High pressure assisted thermal processing of pumpkin purée: effect on microbial counts, color, bioactive compounds and polyphenoloxidase enzyme

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

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

*Corresponding author: M. Rosario Ramírez.

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

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

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

ABSTRACT

The effect of high pressure assisted thermal processing (HPTP) to preserve the pumpkin purée was evaluated. Pumpkin cv. *Butternut* purée was submitted to 9 different combinations of pressure and temperature of HPTP (300, 600, 900 MPa/60, 70, 80 °C/1 min). Native microorganisms', instrumental color, polyphenol oxidase (PPO) enzyme activity, carotenoids content, total antioxidant activity and individual and total polyphenols content were examined after processing and compared to non-processed purée. In treated purées, the inactivation of native microorganisms increased proportionally with pressure and temperature; CIE L* increased while CIE a* decreased respect to control purée. Moreover, most of the treatments applied reduced markedly the PPO activity, although the treatment at 600 MPa/70 °C was the most effective to reduce its activity. All treatments applied were effective to maintain or increase the content of carotenoids and phenolic compounds in the purée. The combination of 600 MPa/70 °C would be the treatment of choice since it increased the contents of bioactive compounds and the antioxidant activity of the purées, while caused the largest reduction of PPO activity.

Keywords: high pressure assisted thermal processing, bioactive compounds, carotenoids, polyphenols, pumpkin.

Chemical compounds studied in this article

Lutein (PubChem CID: 5281243); β -carotene (PubChem CID: 5280489); α -carotene (PubChem CID: 6419725); Syringic acid (PubChem CID: 10742); Chlorogenic acid (PubChem CID: 1794427)

INDUSTRIAL RELEVANCE

The combination of pressure and heating for short times (high pressure assisted thermal processing or HPTP), could be a new alternative to extend the shelf-life of vegetable products. Native microorganisms were significantly reduced after HPTP that demonstrated effectiveness of this treatment for the microbial inactivation. HPTP processing could improve the

bioavailability of bioactive compounds and the nutritional value of products like pumpkins, which need intense heat treatments to reach an adequate stability for their commercialization due to their high pH. Combined treatment of high pressure and temperature applied in pumpkin purée increased the extraction of carotenoids and phenolics compounds. This treatment would be appropriate to maintain and even increase the bioactive compounds of pumpkin purée.

1.-INTRODUCTION

Conventional methods for food pasteurization and sterilization are based on thermal processing. The application of high temperatures affects adversely to the quality of foods since they cause the degradation of some nutritional compounds such as vitamins, carbohydrates, fatty acids and/or proteins. The increase of chemical reactions leading to off-flavors, destruction of health-related compounds in foods such as pigments, polyphenols and antioxidant compounds; and color changes, among others, are particularly important disadvantages of conventional thermal processing technologies (Patras et al., 2009).

Because of these limitations, and the increased perception of consumers towards foods with higher nutritional and functional quality, food industry is interested in alternative approaches for food preservation such as emerging food processing technologies (Nielsen et al., 2009). Nowadays there is a growing interest in novel technologies that applies hydrostatic high pressures for food preservation. High pressure assisted thermal processing (HPTP) can be used for food products sterilization or pasteurization as a function of the temperature of treatment (Van Der Plancken et al., 2012). HPTP utilizes moderate initial temperatures between 60°C and 90°C in which, through internal compression heating at pressures of 600 MPa or greater, the process temperatures can reach 90°C to 120°C. The process has been proposed as a high-temperature short-time process, where both pressure and compression heat contribute to the treatment.

The effects of combined treatments of high pressures with heating have been widely studied in microorganisms and microbial spores (Nguyen et al., 2013). In general, combined pressure and temperature is applied to inactivate the vegetative cells and bacterial spores who are resistant to the application of high pressures at low-room temperatures (Mújica-Paz et al., 2011). Benefits of HPTP in preserving the quality attributes of foods like color or health-related compounds are quite less studied than microbiological aspects. Regards carotenoids, which are pigments that give a coloration that ranges from yellow to red, some authors (Gupta et al., 2010) have evaluated the impact of HPTP on their retention or bioavailability in tomato and carrot.

