1 Effect of a different high pressure thermal processing
2 compared to a traditional thermal treatment on a red flesh
3 and peel plum purée

4
5
6 Authors: García-Parra, J.; González-Cebrino, F., Cava, R. and Ramírez, R*.

7 CICYTEX (Centro de Investigaciones Científicas y Tecnológicas de Extremadura).
8 Technological Agri-Food Institute (INTAEX), Avda Adolfo Suárez s/n, 06071, Badajoz, Spain.

9
10
11
12
13
14 *Corresponding author: M. Rosario Ramírez.

15 Technological Agri-Food Institute (INTAEX), Avda. Adolfo Suárez s/n, 06071. Badajoz, Spain.

16 Tel.: +34 924 012660. Fax: +34 924 012674.

17 E-mail address: mariarosario.ramirez@gobex.es

ABSTRACT

The effect of the application of different high pressure thermal (HPT) treatments on the instrumental colour, the activity of the polyphenoloxidase (PPO) enzyme, bioactive compounds (total polyphenols and anthocyanins) content and the antioxidant activity were evaluated after processing a red flesh and peel plum purée and compared to an equivalent thermal treatment (TT) from a microbiological point of view. Different pressure levels (300, 600, and 900 MPa) at different initial processing temperatures (60, 70, and 80°C) were applied with the same holding time (1 min). HPT treatments produced a similar inactivation of the PPO as the TT (~ 50%). In addition, HPT treatment was more effective than the TT in preserving the original colour, the anthocyanins content (HPT treatments were between 2.33 to 3.06 mg 100 g⁻¹ fresh weight while TT was 2.35 mg 100 g⁻¹ fresh weight, being 3.03 mg 100 g⁻¹ fresh weight content of control purées) and the antioxidant activity (HPT treatments were between 94.7% to 119.2% while TT value was 80.4% from original purée content) of the processed purées. The application of pressures at 600 MPa combined with initial temperatures of 70°C was the treatment which gave the highest inactivation of the polyphenoloxidase and best maintained the bioactive compounds of purées.

Keywords: high pressure thermal treatment, plum purée, enzyme activity, bioactive compounds,

Chemical compounds studied in this article

Cyanidin 3-O-Glucoside (PubChem CID: 441667); Cyanidin 3-Rutinoside (PubChem CID: 29231)

INDUSTRIAL RELEVANCE

The exploration of new technologies, such as the combination of pressure and heating for short times (high pressure thermal or HPT treatments), could be a new way to extend the shelf-life of fruit-derived products. HPT treatments can better preserve bioactive compared to most traditional thermal treatment. While there are currently no HPT-processed shelf-stable foods commercially available, it's possible to provide a viable process for heat-sensitive products such

as fruit-derived products that would suffer a severe loss of quality by the application of a traditional thermal process. A previous study (González-Cebrino et al., 2013) showed that high pressure processing (HPP) did not inactivate polyphenoloxidase enzyme in plum purée. Results of this paper could allow production of a plum purée with an enzyme activity similar to a thermally treated purée.

1.- INTRODUCTION

High pressure thermal (HPT) treatments consist of a combination of high pressure (500 MPa to 900 MPa) and temperature (70 to 120°C) over a short holding time. The rapid temperature increase during compression and temperature decrease in the product upon decompression could help to reduce the hardness of thermal effects encountered in conventional thermal technologies. HPT treatments offer several advantages like a quick elimination of microorganisms, a uniform and rapid increase of temperature of the product, a reduced thermal impact and processing time, and a shelf life extension of the processed food (Subramanian et al, 2006). Some authors have reported that HPT treatments provided better colour, flavor and aroma retention compared to traditional thermally treated products (Gupta et al, 2010). It accelerates enzymatic inactivation, reducing the processing time at high temperature, decreasing the severity of processing in comparison with the conventional heat treatment and improving the final quality of the food (Matser et al., 2004).

Japanese plum (*Prunus Salicina* Lindl.) is an important stone fruit marketed worldwide because of its degree of acceptance by consumers. This plum has been also cultivated commercially in Spain, where a large number of varieties have reached a major expansion and specialization (Lozano et al., 2009). One of the more remarkable characteristics of the plums is the capacity as an intestinal regulator, an effect of its dietary-fiber content, which helps to maintain and develop intestinal bacterial flora, and contribute to glycemic-index reduction (Stacewicz-Sapuntzakis et al., 2001). Traditional methods of commercial preservation of this fruit are normally carried out in an autoclave or in a hot water bath.

Red flesh and peel plums are very interesting fruit due to their high content on bioactive compounds, such as the anthocyanins and other polyphenolic compounds with a high

antioxidant capacity (Díaz-Mula et al, 2009). These substances of natural origin are interesting compounds due to their role in preventing diseases such as diabetes or cancer (Seeram et al., 2006; Zafra-Stone et al., 2007). In addition, these compounds have health-promoting properties, acting as antioxidants to eliminate free radicals, which are implicated in most degenerative diseases (Cantín et al, 2009). The 3-rutinoside and 3-glucoside of cyanidin are the major anthocyanins present in red flesh and peel plums, with smaller amounts of the corresponding peonidin derivatives (Tomás-Barberán et al., 2001).

During food processing, physical and biological factors, such as increased temperature of processing and the maintenance of the enzyme activity after the treatment may result in the destruction of phenolic compounds such as phenolic acids and anthocyanins, which reduces their functional properties. In addition, anthocyanins provide the characteristic red colour, an attribute much appreciated by consumers. The conventional heat treatments are intensive enough to degrade in a significant extent these beneficial compounds. Enzymes such as polyphenoloxidase are responsible for biological browning reactions of phenolic compounds, since during food processing, physical processes can occur on the phenolic compounds to produce brown pigments (Kim and Padilla-Zakour, 2004).

The exploration of new technologies for food processing can help to maintain and preserve these compounds in quantities similar to the fresh product. While there are currently no HPT processed shelf-stable foods commercially available, it would be of interest to provide to food industry a viable process for heat-sensitive products such as fruit-derived products that would suffer a severe loss of quality by traditional thermal processing.

