB}	5.28 ± 0.07 ^{aC}	/	/	/	/
	300	5.68 ± 0.03 ^{abA}	5.65 ± 0.07 ^{bA}	5.53 ± 0.05 ^{bB}	5.52 ± 0.05 ^{bB}	5.50 ± 0.02 ^{bB}	5.46 ± 0.08 ^{aB}	5.37 ± 0.04 ^{aC}	/	/
	400	5.71 ± 0.16 ^{bA}	5.68 ± 0.08 ^{bcA}	5.67 ± 0.06 ^{cA}	5.62 ± 0.06 ^{cAB}	5.65 ± 0.05 ^{cAB}	5.59 ± 0.10 ^{abAB}	5.50 ± 0.02 ^{bBC}	5.38 ± 0.08 ^{aC}	/
	500	5.73 ± 0.06 ^{bA}	5.75 ± 0.05 ^{cA}	5.69 ± 0.04 ^{cA}	5.68 ± 0.04 ^{cA}	5.69 ± 0.07 ^{cA}	5.65 ± 0.02 ^{bA}	5.48 ± 0.02 ^{bB}	5.44 ± 0.07 ^{aB}	5.34 ± 0.08 ^{C}

^{a,b,c}Different superscripts in the same column indicate significant difference ($P < 0.05$) between different treatments.
$^{A, B, C}$Different superscripts in the same row indicate significant difference ($P < 0.05$) in the same treatment over time.
(/) indicates analysis was not determined on this day as end of shelf life was reached.
* Values are Mean ± standard deviation.

(Table 3). No significant differences were observed between the MPC high pressure processed at 300 or 400 MPa in comparison to the untreated control or 500 MPa MPC. This indicates that only when HPP is applied at 500 MPa, the MPC become significantly tougher compared to control samples. It was reported that pressures up to 1000 MPa can influence meat protein conformation and induce protein denaturation, aggregation or gelation which can result in meat becoming either tenderised or toughened and these outcomes depend on the meat protein system, the temperature used, the pressure applied and the holding time (Sun & Holley, 2010). The results found in this study are in agreement with the findings of Kruk et al. (2011) and Zamri, Ledward, and Frazier (2006)) who found that hardness in chicken breast fillets increased proportionally with increasing pressure levels up to 600 MPa while McArdle et al. (2011) and Ma and Ledward (2004) reported higher WBSF and hardness values in beef high pressure processed at 600 MPa than in beef treated at 400 MPa. Similarly Rodrigues et al. (2016) reported that marinated beef that high pressure processed at 300, 450 or 600 MPa increased significantly the WBSF as the pressure level increased and that samples high pressure processed at 600 MPa resulted in the toughest samples compared to the other treatments. The increased toughness with pressure has been attributed to an increasing incidence of sarcomeres, in which thick filaments have been compressed onto the Z-line, thus removing the I-band as a zone of weakness (MacFarlane, McKenzie, & Turner, 1980).

Throughout storage time, in both untreated control and high pressure processed marinated pork chops, WBSF values decreased significantly ($P < 0.05$) resulting in marinated pork chops becoming more tender. The decrease in untreated control marinated pork chops was noticed after 16 days and on day 16, 23 or 11 for marinated pork chops that were high pressure processed at 300, 400 or 500 MPa, respectively. On day 1, significantly ($P < 0.05$) tougher samples were noticed in marinated pork chops that were high pressure processed at 500 MPa in comparison to untreated samples (0.1 MPa); however, from day 7 until the end of their respective shelf life, there were no significant differences in toughness between untreated control and high pressure processed marinated pork chops which suggests the ability of higher pressure levels (HPP ≥400 MPa) to not only accelerate marinade absorption but also to accelerate the rate of tenderisation in the tougher samples. These results highlight the potential of the combination of marinades and HPP at higher levels (≥400 MPa) to tenderise meat which has become tougher due to the application of HPP.

Many authors have demonstrated the ability of marinades to tenderise meat products such as beef, chicken and pork (Aktaş et al., 2003; Berge et al., 2001; Burke & Monahan, 2003; Lewis & Purslow, 1991; Oreskovich et al., 1992, Bowker et al., 2010; Birk et al., 2010; Burke & Monahan, 2003; Wang et al., 2015). Similar to our results, Rodrigues et al. (2016) reported that the WBSF decreased ($P < 0.05$) during storage in low-salt beef marinated in citric acid that were high pressure processed at 600 MPa which may be due to tenderising effect of the marinade. The tenderisation of meat using marinades was attributed to marinade uptake by muscle proteins and also to solubilisation of collagen (Burke & Monahan, 2003).