Pumpkin is a vegetable highly appreciated for its content of health-related compounds, such as the carotenoids and other polyphenols compounds which contribute to the antioxidant capacity (Murkovic et al., 2002). The consumption of carotenoids lowers the risk of degenerative and cardiovascular diseases, cataracts, macular degeneration as well as certain types of carcinomas (Rao & Rao, 2007). Chlorogenic acid, a representative phenol acid compound present in this vegetable pulp (Dragovic-Uzelac et al., 2005), have antioxidative activities for human low-density lipoprotein (Nardini et al., 1995) and for reactive species of oxygen and nitrogen (Kono et al., 1997), antimutagenic and anticarcinogenic effects (Friedman, 1997). It is very important to determine the content of these major phenolic acids to characterize the nutritional value of pumpkins.

Enzymes like the polyphenoloxidase, which are responsible for biological browning reaction of phenolic compounds, was found to be partially inactivated by HPTP on carrots (Kim et al., 2001). Heat-sensitive products, such as purées from pigmented fruit or vegetable, which would suffer a severe loss of quality by traditional thermal processing, would be the target of this novel technology. HPTP is investigated for the preservation of low-acid foods such as pumpkin, which pH is generally around 6-7. For this reason, the preservation of this vegetable by high pressure processing applied at low temperatures, would not reach a sufficient shelf-life and more intense treatments need to be applied.

Contador et al. (2012) and Zhou et al., (2014) studied the effect of high pressure processing on pumpkin. However, as far as we know, none study has evaluated the effect of HPTP on pumpkin purée. The aim of this research was to evaluate the impact of HPTP into bioactive compounds such as carotenes, polyphenols and antioxidant activity as well as the polyphenoloxidase enzyme, on a pumpkin purée.

2. -MATERIAL AND METHODS

2.1.-MATERIAL

2.1.1.- Purée manufacture. Pumpkins cv. *Butternut* were collected at commercial ripening stage from Finca La Orden (Badajoz, Spain), an experimental agronomic center. Pumpkins were washed, removed seeds and chopped with an electric blender (Thermomix; Vorwerk,

Madrid, Spain) for 5 min at maximum speed. The purée obtained had a soluble solid content of 10.1 °Brix, pH of 6.60 and the titratable acidity was 0.12 g malic acid per 100 g of sample weight. The purée was packaged, taking care to exclude air as much as possible, in plastic polyethylene (9.3 mL O₂/m²/24h at 0 °C) pouches with 15 g of purée which were vacuum heat-sealed (ILPRA, UM 18-GAS DIG. Barcelona, Spain). Pouches of purée were processed immediately after being filled and closed.

2.1.2.- High pressure thermal process conditions. A multi-vessel Resato unit (FPU-100-50, serial no. 14685/42798. Roden, Netherland) was used. The equipment had 6 vessels. Three vessels were pressurized each time (1 pouch per vessel). Before the application of the treatments, the samples were equilibrated at the initial working temperature for 1 min. This time was necessary to equilibrate the temperature of ethylene glycol (pressure-transmitting medium) when the sample was introduced into the vessel. This operation was repeated for all samples. The unit is equipped with a thermostatic jacket for the control of temperature. 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.

Three pouches per treatment were analysed and were compared with control non-processed purée. Therefore, a total of 30 bags were analysed. The increases of pressure and temperature during the treatments applied are shown in figure 1. Graphics show the temperature of the pressurisation media, and the pressure measured inside the vessels during the treatments applied to pumpkin purée. The temperature probe is located inside the cap of the vessel, in contact with the pressurization medium. The adiabatic heating is responsible of the fast increase of temperature during pressure building up, while when pressure application finishes a rapid decrease of temperature is observed. After processing, the pouches were submerged in ice-water bath to rapidly reduce the purée temperature. Then, samples (processed and unprocessed) were frozen at -40 °C until the analysis was performed.

2.2.-METHODS

2.2.1.- Microbiological analysis. Decimal dilutions of 10 g pumpkin purée were prepared in sterile 0.1% (w/w) peptone solution. Aerobic total counts were measured in plate count agar (Merck) at 30 °C for 72 h. Total Coliforms were determined in violet red bile agar (Merck) at 37 °C for 48 h. *Escherichia coli* counts were measured in Chromocult® Coliform agar (Merck) incubated at 37 °C for 24 h. The quantity of spores of *Clostridium perfringens* and *Bacillus* counts were measured in SPS-agar (Merck) at 37 °C for 24-48 h and plate count agar (Merck) at 37 °C for 48 h, respectively, from a peptone samples solution heated at 80 °C for 10 min. Moulds and yeasts counts were measured in yeast extract glucose chloramphenicol agar (Merck) at 25 °C for 5 days.