HPT treatments are normally applied to preserve low-acid foods. However, in a previous study that we recently published (González-Cebrino et al., 2013) about the effect of high pressure processing (HPP) on a red pulp and flesh plum purée, the application of the treatment at commercial conditions did not reduce polyphenoloxidase activity that could decrease the shelf-life of the processed purées if no additives or other pretreatments are applied to prevent browning-related reactions. The exploration of new technologies, such as the combination of pressure and heating at short times of treatment (HPT treatments), could be a new way to

extend the shelf-life of acid foods from fruits. This type of products do not need this sort of processing from a microbiological point of view, although its application could be interesting for the inactivation of enzymes. Therefore, the aim of this research was to evaluate the effect of the HPT treatments on the colour, bioactive compounds content and the inactivation of the enzyme polyphenoloxidase on a red flesh and peel plum purée and compare the effect with a conventional thermal treatment (TT).

2.- MATERIAL AND METHODS

2.1.-MATERIAL

2.1.1.-Purée manufacture. Red flesh and peel plums cv. *Crimson Globe* were collected at commercial ripening stage from Tanynature S.L. (Zurbarán, Badajoz, Spain), a fresh fruit packing station. Plums were washed, stoned and chopped without peeling in an electric blender (Thermomix; Vorwerk, Madrid, Spain) for **1 min**. The purée obtained had a soluble solids content of 15.8°Brix, pH of 3.4 and the titratable acidity was 1.3 g malic acid per 100 g of sample weight. The purée was packaged, taking care to exclude as much air as possible, in plastic polyethylene (9.3 mL O₂/m²/24h at 0°C) pouches (10x5 cm) with 15 g of purée which were vacuum heat-sealed (ILPRA, UM 18-GAS DIG. Barcelona, Spain). Pouches of purée were quickly processed.

2.1.2.-Processing conditions.

Processing conditions were selected on equivalent basis according to the study of Timmermans et al. (2011) who compared a traditional thermal treatment with novel processing technologies. For a product of pH<4.6 y a_w>0.85, 5-log reductions of a target microorganism are required. In this case, the most resistant pathogen could be *E. coli* O157:H7 (FDA, 2004). Equivalent microbiological inactivation conditions were selected. We have analysed the log reduction of an *E. coli* O157:H7 non-toxigenic strain (Colección Española de Cultivos Tipo, CECT 5947) in all the HPT and TT applied and we have found more than 8-log reductions after all the treatments applied (data not shown). More intense processing conditions were applied to ensure the stability of the purée after processing. For the application of the thermal treatment, purées pouches were heated in a water bath equipped with a thermoregulator Techne TE-10A (Bibby

Scientific Limited, UK) until they achieved a core temperature of 85°C for 5 min. These conditions were also selected according to the local industrial conditions for pasteurisation of fruit purées. Purées samples core temperature profile was recorded using an Ellab Copenhagen (mod. Ctf. 84) data module. The register of the increase of temperature during the treatments applied is shown in figure 1.

For the application of the high pressure thermal (HPT) processing, a multi-vessel Resato unit (FPU-100-50, serial no. 14685/42798. Roden, Netherland) was used. The equipment has six vessels. Three vessels were pressurized each time (1 pouch per vessel). Before the application of the treatments, pouches were 1 min inside vessel to equilibrate temperature at the initial working temperature. The machine uses ethylene glycol as pressure-transmitting medium, and was equipped with thermostatic jacket for the temperature control. The initial temperature of the assays were 60, 70 and 80°C and the pressures applied were 300, 600, 900 MPa. The rate of pressure build-up was 10 MPa s⁻¹ and the holding time was 1 min. This holding time was chosen because during this period the temperature inside the vessel was quite constant and no great losses of heat were observed. The increases of pressure and temperature during the treatments applied are showed in figure 1. Graphics shows the temperature of the pressurization medium, and the pressure measured in the vessels during the treatments applied to plum purée. A rapid increase of temperature was observed when pressure increases due to the adiabatic heating, while when pressure decreases a fast decrease of temperature was observed which allows a fast cooling of the product. We have measured the temperature changes of the pressurization medium, so temperature of purée pouches should be lower. However due to the low amount of purée in each pouch differences of temperature between the purée and the pressurization medium should not be very different.

After processing (TT and HPT), the pouches were submerged in an ice-water bath to rapidly reduce the purée temperature. Then, samples were frozen at -40°C until the analysis was performed. Three pouches per treatment (TT and HPT) were analysed and were compared with non-processed purée (also 3 bags). Therefore, a total of 33 bags were analysed.

2.2.-METHODS

2.2.1.-Polyphenoloxidase (PPO) enzyme activity. A plum purée sample (5 g) was mixed with 10 mL of the extraction solution (0.2 M phosphate buffer, 1 M NaCl, 4% polyvinylpyrrolidone, 1% Triton X-100, adjusted to pH 6.5) for 3 min and centrifuged (Centrifuge Allegra 25R, Beckman Coulter, Inc.) at 22,300xg at 4°C for 30 min. The supernatant was filtered through a Whatman#1 paper to obtain the enzyme extract. The activity of the enzyme was determined by spectrophotometry adding 100 µL of the enzyme extract to a 3 mL of 0.1 M catechol in 0.05 M sodium phosphate buffer (pH = 6.5) solution. The tests were carried out by recording the colour changes at 400 nm for 1 min using an UV-Vis spectrophotometer (UV-2450, Shimadzu, Japan). The relative activity of the PPO was calculated according to the equation (1):

$$(1) \quad RA = A/A_0 * 100$$

where A and A₀ are the absorbance of the pressure-treated and the control samples, respectively.

2.2.2.-Instrumental colour. Instrumental colour was measured in a Konica Minolta spectrophotometer CM- 3500d. Purée samples were filled into a petri plate (10 mL) taking care to exclude air bubbles, and were placed into the 30 mm aperture masks. Reflectance measurements were made using a geometry d/8 (diffuse illumination and 8° viewing angle) with the primary illuminant D65. In addition, Hue (H) angle and Chroma (C) were calculated.

