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Impact of long-term storage at ambient temperatures on the total quality and stability of high pressure-processed tomato juice.

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ABSTRACT

High pressure processing (HPP) can produce tomato juice of high quality and safety with a short shelf life under refrigeration temperatures. Long-term higher temperature storage studies are rare and temperature tolerant products are challenging to develop. The effect of high pressure processing (HPP) on the total quality (colour, microbial counts, phytochemical levels, antioxidant and enzymatic activities) and stability (retention over time) of tomato juice during long-term storage was investigated. Thermal processing (TP) was used as a control treatment and overall, two different ambient conditions (20°C and 28°C) were tested. Immediately after processing, HPP products proved superior to TP ones (enhanced redness, total carotenoids and lycopene, stable total phenols, and inactivation of pectin methyl esterase). During initial storage (30 d) most quality attributes of HPP juice remained stable. Prolonged storage, however, led to losses of most quality attributes, although HPP (20°C) showed lower quality degradation rate constants comparison to TP and HPP (28°C).

Keywords: tomato, high-pressure, thermal processing, nutritional, phytochemical, quality, storage

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the second most widely grown vegetable crop in many countries across the globe and is consumed in large quantities partly due to its nutritional, functional and health properties (Clinton, 1998; Takeoka, Dao, Flessa, Gillespie, Jewell, & Huebner, 2001). Awareness of healthy eating has grown amongst modern consumers who are seeking nutrient dense and convenient foods such as tomato juice. Processing is essential to ensure the total quality of tomato juice and to maintenance or stabilise these qualities until consumption. Conventional processing techniques, such as heating, may induce undesirable changes in organoleptic properties and reduce the bioavailability of micronutrients (Patras, Brunton, Pieve, Butler, & Downey, 2009). Non thermal processing technologies are emerging as alternatives which claim no compromise in safety while ensuring higher retention of important nutrients. There is an opportunity for the food industry, in particular tomato processors, to adapt and develop new safe products that guarantee unprecedented quality and 'freshness' characteristics.

As a non-thermal processing technique, high pressure processing (HPP) applies a pressure between 200-600 MPa to inactivate vegetative microorganisms, some enzymes and to preserve quality attributes. Many authors have assessed the effect of HPP on quality and micronutrients of tomato juice and purée in comparison to thermal processing (TP) and it has proved useful for preserving the quality of tomato juice and purée after treatment (Dede, Alpas, & Bayindirli, 2007; Hsu, 2008; Krebbers, Matser, Hoogerwerf, Morzelaar, Momassen, & Van den berg, 2003; Patras et al., 2009; Porretta, Birzi, Ghizzoni, & Vicini, 1995; Sanchez-Moreno, Plaza, Ancos, & Cano, 2006). Alongside nutrient retention inactivation of two enzymes, pectin methyl esterase (PME) and polygalacturonase (PG) is very important to maintain the desired viscosity of tomato juice and purée. Several researchers have investigated the effect of HPP on PME and PG activities at various temperatures (Boulekou,

Mallids, Taoukis, & Stoforos, 2011; Crelier, Robert, Claude, & Juillerat, 2001; Fachin, Van Loey, Nguyen, Verlent, Indrawati, & Hendrickx, 2003; Hsu, 2008; Krebbers et al., 2003; Verlent, Van Loey, Smout, Duvetter, & Hendrickx, 2004) and they found total inactivation of PG and contradictory behaviour of PME at some pressure/temperature conditions (500-800 MPa/ 20-90°C). Colour retention is also very important quality attribute of tomato juice. It is established that pH has an important effect on pigments and responsible for the colour of fruits and vegetables during processing (Andres-Bello, Barreto-Palacios, Garcia-Segovia, Mir-Bel, & Martinez-Monzo, 2013) but the impact of reducing pH on the maintenance of tomato juice quality has not been widely investigated.

Maintaining quality and nutritional properties of tomato juice throughout long-term storage at higher temperatures is very challenging, but would be extremely useful especially for tropical countries of high ambient temperatures, where low temperature storage facilities are not adequately available, to preserve large quantities of tomatoes produced during the glut season. Surprisingly few researchers have reported the effect of HPP on quality attributes during long-term storage under such conditions and the results are contradictory. Gupta, Balasubramaniam, Schwartz, & Francis (2010) claimed that HPP (500-700 MPa) can result in a microbiologically safe tomato juice product for 52 weeks at 25 and 37°C whereas Dede et al., 2007) showed more moderate results after 4 weeks at 25°C using much lower pressures (150-250 MPa). As well as microbiological stability, the fate of minor constituents and other crucial quality parameters of tomato juice during storage at ambient temperature have not been well studied. Only one study reported on the complete antioxidant activity, ascorbic acid content and colour degradation after HPP treatment, although only for limited amount of time (Dede et al, 2007). Their results showed better retention of ascorbic acid, antioxidant activity and colour than the conventional, thermally processed samples. The long-term, high temperature storage study of Gupta et al. (2010) considered some quality parameters (colour,

lycopene content) and found better retention throughout storage compared to heat treatment, although the effect on other important nutritional parameters such as ascorbic acid, total phenol content and related antioxidant activities and enzyme activities that are essential in assessing the stability of long storage products was not addressed.

Most importantly, most of the HPP studies cited, including the two above, have been conducted using laboratory scale HPP equipment where the capacity is below 5 L. Results obtained using this type of experimental equipment may not necessarily reflect results acquired using industrial processors and usually significant charges are required for scaling up.

Therefore, studying the effect of HPP on the quality of tomato juice during long-term storage is an important consideration in evaluating novel processing methods in comparison to existing practices. The aim of this study was to evaluate the effects of industrial scale HPP on microbiological quality, appearance and nutrient retention of tomato juice in comparison to TP during long-term storage at two different ambient conditions (20°C and 28°C). The effect of juice pH on the quality parameters was also investigated.

