

Influence of Selected Packaging Materials on Quality Aspects of High Pressure Processed Boccoli Puree

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Abstract
In the present study, role of packaging properties on pressure and thermal processed broccoli puree quality were investigated. Samples were packaged in two multilayer packaging pouches (PET/PA/LLLDPE and BOPP/LLDPE). Packaging type significantly influenced the product colour. PET/PA/LLLDPE package best preserved broccoli puree quality. Thermal processes at both intensity levels, had a significant effect on quality properties of broccoli puree. Tensile strength of both packaging materials were improved by severe HP and thermal pasteurization. The shape of endothermic heating curves of both packaging materials as well as Tg value of BOPP/LLDPE, compromised by conventional thermal treatments. High pressure processing at 600 MPa (holding time: 10 min/initial temperature: 10 °C) improved the colour ($a*$ and $L*$ values) compared to severe HP pasteurization and thermal pasteurization processes. Both packaging materials resulted in inactivation of the inoculated *Listeria innocua* to a level below the detection limit at HP and thermally processed broccoli pure.
Keywords: Packaging material, Broccoli, Puree, Thermal processing, High pressure processing, Barrier properties

1. Introduction

Broccoli is one of the most popular *Brassica* vegetables. It has received significant attention in recent years due to its flavour and health promoting properties. The health properties of broccoli have been associated with its unique micronutrients, photochemicals, and anticancer or antioxidant properties which have been mainly attributed to glucosinolates and phenolic compounds (Ares, Nozal, & Bernal, 2013). Broccoli puree is a relatively new kind of high quality functional food product which provides an opportunity to use broccoli in more recipes. Typically, a thermal treatment is employed to extend the shelf life of vegetable purees. However, during the thermal processing, photochemicals may undergo thermal degradation (Alvarez-Jubete, Valverde, Patras, Mullen, & Marcos, 2013; Lopez-Sanchez et al., 2014). In this respect, it has become necessary to explore and adopt alternate processing methods such as high pressure processing (HPP). The food technology community has witnessed numerous studies presenting the effect of high pressure processing on food quality characteristics with that of conventional thermal processing. Most research claim high pressure treated foods generally have superior quality compared to their thermally treated counterparts without conducting the HP and thermal experiments in a similar condition (Liu et al., 2014; Zhao et al., 2013). In a recent research (Vervoort et al., 2013) presented a comparison between HP and thermal processing based on principles of equivalence. They aimed to guarantee a fair comparison based on equivalent microbial safety.

Prior to conducting most of the food processing methods, food products must be vacuum packaged. In particular, the success of most preservation methods depends on the possible effect of treatment conditions on the mechanical, morphology and barrier properties of the packaging material. The modifications in properties of the packages may have an effect on quality characteristics of the packaged food products such as oxidation, microbial growth, package damage or even safety problems (Rivas-Cañedo, Nuñez, & Fernández-García, 2009). Also, food packaging should maintain physical integrity, flexibility and withstand the mechanical and thermal loading associated with HPP and thermal processing (Bermúdez-Aguirre & Barbosa-Cánovas, 2010). Single and combinations of LLDPE, OPP, PET, PA and EVOH films are a number of commonly used packaging materials for thermal and HP processes. Polymeric films with adhesive laminated films on a polymer base are also adopted (Ayvaz et al., 2012; Richter, Sterr, Jost, & Langowski, 2010). However, delamination has been reported to occur in multilayer structures, including aluminium foils or metalized layers particularly after HPP(Morris et al., 2007; Sansone, 2008). Colour, texture and sensory properties are very important criteria of broccoli puree products and are most obviously damaged by thermal pasteurization. Motivated by these premises, this research aims at evaluation the impact of packing material, high-pressure and thermal processing on the quality of broccoli puree.

2. Materials and methods

2.1. Broccoli puree preparation

Broccoli (Brassica oleracea cv. Italica) were purchased from a local supermarket and stored at 4 °C until processing. Broccoli were washed, and stem and florets were separated (approximately 2 cm from top). The samples were blanched in a water bath (WB5, Grant Instruments Ltd., Shepreth, Cambridgeshire, U.K.) at 95 °C for 5 min. The temperature in the core of the pieces was monitored using thermocouple (HI 98804, HANNA instruments, USA) attached to a data logger. After heating, the broccoli were immediately cooled down in ice water, followed by blending of the samples using a laboratory blender (Multipro FP732, Kenwood, Havant, UK), operating the first 20 s at low speed and the next 40 s at high speed. Broccoli puree was immediately packed in multilayer polymer pouches, and kept in a refrigerated room (4 °C) until HPP and conventional heat pasteurization.

2.2. Packaging materials

Two commercial multilayer polymer films supplied by the Plastic Mashin Alvan (PLA) Industry (Tehran, Iran) were analysed: PET (12 µm)/ PA (40 µm)/ LLDPE (40 µm) and OPP (25 µm)/ LLDPE (35 µm). The packaging films were used to fabricate flexible pouches of 12 cm × 10 cm surface dimensions. Pouches were obtained by sealing the three sides of the films using an impulse sealer PAC Seal (IS300C, 500W). The pouches were filled with 20 ml distilled water (food simulant) and heat sealed. As much air as possible was excluded from the pouches. The pouches for the control experiment were not treated and characterized for their mechanical, structural and barrier properties.