Total colour differences (ΔE) were calculated using the equation:

$$(2) \quad \Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

where L^{*}₀, a^{*}₀, b^{*}₀ are the control values for unprocessed purées (in order to evaluate changes after processing).

2.2.3.- Individual anthocyanins determination. Extractions of anthocyanins were carried out as described by Mullen et al, (2002) with some modifications. A filtered supernatant from 10 g of plum purée blended with 10 mL methanol 1% chlorhydric acid and centrifuged (Centrifuge

Allegra 25R, Beckman Coulter, Inc.) at 22,300xg at 4°C for 10 min were used to obtain the anthocyanins extracts. The process was repeated twice. The joined extracts were purified with a Sep-Pak C18 preactivated with water and methanol.

Determinations of anthocyanins were carried out as described by González-Gómez et al, (2010). 5 µL aliquots of plum purée extracts were separated using a Phenomenex Aqua C18 column (150x4.6 mm; 5 µm I.D.) and eluted at 0.5 mL min⁻¹ with a 45 min gradient of 10–30% acetonitrile in water containing 0.1% trifluoroacetic acid (v/v). The chromatographic system was coupled to a single quadrupole mass spectrometer equipped with an electrospray ionization interface which was used to identify and confirm peak compounds. The mass spectrometer was operated in positive ion mode. Once the compounds were identified, a diode array detector was used for the quantifications of the registered peak areas at 520 nm and coinjected of standard solutions.

2.2.4.-Total polyphenols content. The phenolic extract was obtained from the filtered supernatant from 5 g of purée centrifuged (Centrifuge Allegra 25R, Beckman Coulter, Inc.) at 22,300xg and 4°C for 15 min. Folin-Ciocalteu reagent was added to determinate total polyphenols content (TPC) according to the method of Singleton and Rossi (1965) with some modifications. The absorbance was measured at 760 nm using Gallic acid as a standard. Results were expressed as mg Gallic Acid Equivalent (GAE) 100 g⁻¹ fresh weight.

2.2.5.-Total antioxidant activity. Total antioxidant activity (TAA) was estimated according to the method established by Cano et al, (1998) with some modifications. The antioxidant capacity was measured by means of the ABTS (2,2'-azino-bis-(3-ethyl-benzothiazoline-6-sulphonate) diammonium salt (Sigma) radical cation assay. The measurements were carried out in UV-Vis spectrophotometer (UV-2450, Shimadzu, Japan). The results were expressed as mg of Trolox 100 g⁻¹ of fresh weight.

2.2.6.-Statistical analysis. SPSS Version 17.0 was used (SPSS Inc., Chicago, IL) for the statistical studies. 3 samples (individually processed bags) per treatment were analyzed (n=3). One-way analysis of variance (ANOVA) of the effect treatment applied was performed. HSD Tukey's test was applied to compare the mean values when ANOVA showed significant

differences. Correlations were estimated with the Pearson test at $p < 0.05$. The relationships between parameters were assessed by the calculation of Principal Components Analysis (PCA). Mean values with standard error of the mean (SEM) were reported.

3.-RESULTS AND DISCUSSION

3.1.- Inactivation of polyphenoloxidase (PPO) enzyme in plum purée after processing (HPT and TT).

All treatments applied significantly reduced the PPO activity of the plum purée (Figure 2). The percentage of activity to the PPO of processed purées compared to control purée ranged from 87% (300 MPa/60°C) to 39% (900 MPa/70°C). Intermediate values were obtained at 600 MPa throughout the range of temperatures studied. The most effective treatment to reduce the PPO activity was the combination 900 MPa/70°C. The thermal treatment reduced the activity of the enzyme similarly to the most effective high pressure thermal (HPT) treatments. So, these treatments were also quite effective to reduce the PPO activity. The least effective treatment to inactivate the PPO was the HPT treatment at 300 MPa/60°C. In general, in the HPT treatments, the increase of pressure was more effective to reduce the residual PPO enzyme activity than the increase of temperature. An increase of temperature at the same pressure intensity had no apparent effect on the inactivation of the PPO. The application of intermediate temperatures (70°C) at 300 MPa and 900 MPa, was more effective than initial temperatures of 60 and 80°C. However, at 600 MPa the three temperatures tested reached a similar enzyme inactivation. Rapeanu et al. (2005) found a similar behavior in the PPO of *Victoria* grapes. The application of mild temperatures and pressures (500 MPa/55°C) reduced the enzyme activity up to 39%, while at the same temperature and higher pressures (800 MPa) reduced the activity of the enzyme up to 80%.

Exposure time and temperature required by a thermal treatment for the inactivation of this enzyme are quite variable among different plant sources. Although PPO is generally considered to be the most thermostable enzyme in plants, temperature affects integrity of the three-dimensional structure of the enzyme molecule and resulting in disruption and denaturation (Whitaker, 1994). PPO in mango skin was also relatively thermostable, requiring more than 15

min at 80°C for 50% loss of activity (Robinson et al. 1993). Studies on thermal stability of a grape PPO indicated that the enzyme shows about a 50% reduction in activity at 65°C after 20 min and complete inactivation can be achieved at 75°C after 15 min (Valero et al. 1988).

The effect of high pressure treatment on the inactivation of enzymes was highly dependent of the food matrix (Ludikhuyze et al., 2003). Thus, while a 600-900 MPa treatment at room temperature substantially decreased the activity of PPO in apple, grape, and avocado, this treatment didn't appear to be effective in the case of pear or plum PPO. Weemaes et al. (1998) showed that the PPO of plums *Red Beauty* was not inactivated by applying treatments of high pressure at room temperature, so that combination would require more intense treatments to reduce the browning effects. In general, plum enzymes were quite resistant to pressure. Moreover, Gonzalez-Cebrino et al. (2013) studied the effect of HPP in *Crimson Globe* plum purée. HP treatment assayed (400, 500, 600 MPa, for 1, 150, and 300 s, at 10°C) did not modify the initial PPO activity of the plum purée. The initial activity of the enzyme was maintained after processing, so this fact could reduce the shelf life of purées during storage. At industrial level, this problem is sometimes solved by the use of additives such as ascorbic acid. According to our results, the application of HPT treatments could be an alternative to solve this problem since the inactivation of the PPO enzyme was quite effective. Therefore, HPT treatments would allow the production of stable plum purée with no additives (this needs be confirmed by a study of stability during storage of the processed purées).