All culture media were prepared following manufacturer indications. After HPTP, only samples treated at initial temperatures of 60 or 80 °C were analysed. Only extreme temperatures were studied because intermediate treatments would probably produce an intermediate effect between the previous ones.

2.2.2.- Instrumental color. Instrumental color was measured in a Spectrophotometer CM-3500d (Konica Minolta Inc., Japan). Purée samples were filled into a Petri plate taking care to exclude air bubbles, and placed into the 30 mm aperture masks. Reflectance measurements were made using geometry d/8 (diffuse illumination and 8° viewing angle) with the primary illuminant D65. In addition, Hue (H) angle was calculated ($H = \tan^{-1} \frac{b^*}{a^*}$) as well as the Chroma (C) ($C = \sqrt{a^{*2} + b^{*2}}$).

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

$$(1) \quad \Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

where L_0^* , a_0^* , b_0^* are the control values for control purées (in order to evaluate changes after processing).

2.2.3.- Carotenoids determination. Carotenoids (lutein, α -carotene and β -carotene) were extracted with acetone in conditions of restricted lighting. Samples were analyzed using an Ultra High Performance Liquid Chromatographer Agilent 1290 (Agilent Technologies, USA) with a

Zorbax Eclipse Plus C18 (column 50x2.1 mm; 1.8 µm particle size) coupled to a DAD detector (measuring at 460 nm). The gradient mobile phase was a mix of acetonitrile/methanol 85:15 (solvent A) and acetonitrile/methanol/ethyl acetate 60:20:20 (solvent B) containing 0.1% BHT (butylated hydroxytoluene) and 0.05% TEA (Tetraethylammonium): initially of 100% solvent A for 4 min, then 5 min with 100% solvent B and finally 100% solvent A until the end of the run. Flow rate was 0.3 mL/min and column temperature was maintained at 28 °C using a column oven. Carotenoids were identified and quantified by their retention times using standard solutions. Results are expressed as mg 100 g⁻¹ of sample weight.

2.2.4.- Polyphenol oxidase (PPO) enzyme activity. The extraction and the assay of the enzyme activity were carried out following the method of Terefe et al., (2010) with some modification. A pumpkin purée sample (5 g) was mixed with 10 mL of the extraction solution (0.2 M phosphate buffer, 1M NaCl, 4% polyvinylpolypyrrolidone, 1% Triton X-100, adjusted to pH 6.5) for 3 min and centrifuged (Centrifuge Allegra 25R, Beckman Coulter Inc., USA) 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 spectrophotometrically 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 color changes at 400 nm for 1 min using an UV-Vis spectrophotometer (UV-2450, Shimadzu, Japan). The relative activity (RA) of PPO was calculated according to the equation (2):

$$(2) \quad RA = \frac{A}{A_0} * 100$$

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

2.2.5.- Total and individual polyphenols content. For total phenolic compounds determination 5 g of purée were centrifuged (Centrifuge Allegra 25R, Beckman Coulter Inc., USA) at 22300xg and 4 °C for 15 min and filtered by glass wool. To determinate total polyphenols content (TPC) Folin-Ciocalteu reagent was used according to the method of Singleton and Rossi (1965). The absorbance was measured at 760 nm using gallic acid as a standard. Results were expressed as mg Gallic Acid Equivalent (GAE) 100g⁻¹ fresh weight.

For individual phenolic compounds determination, previous phenolic extract were injected in a HPLC-MS. A Phenomenex C18 HPLC column (150x4.6 mm, 5 μ m) heated at 35 °C was used for chromatographic separation. The mobile phase used for the separation was composed of aqueous TFA (trifluoroacetic acid) 0.1% (A) and acetonitrile (B) in gradient mode set as follows: initial conditions 10% B; from 0 to 3 min 10% B; from 3 to 15 min 15% B; from 15 to 20 min the composition was kept constantly at 15% B; from 20 to 25 min 18% B and from 25 to 40 min 30% of B. The flow was fixed at 0.5 mL/min and the injected volume was 50 μ L. The chromatographic system was coupled to a DAD and an API-ESI single quadrupole mass spectrometer, operated in the positive-ion mode. Peaks identification was accomplished using mass spectrometer scan mode and retention time of standards. DAD was used for the quantifications with the peak areas, and external standard method.