3.1.5. pH

The results for pH showed that in raw marinated pork chops, the level of HPP increased the pH proportionally as untreated control samples had the lowest ($P < 0.05$) pH values and 500 MPa samples had the highest ($P < 0.05$) pH values. The pH of the piri-piri marinade was 4.4 and due to the higher marinade absorption in samples which were high pressure processed at 400 or 500 MPa it would be expected that these samples would also have a lower ($P < 0.05$) pH compared to untreated control and 300 MPa marinated pork chops which had lower ($P < 0.05$) marinade absorption; however, independent of the pressure applied HPP increased the pH of the marinated pork chops regardless of the level of marinade absorption.. Increase in pH after HPP has been attributed to a decrease in available acidic groups in the meat as a result

Table 3
Changes in colour, pH and hardness over the shelf life of cooked marinated pork chops.*.

	Treatment (MPa)	Day 1	Day 7	Day 9	Day 11	Day 16	Day 23	Day 30	Day 37	Day 44
Lightness	0.1	51.74 ± 1.99^{aA}	52.20 ± 3.37^{aA}	51.48 ± 3.52^{aA}	47.44 ± 2.27^{aB}	47.15 ± 4.11^{aB}	/	/	/	/
	300	58.82 ± 2.00^{bA}	58.61 ± 3.89^{bA}	58.91 ± 3.55^{bA}	58.43 ± 3.37^{bA}	57.27 ± 4.03^{bAB}	54.80 ± 2.57^{aB}	53.80 ± 2.84^{aB}	/	/
	400	59.62 ± 3.45^{bA}	61.30 ± 3.01^{bA}	58.98 ± 2.87^{bA}	58.25 ± 2.91^{bAB}	54.47 ± 2.64^{bBC}	54.59 ± 1.78^{aBC}	53.44 ± 1.25^{aC}	53.34 ± 3.24^{aC}	/
	500	60.99 ± 3.08^{bA}	61.52 ± 3.29^{bA}	60.33 ± 3.17^{bAB}	60.97 ± 3.81^{bA}	55.73 ± 2.13^{bC}	56.26 ± 1.66^{aBC}	55.92 ± 3.04^{aC}	55.70 ± 1.94^{aC}	54.75 ± 2.85^{C}
Redness	0.1	8.96 ± 1.73^{aA}	8.89 ± 1.16^{aA}	8.26 ± 1.43^{aA}	8.92 ± 1.18^{aA}	8.67 ± 1.59^{aA}	/	/	/	/
	300	8.66 ± 2.18^{aA}	8.76 ± 1.35^{aA}	8.45 ± 2.14^{aA}	8.81 ± 1.36^{aA}	8.67 ± 1.24^{aA}	8.78 ± 1.18^{aA}	8.91 ± 1.20^{aA}	/	/
	400	8.67 ± 1.69^{aA}	9.14 ± 1.47^{aA}	8.03 ± 1.62^{aA}	8.54 ± 1.87^{aA}	8.64 ± 1.04^{aA}	9.10 ± 1.39^{aA}	9.04 ± 1.93^{aA}	8.49 ± 2.03^{aA}	/
	500	8.38 ± 2.31^{aA}	8.28 ± 1.74^{aA}	8.07 ± 1.80^{aA}	8.71 ± 2.14^{aA}	8.33 ± 1.20^{aA}	9.06 ± 1.90^{aA}	8.64 ± 2.33^{aA}	8.27 ± 1.77^{aA}	8.19 ± 2.12^{A}
Yellowness	0.1	30.83 ± 3.35^{aA}	30.59 ± 3.97^{aA}	32.07 ± 3.02^{aA}	31.19 ± 2.64^{aA}	32.77 ± 3.02^{aA}	/	/	/	/
	300	30.42 ± 3.73^{aA}	28.52 ± 2.82^{aA}	30.87 ± 2.98^{aA}	29.62 ± 2.71^{aA}	30.76 ± 3.34^{aA}	30.04 ± 2.82^{aA}	30.24 ± 3.51^{aA}	/	/