2. MATERIALS AND METHODS

2.1 Materials

All chemicals and standards except for nutrient agar and maximum recovery diluent (Oxoid, Basingstoke, UK) were purchased from Sigma Aldrich (Dorset, UK) and were of analytical grade. Fully ripe tomatoes (cv *Pitenza*, cultivated in Spain) were purchased at 3 different occasions from local stores in Northern Ireland (UK) between April to May 2013. In total, 45 kg of tomatoes were purchased and graded before processing (mean weight, 85 ± 5 g and mean circumference, 15 ± 1 cm), odd shape and size tomatoes were excluded. Cheese cloth

 $(100 \times 100 \text{ cm})$ was purchased in the local market and standard packaging materials (polyethylene/polyamide film) were obtained from Scobie & Junor (Mallusk, UK).

2.2 Sample preparation

Tomatoes were washed, cut into pieces and blended using a household blender. The seeds and skin of the tomato were removed by passing the resulting juice through a cheese cloth. The juice was divided into three aliquots and the pH of one aliquot of tomato juice was altered to pH 3.93 by adding citric acid. The pH of the other two aliquots was left unaltered, at the original pH of 4.3. Samples of juice (50 ml) were transferred into polyethylene/polyamide pouches (15×10 cm), heat sealed and kept under refrigeration (4°C) until processing.

2.3 High pressure (HP) and thermal processing (TP) and storage study design

Tomato juices of altered and unaltered pH were blanched using hot break method at 90°C for 2 min (Hayes *et al*, 1998) using a water bath (Grant, GD 100, UK). The temperature of the juice during blanching was monitored using a digital thermometer (HI 98804, Hanna Instruments, UK) fitted with a k-type thermocouple and samples were cooled in iced water immediately after blanching. The samples for HPP were treated using an Avure Quintus 35L (Avure Technologies, Middletown, OH, USA) with a heat-controlled vessel and a capacity of 35 L (internal diameter: 18 cm, length: 1.2 m) and pressurised (600 MPa /1 min) at ambient temperature (approximately 16°C). The temperature increase due to adiabatic heating was approximately 3°C per 100 MPa. Time taken to reach the target pressure was approximately 2 min and decompression took approximately10 sec. For comparison with conventional heat processing, heat sealed pouches of tomato juice were subjected to thermal processing using a water bath at 95°C for 20 min (Hayes, Smith, & Morris, 1998), as described above. The entire

experiment was conducted on three separate batches, on three consecutive days to produce three replicate samples.

HPP and TP samples were stored for up to 12 months at two different ambient temperature (20°C and 28°C) conditions and analysed after 0, ½, ¾, 1, 2, 3, 6, 9 and 12 months storage for microbial counts, colour, phytochemicals, antioxidant activity and enzyme activity. Phytochemicals, antioxidant activity and enzyme activity analyses were carried out on freeze-dried samples, taken at each sampling time but processed all at the same time (i.e. after 12 months). Freeze drying was achieved using a freeze dryer (Christ-Alpha 1-4 LD, Germany).

2.4 Microbiological analysis

Total viable count (TVC) was determined using Nutrient agar. Tomato juice (1 ml) was aseptically transferred to 9 ml maximum recovery diluent and serial dilutions prepared. Aliquots (100 μ l) of appropriate dilutions were spread plated on duplicate nutrient agar plates. After incubation at 30°C for 24 h, colonies were counted and results were expressed as CFU/ml of tomato juice.

2.5 Colour measurements

Colour was measured using a Konica Minolta portable colorimeter (CR-410, Japan). A standard white tile (X= 87.01, Y=0.3185, Z=0.3365) was used to calibrate the instrument and L*, a* and b* values were directly taken from the colorimeter. The Hue value (a/b) and the overall colour change ($\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$) were calculated based on measured L*, a* and b* values.

2.6 Quantification of total carotenoids and lycopene content

Total carotenoids were determined according to Koca, Burdurlu, & Karadeniz (2007) with some modifications. Freeze-dried tomato samples were extracted with hexane:acetone (7:3) (0.5 g/25 ml) and total carotenoids were quantified with colorimetric detection at 450 nm using UV-vis spectrophotometer (JENWAY 6305, UK) and β -carotene as a standard (0.5-10 µg/ml). Lycopene content was determined colorimetrically (472 nm) using the method described by Sadler, Davis, & Dezman (1990) after hexane:ethanol:acetone (2:1:1) extraction. Concentration of lycopene was calculated using the equation: C = A x10⁴/ E[%]_{1 cm} = extinction x *l*, where: C = lycopene concentration (µg/ml), A=absorbance at 472 nm, E[%]_{1 cm} = extinction coefficient (3450 for lycopene in hexane) and *l* the path length (1 cm).

2.7 Quantification of total phenolic content

The total phenolics were conventionally quantified colorimetrically using a modified Folin-Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999). The reaction mixture, consisting of 125 μ l of methanolic tomato extract (0.1 g/10 ml), 0.5 ml of distilled water and 125 μ l of Folin-Ciocalteu reagent, was allowed to stand for 6 min before adding 1.25 ml of a 7% sodium carbonate and final volume was adjusted to 3 ml with distilled water. Absorbance was measured at 760 nm, after 90 min and a standard curve was obtained with gallic acid (50-600 μ M).

2.8 Quantification of ascorbic acid

Ascorbic acid content was determined according to the modified 2,6dichlorophenolindophenol (DIP) method (Klein and Perry, 1982). Freeze-dried samples were extracted with 1% metaphosphoric acid (0.1 g/10 ml) and centrifuged ($3000 \times g/15$ min) (SORVALL Legend RT, Germany). An aliquot of 0.5 ml of the supernatant was added to 4.5 ml of 0.05 mM DIP, mixed for 15 sec and measured colorimetrically at 515 nm. L-ascorbic acid was used as a standard (0-400 µg/ ml).