3.2.- Instrumental colour changes in plum purée after processing (HPT and TT).

Instrumental colour changes of plum purée after processing are shown in Table 1. The lightness (CIE L*) of all processed purées was significantly higher than in control samples ($p < 0.001$). Purées after the TT had highest value while purées after the HPT treatments showed intermediate values respect to the control.

The redness (CIE a*) and Chroma (C) of plum purées were significantly higher in control purées than in purées processed by HPT at the less intense treatments (300 MPa/60°C for CIE a* and 300 MPa/60 and 70°C; 900 MPa/60°C for Chroma). The rest of treatments (TT and HPT)

showed intermediate values. Therefore, the red colour of the control purées was especially reduced after HPT at 60°C, independently of the pressure intensity applied.

The CIE b^* and Hue were significantly higher in thermally treated purée than in some HPT treatments at 600 and 900 MPa. Control purées and the rest of HPT processed purées showed intermediate values.

In order to make easier to obtain general conclusions about colour modifications after processing, the parameter (ΔE) was calculated (Table 1). In addition, the significant differences reported for individual colour parameters cannot indicate whether a difference in colour is perceptible by humans. Differences in perceivable colour could be estimated such as not noticeable (0 to 0.5), slightly noticeable (0.5 to 1.5), noticeable (1.5 to 3.0), well visible (3.0 to 6.0), and great (6.0 to 12.0). In this case, ΔE values between 4 and 5 were found for HPT treated purées which indicate that purées suffered colour modifications after processing perceptible by most people (Cserhalmi et al., 2006). For the thermally treated purée, the ΔE was around 6 which indicated that great differences were found between control and thermally treated purées. Therefore, the most intense colour modifications were found in purées after the TT.

Regarding changes in HPT treated purées, an increase in ΔE takes place when applying higher initial temperatures within the same pressure intensity. However, the increase of pressure intensity with a same initial temperature of processing did not produce an increase in ΔE , despite that increasing pressure treatments also increases the processing temperatures due to the adiabatic heating during food pressurization.

González-Cebrino et al., (2013) reported lower ΔE values (between 1-3) than in the present study when applied HPP in red plum purée. As expected, HPP maintained the original colour of red plum purée, while the HPT treatment and specially the TT applied in this study slightly affected the original colour of plum purée. The effect of HPT on the colour of red fruits depends on the matrix and the combination of pressure and temperature applied. Rodrigo et al., (2007) found that at mild temperature (65°C) and a range of 300/700 MPa pressure processing, the colour of strawberry juice was slightly affected, while Ferrari et al. (2010) have found in

pomegranate juice that the combinations of 600 MPa/50°C have a great influence in the colour perception.

The CIE L* values are generally associated to the appearance of browning in fruits and vegetables, so this parameter has been used as a browning indicator (Soliva-Fortuny et al., 2002). High pressure has been found to induce discolouration in mushrooms and onions because of the activity of the PPO enzyme, responsible for browning (Butz et al., 1994). In the present study, a negative correlation were found between CIE L* and %RA PPO in the processed samples ($r = -0.408$; $p < 0.05$). That would indicate that purées with higher PPO activity had also lower values of CIE L*, which would be in line with results reported in the literature.

3.3.- Changes in anthocyanins in plum purée after processing (HPT and TT).

Anthocyanins are a group of phytochemicals that provides red, blue and purple pigments in plant tissues. In dark-purple plums, these compounds are present in the skin and pulp. Anthocyanins are glycosylated anthocyanidins in which sugars are normally linked to the 3-hydroxyl position of anthocyanidins. The main anthocyanin reported in plum are cyanidin 3-glucoside followed by cyanidin 3-rutinoside (Tomás-Barberán et al., 2001) which are the anthocyanins found in this purée. Literature data on the effect by HPT treatments of individual anthocyanins is very limited in this fruit matrix.

Anthocyanins changes in plum purée after processing are shown in Table 2. In general, control purées showed higher anthocyanins content than purées thermally treated. The treatment at 600 MPa/70°C maintained the levels of the two anthocyanins measured similar to the control purées. So, this treatment would be the most adequate to preserve these compounds. In contrast, the least intense HPT treatments (ie. treatments at 300 MPa or/and at an initial temperature of 60°C) showed lower anthocyanins content than initial purée and the values were similar to purées thermally treated. Although pressure plays an additional role in enhancing the degradation rate of anthocyanins, the shorter treatment times in HPT treatments, result in a lower impact process in comparison to an equivalent thermal process (Plancken, et al., 2012). Total anthocyanin contents were reduced after applying HPP in plum purée (González-Cebrino et al., 2013). So the application of HPT treatments could be more effective even to HPP to

preserve these bioactive components. Probably the no inactivation of the PPO after HPP would have caused these different results.

In pomegranate juice, Ferrari et al., (2010) have also reported that anthocyanins were similar or higher than the content estimated for the fresh juice after a treatment at 600 MPa at 50°C. In contrast to our results, Verbeyst et al., (2010) reported that anthocyanins degradation was enhanced by the increase of heat and pressure (95–130°C, 0.1–700 MPa) in strawberry paste, although the effect of pressure was more important than the effect of temperature.

Our results show that pressures above 600 MPa combined with temperatures over 70°C was the best maintained the anthocyanins contents, so it could indicate that the mechanisms of the degradation of anthocyanins were modified, affecting the molecules involved in the kinetics of reaction, such as enzymes.