	400	31.06 ± 3.43^{aA}	31.65 ± 2.47^{aA}	31.49 ± 2.67^{aA}	30.57 ± 3.54^{aA}	31.44 ± 3.44^{aA}	33.65 ± 3.24^{aA}	32.06 ± 2.47^{aA}	32.54 ± 3.84^{aA}	/
	500	30.12 ± 2.35^{aA}	31.82 ± 4.48^{aA}	34.16 ± 3.98^{aA}	32.02 ± 4.39^{aA}	30.06 ± 2.32^{aA}	32.94 ± 3.45^{aA}	33.24 ± 3.45^{aA}	33.94 ± 2.48^{aA}	33.28 ± 3.41^{A}
pH	0.1	5.86 ± 0.06^{aA}	5.87 ± 0.04^{aA}	5.83 ± 0.07^{aA}	5.79 ± 0.09^{aA}	5.78 ± 0.08^{aA}	/	/	/	/
	300	5.87 ± 0.07^{aA}	5.83 ± 0.06^{aA}	5.79 ± 0.08^{aA}	5.80 ± 0.06^{aA}	5.84 ± 0.07^{aA}	5.79 ± 0.06^{aA}	5.80 ± 0.05^{aA}	/	/
	400	5.91 ± 0.06^{aA}	5.86 ± 0.04^{aA}	5.85 ± 0.04^{aA}	5.85 ± 0.06^{aA}	5.87 ± 0.05^{aA}	5.84 ± 0.06^{aA}	5.85 ± 0.08^{aA}	5.84 ± 0.05^{aA}	/
	500	5.90 ± 0.07^{aA}	5.83 ± 0.04^{aA}	5.79 ± 0.05^{aA}	5.83 ± 0.07^{aA}	5.83 ± 0.05^{aA}	5.80 ± 0.05^{aA}	5.84 ± 0.06^{aA}	5.87 ± 0.05^{aA}	5.79 ± 0.07^{A}
Shear force (N)	0.1	14.71 ± 2.82^{aA}	14.25 ± 3.01^{aAB}	14.84 ± 1.67^{aA}	12.66 ± 1.59^{aAB}	12.07 ± 1.96^{aB}	/	/	/	/
	300	15.08 ± 2.37^{abA}	15.63 ± 1.97^{aA}	16.39 ± 2.23^{aA}	14.82 ± 2.06^{aA}	12.46 ± 1.52^{aB}	12.17 ± 1.43^{aB}	11.67 ± 1.38^{aB}	/	/
	400	16.52 ± 3.02^{abA}	14.00 ± 2.89^{aAB}	14.68 ± 2.23^{aAB}	14.30 ± 2.75^{aAB}	13.94 ± 2.62^{aAB}	11.96 ± 1.90^{aB}	11.89 ± 2.26^{aB}	12.04 ± 1.87^{aB}	/
	500	18.83 ± 2.16^{bA}	15.82 ± 3.64^{aAB}	15.50 ± 1.77^{aAB}	14.05 ± 3.43^{aBC}	14.15 ± 3.61^{aBC}	12.91 ± 2.90^{aBC}	11.21 ± 2.65^{aC}	12.32 ± 2.16^{aBC}	11.04 ± 2.57^{C}

[a, b]Different superscripts in the same column indicate significant difference ($P < 0.05$) between different treatments.
[A, B, C]Different superscripts in the same row indicate significant difference ($P < 0.05$) in the same treatment over time.
(/) indicates analysis was not determined on this day as end of shelf life was reached.
* Values are Mean ± standard deviation.

of conformational changes associated with protein denaturation (McArdle et al., 2011; Angsupanich & Ledward, 1998). Rodriguez-Calleja et al. (2012) found that the pH values of chicken high pressure processed at 300 MPa for 5 min were significantly higher than control samples and Wang et al. (2015) also found that the pH was higher ($P < 0.05$) in honey garlic pork chops treated at 450–600 MPa for 3 min compared to control samples. Similar results on increased pH on muscle food products were reported by McArdle et al. (2011) and Cruz-Romero, Kelly, and Kerry (2007)).