2.9 Quantification of ABTS and ORAC antioxidant activities

Antioxidant activities were measured using two methods. Firstly, the 2,2-azino-di-3ethylbenzothialozine-6-sulphonic acid diammonium salt (ABTS) decolouration method was applied with some modifications (Re, Bramley, & Rice-Evans, 1999). Freeze-dried tomato powder was extracted with 80% ethanol (0.25 g/10 ml) and centrifuged ($3116 \times g / 10 min$). The total antioxidant activity was measured in the supernatant. Absorbance readings were taken 1 min after initial mixing of the reaction mixture (sample extract (10 ml) and 1.1 ml ABTS⁺) and a standard curve was obtained using Trolox (100-800 µM). For the oxygen radical absorption capacity (ORAC), the method of Huang, Ou, Hampsch-Woodill, Flanagan, & Prior (2002) was used with some modifications. Appropriately diluted tomato extract (20 μ l, 0.05 g/40 ml, 80% methanol) and 8.16 x 10⁻⁵ mM fluorescein (40 μ l) solutions were loaded on a Greiner flat bottom 96-well plate and pre-incubated for 30 min at 37°C within the plate reader (TECAN Safire2 monocromator). After incubation, 140 µl of 0.5 M 2,2'-Azobis (2-methylpropionamidine) dihydrochloride (AAPH) solution was added and immediately the loss of fluorescence was followed at 1 min intervals for 100 min. The standard curve was obtained using Trolox within the linear range of 0-64 µM and all reagents were prepared using 75 mM phosphate buffer (pH 7.4).

2.10 Quantification of PME and PG enzymatic activities

Pectin methyl esterase (PME) activity was assayed according to the method applied by Hagerman and Austin (1986) with modifications. Briefly, the enzyme was extracted (0.25 g/10 ml, 8.8% NaCl) from the freeze-dried tomato powder and centrifuged ($4369 \times g / 25$ min). The extracted supernatant, reagents of citrus pectin solution (0.5% w/v in 0.1 M NaCl) and bromothymol blue indicator dye (0.01% w/v in 0.003 M potassium phosphate buffer, pH 7.5) were adjusted to pH 7.5 using NaOH (1M). Initial absorbance was measured after

mixing the pectin solution (1 ml) with 0.1 ml of bromothymol blue and the decrease of absorbance (620 nm) at 30°C was recorded 5 min after adding the enzyme extract (20 μ l) to the mixture. D-galacturonic acid (0-50 μ M) was used to obtain a standard curve.

Polygalacturonase (PG) was extracted from the freeze-dried tomato powder using a modified method of Pressey (1986). The supernatant was adjusted to pH 4.4 and assayed for PG activity using the method applied by Gross (1982). The reaction mixture, consisting of enzyme extract (100 μ l), 0.2% polygalacturonic acid (50 μ l) and 50 mM sodium acetate (150 μ l), was incubated at 30 °C for 1 h. Subsequently, 2 ml borate buffer (pH 9) and 400 μ l of 1% cyanoacetamide were added to the reaction mixture, boiled for 10 min and the absorbance was measured at 276 nm after equilibrium at room temperature. D-galacturonic acid (0-250 μ M) was used to obtain a standard curve.

2.11 Statistical analysis

The data were statistically analysed by two-way ANOVA with SPSS 22 (IBM, UK) and means were compared using the Tukey post hoc test to explore which means are significantly different from each other. Statistical significance was accepted at a level of 95%.

3. RESULTS AND DISCUSSION

3.1 Microbial quality of tomato juice

The mean TVC of the raw tomato juice was 6.69 \log_{10} CFU/ml and all HPP treatments (600 MPa/1 min) and thermal processing (95°C/20 min) were effective in reducing the TVC to below the detection limit (< 1 \log_{10} CFU/ml) as shown in Table 1. Lower initial microbial populations (3.62 and 4.5 \log_{10} CFU/ml) and similar behaviour after processing, albeit at lower pressures (500 MPa/10 min; 250 MPa/15 min, respectively), were reported by Dade et

al. (2007) and Hsu, Tan, & Chi (2008). Krebbers et al. (2003) observed that HPP treatment up to 500 MPa at ambient temperature was sufficient for moderate inactivation of naturally present microorganisms, but treatment at 700 MPa was needed to reduce the natural microflora to below the detection limit. With regards to effect of storage, results showed that HPP (600 MPa/1 min/35°C) was effective in maintaining the microbial count below the detection limits throughout the storage period irrespective of storage temperature and juice pH (data not shown); the same situation was observed for thermal processing. Porretta et al. (1995) also tested tomato juice with different pH levels ranging from 4-5 and observed that HPP (500 MPa/3 min/25°C) was sufficient to develop products with no microbes detected, irrespective of pH level. Daryaei & Balasubramanium (2013) showed that inactivation of B. coagulans spores by a combination of pressure and high temperature is needed in order to develop an ambient-stable product. In the present study, the blanching treatment (90°C/2 min) conducted prior to HPP must have contributed to the maintenance of ambient-stable product. Gupta et al. (2010) created a product that was microbiologically stable after 52 weeks storage at high ambient temperatures (25 and 37°C), using HPP (700 MPa/10 min/45°C) or pressureassisted thermal processing (PATP) (600 MPa/10 min/100°C) instead of blanching. Others have claimed microbiologically stable products after a short storage period (12 weeks at 37°C, 28 days at 4°C, 8 weeks at 4°C) after treatment at 600 MPa/10 min/75°C, 500 MPa/10 min/25°C and 700 MPa/2 min/20°C, respectively (Daryaei & Balasubramanium, 2013; Hsu, 2008; Krebbers et al., 2003), using laboratory-scale equipment.