The degradation of anthocyanins involves a decrease in the intensity of red colour and might influence colour changes, so an attempt to correlate colour changes with the main anthocyanins present in the plum purée was made. Pearson's correlation coefficient was calculated and significant positive correlations ($p < 0.01$) were found between the parameter CIE a^* and the content of anthocyanins ($r = +0.49$ and $+0.56$ for Cyanidin-3-O-Glucoside and Cyanidin-3-O-Rutinoside, respectively). This could indicate that the changes in CIE a^* after processing would be associated to changes in the red pigments content in the plum purées.

3.4.- Total antioxidant activity and polyphenols content in plum purée after processing (HPT and TT).

The effect of HPT and TT on the total antioxidant activity (TAA) and the total polyphenols content (TPC) of plum purée is shown in Table 2. TPC and TAA were higher than those reported by González-Cebrino et al., (2013) in the same plum variety. Differences could be associated to the different manufacture process of the purées of both studies: purées were sieved in that study and probably part of the antioxidant present in the peel were removed.

All treatments applied increased or maintained the original levels of TPC. The highest TPC was found in purées after the TT and HPT treatment at 600 MPa/70°C. The other HPT treatments

assayed did not modify TPC found in control purée. The HPT treatment at 600 MPa/70°C was also that best preserved the anthocyanins content of plum purée, so it could be the treatment of election for the preservation of bioactive compounds of plum purée.

The effect of HPP on TPC content have also shown very different responses depending on the type of vegetable and the high pressure conditions (Oey et al., 2008). González-Cebrino et al., (2012) reported important increases of polyphenols content in a *Songold* plum purée after the application of a TT and HPP (400-600 MPa /10°C). TPC content decreased when strawberries were treated at 300–600 MPa and 20–60°C for 2–10 min (Terefe et al., 2009). Processing (400 MPa/5–50°C/30 min) did not modify TPC in onions (Roldán-Marín et al., 2009) while HPP of strawberry purée (600 MPa/20°C/ 15 min) showed increased TPC values (Patras et al., 2009). Corrales et al., (2008) reported that HPT treatment at 600 MPa/70°C/60 min in grape by-products produced an increased phenolic compounds recovery approximately two fold higher than the control extraction. This increase in total phenols may be associated with cell disruption entailed by the combination of temperature and pressure, which would release a large number of polyphenols compounds of plasma membranes which, usually, are combined or occluded with proteins and other macromolecular compounds and promote changes in total phenols content (Patras et al., 2009; Barba et al., 2013).

The TAA of purées was differently modified according to the processing applied. TT reduced the TAA of purées while HPT treatment maintained or increased the initial activity of the original purée. The highest values of TAA were found in purées treated at 900 MPa/80°C while the lowest activity was found in the TT treated purée.

The different trend found in the preservation of TPC and TAA in the TT could be associated to the degradation to the anthocyanins after the TT which could reduce the TAA of processed purées. Lozano et al., (2007) found that red flesh and peel plum varieties with high total antioxidant activity showed strong correlations with the phenolic and anthocyanin contents.

In addition, other antioxidants that have not been evaluated in this study such as ascorbic acid could also play an important role in these results. Ascorbic acid is generally added to preserve TPC after processing and during the manufacture process. Arias et al. (2007a, b) described the

hypothesis that ascorbic acid could act not directly on the PPO but on the oxidized products of the reaction, retarding the appearance of browning.

It is important to note that, although the application of heating was negative to preserve the antioxidant activity, the combination of high pressure and high temperature produced a positive effect on the TAA. The increase in the antioxidant activity after HPT treatments has been previously reported in other vegetable matrices such as in tomato purée processed at 400 MPa/25°C/15 min (Sánchez-Moreno et al., 2006) and in carrot juice treated at 100–800 MPa/from 30° up to 65°C/90 min (Oey, et al. 2004). This effect could be associated to an increase in the extraction of antioxidant components after the treatment due to cell membrane disruption as a result of high pressure, which also improves the bioavailability of antioxidant complexes present in vegetables by breaking down cell walls.

3.5.- Principal component analysis (PCA)

PCA is a statistical analysis for resolving sets of data into orthogonal components, whose linear combinations (principal components, PC) approximate the original data to any desired degree of accuracy. Figure 3 shows the loadings of the first principal components (PC1 and PC2) after PCA which explains the 38% and 28% of the variability, respectively. With positive loadings on PC1 are located far from the origin, the anthocyanins content and CIE a* while in the negative part of this PC are located CIE L* and Hue. In the positive part of PC2 is located CIE b* while in the negative part are located the TAA and the anthocyanins content.

Regarding of distribution of the scores on the first two PCs, PC1 and 2 explained part of the differences due to the effect of TT versus HPT treatments. Thermally treated plum purée are located in the negative area of PC1 in the same side of colour parameters like CIE b* or CIE L*. In the positive area of PC1 are located HPT treated purées (the most intense treatments) and control samples which could be related to parameters like individual anthocyanins content. PC2 also separated the thermally treated purées to the control and HPT processed purées. Most HPT treated purées were in the negative part of the PC2, associated to the TAA, while thermally treated purées were in the positive side of this PC. In general, control and HPT treated purées showed the highest levels of anthocyanins, the reddest colour and the highest TAA and this

determined the differences with the thermally treated plum purées. Non-processed purée was close to some HPT treated purées, so this would indicate that the effect of the HPT treatment maintained better the original quality of the purée than the TT.