2.2.6.- Total antioxidant activity. The anti-oxidative capacity was estimated according to the method established by Cano et al. (1998) and it was measured by means of the ABTS (2,2'-azino-bis-(3-ethyl-benzothiazoline-6-sulphonate) diammonium salt radical cation assay. Determinations were carried out using a UV-Vis spectrophotometer (UV-2450, Shimadzu, Japan). Results were expressed as mg of Trolox 100 g⁻¹ of raw matter.

2.2.7.- Statistical analysis. Three samples per treatment were analyzed (n=3). Statistical analyses were performed using SPSS Version 17.0 statistical package (SPSS Inc., USA). To determine the effect of treatments on studied traits a one-way analysis of variance (ANOVA) was performed. HSD Tukey's test was applied to compare the mean values when ANOVA showed significant differences. Mean values with standard error of the mean (SEM) are reported. Correlations were estimated with the Pearson test at p<0.05.

3.-RESULTS AND DISCUSSION

3.1.- Changes in microbiological counts of a pumpkin purée.

The effect of HPHT on the native microorganisms of pumpkin purée was compared to non-processed purée (Table 1). Microbiological analyses were performed to evaluate the efficacy of treatments on inactivation of microorganisms.

Mesophilic bacteria, total coliforms, viable spores of *Bacillus spp*, moulds and yeasts counts were significantly reduced after the HPTP. Microbial counts of *Clostridium perfringens* and *E. coli* were under the limit of detection of the method ($<1 \text{ Log UFC g}^{-1}$) in all pumpkin purées.

The effectiveness of HPTP on the inactivation of native microorganisms directly increased with pressure and temperature. As a result, the most intense HPHT (900 MPa at 60 and 80 °C), resulted in the inactivation of coliforms, moulds and yeasts, also caused reduction of viable spores of *Bacillus spp* counts (counts were under detection limit of method). Mesophilic bacteria were also inactivated although counts were above the detection limit at 900 MPa/60 °C ($1.89 \text{ log CFU g}^{-1}$); when the temperature of the treatment increased at 80 °C the reduction was under detection limit. In addition, HPTP (600 MPa at 80 °C and 900 MPa at 60 and 80 °C) reduced viable spores of *Bacillus* contamination level at least in 2 log units.

Pressure acts on vegetative microbial cells via inhibition of protein synthesis, enzyme denaturation and decrease of lipid membrane fluidity (Bartlett, 2002). In HPTP, the temperature rise during the treatment is a significant factor to establish the efficiency of inactivation. Kalchayanand et al., (1998) reported that the combination of pressure in the range of 200-500 MPa with a temperature greater than 50 °C could drastically reduce the number of vegetative bacteria in a wide range of food products. Leadley et al., (2008) found the absence of detectable aerobic colony counts in green beans after HPTP at 700 MPa/ 86 °C in two cycles of treatment of 2 min each one. Kim et al., (2001) found no viable cells in the carrot juice pressurized above 400 MPa, being 300 MPa, 50 °C, for 10 min the minimal condition for combined treatment of pressure and mild heat to maintain bioactive compounds like carotenoids and destroy vegetative forms. Sohn and Lee (1998) reported that pressure treatment above 400 MPa could inactivate the yeasts and the moulds in carrot juice.

Thus the thermal impact may become a deciding factor for the application of HPHT, since certain conditions, such as the initial temperature, heat capacity of foods, etc., affect pathogen inactivation. Therefore, it is highly recommended that the food temperature profile during HPTP should be properly controlled to analyze microbiological results.

According to the literature (Wilson et al., 2008; Bull et al., 2009) treatments applied in this study would not be sufficient to eliminate spores and could germinate at ambient temperatures in a low acid product. So processed pumpkin purées at these conditions would not be sterile and needs storage at refrigerated temperature.

3.2.- Changes in instrumental color of a pumpkin purée.