3.2 Colour change of tomato juice

An attractive red colour is one of the main quality characteristics of tomato products. The raw juice had the lowest redness (a/b) values, as a result of the typical pinkish red colour of tomato juice before processing (Table 1). ΔE represents the change of juice colour in

comparison to fresh juice and all processed samples showed higher redness values (large ΔE values) due to the leakage of lycopene from broken cellulose structure of the tomato tissue during processing (Lin & Chen, 2005). The instrumental colour results (Table 1) were in agreement with those of Krebbers et al. (2003) and Poretta et al. (1995) who also observed enhancement of the redness of tomato puree after processing. Furthermore, tomato juice with natural pH (4.3) subjected to HPP showed significant (p<0.05) red colour retention compared to the juice with the pH altered, which was also previously reported by Porretta et al. (1995). This is possibly due to the lower level of ascorbic acid content in the unaltered pH sample compared to the pH altered, which is involved in the non-enzymatic browning reaction in tomato juice (Mudahar, Sidhu, & Minhas, 1986). All treatments were equally ineffective to retain the initial colour of tomato juice, resulting in brown coloured products after 12 month period; this was more intense in TP samples and in samples stored at 28°C. In contrast, Gupta et al. (2010) observed better colour retention of HP and PATP samples compared to TP samples during storage (52 weeks at 4, 25 and 37°C), but this may have been due to vacuum packaging. The kinetic behaviour of the colour characteristics of tomato juice samples throughout storage was determined. The change of redness as a function of storage time was described by a first-order kinetic model with high determination coefficients (R^2 =0.97-0.79). The first order model rate constants ranged from 0.0735 (TP at 28°C) to 0.0348 (HPP, pH 3.93 at 20°C), emphasising higher red colour degradation in TP samples and the effectiveness of reducing the pH in tomato juice (lower rate constants were obtained for the pH-altered samples). The pattern of colour change (ΔE) in pH-altered HPP juice was not significantly different for the two storage temperatures for up to 9 months of storage, while both the juice with unaltered pH that had been treated with HPP and the TP samples showed significant differences between storage temperatures after 3 months of storage (Figure 1). The redness (a/b) of tomato juice can be correlated with lycopene content throughout storage, and the

change in red colour as a function of lycopene and carotenoid content is fairly linear (Pearson coefficients 0.85 and 0.80, respectively) (Table 2). Krebbers et al. (2003) reported little dependence on change in lycopene concentration for instrumental colour, in samples processed by thermal and combined pressure-thermal treatments.

3.3 Phytochemicals retention after processing and during storage

3.3.1 Ascorbic acid content

Ascorbic acid (vitamin C), as well as an indicator of the nutritional quality in fruit juices can be used as benchmark for the retention of other micronutrients during processing and storage because it is the most heat labile vitamin. The fresh (raw) tomato juice was found to be very rich in ascorbic acid containing 330.8 ± 4.5 mg/100 g (Table 1), which degraded rapidly after processing and during storage. In fact, ascorbic acid levels were shown to be much more susceptible to degradation than any other nutrient in the present study. Immediately after processing, HPP treatments of both 4.3 and 3.93 pH samples provided significantly (p<0.05) better ascorbic acid retention (66 and 69%, respectively) compared to TP (57%) (Table 1). Similar results have been reported by Hsu et al. (2008) and Sanchez-Moreno et al. (2006). Even higher retention (>90%) was claimed in low (250 MPa) (Dede et al. (2007) and high (600 MPa) pressure treated juices (Patras et al., 2009). Processing losses are highly dependent on the HPP equipment, conditions and blanching procedures, if used. The low retention of ascorbic acid in this study is most likely due to the blanching treatment (90°C/2 min) that was applied prior to HPP. These losses may have been exacerbated by the equipment used (water bath) leading to long come-up times to reach the target temperature.

During long-term storage, substantial depletion of ascorbic acid was observed during the first 4 weeks in all treatments resulting in very low retention (4%) at the end of storage (Table 3). The latter might be due to the presence of residual oxygen in the packaged juice (Lin & Chen,

2005). The rate constants, obtained from the developed first order kinetic model, which was fairly linear ($R^2>0.75$), varied from 0.2757 (TP, at 20°C) to 0.3761 (HPP, pH 3.9 at 20°C). Higher ascorbic acid retention in unaltered pH samples, in comparison to altered pH, could be observed at the end of storage. This is also confirmed by the obtained lower rate constants (Table 4). Similar long term studies could not be found in the literature. Dede et al. (2007) reported 70 and 45% ascorbic acid retention in HPP tomato juice after only 30 days of storage at 4 and 25°C, respectively. Hsu et al. (2008) observed more stable ascorbic acid content (70%) during 28 days chill storage. In the present study between 8 and 19% of ascorbic acid was retained over the same time period might be due to the accelerated oxidation rates at higher storage temperatures.

3.3.2 Total phenol content

Phenols appeared to be relatively resistant to the effects of processing, with the exception of HPP/unaltered pH tomato juice, which had a total phenol content of 4.83 mg gallic acid/g, representing a significant difference (p<0.05) from fresh juice (Table 1). This agrees with Dewanto, Wu, Adom, & Liu (2002), who also observed that total phenol content did not change during processing. In contrast, Patras et al. (2009) observed a significant increase in phenol content in tomato after HPP (600 MPa/15 min). Here, long-term storage TP samples (at 28°C and 20°C) resulted in higher rate constants (0.0631 and 0.0552, respectively) in comparison to HPP samples, according to the developed first order kinetic model (Table 3). Higher total phenol content could be observed after 6 months storage (28°C) in HPP/ unaltered pH tomato juice samples with the lowest rate constant (0.0454), when contrasted with the HPP/ altered pH samples (rate: 0.0490). Similar behaviour was noticed in the treatments stored at 20°C. All treatments, however, were effective in retaining a total phenol content of more than 50% at the end of 12 month storage (Table 3). In another study, tomato juice treated at 90°C for 30 and 60 sec also retained the initial total phenol content for a

period of 2 and 3 weeks, respectively, at 4°C (Odriozola-Serrano, Solivia-Fortuny, & Martin-Belloso, 2008). Maintenance of total phenol content during long-term storage might be due to the inactivation of the enzymes responsible for their degradation.