Throughout storage time, independent of the treatment applied to raw marinated pork chops, the pH decreased ($P < 0.05$) which may have been due to the production of lactic acid through LAB metabolism (Farber, 1991). In general, the pH decrease occurred when LAB reached ˜Log 4 CFU/g of sample. These results are in agreement with the findings of Kruk et al. (2011) who observed significant ($P < 0.05$) reductions in pH values throughout the storage period for raw chicken breast fillets.

After cooking of marinated pork chops, the pH increased ($P < 0.05$) in all treatments; however, the increase was not significantly different between untreated or high pressure processed marinated pork chops at day 1 or throughout storage time. Increase in pH due to cooking may be due to the decreased number of acidic groups in muscle proteins as proteins unfold (Hamm & Deatherage, 1960). In the high pressure processed marinated pork chops, the effects of the combined application of cooking and HPP were not additive in regards to increasing of pH and therefore no significant differences were observed compared to untreated control samples which were cooked but not high pressure processed. This may be due to increased severity of the cooking process in comparison to the milder process of HPP. A similar effect on pH was also observed in high pressure processed cooked beef muscle compared to untreated cooked samples (Ma & Ledward, 2004).

3.1.6. Lipid oxidation

From the sensory point of view, lipid oxidation cause rancidity problems which are considered unpleasant for consumers (Jeremiah, 2001). The results for TBARS showed that HPP increased ($P < 0.05$) the lipid oxidation of the marinated pork chops and this increase was proportional to the HPP level applied as the control untreated marinated pork chops had the lowest TBARS values and that the marinated pork chops high pressure processed at 500 MPa had the highest TBARS values (Fig. 2). These results are in agreement with Cheah and Ledward (1996); Cheah & Ledward, 1997 who reported that the effect of HPP on the oxidative stability of lipids in pork meat depends on the applied pressure with a value between 300 and 400 MPa constituting the critical pressure to accelerate lipid oxidation. Similarly, it was reported that the pressure level and holding time increased the extent of lipid oxidation in meat products such as dry-cured Iberian ham, pork loin, chicken breast fillets and pork (Cava, Ladero, González, Carrasco, & Ramírez, 2009, Kruk et al., 2011, Souza et al., 2011 Increased rates of lipid oxidation due to HPP has been attributed to pressure-induced protein denaturation which leads to the release of free-radicals catalysing oxidation (Cheftel & Culioli, 1997) and also has been attributed to the release of metal ions from iron complexes promoting auto-oxidation of lipids in high pressure processed meat and also due to membrane damage (Angsupanich & Ledward, 1998; Cheah & Ledward, 1996, 1997; Chevalier, Le Bail, & Ghoul, 2001). Marinades can provide antioxidant benefits in fresh meat (Kim et al., 2010; Tang & Cronin, 2007; Yusop et al., 2011). In a study carried out by Kim et al. (2010) it was concluded that garlic and onion juice provide antioxidant benefits to fresh pork during cold storage and the effects are concentration-dependent. However in our study the ability of HPP to significantly increase lipid oxidation linearly was not reduced by the piri-piri marinade which was applied at the same concentration in all treatments.

Over storage time, the TBARS values increased significantly ($P < 0.05$) in untreated control and high pressure processed marinated pork chops (Fig. 2). At the end of their respective shelf life, the TBARS differences observed on day 1 between untreated control and high pressure processed marinated pork chops (TBARS increased as HPP level increased) were similar. However, throughout storage, TBARS values in all samples were below the maximum

acceptable limit for TBARS of 1 mg/kg (Warriss, 2000) which is regarded as the limit beyond which meat products will normally develop objectionable odours/tastes. In agreement with the results found in this study Rodrigues et al. (2016) and Grossi, Bolumar, Søltoft-Jensen, and Orlien (2014)) reported significantly increased TBARS values during storage of marinated beef or brine injected pork meat that were high pressure processed at 600 MPa.

3.2. Sensory analysis

The results for sensory analysis showed that at day 1 there were no significant differences between untreated control or high pressure processed marinated pork chops in terms of appearance (raw in packaging), appearance (cooked), juiciness or OSA; however, significant differences ($P < 0.05$) were observed in flavour, texture and tenderness (Table 4). In terms of flavour, the control untreated marinated pork chop was the least ($P < 0.05$) preferred and the 500 MPa sample was the most ($P < 0.05$) preferred which may be attributed to HPP's ability to increase the marinade absorption which in turn may have improved the flavour of the cooked marinated pork chops.