4.-CONCLUSIONS

The application of high pressure thermal (HPT) treatments would not be necessary in very low-acid foods such as plum (pH=3.4) from a microbiological point of view. However, this type of processing was quite effective to inactivate polyphenoloxidase. This enzyme was resistant to application of high pressure at low temperature (HPP) (González-Cebrino et al., 2013), and that could limit the shelf-life of processed plum purées. However, the combination HPT treatments produced a similar inactivation to that obtained with a traditional thermal treatment (TT). In addition, HPT treatment was more effective than the TT to preserve the original colour of plum purée and the bioactive compounds content of processed purées such as the anthocyanins content and the antioxidant activity. The application of pressures at 600 MPa at initial temperature of 70°C was the combination which reached a highest inactivation of the polyphenoloxidase and best maintained the bioactive compounds of purées. Future studies comparing this technology with high-temperature-short-time processes should be carried out because differences between both treatments may be lower than in the current study

5.-ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial support by the project INIA RTA2010-00079-C02 (Obtención de purés de frutos de alto valor funcional mediante tecnologías innovadoras de procesado). J. Garcia-Parra thanks the MICINN his employment (BOE 05/12/11). R. Ramírez thanks to the Gobierno de Extremadura her employment (DOE 12/06/13).

6.-REFERENCES

Arias, E., González, J., Oria, R. & López-Buesa, P. (2007a). Ascorbic acid and 4-Hexylresorcinol effects on pear PPO and PPO catalysed browning reactions. *Journal of Food Science*, 72, C422–C429.

434 Arias, E., González, J., Peiró, J.M., Oria, R. & López-Buesa, P. (2007b). Browning prevention
 435 by ascorbic acid and 4 Hexylresorcinol: different mechanisms of action on the polyphenol
 436 oxidase in the presence and in the absence of substrates. *Journal of Food Science*, 72,
 437 C464–C470.

438 Barba, F. J., Esteve, M. J., & Frigola, A. (2013). Physicochemical and nutritional characteristics
 439 of blueberry juice after high pressure processing. *Food Research International*, 50(2), 545–
 440 549.

441 Butz, P., Koller, D., & Tauscher, B. (1994). Ultra-high pressure processing of onions: Chemical
 442 and sensory changes. *LWT - Food Science and Technology*, 27, 463–467.

443 Cano, A., Hernández-Ruiz, J., García-Cánovas, F., Acosta, M., & Arnao, M.B. (1998). An end-
 444 point method for estimation of the total antioxidant activity in plant material. *Phytochemical*
 445 *Analysis*, 9, 196–202.

446 Cantín, C. M., Moreno, M. A., & Gogorcena, Y. (2009). Evaluation of the Antioxidant Capacity,
 447 Phenolic Compounds, and Vitamin C Content of Different Peach and Nectarine [*Prunus*
 448 *persica* (L.) Batsch] Breeding Progenies. *Journal of Agricultural and Food Chemistry*, 57(11),
 449 4586–4592.

450 Corrales, M., Toepfl, S., Butz, P., Knorr, D., & Tauscher, B. (2008). Extraction of anthocyanins
 451 from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric
 452 fields: A comparison. *Innovative Food Science and Emerging Technologies*, 9, 85–91.

453 Cserhalmi, Z., Sass-Kiss, Á., Tóth-Markus, M., & Lechner, N. (2006). Study of pulsed electric
 454 field treated citrus juices. *Innovative Food Science and Emerging Technologies*, 7, 49–54.

455 Díaz-Mula, H. M., Zapata, P. J., Guillén, F., Martínez-Romero, D., Castillo, S., Serrano, M., &
 456 Valero, D. (2009). Changes in hydrophilic and lipophilic antioxidant activity and related
 457 bioactive compounds during postharvest storage of yellow and purple plum cultivars.
 458 *Postharvest Biology and Technology*, 51(3), 354–363.

459 FDA (2004). Guidance for industry: Juice HACCP hazards and controls guidance Final
 460 Guidance, March 3, 2004 (First ed). U.S. Department of Health and Human Services, Food
 461 and Drug Administration, Center for Food Safety and Applied Nutrition (CFSAN).
 462 [http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/uc](http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm072557.htm)
 463 [m072557.htm](http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm072557.htm)

464 Ferrari, G., Maresca, P., & Ciccarone, R. (2010). The application of high hydrostatic pressure for
 465 the stabilization of functional foods: Pomegranate juice. *Journal of Food Engineering*, 100(2),
 466 245–253.

467 González-Cebrino, F., García-Parra, J., Contador, R., Tabla, R., & Ramírez, R. (2012). Effect of
 468 the application of hydrostatic high pressure and thermal treatment on a “Songold” plum
 469 purée manufactured with and without an initial thermal blanching and stability during
 470 refrigerated storage. *Journal of Food Science*, 77(8), 866-873.

471 González-Cebrino, F., Durán, R., Delgado-Adámez, J., Contador, R., & Ramírez, R. (2013).
 472 Changes after high-pressure processing on physicochemical parameters, bioactive
 473 compounds, and polyphenol oxidase activity of red flesh and peel plum purée. *Innovative*
 474 *Food Science and Emerging Technologies*, 20, 34–41.

475 González-Gómez, D., Lozano, M., Fernández-León, M. F., Bernalte, M. J., Ayuso, M. C., &
 476 Rodríguez, A. B. (2010). Sweet cherry phytochemicals: Identification and characterization
 477 by HPLC-DAD/ESI-MS in six sweet-cherry cultivars grown in Valle del Jerte (Spain). *Journal*
 478 *of Food Composition and Analysis*, 23(6), 533–539.

479 Gupta, R., Balasubramaniam, V. M., Schwartz, S. J., & Francis, D. M. (2010). Storage stability
 480 of lycopene in tomato juice subjected to combined pressure-heat treatments. *Journal of*
 481 *Agricultural and Food Chemistry*, 58(14), 8305–13.

482 Kim, D.-O. & Padilla-Zakour, O.I. (2004). Jam Processing Effect on Phenolics and Antioxidant
 483 Capacity in Anthocyanin-rich Fruits Cherry Plum and Raspberry. *Journal of Food Science*,
 484 69(9), 395–400.

485 Lozano, M., Vidal-Aragón, M. C., Hernández, M. T., Ayuso, M. C., Bernalte, M. J., García, J.,
 486 Velardo, B. (2008). Physicochemical and nutritional properties and volatile constituents of six
 487 Japanese plum (*Prunus salicina* Lindl.) cultivars. *European Food Research and Technology*,
 488 228(3), 403–410.