3.3.3 Total carotenoid and lycopene content

Prior to processing, total carotenoid and lycopene content of raw tomato juice were 83.7 and 34.9 mg/100 g, respectively (Table 1), which are typical values for this product (Krebbers et al., 2003). After TP and HPP (at pH 4.3 and 3.93), total carotenoid contents were significantly increased by 255%, 197% and 278%, respectively. These results were higher than those reported by Patras et al. (2009) who observed 172% increases in total carotenoid contents using similar pressures. Hsu et al. (2008) observed no change in total carotenoids in TP samples and 60% increase in HPP samples. It has been reported that HP treatment is effective in enhancing the lycopene content (Krebbers et al., 2003) and the total carotenoid content (Sanchez-Moreno et al., 2006) of tomato purée. This enhancing effect has been attributed to pressure-induced extraction, as a result of effects on the membranes in plant cells (Shi & Le Maguer, 2000). During long-term storage of the tomato juice, carotenoid and lycopene content depleted substantially (>35%) within the first four weeks irrespective of treatment. This agrees with Gupta et al. (2010) who reported rapid degradation of lycopene during the first 5 weeks of storage. The degradation was more severe at 28°C, as confirmed by higher rate kinetics obtained for both carotenoids (>0.3739 for 28°C and <0.2783 for 20°C) and lycopene (>0.1819 for 28°C and <0.1511 for 20°C) using the existing experimental data (Table 4). HPP-treated tomato juice stored at 20°C retained a higher total carotenoid content (>18%) compared to all the other treatments, which resulted in less than 11% retention at the end of 12 month storage. With regards to the effect of pH, tomato juice samples behaved in different ways at two temperature conditions: pH altered samples showed higher rate constants for total carotenoids and lycopene contents at 28 °C and 20 °C,

respectively. while, pH unaltered samples showed higher rate constants for total carotenoids and lycopene contents at 20 °C and 28 °C, respectively (Table 4). The loss of carotenoids during storage may be explained by isomerisation or oxidative degradation due to the presence of residual oxygen in the sample (Lin & Chen, 2005).

3.4 Antioxidant activity of tomato juice extracts

HPP successfully retained antioxidant activity, as assessed by ABTS, without significant change, while TP resulted in a significant decrease of 15% (p<0.01) during processing, in comparison to raw tomato juice (Table 1). Dede et al. (2007) and Sanchez-Moreno et al. (2006) also reported that total scavenging activity (DPPH) in tomato purée and juice was unaffected after HPP (400 MPa/15min/25°C and 250 MPa/15min/35°C), while Patras et al. (2009) observed increased antioxidant activity after HPP (600 MPa). The antioxidant activity of tomato juices was substantially depleted during the first few weeks of storage but then decreased more slowly, reaching values of $>322 \mu M/100$ g in all treatments at 12 month storage (data not shown). The developed first order kinetic model predicted the antioxidant activity based on the ABTS well, as indicated by determination coefficients ($R^2 = 0.79$ to 0.95). In contrast to other quality parameters, the best retention (more than 35%) of antioxidant activity was seen in the pH unaltered juice (pH 4.3), treated with HPP and stored at 20°C with the lowest rate constant (0.0999, R²=0.85). On the other hand, pH altered tomato juice showed higher retention in antioxidant activity (377 μ M/100 g) with lower rate constant (0.1139, R²=0.79) after 12 months storage at 28°C in comparison to the values of the pH unaltered juice sample (Table 4). In practical terms, antioxidant activity in tomato products relates to the vitamin C, polyphenols, vitamin E and carotenoids contents (Takeoka et al., 2001). In this study positive strong correlations (>0.87) were found between ABTS and ascorbic acid, total phenol, total carotenoids and lycopene in all treatments during storage

(Table 2). This implies that the decrease in total antioxidant activity is mainly attributed to the decrease in all of these nutrients during storage. Dede et al. (2007) also demonstrated 20 and 70% loss of antioxidant activity in HPP and TP tomato juices respectively, during 30 days storage at 4 and 25°C.

With regards to the ORAC assay, the raw juice had the highest antioxidant value (4084.8 μ M/100 g) and this was significantly reduced (p<0.01) in all processed samples (Table 1). The first order kinetic model poorly fitted the antioxidant capacity based on ORAC as indicated by low determination coefficients ($R^2 = 0.15$ to 0.07). However, it showed higher rate constants for 28 °C stored HP treated samples in comparison to those stored at 20°C. Furthermore, lower rate constants showed pH unaltered juice at 28°C and pH altered juice at 20°C (Table 4). ORAC results correlated with the ascorbic acid and total phenol content (0.97 and 0.77), but not with total carotenoids and lycopene contents. Lipophylic compounds such as lycopene are responsible for lipophylic antioxidant activity in tomatoes, but their contribution to total antioxidant activity is considered low (Wu, Beecher, Holden, Haytowitz, Gebhardt, & Prior, 2004). Different ORAC and ABTS antioxidant activities were found in this study for all juice samples which is expected because they are based on a different principle (Frankel & Meyor, 2000). Thus, the correlation between ORAC and ABTS was poor (0.28) demonstrating how a single assay is not sufficient to evaluate the total antioxidant activity. Changes in antioxidant activity, measured by ORAC, were not significant in all samples throughout the storage and very poor correlations were shown between ORAC values and nutritional parameters (Table 2). The antioxidant activity remained above 1166 μ M/100 g after 12 month storage period (data not shown).

3.5 Enzyme activity

Inactivation of pectin methyl esterase (PME) is desirable for cloudy juices to prevent the enzyme acting on pectin present in the food matrix, leading to a loss of cloud. Raw tomato juice showed high enzyme activity and all of the processing methods caused a decrease in the activity; 94.3, 91.2 and 97.5%, respectively, in TP and HPP (pH 4.3 and 3.93) samples (Table 1). These results agree with those of De Sio, Dipollina, Villari, Loiudice, Laratta, & Castaldo (1995) who reported efficient inactivation of tomato PME by thermal treatment (88°C /20 sec). In contrast, some researchers have observed increased PME activity in un-blanched tomato juice after HPP (Boulekou et al., 2011; Hernandez & Cano, 1998; Hsu, 2008; Krebbers et al., 2003), while Hsu et al., 2008 have reported efficient inactivation by using low pressure-mild temperature. In the present study the residual PME activity of treated tomato juice remained constant during storage with few fluctuations (data not shown) and samples treated with TP and HPP (pH 3.93) and stored at 28°C, maintained the highest (<11 %) and lowest (<2.5 %) residual activities, respectively, throughout storage. This more stabilised behaviour of PME activity during storage was well described by the poor determinant coefficients (R^2 =0.53-0.06) of the first order kinetic model. Aguilo-Aguayo, Solivia-Fortuny, & Martin-Belloso (2008) also observed stabilised 14% residual activity in tomato juice throughout storage (77 days at 4°C).