The score of acceptance for OSA is 4.5 as this represents the midpoint of the 9 point scale and products scoring beyond this threshold are considered acceptable". In this study, all marinated pork chops were

Table 4
Sensory changes over the shelf life of cooked marinated pork chops*.

Sensory Attribute	Treatment (MPa)	Day 1	End of shelf life
Appearance (raw in packaging)	0.1	6.72 ± 1.24 aA	6.46 ± 1.33 aA
	300	6.78 ± 1.39 aA	6.51 ± 1.08 aA
	400	6.54 ± 1.15 aA	6.34 ± 1.41 aA
	500	6.70 ± 1.42 aA	6.52 ± 1.27 aA
Appearance (cooked)	0.1	7.48 ± 0.59 aA	7.25 ± 0.61aA
	300	7.25 ± 0.45 aA	7.42 ± 0.62 aA
	400	7.22 ± 1.24 aA	7.14 ± 0.67 aA
	500	7.35 ± 0.66 aA	7.15 ± 0.84 aA
Flavour	0.1	6.08 ± 1.56 aA	6.24 ± 1.65 aA
	300	6.78 ± 1.63 abA	6.36 ± 1.37 aA
	400	6.91 ± 1.22 abA	6.94 ± 0.91 abA
	500	7.54 ± 1.05 bA	7.61 ± 0.77 bA
Texture	0.1	6.30 ± 0.90 aA	7.03 ± 0.89 aA
	300	5.84 ± 1.16 aA	6.12 ± 1.44 aA
	400	3.99 ± 0.78 bA	6.01 ± 0.92 aB
	500	3.82 ± 0.84 bA	6.10 ± 1.11 aB
Juiciness	0.1	5.82 ± 0.90 aA	5.86 ± 1.15 aA
	300	5.33 ± 2.25 aA	5.68 ± 0.81 aA
	400	5.59 ± 1.74 aA	5.86 ± 1.03 aA
	500	5.49 ± 1.81 aA	5.62 ± 0.89 aA
Tenderness	0.1	6.44 ± 0.85 aA	6.73 ± 1.14 aA
	300	6.29 ± 0.99 aA	6.63 ± 1.24 aA
	400	4.34 ± 0.97 bA	6.37 ± 0.91 aB
	500	4.45 ± 1.10 bA	6.26 ± 1.22 aB
OSA	0.1	6.84 ± 1.18 aA	6.63 ± 0.64 aA
	300	6.83 ± 1.28 aA	6.85 ± 1.07 aA
	400	7.08 ± 1.32 aA	6.93 ± 1.20 aA
	500	6.78 ± 1.25aA	6.46 ± 0.94 aA

a, b Different superscripts in the same column indicate significant difference ($P < 0.05$) between different treatments.
A, B Different superscripts in the same row indicate significant difference ($P < 0.05$) in the same treatment over time.
* Values are Mean ± standard deviation.

therefore acceptable in terms of OSA.

Regarding the texture of the marinated pork chops, at day 1, there were no significant differences in the texture acceptability of control untreated and marinated pork chops high pressure processed at 300 MPa; however, marinated pork chops that were high pressure processed at 400 MPa or 500 MPa were the least ($P < 0.05$) preferred compared to untreated control or samples that were high pressure processed at 300 MPa. Similar to the WBSF values, untreated control samples had the lowest WBSF values and were therefore the most tender (correlation shown in PCA plot – Fig. 3). The ability of HPP to increase the toughness of post rigor meat has been well documented (Kruk et al., 2011; Ma & Ledward, 2004; Del Olmo, Morales, Ávila, Calzada, & Nuñez, 2010; Zamri et al., 2006; Jung, Ghoul, & de Lamballerie-Anton, 2000; Grossi et al., 2014).

Over storage time, there were no significant differences in terms of appearance (raw in packaging), appearance (cooked), flavour, juiciness or OSA in untreated control and high pressure processed marinated pork chops; however, marinated pork chop samples that were high pressure processed at ≥400 MPa became more tender ($P < 0.05$) and as a result the liking of texture increased ($P < 0.05$). These results are in agreement with findings on the instrumental WBSF results which decreased significantly over time, subsequently increasing tenderness. Despite increased tenderness in marinated pork chops that were high pressure processed at 400 or 500 MPa, no significant differences on the OSA were observed over storage time. According to Burke and Monahan (2003), marination has been reported to increase tenderness due to marinade uptake by muscle proteins and through solubilisation of collagen.