Table 2 shows instrumental color changes after HPTP of pumpkin purée. Apart from HPTP, changes in color could be also caused by the preheating time inside the vessel (impossible to avoid in each experimental run), and because color measurements were carried out in the presence of oxygen. Although these factors affected all samples it is possible that final color of purées would be better avoiding these conditions.

Significant differences were found for L^* , a^* and Hue values while b^* and C were not affected by treatments. Lightness (CIE L^*) was significantly higher in processed purées than in control samples. Purées treated at 600 MPa/60 °C had the highest value of L^* , while other treatments had intermediate values respect to the control. Within the same pressure of treatment, the lightness was not significantly affected by the temperature.

The CIE a^* value was significantly highest in untreated purées and HPTP at lower temperatures and pressure (300 MPa/60 °C). Within the same pressure level, CIE a^* value tended to decrease with increasing temperature, so purée treated at 900 MPa/80 °C had the lowest CIE a^* value. Hue angle was significantly higher in purées treated at 300 MPa/80 °C than in control and those processed at the same pressure level and lower temperature. Contador et al., (2012) did not find differences between untreated pumpkin purée and 400 MPa/5 min treated purée (refrigerated temperature) for CIE $L^*a^*b^*$ parameters, while more intense pressures at 600 MPa/5 min decreased the parameters CIE L^* , a^* and b^* value. The differences in the values of CIE L^* and a^* which undergoes pumpkin purée in our study may be due to the increase of pressure, rather than to the increase of temperature within the same pressure intensity.

The significant differences in individual color parameters cannot indicate whether a difference in color is perceptible by humans or not. For that reason, total color differences (ΔE) were calculated (Table 2). Differences in perceivable color can be classified analytically 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). Therefore, ΔE values of at least 3 indicate a color difference perceptible by most people (Cserhalmi et al., 2006). Since ΔE values after processing pumpkin purée (Table 2) were around 3-4, color changes could be considered perceptible by the human eye, indicating that HPTP cause color changes regarding to untreated purées.

At 300 and 900 MPa, ΔE increased when initial temperature of processing increased, so initial temperature of treatment could be determinant for the preservation of the original color of pumpkin purées. The greatest color changes occurred with a combined treatment of high pressure and mild temperature (600 MPa/60 °C). Oey et al., (2008b) reported that high pressure/low and moderate temperatures of processing have a limited effect on pigments (chlorophyll, carotenoids or anthocyanins) responsible for the color of fruits and vegetables. Anyway, all processing conditions caused a perceptible change of color, which is consistent with previous studies in other vegetables with similar characteristics as pumpkin such as carrots (Nguyen et al., 2007). These authors studied carrot samples treated at 500-700 MPa/ 95-121 °C/5 min, and found the maximum color change ($\Delta E=6$) at 700 MPa/121 °C, that was lower than color changes values found in conventional thermal treatment at 121 °C. Combined treatment of lower pressure and temperature (500 MPa/95 °C) achieved a lower color difference.

3.3.- Changes in carotenoids of a pumpkin purée.

Carotenoids found in pumpkin purée were lutein, α -carotene and β -carotene (Table 3). The contents of lutein and β -carotene were significantly altered after HPTP ($p<0.05$).

Lutein content increased after HPTP at 300 MPa/60 °C, being this carotene which suffered the highest increase in treated purées. Respect to β -carotene content, purées treated at 600 MPa and 900 MPa had higher levels than those treated at 300 MPa, although differences were not statistically different except for purée processed at 300 MPa at 60 °C that had the lowest β -carotene content.

In general, the application of HPTP was effective to maintain and/or increase the content of individual carotenoids, especially at 600 and 900 MPa. The temperature of the treatment at 600 or 900 MPa did not change the content of individual carotenes, but 70 °C seems to be the

temperature at which the highest levels of total carotenoids were found. In contrast, at lower pressure treatments (300 MPa) changes in carotenoid contents were dependent on the temperature of processing.