On the other hand, polygalacturonase activity (PG) was found to be more stable to both thermal and pressure treatments than PME. TP resulted in a significant reduction (26%) after tomato juice processing while HPP treatments caused a small but non-significant decrease in PG activity (Table 1). Crelier et al. (2001) also found a smaller inactivation of PG by heat (60-105°C) while complete and more than 98% inactivation of PG by hot break treatment has also been reported; 93°C, 3 min (Fachin et al., 2003) and 95°C, 2 min (Boulekon et al., 2011). PG is present in tomato fruit in two forms (PG1 and PG2) and depending on the extraction method used, a different ratio of PG 1 and PG2 is often obtained, with different overall

thermal stability (Crelier et al., 2001). Literature emphasises the pressure resistance of PG at certain temperatures is due to reversible configuration of the enzyme (Fachin et al., 2003; Krebbers et al., 2003). Hsu (2008) reported 12 and 98% PG activity reduction at 250 MPa and 400-600 MPa, respectively. Furthermore, PG in tomato-based products could be totally inactivated at some pressure/temperature combinations; 500-550 MPa/60-75°C (Fachin et al., 2003; Verlent et al., 2004). Here, significant inactivation of PG activity was found in all treatments during long-term storage, irrespective of processing method and temperature (data not shown). Aguilo-Aguayo et al. (2008) also observed decaying PG activity in tomato juice during storage at 4°C for 77 days.

4. CONCLUSIONS

Our results highlight the importance of high pressure processing (HPP) in comparison to conventional thermal processing (TP) in order to retain quality and most of the important tomato juice micronutrients, while resulting in a microbiologically stable product. However, the maintenance of these favourable properties over long-term elevated temperature storage is challenging due to stability issues over time. First order kinetics model revealed that nutrients decayed at a higher rate at 28°C in comparison to 20°C, and altering of juice pH positively affected colour preservation but negatively affected ascorbic acid retention during storage, which uniquely shows the complexity of the chemical and biochemical phenomena occurring during storage of a fresh product. HPP technology, in combination with blanching, could be a viable alternative to conventional thermal treatment for tomato juice when an ultra-high quality and nutritious product is expected. Furthermore, the results of this study could be of economic importance for the tomato juice processors by supporting the development of new added-value products which are stable in ambient temperatures and, in turn, reduce refrigeration costs under specific conditions. Nevertheless, further research is needed in order

to maintain a wider range of quality attributes after processing and during long-term storage especially if high ambient temperature tolerant products ($\geq 28^{\circ}$ C) are required in specific parts of the world.

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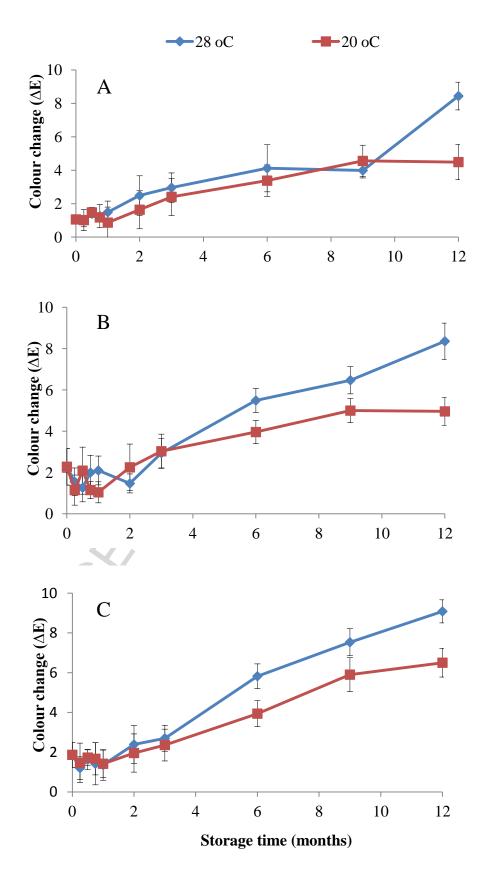


Figure 1: Effect of temperature on colour change of high pressure processed pH altered (pH 3.93 (A), unaltered (pH 4.3) (B) and thermally processed (C) tomato juice during storage.

Table 1. Initial effects of thermal and high pressure processing on microbial levels, colour, nutritional quality, and antioxidant and enzymatic activities of tomato juice.

Parameter assessed	Raw juice	Treatment applied			
			0		
	-	TP	HPP (pH 4.3)	HPP (pH 3.9)	
			2-		
MICROBIAL LEVELS)		
TVC (log cfu/ml)	6.69±0.01a	<2.00±0.00b	<2.00±0.00b	<2.00±0.00b	
COLOUR		~			
	7				
Lightness (L value)	32.76±0.34 ab	33.41±0.21 a	31.55±0.88 b	33.4±0.08 a	
	6				
Redness (a/b)	1.39±0.09 b	1.51±0.08 ab	1.66±0.05 a	1.46±0.01 b	
Total colour difference (ΔE)	0.00±0.00b	1.87±0.63 a	d2.28±0.89 a	1.06±0.11 ab	
PHYTOCHEMICAL LEVELS					
Ascorbic acid (mg/100 g)	330.7±4.5a	191.5±4.3c	220.3±3.8b	230.3±10.3b	
Total phenol content	6.5±0.8a	5.1±0.4ab	4.8±0.5b	6.1±0.2ab	
(mg gallic acid/g)					
Total carotenoid	83.7±12.9c	213.7±13.7a	165.0±13.5b	232.8±16.4a	
(mg β -carotene/100g)					
Lycopene (mg /100g)	34.9±0.4b	38.4±1.3ab	41.1±2.0a	35.9±1.5b	
ANTIOXIDANT ACTIVITY					
ABTS (µM Trolox/100 g)	1294.0±5.0a	1100.0±77.8b	1270.6±55.7a	1360.0±20.0a	