Conversely, Díaz, Nieto, Garrido, and Bañón (2008)) reported that sensory spoilage preceded microbiological spoilage of sous vide pork loin and this loss of acceptance was mainly due to the deterioration of meaty flavour and odour, although the loss of appearance, juiciness and toughness also contributed. In that case, the sensory analysis was the most effective method for determining the shelf life of the sous vide pork.

Cheftel and Culioli (1997) suggested that HPP of fresh meat causes drastic changes, especially in redness, and thus cannot be suitable of practical applications. Souza et al. (2011) also stated that consumers' purchasing preferences are highly based on fresh meat colour and HPP caused meat to appear lighter meaning that more work is needed to investigate meat colour preservation. However, in the current study the addition of the piri-piri marinade masked the significant colour changes of the raw pork meat after HPP as there were no significant differences in the sensory attribute of appearance (vacuum packaged raw marinated pork chops) between samples that were high pressure processed and untreated control samples even though instrumental colour results showed a significant ($P < 0.05$) increase in lightness and yellowness and a decrease in redness in raw marinated pork chops that were high pressure processed. These results indicate the potential of marinades to mask the whitening effect/discolouration of HPP on raw meat which can decrease consumer acceptability. Similarly, Wang et al. (2015) concluded that the application of honey garlic marinade partially masked meat discolouration due to the application of HPP up to 600 MPa.

In the present study, as TBARS values were below the acceptability limits throughout storage and sensory acceptability did not change significantly; the end of shelf life for all marinated pork chop samples was determined based on the recommended microbiological limits for raw meat products.

3.3. Principle component analysis

The PCA plot (Fig. 3) is a graphical representation of the degree of existing correlations between the MPC samples and the physicochemical and sensory responses on day 1. The plot shows that the 0.1 MPa MPC were the most closely related to redness (raw) and sensory attributes including liking of texture and tenderness. These results correspond with the descriptive results presented in Tables 2 & 4 as control samples (0.1 MPa) had the highest redness values and highest scores for liking of texture and tenderness. In terms of the other MPC treatments, the closest sample to control samples (0.1 MPa) was the 300 MPa MPC both on the PCA plot and also based on data shown in all tables.

The plot also shows that the 500 MPa MPC was most closely related to the 400 MPa MPC and was also correlated with the physicochemical characteristics of marinade absorption, shear force, lightness (raw & cooked), yellowness (raw), pH (raw) and also the sensory attribute of flavour. These results also correspond with the descriptive results presented in Tables 1–4 as MPC that were high pressure processed at 500 MPa had the highest marinade absorption, pH, shear force values as well as the highest degree of colour change in both raw and cooked MPC. Highest sensory scores for flavour were also observed in the 400 MPa and 500 MPa MPC.

Based on their location the plot, the sensory attributes of texture and tenderness were shown to be negatively correlated with the physicochemical response of shear force.

The substantial distance on the plot between the control (0.1 MPa) and 500 MPa MPC indicates that they were the most different in terms of MPC treatments and this is also evident in the data presented in Tables 1–4.

3.4. Microbiological analysis

The microbiological changes for TVC and LAB during vacuum packed chilled storage at 4 °C in untreated control and marinated pork chops that were high pressure processed is shown in Fig. 1. The following recommended microbiological limits are applied for fresh meat products: Aerobic plate counts < 5×10^6 CFU/g of product; *E. coli* < 10 CFU/g of product; LAB < 10^9 CFU/g of product, *Salmonella*: absent in 25 g of product (FSAI, 2015). For this study, the recommended microbiological limits of acceptability for the raw marinated pork chops