489 Ludikhuyze, L., Van Loey, A., Smout, C., Oey, I., & Hendrickx, M. (2003). Effect of combined
 490 pressure and temperature on enzymes related to quality of fruits and vegetables: from kinetic
 491 information to process engineering aspects. *Critical reviews in Food Science and Nutrition*,
 492 43(5), 527-586.

493 Matser, A. M., Krebbers, B., van den Berg, R. W., & Bartels, P. V. (2004). Advantages of High
 494 Pressure Sterilization on Quality of Food Products. *Trends in Food Science Technology* 15,
 495 79–85.

496 Mullen, W., Stewart, A. J., Lean, M. E. J., Gardner, P., Duthie, G. G., & Crozier, A. (2002).
 497 Effect of Freezing and Storage on the Phenolics, Ellagitannins, Flavonoids, and Antioxidant
 498 Capacity of Red Raspberries. *Journal of Agricultural and Food Chemistry*, 50(18), 5197–
 499 5201.

500 Oey, I., Van Loey, A., & Hendrick, M. (2004). Pressure and temperature stability of water-
 501 soluble antioxidants in orange and carrot juice: A kinetic study. *European Journal of Food*
 502 *Research Technology*, 219,161–166.

503 Oey, I., Lille, M., Van Loey, A., & Hendrickx, M. (2008). Effect of high-pressure processing on
 504 colour, texture and flavour of fruit- and vegetable-based food products: a review. *Trends in*
 505 *Food Science & Technology*, 19, 320–328.

506 Patras, A., Brunton, N. P., Pieve, S. Da, & Butler, F. (2009). Impact of high pressure processing
 507 on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of
 508 strawberry and blackberry purées. *Innovative Food Science and Emerging Technologies* 10,
 509 308–313.

510 Plancken, I. Van der, Verbeyst, L., Vleeschouwer, K. De, Grauwet, T., Heiniö, R.-L., Husband,
 511 F. A., Lille, M., Mackie, A. R., Van Loey, A., Viljanen, K., & Hendrickx, M. (2012).
 512 (Bio)chemical reactions during high pressure/high temperature processing affect safety and
 513 quality of plant-based foods. *Trends in Food Science & Technology*, 23, 28–38.

514 Rapeanu, G., Van Loey, A., Smout, C., & Hendrickx, M. (2005). Thermal and High-Pressure
 515 Inactivation Kinetics of Polyphenol Oxidase in Victoria Grape Must. *Journal of Agricultural
 516 and Food Chemistry*, 53, 2988–2994.

517 Rodrigo, D., Van Loey, A., & Hendrickx, M. (2007). Combined thermal and high pressure colour
 518 degradation of tomato puree and strawberry juice. *Journal of Food Engineering*, 79, 553–
 519 560.

520 Robinson, S.P., Loveys, B.R., & Chacko, E.K. (1993). Polyphenol oxidase enzymes in the sap
 521 and skin of mango fruit. *Aust. Journal of Plant Physiology*, 20, 99-107.

522 Roldán-Marín, E., Sánchez-Moreno, C., Lloría, R., De Ancos, B., & Cano, M. P. (2009). Onion
 523 high-pressure processing: Flavonol content and antioxidant activity. *LWT - Food Science
 524 and Technology*, 42, 835–841.

525 Sánchez-Moreno, C., Plaza, L., De Ancos, B. & Cano, M.P. (2006). Impact of high-pressure and
 526 traditional thermal processing of tomato puree carotenoids , vitamin C and antioxidant
 527 activity. *Journal of the Science of Food and Agriculture*, 86, 171–179.

528 Seeram, N. P., Adams, L. S., Zhang, Y. J., Lee, R., Sand, D., Scheuller, H. S., & Heber, D.
 529 (2006). Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry
 530 extracts inhibit growth and stimulate apoptosis of human cancer cells in vitro. *Journal of
 531 Agricultural and Food Chemistry*, 54(25), 9329–9339.

532 Singleton, V., & Rossi, J. (1965). Colorimetry of total phenolics with
 533 phosphomolybdicphosphotungstic acid reagents. *American Journal of Enology and
 534 Viticulture*, 16, 144–158.

535 Soliva-Fortuny, R. C., Elez-Martínez, P., Sebastián-Calderó, M., & Martín-Belloso, O. (2002).
 536 Kinetics of polyphenol oxidase activity inhibition and browning of avocado purée preserved
 537 by combined methods. *Journal of Food Engineering*, 55, 131–137.

538 Stacewicz-Sapuntzakis, M., Bowen, P.E., Hussain, E.A., Damayanti-Wood, B.I., Farnsworth,
 539 N.R. (2001). Chemical Composition and Potential Health Effects of Prunes: A Functional
 540 Food? *Critical Reviews in Food Science and Nutrition*, 41(4), 251-286.

541 Subramanian, A., Ahn, J., Balasubramaniam, V. M., & Rodriguez-Saona, L. (2006).
 542 Determination of spore inactivation during thermal and pressure-assisted thermal processing
 543 using FT-IR spectroscopy. *Journal of Agricultural and Food Chemistry*, 54(26), 10300–
 544 10306.

545 Terefe, N. S., Matthies, K., Simons, L., & Versteeg, C. (2009). Combined high pressure-mild
 546 temperature processing for optimal retention of physical and nutritional quality of
 547 strawberries (*Fragaria x ananassa*). *Innovative Food Science and Emerging Technologies*,
 548 10, 297–307.

549 Timmermans, R. A. H., Mastwijk, H. C., Knol, J. J., Quataert, M. C. J., Vervoort, L., Plancken, I.
 550 Van der, Hendrickx, M., & Matser, A. M. (2011). Comparing equivalent thermal, high
 551 pressure and pulsed electric field processes for mild pasteurization of orange juice. Part I:
 552 Impact on overall quality attributes. *Innovative Food Science and Emerging Technologies*,
 553 12, 235–243.