 $ORAC (\mu M \ Trolox/100 \ g) \qquad 4084.0 \pm 139.5 \quad 1615.0 \pm 311.3 b \quad 1638.3 \pm 162.5 b \quad 1809.0 \pm 109.0 b$

ENZYME ACTIVITY		K	
PME (mmol/min/g)	23.3±1.0a	1.3±0.6b 2.0±0.6b	0.5±0.1b
PG (mmol/min/g)	28.4±1.0a	21.1±2.1b 25.6±3.4ab	26.3±2.0 ab

Values are means \pm standard deviation and the same letter following the value within the each raw indicates no significant difference at p<0.05, n=3, expressed on dry weight basis.

A CLAN

Parameters	Colour	Carotenoids	Lycopene	Ascorbic	Total phenols	ABTS	ORAC
				acid	0		
Colour-redness	1	0.80**	0.85**	0.70**	0.70**	0.80**	0.33**
Carotenoid		1	0.89**	0.88**	0.90**	0.91**	0.12
Lycopene			1	0.83**	0.86**	0.89**	0.22
Ascorbic acid			4		0.85**	0.91**	0.01
Total phenols			S		1	0.87**	0.05
ABTS						1	0.08
ORAC							1
Significant** at p<0.	.01	C	<u> </u>				
		V	~				

Table 2: Pearson correlation between multiple quality parameters of tomato juice during long term storage at 28°C and 20°C.

Table 3: Effect of high pressure processing in comparison to thermal processing on phytochemical levels of tomato juice during storage at 28°C

X

and 20°C.

				0				
Parameter	Treatment		Storage time (months)					
	-	0	1	2	3	6	9	12
Ascorbic acid	28°C TP	191.5±4.3a	16.1±2.8b	10.3±2.6b	19.5±1.2b	14.8±0.7b	10.7±3.7b	12.4±0.7b
	28°C HPP (pH 4.3)	220.3 ±3.8a	43.2±7.6b	16.5±3.6c	18.2±3.3c	13.2±1.2c	8.2±3.3d	9.0±0.7d
	28°C HPP (pH 3.93)	230.3±10.0a	22.8±0.7b	23.2±4.5b	18.6±0.7b	12.0±1.2c	9.0±0.7cd	5.3±0.7d
	20°C TP	191.5±4.3a	23.2±5.4b	21.5±4.7b	22.4±1.9b	19.0±1.9b	9.9±2.6c	10.7±3.7c
	20°C HPP (pH 4.3)	220.3±3.8a	22.8±0.7b	17.0±1.2c	16.5±1.9c	12.8±1.9d	9.9±1.4e	13.2±1.2d
	20°C HPP (pH 3.93)	230.3±10.0a	27.4±1.9b	22.8±1.9b	19.0±0.7b	16.5±2.6c	14.0±0.7c	7.4±4.0d
Total phenols	28°C TP	5.1 ±0.4a	3.4±0.3b	3.1±0.2b	3.1±0.1b	3.1±0.1b	2.6±0.2b	2.6±0.1b
	28°C HPP (pH 4.3)	4.8±0.5a	3.7±0.1b	3.0±0.4b	3.3±0.5b	3.5±0.1b	3.1±0.4b	3.2±0.3b
	28°C HPP (pH 3.93)	6.1±0.2a	3.6±0.4b	3.3±0.4b	3.0±0.4b	3.2±0.1b	3.7±0.3b	3.9±0.1b
	20°C TP	5.1±0.4a	3.6±0.4a	3.5±0.6a	3.5±0.4a	3.1±0.3a	3.2±0.4a	3.1±0.5a
	20°C HPP (pH 4.3)	4.8±0.5a	4.0±0.1a	3.8±0.1c	3.0±0.1d	3.1±0.3d	3.2±0.3d	3.2±0.3d
	20°C HPP (pH 3.93)	6.1±0.2a	3.7±0.4b	3.9±0.1b	3.4±0.3c	3.4±0.5c	3.3±0.2c	3.1±0.4c

Total	28°C TP	213.7±13.7a	58.4±14.5b	46.6±16.0b	11.0±1.7c	6.3±2.4d	4.0±1.1d	4.4±1.0d
carotenoids	28°C HPP (pH 4.3)	165.0±13.5a	83.2±6.9b	57.7±4.8c	45.2±10.4d	4.0±1.1f	5.6±1.0f	11.5±2.7e
	28°C HPP (pH 3.93)	232.8±16.4a	86.9±11.9b	74.6±4.3c	33.6±2.7d	26.5±5.3e	2.8±1.7g	6.3±2.5f
	20°C TP	213.7±13.7a	70.0±14.3b	65.9±7.1b	53.4±3.3c	44.5±11.5c	26.8±8.4d	8.1±1.3e
	20°C HPP (pH 4.3)	165.0±13.5a	50.9±4.0b	53.8±0.6b	35.6±5.7c	27.2±3.4d	22.4±2.4e	18.1±1.4f
	20°C HPP (pH 3.93)	232.8±16.4a	54.8±6.8b	40.2±4.1c	41.1±2.5c	41.8±6.1c	35.0±1.7d	38.4±5.8cd
Lycopene	28°C TP	38.4±1.3a	14.8±0.9b	13.2±1.0c	12.4±0.3c	10.2±1.6d	5.9±0.8e	3.9±0.3f
	28°C HPP (pH 4.3)	41.1±2.0a	20.0±1.0b	15.1±0.5c	11.4±2.6d	8.5±0.1e	9.1±1.1e	6.2±1.2f
	28°C HPP (pH 3.93)	35.9±1.5a	12.8±1.6c	13.6±1.4c	15.3±1.3b	9.2±1.7d	10.6±1.3d	6.5±1.5e
	20°C TP	38.4±1.3a	25.17±2.1b	19.0±1.3c	16.7±0.9d	13.1±0.9e	12.0±1.3e	9.0±1.8f
	20°C HPP (pH 4.3)	41.1±2.0a	21.5±0.1b	21.4±2.7b	17.6±2.1c	14.2±1.1d	15.6±2.3cd	13.6±1.9de
	20°C HPP (pH 3.93)	35.9±1.5a	17.0±1.6c	21.4±1.1b	15.2±2.0c	11.8±1.4d	10.7±1.0d	9.2±0.6e

Values are means \pm standard deviation and the same letter following the value within the each raw is not significantly different as for the table above at p<0.05, n=3, expressed on dry weight basis.