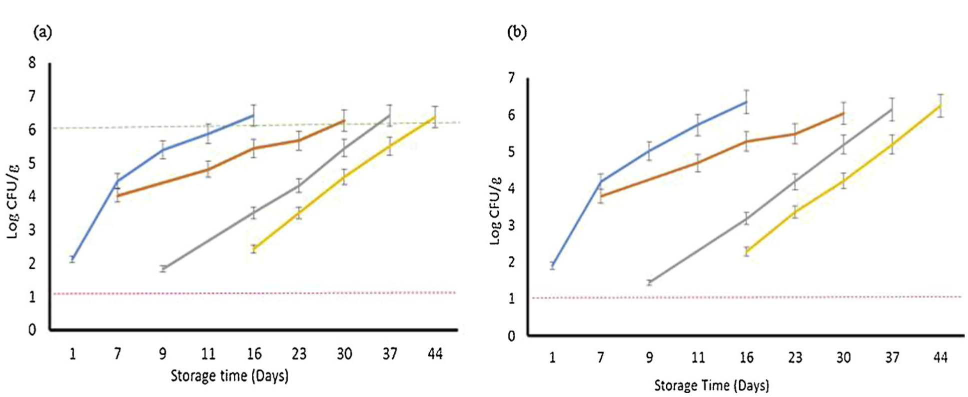

Fig. 1. Microbiological changes of (a) Total viable count and (b) Lactic acid bacteria during chilled storage at 4 °C of control raw marinated pork chops (0.1 MPa) (blue) or or raw marinated pork chops HPP at 300 MPa (orange), 400 MPa (grey) or 500 MPa (yellow) for 3 min. The dotted lines show the limits of detection (red) and acceptability (green).

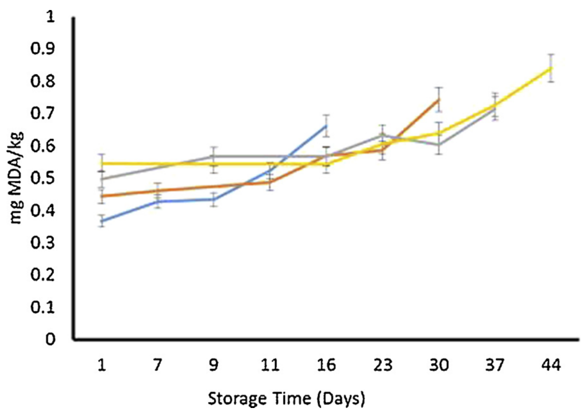

Fig. 2. – Lipid oxidation (TBARS) changes during chilled storage at 4 °C of control raw marinated pork chops (0.1 MPa) (▬) or raw marinated pork chops HPP at 300 MPa (▬), 400 MPa (▬) or 500 MPa (▬) for 3 min.

were set as above with reference to TVC, *E. coli* and *Salmonella.* The initial microbiological quality of the untreated marinated pork chops were of good quality (Fig. 1). After HPP the marinated raw pork meat samples were below the limit of detection for TVC and *E.Coli* (< 10 CFU/g) and there was an absence of *Salmonella* in 25 g of sample. Untreated control samples (0.1 MPa) had an initial TVC of 2 log (CFU/g), however, *E. coli* was also below the detection limit an there was an absence of *Salmonella* in 25 g of sample. Throughout storage *Salmonella E.Coli* & coliforms remained absent in all samples.

For untreated control raw marinated pork chop samples which contained 0.3% Inbac™, the limit of acceptability in terms of TVC was reached after 14 days of storage while the limit of acceptability for raw marinated pork chop samples that were high pressure processed at 300, 400 or 500 MPa and contained 0.3% Inbac™ was reached after 30, 36 or 43 days, respectively, indicating that the shelf life significantly increased by 114, 157 and 207% when the raw marinated pork chops were high pressure processed at 300, 400 or 500 MPa, respectively compared to untreated control samples (Fig. 1a). The unmarinated pork meat supplied for this study had a use by date of 14 days which corresponds with the end of shelf life for the untreated control raw marinated pork chop samples in which the limit of acceptability in terms of TVC was reached after 14 days. This indicates that the marinade had no effect in the reduction of microbial load or shelf life extension.

Apparently, for untreated control and marinated raw pork chops that were high pressure processed, the main spoilage microorganism was LAB (Fig. 1b) which increased significantly ($P < 0.05$) over storage time at a rate similar to TVC. It is well known that LAB is the major group associated with spoilage of refrigerated vacuum or modified atmosphere packed meat products (Korkeala & Björkroth, 1997) and vacuum packed high pressure processed meat products (Pietrasik, Gaudette, & Johnston, 2017; Yanqing et al., 2009).