Oey et al., (2008a) reported that the total carotenoids content of fruits and vegetables was either unaffected or increased by high pressure treatments. Intense treatments could result in physicochemical changes that affect the extractability of carotenoids, as a consequence of pressure-induced structural changes at cell membrane. This can result in different release of carotenoids as has been reported for nectarine purée submitted to high pressure processing (HPP) (600 MPa/10 °C/5 min) (García-Parra et al., 2011). The effect is related to the intensity of pressure applied and type of matrix, as it has been reported for different fruits and vegetables such as orange juice, carrot and tomato purée, in which the extractability of carotenoids was dependent on the intensity of pressure applied (Oey et al., 2008b). Similarly, Contador et al. (2012) described an increase in the extraction of carotenoids in acidified pumpkin purée treated by HPP (600 MPa/5 min/10 °C).

When pressure is combined with high temperatures, the carotenoids are also well preserved or even increased. In fact, in some fruits, HPTP increases the extraction of carotenoids when pressure is applied by the heating (Oey et al. 2008b) or even if both treatment applied separately, first heated and then pressurized (Contador et al., 2012). In this respect, Tauscher, (1998) found in carrot based product a very low carotene loss (less than 5%) at 600 MPa/75 °C/40 min. These results would be in line with those reported for pumpkin purée.

These results are contributing to give advantage to other recent applications of different high pressure food preservation. Lately, there is a great interest to assess it is possible to increase the extraction of functional compounds from vegetable by-products by pressurizing it prior to the extraction of compounds. Also due to the high resistance to heat treatment of carotenoids (Sánchez-Moreno et al., 2005), the combination of high pressure and heating may enhance the recovery of these compounds in matrixes such as pumpkin in carotenoids.

Sánchez et al., (2014) found that carotenoids content of commonly consumed vegetables (carrot, tomato or red pepper) was not significantly influenced by HPP (625 MPa, 5 min, 20 °C)

or HPTP (625 MPa, 5 min, 70 °C and 625 MPa, 5 min, 117 °C). Similarly, Van Der Plancken et al. (2012) did not find major losses in α -carotene or β -carotene in carrot purée treated at 800 MPa and 40–74 °C. At higher treatments temperatures, Vervoort et al. (2012) did not point out significant changes in α -carotene and β -carotene in carrot after high pressure sterilization at 700 MPa and 124.8 °C for 3 min. Nguyen et al. (2007) also reported a 92% carotene retention in carrot after 700 MPa and 121 °C treatment for 1 min.

There was a significant positive correlation ($r = +0.543$, $p < 0.05$) between lutein content and CIE b^* values as a direct reflection of carotenoids content which also increased significantly at pressure above 600 MPa. Some authors (Murkovic et al., 2002; Gliemmo et al., 2009) found correlations between the carotenoid profile and fresh pumpkin color. These authors stated that pumpkin varieties with high carotenoid content have a more orange color. On the other hand, Kim et al., (2001) reported a decrease of β -carotene and α -carotene in carrot juice treated at 500 MPa and 70 °C (65% to 78%). This degradation could be due to the loss of the vegetable structure during the production of carrot juice. However, in processed product, for example in the form of purée, is difficult to establish this kind of relationship because certain treatments applied (such as mechanical homogenization or high pressure treatments) enhance the extractability of carotenoids from vegetable matrices retained (Oey et al., 2008b).

3.4.- Relative activity of enzyme Polyphenoloxidase (PPO) of a pumpkin purée.

The effect of HPTP of pumpkin purée on the residual activity of PPO is shown in Figure 2. The percentage of residual activity of PPO compared to control pumpkin purée varied as a result of HPTP between 112% (300 MPa at 70 °C) and 40% (900 MPa at 70 °C). All treatments significantly reduced the PPO activity with the exception of the treatment at 300 MPa/70 °C, which increased the PPO activity. At 600 and 900 MPa, the inactivation of the PPO was similar for all combinations of pressure and temperature tested, whereas the effect of processing at 300 MPa was related to the initial temperature of processing. At 300 MPa, treatments performed at 60 and 80 °C achieved an intense enzyme inactivation (especially at 80 °C), while the treatment at 70 °C enhanced the activity of the PPO.

The combination of 600 MPa with an initial temperature of 70 °C achieved greater inactivation of polyphenoloxidase enzyme of processed pumpkin purée. This combination was also that better preserved the quality of red flesh and pulp plum purée; it reached a highest inactivation of the PPO and best maintained the bioactive compounds of plum (García-Parra et al., 2014). Probably a similar inactivation of the PPO enzyme on both fruit purées would be responsible of the equivalent results.