554 Tomás-Barberán, F. A., Gil, M. I., Cremin, P., Waterhouse, A. L., Hess-Pierce, B., & Kader, A.
 555 A. (2001). HPLD–DAD–ESI/MS analysis of phenolic compounds in nectarines, peaches,
 556 and plums. *Journal of Agricultural and Food Chemistry*, 49, 4748–4760.

557 Valero, E., Varon, R., & Garcia-Carmona, F. (1988). Characterization of polyphenol oxidase
 558 from Airen grapes. *Journal of Food Science*, 53, 1482-1485

559 Verbeyst, L., Oey, I., Van der Plancken, I., Hendrickx, M., & Van Loey, A. (2010). Kinetic study
 560 on the thermal and pressure degradation of anthocyanins in strawberries. *Food Chemistry*,
 561 123(2), 269–274.

562 Weemaes, C. A., Ludikhuyze, L. R., Van den Broeck, I., Hendrickx, M., & Tobback, P. P. (1998).
 563 Activity, electrophoretic characteristics and heat inactivation of polyphenoloxidases from
 564 apples, avocados, grapes, pears and plums. *LWT - Food Science and Technology*, 31, 44–
 565 49.

566 Whitaker, J.R. (1994). Principles of Enzymology for the Food Sciences, Second Ed., pp. 271-
 567 556, Marcel Dekker, New York.

568 Zafra-Stone, S., Yasmin, T., Bagchi, M., Chatterjee, A., Vinson, J. A., & Bagchi, D. (2007). Berry
 569 anthocyanins as novel antioxidants in human health and disease prevention. *Molecular*
 570 *Nutrition and Food Research*, 51(6), 675–683.

Figures 1: A, B, C, D. Pressure and temperature changes during the high pressure–high temperature treatments applied in a plum purée for 60 s at 300 MPa (figure A), 600 MPa (figure B), 900 MPa (figure C) and thermal treatment (85°C/5 min; figure D). T1: initial temperature of treatment at 60 °C; T2: initial temperature of treatment at 70 °C; T3: initial temperature of treatment at 80 °C.

Figure 2. Effect of combined treatments of high pressure and temperature and thermal treatment in the % Relative Activity of the enzyme polyphenoloxidase (%RA PPO) in a plum purée.

TT: thermal treatment. a, b, c, d: Different letters indicate significant statistical differences due to the applied treatment (Tukey's Test, $p < 0.05$).

Figure 3. (Figure A) Loading plots after principal component analysis of the variables in the plane defined by the two first principal components (PC1: principal component 1; PC2: principal component 2). (Figure B): Score plot after principal component analysis of the individuals in the plane defined by the two first principal components (PC1: principal component 1; PC2: principal component 2).

Total Ant.: Total anthocyanins; CyRut: Cyanidin-3-O-Rutinoside; CyGlu: Cyanidin-3-O-Glucoside; TAA: Total antioxidant activity; TPC: Total polyphenolic content; %RA PPO: % Relative activity of Polyphenoloxidase.

Table 1. Instrumental colour changes after applying different treatments to a plum purée.

	Control	TT	300 MPa			600 MPa			900 MPa			SEM	<i>p</i> _{value}
			60°C	70°C	80°C	60°C	70°C	80°C	60°C	70°C	80°C		
L*	21.4c	27.3a	25.1b	25.1b	26.3ab	25.6ab	25.8ab	26.2ab	24.3b	25.1b	26.4ab	0.3	0.001
a*	25.0a	24.2abc	22.3c	23.0abc	24.7ab	23.0abc	23.7abc	24.3abc	22.7bc	23.4abc	24.0abc	0.2	0.003
b*	9.8ab	10.2a	9.3abc	8.6bc	9.3abc	9.1abc	8.4c	8.6bc	8.6bc	8.2c	8.7bc	0.1	0.001
C	26.9a	26.2ab	24.2b	24.6b	26.4ab	24.7ab	25.1ab	25.8ab	24.3b	24.8ab	25.5ab	0.2	0.003
H	21.3abcd	22.8a	22.6ab	20.6bcd	20.7bcd	21.6abc	19.5d	19.4d	20.8bcd	19.3d	19.9cd	0.2	0.001
ΔE (control-processed purée)	-	6.02	4.63	4.38	4.96	4.73	4.85	4.99	3.93	4.33	5.25		

a, b, c, d: Different letters in the same row indicate significant statistical differences due to the applied treatment (Tukey's Test, $p < 0.05$). SEM: standard error of mean. TT: thermal treatment.

Table 2. Concentration of individual anthocyanins (mg 100 g⁻¹ fresh weight), total phenolic content (mg Gallic ac.100g⁻¹ fresh weight) and TAA (mg Trolox 100g⁻¹ fresh weight) after applying different treatments to a plum purée.

	Control	TT	300 MPa			600 MPa			900 MPa			SEM	p _{value}
			60°C	70°C	80°C	60°C	70°C	80°C	60°C	70°C	80°C		
Cy-Glu	1.31ab	0.97d	1.05cd	1.07bcd	1.19abcd	1.02cd	1.33a	1.22abc	1.04cd	1.23abc	1.19abcd	0.02	0.001
Cy-Rut	1.72a	1.38b	1.37b	1.40b	1.54ab	1.31b	1.73a	1.56ab	1.33b	1.59ab	1.53ab	0.03	0.001
AntTotal	3.03a	2.35b	2.42b	2.47b	2.74ab	2.33b	3.06a	2.79ab	2.37b	2.82ab	2.71ab	0.05	0.001
Phenolic content	65.98bc	76.28a	67.34bc	65.23bc	72.46ab	65.40bc	77.42a	69.88abc	61.11c	64.79bc	72.49ab	0.99	0.001
TAA	192.5def	154.77g	185.6ef	182.3f	184.8ef	201.5cd	197.5de	184.9ef	213.5bc	217.6ab	229.4a	3.53	0.001

a-g: Different letters in the same row indicate significant statistical differences due to the applied treatment (Tukey's Test, p<0.05). SEM: standard error of mean. TT: thermal treatment.