These results are in agreement with the results reported by Kruk et al. (2011) who found that HPP at 600 MPa for 5 min reduced the total bacterial count by 6–8 log (CFU/g) improving shelf life for 7–14 days in raw chicken breast fillets. Similarly, Rodriguez-Calleja et al. (2012) reported that a combination of HPP at 300 MPa for 5 min and an edible antimicrobial coating Articoat™ reduced the bacterial load on raw chicken breast fillets below the detection limit and the shelf life of skinless chicken fillets was extended up to four weeks with LAB constituting the main spoilage microorganism. Garriga et al. (2004) also reported that HPP at 600 MPa for 6 min of vacuum-packed marinated beef loin samples reduced at least 4 log cycle for aerobic, psycrothrophic, and LAB counts and that *E. coli* and *Staphylococcus aureus* were kept below the detection limit (< 10 or < 10^2 CFU/g), respectively, during the chilled storage for 120 days. Wang et al. (2015) reported that HPP at pressures ≥450 MPa for 3 min significantly extend the shelf life of honey garlic marinated pork chops from 10 days to 31 days based on results for TVC.

The results presented in this study indicated that a combined effect of HPP and Inbac™extended the shelf life of marinated pork chops and that the shelf life extension depended on the pressure level applied. The results presented in this study also indicated the effectiveness of the combined effect of HPP and a mix of organic acids not only in improving the safety and shelf life of marinated pork chops but also, the effectiveness of HPP at pressures ≥ 400 MPa in accelerating the marinade absorption of pork chops which in turn improved the flavour acceptability, masked the discolouration caused by HPP and accelerated the rate of tenderisation of the marinated pork chops over storage time.

4. Conclusion

Pressures higher than 400 MPa were required to significantly accelerate ($P < 0.05$) the piri-piri marinade absorption in pork chops and improve the flavour acceptability; therefore compensating in terms of OSA for the negative textural effects caused by HPP. A symbiotic effect between HPP and the piri-piri marinade was observed as HPP increased marinade absorption and in turn the marinade increased the flavour acceptability and accelerated the rate of tenderisation of the high pressure processed marinated pork chops over storage time. The highly pigmented piri-piri marinade also masked the whitening effect on raw pork due to HPP which can decrease consumer acceptability.

The results found in this study indicated that the combination of HPP and antimicrobial Inbac™ increased the safety and shelf life of piri-

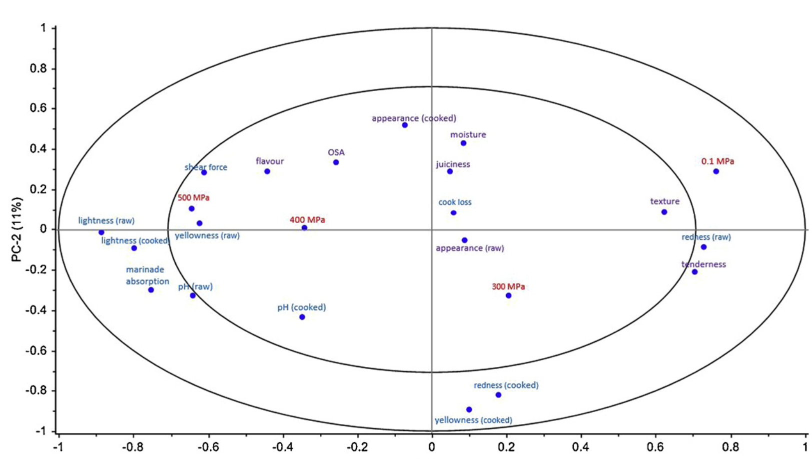

Fig. 3. Principle component analysis of the physicochemical and sensory attributes of marinated pork chops.

piri marinated pork chops and that the shelf life compared to control untreated samples was increased proportionally to the pressure level applied resulting in a shelf life extension of 16, 22 or 29 days, for samples that were high pressure processed at 300, 400 or 500 MPa, respectively. LAB apparently was the main spoilage micro-organism.

Herein we have demonstrated that HPP can improve the flavour of marinated pork chops by accelerating the marinade absorption and in combination with the commercial antimicrobial Inbac™ can extend significantly the shelf life of marinated pork chops without compromising the physicochemical or sensory quality of the pork meat. The extended shelf life can enhance sustainability by reducing food waste of these meat products and also offers potential benefits to meat processors, retail food service suppliers and consumers.

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