Crelier et al., (2001) applied different HPTP on tomato and found that the ratio of inactivation of the enzymes tested, like polygalacturonase and pectinmethylesterase, was reduced at pressures between 300 and 600 MPa at 70 °C of temperature at the end of the pressure build-up phase. In our study, the applied conditions were similar, although the results were different because the rate of inactivation of PPO increased with increasing pressure intensities. However, the effect of high pressure treatment on the inactivation of enzymes is highly dependent of the food matrix (Ludikhuyze et al., 2003) and that could explain the differences in the response of each product to the treatments applied.

The result of the PPO the treatment at 300 MPa with an initial temperature of 70 °C is unexpected. It is difficult to hypothesize about the activation or the increase of the extractability of the PPO under those conditions. Other authors have also described increases in this enzyme activity after application of some high pressure treatments, but we have not found an explanation for HPTP. Hendrickx et al., (1998) reported an increase in the PPO enzyme activity in apple (300-500 MPa/ room temperature/ 1 min), onion (pressure above 300 MPa/ 25 °C/ 10min) or pear (600 MPa/ 20 °C/ 6h) and Gomes et al., (1996) tested in mushrooms different pressure intensities and the extracts processed at 400 MPa at room temperature for 10 min showed an activity of PPO up to 140% and even after 10 min at 800 MPa remained about 60% of the original value.

The resistance of endogenous enzymes to HPTP emphasizes the necessity of combining this treatment with other techniques, such as storage at low temperature or naturally occurring protein inhibitors to maintain food quality and extend shelf- life. In this case, as the final residual activity of the enzyme was between 50-60%, the utilization of other methods to avoid the activity of PPO would be recommended.

3.5.- Total and individual polyphenols content of a pumpkin purée.

The effect of HPTP on total polyphenolic content (TPC) of pumpkin purée is shown in table 4. All treatments applied increased the initial levels of TPC. The highest TPC was found in purées after HPTP at 600 MPa/70 °C with a 65% of increment compared to control purée while other HPTP increased TPC between 39% to 60%.

The absence of studies that evaluate the effect of HPTP on TPC of vegetables makes difficult to discuss this results. However, some studies have evaluated the effect of HPP on pumpkin. Zhou et al., (2014) reported an increment of TPC in pumpkin purée by HPP respect to control purée (550 MPa/10 min/room temperature). Treatments at similar HPP conditions (400 and 600 MPa/5 min/ 10 °C) maintained or decreased the TPC content of pumpkin purée (Contador et al., 2012) but this purée was industrially obtained and contained ascorbic and citric acids. On the other hand, generally the thermal treatment applied for food processing led to an increase in phenol concentration in green vegetables (Turkmen et al., 2005).

The thermal pasteurization well preserved the TPC content of pumpkin purée (Contador et al., 2012). In our results, the important increase of TPC in pumpkin purée after HPTP could be enhanced by the combination of both effects (heating and high pressure). These results suggested that treatments might produce changes in antioxidant extractability, like cellular disruption and dissociation of some phenolic compounds from biological structures or alteration their chemical structure to conversion of insoluble phenolic into more soluble forms (Dini et al., 2013).

Individual phenolic compounds present in control and purée by HPTP were quantified by HPLC (Table 4). Compounds detected were two phenolic acids such as syringic acid and chlorogenic acid, being syringic acid the most abundant phenolic compound. Syringic acid content was modified after HPTP showing significant differences. Pumpkin purées treated at 900 MPa/60 °C had the lowest content of chlorogenic acid while control presented a higher concentration. Chlorogenic acid did not show significant differences due to the treatment applied, although means were statistically different.

The differences between results of TPC and individuals polyphenols detected could be associated to changes in other phenolic compounds different to the two isolated in our study and which could have determined the changes of total compounds. In this respect, Dragovic-Uzelac et al., (2005) detected four phenolic compounds, namely chlorogenic acid, *p*-coumaric acid, caffeic acid and syringic acid in *Cucurbita moschata* and other pumpkin varieties. TPC could be also related to a better extractability of some antioxidant components after the treatment, such as amino acids and/or protein with phenolic hydroxyl group.