Table 4: First order kinetics rate constants (k) and determination coefficients (\mathbb{R}^2) for the degradation of the colour and micronutrients of processed tomato juice during storage at 20 °C and 28 °C

Parameters	Storage	Treatment	Co	k	R^2
	temperature				
Colour-redness	28 °C	TP	0.5762	- 0.0953	0.83
		HPP (pH 4.3)	0.5812	- 0.0874	0.86
		HPP (pH 3.93)	0.4965	- 0.0649	0.67
	20 °C	ТР	0.4529	- 0.0637	0.77
		HPP (pH 4.3)	0.5225	- 0.0649	0.94
		HPP (pH 3.93)	0.4400	- 0.0479	0.84
Ascorbic acid	28 °C	ТР	4.9263	- 0.2979	0.75
		HPP (pH 4.3)	5.5127	- 0.3654	0.95
	0	HPP (pH 3.93)	5.2787	- 0.3637	0.83
	20 °C	TP	4.8956	- 0.2757	0.86
	5	HPP (pH 4.3)	5.3445	- 0.3440	0.81
C)	HPP (pH 3.93)	5.6617	- 0.3761	0.87
A					
Total phenol content	28 °C	TP	1.5677	- 0.0631	0.86
		HPP (pH 4.3)	1.6356	- 0.0490	0.49
		HPP (pH 3.93)	1.5506	- 0.0454	0.78
	20 °C	TP	1.6413	- 0.0552	0.86
		HPP (pH 4.3)	1.5840	- 0.0489	0.69
		HPP (pH 3.93)	1.6600	- 0.0552	0.76
Total carotenoid	l 28 °C	TP	5.9682	- 0.4694	0.94

content		HPP (pH 4.3)	5.7041	- 0.3739	0.75
		HPP (pH 3.93)	6.1000	- 0.4195	0.83
	20 °C	TP	5.5982	- 0.2783	0.85
		HPP (pH 4.3)	5.2986	- 0.2450	0.96
		HPP (pH 3.93)	5.3187	- 0.2026	0.85
			R		

Lycopene	28 °C	ТР	3.6702	- 0.2002	0.90
		HPP (pH 4.3)	3.8937	- 0.2012	0.98
		HPP (pH 3.93)	3.7958	- 0.1819	0.91
	20 °C	TP	3.8168	- 0.1511	0.95
		HPP (pH 4.3)	3.6734	- 0.1121	0.92
		HPP (pH 3.93)	3.6117	- 0.1353	0.92

ABTS	28 °C	TP	6.9779	- 0.1067	0.93
		HPP (pH 4.3)	6.9937	- 0.1208	0.82
		HPP (pH 3.93)	6.9750	- 0.1139	0.79
	20 °C	TP	7.0190	- 0.1141	0.95
	\mathbf{G}	HPP (pH 4.3)	7.0295	- 0.0999	0.85
	A	HPP (pH 3.93)	7.1366	- 0.1294	0.91

ORAC	28 °C	TP	7.5605	- 0.0139	0.07
		HPP (pH 4.3)	7.6238	- 0.0267	0.14
		HPP (pH 3.93)	7.6955	- 0.0467	0.21
	20 °C	TP	7.6483	- 0.0270	0.13
		HPP (pH 4.3)	7.6107	- 0.0258	0.15
		HPP (pH 3.93)	7.5236	- 0.0212	0.09

PME	28 °C	ТР	0.5142	0.0437	0.27
		HPP (pH 4.3)	1.2436	0.2120	0.58
		HPP (pH 3.93)	0.5707	- 0.0964	0.23
	20 °C	ТР	0.8937	- 0.1060	0.25
		HPP (pH 4.3)	0.7126	- 0.0829	0.14
		HPP (pH 3.93)	-0.2864	- 0.0273	0.03
PG	28 °C	ТР	3.5497	- 0.3499	0.98
		HPP (pH 4.3)	3.4864	- 0.2780	0.89
		HPP (pH 3.93)	3.4632	- 0.2951	0.88
	20 °C	ТР	3.4858	- 0.2988	0.97
		HPP (pH 4.3)	3.2975	- 0.3026	0.88
		HPP (pH 3.93)	3.2357	- 0.4116	0.88
	X				

Industrial relevance

There is a demand for ambient stable tomato products, especially in some parts of the world, and current industrial practices (canning, pasteurisation) either compromise in product quality or require refrigeration conditions. High pressure processing have been investigated as milder technology, with a potential to deliver superior quality. The drawback is that is also requires chill storage. The results of this study show how quality parameters behave in a high pressured tomato product and pave the way for further development that could optimise this technology. This could be of economic importance for the tomato juice industry to develop new products stable in ambient temperatures and perhaps beneficial for cutting down the refrigeration costs under specific conditions.

Highlights

- High pressure processing (HPP) on storage quality of tomato juice was studied.
- Lowering pH is beneficial in maintaining the colour of tomato juice during storage.
- Total quality of HPP tomato juice during the 1st month is superior to conventional.
- Quality degradation of the juice during storage is higher at 28°C compared to 20°C.
- Long term stability of processed tomato juice in ambient temperatures is challenging.