Relative activity (%) of PPO showed an inverse correlation with TPC ($r=-0.431$, $p<0.05$). This could indicate that the changes in total polyphenols content after HPTP would be associated to changes in the PPO activity in pumpkin purées. A higher inactivation of the PPO could maintain better the levels of TPC, since the enzyme oxidized the phenol compounds. In addition, a significant positive correlation was found between CIE L*-value and TPC values ($r=+ 0.844$, $p<0.001$). CIE L*-value increased significantly ($p<0.001$) when subjected to HPTP respect to control purées. These correlations could indicate that the color of the purée became lighter probably due to the higher inactivation of PPO, which could induce an enzymatic browning of phenolic compounds in the pumpkin purée, leading a darker purée due to the formation of brown pigments. This is confirmed by a negative correlation of CIE L*-value with relative activity of PPO ($r=-0.478$; $p<0.01$) where higher lightness, lower PPO activity. Therefore, high levels of polyphenols would be associated to low PPO activity and to a lighter color of the processed pumpkin purées.

3.6.- Total antioxidant activity (TAA) of a pumpkin purée.

The effect of HPTP on TAA of pumpkin purée is shown in Table 4. TAA in pumpkin purées was altered differently depending on the pressure/temperature of processing. TAA of pumpkin purées treated at 300 MPa was significantly lower than control samples while purées processed at 900 MPa and at 70 and 80 °C were significantly higher than control purées. In addition, the treatment at 600 MPa/70 °C also showed the highest TAA.

The increase in the TAA after HPTP seems to be related to an better extractability of antioxidant compounds from the vegetable matrix instead of an absolute increase (Oey et al. 2008a). The

TAA is a parameter dependent on the matrix under study, according to the review by Oey et al. (2008a), because each vegetable has a different composition of compounds with antioxidant activity and the net effect depends on the stability of these compounds during high pressure treatment.

The major compounds with antioxidant activity in the pumpkin would be phenolics and carotenoids and these compounds should not significantly change during processing and may even increase (Oey et al., 2008a), which is consistent with the results obtained in this work. Moreover, Indrawati et al., (2004) found an increase in the antioxidant activity after processing carrot juice from 100 up to 800 MPa/from 30 up to 65 °C/max for 90 min.

The content of β -carotene showed a positive significant correlation to the TAA ($r = + 0.568$, $p < 0.01$). Probably this compound also contributes to radical scavenging activity of purées due to concentration of this carotenoid in this pumpkin purée. Moreover, %RA PPO had a negative significant correlation ($r = -0.449$, $p < 0.05$) with TAA. This could be explained by the lower levels of phenolic compounds and carotenoids in those purées with more intense PPO activity. These compounds could be mostly responsible for the antioxidant activity of pumpkin purée.

4.-CONCLUSIONS

Mesophilic, total coliforms, *Bacillus* and moulds and yeasts counts were significantly reduced after the HPTP that demonstrated the effectiveness of this kind of treatment on the native microorganisms of pumpkin. Thus the thermal impact may become a determining factor for the HPTP at certain conditions, which include the initial temperature or matrix composition, it makes important to improve understanding of the effects of HPTP on health-related compounds. Other important attributes like color may be affected, increasing CIE L^* values. HPTP of pumpkin purée at moderate temperatures may be a suitable alternative to preserve and even increase the bioavailability of health-related compounds of pumpkin purée since the levels of carotenoids, phenolics acids or antioxidant compounds were increased (better extracted) after processing. The combination of 600 MPa with an initial temperature of 70 °C achieved greater inactivation of polyphenoloxidase enzyme, increased levels of bioactive compounds (carotenoids and polyphenols) and the highest antioxidant activity of processed pumpkin purée.

The resistance of this enzyme to HPTP emphasizes the necessity of combining this treatment with other techniques, such as storage at low temperature or addition of inhibitors to maintain food quality and extend shelf- life. In addition, since intensity of the treatments applied should not reach a sterilization effect, storage at low temperatures should be recommended after processing.

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Figures 1: A, B, C. Pressure and temperature changes during the HPTP applied in a pumpkin purée for 60 s at 300 MPa (figure A), 600 MPa (figure B) and 900 MPa (figure C). T1: initial temperature of treatment of 60 °C; T2: initial temperature of treatment of 70 °C; T3: initial temperature of treatment of 80 °C.