2.3. High-pressure and thermal processing treatments

To obtain a fair comparison for the process impact (Vervoort et al., 2011), HP and thermal processing conditions were selected to result in equivalent microbial safety. Two different intensity levels including mild pasteurisation and severe pasteurisation for each process was implied: HPP at 600 MPa/ holding time: 10 min/ initial temperature:10 °C (H1), HPP at 700 MPa/ holding time: 5 min/ initial temperature: 38 °C (H2), thermal processing at 70 °C/ holding time: 7.5 min/ P_{10}^{70} = 2 min (T1), thermal processing at 90 °C/ holding time: 19.6 min/ P_{10}^{90}=10min (T2). H1 and T1 were selected to achieve mild pasteurization or 6 log reductions of *Listeria monosytogenes* while H2 and T2 were selected to targeting severe pasteurization or 6 log reductions of non-proteolytic *Clostridium botulinum* type E spores. So, there were eight treatments considering two packaging materials (P1: PET/PA/LLDPE and P2: OPP/LLDPE) as follows: H1P1, H1P2, T1P1, T1P2, H2P1, H2P2, T2P1, T2P2. Given H1 and T1 (mild pasteurization) and, H2 and T2 (severe pasteurization) were processing conditions recommended to ensure microbial stability in equal basis (FSAI, 2006). Comparison between these conditions could be considered as a fair comparison (Vervoort et al., 2011).

Thermal treatments were conducted in a thermostated water bath (SAP2, Grant, UK). Timing was started after the slowest heating point in the samples attained the experimental temperature. Samples were heated in water for 2 min and 19.6 min at 70 °C and 90 °C, respectively. High pressure treatments were performed using a Stansted Food Lab 900 high-pressure isostat, capable of operating at 900 MPa (Stansted Fluid Power Ltd., Stansted, UK). The internal diameter of the pressure chamber was 70 mm and the height of the chamber was 203 mm. The pressure transmission fluid was 90% water: 10% Cooledge oil (Castrol, UK) and the temperature increase because of adiabatic heating was approximately 3 °C per 100 MPa. Pressure was increased to 600 and 700 MPa, kept at the desired pressure for 10 and 5 min at initial temperature of 10 °C and 38 °C respectively. The pressure increasing rate was 300 MPa min^{-1}, while depressurisation took 1 min. Temperature in the sample chamber was thermostatically controlled during processing.

2.4. Microbial analysis

A subsample of the broccoli (150 g) was inoculated with 1500 µl of the *L. innocua* suspension (10^8 spores/ml). The inoculated puree and samples of uninoculated puree were packed in PET/PA/LLDPE and BOPP/LLDPE pouches for assessing the effect of packaging material, pressure and thermal treatment effect on microbial inactivation. Samples were stored at 4°C for 24 hours before enumeration. All enumerations were carried out on both a non-selective agar (Tryptone Soya Agar + 0.6% yeast extract (TSAYE) and a selective medium (Oxoid Chromogenic Listeria Agar (OCLA). All plates were incubated at 37 ºC for 48 h. After incubation, microbial colonies were counted and noted as colony forming units (cfu).

2.5. Colour assessment

Colour assessment was conducted at 25±2 °C using a Hunterlab ColorQuest colorimeter (Illuminant D65, Reston, VA, USA). Colour was expressed as L* (lightness; 0=black, 100=white), a* (−a*=greenness, +a*=redness) and hue angel (tan^{-1} b/a). The values determined at a 10° observer angle. The instrument was calibrated using the white tile provided. Broccoli puree samples were transferred into a low reflectance sample container. Three colour measurements were taken per sample and the results were averaged.

2.6. Barrier properties

Water vapour transmission rates (WVTRs)

The water vapour transmission rate (WVTR) was determined using a Permatran 3/33 (Modern Control, Inc., Minneapolis, MN) following the ASTM F1249 method (ASTM, 2006). Film specimens of surface area 50 cm^2

were cut from the polymeric pouches and mounted inside the testing chambers at 100% RH and 25^{o}C. The WVTR of the control (untreated), HP and thermal treated pouches were measured in replicates.

2.7. Differential Scanning Calorimeter (DSC)
HPP and the thermal processing may induce structural changes on thermal transition characteristics of the packaging materials. In order to investigate possible changes DSC test was carried out by using Pyris 6 DSC (Perkin elmer, Waltham, USA). To perform the test, an amount of approximately 10–15 mg taken from untreated, HP and thermal treated pouches, was sealed in nonhermetic aluminium pan and then tested under nitrogen atmosphere. Tests were performed in triplicate. The heating scans were carried out from 25 °C to 190 °C at a heating rate of 10 °C/min for OPP/ LLDPE samples and for PET/PA/LLDPE samples, the scans conducted from 25° to 280 °C at a heating rate of 10 °C/ min, being the typical sample weight around 8 mg. Melting temperature (Tm) and glass transition temperature (Tg) were calculated from the DSC thermograms (TA Universal Analysis). Scans for each sample were carried out in triplicate, and the average values are reported in this paper.

2.8. Statistical analysis
Experimental data obtained was statistically analyzed using statistical software (Littell, Henry, & Ammerman, 1998). Trends were considered significant when means of compared sets differed at $P < 0.05$ (Student's t-test).

3. Results and discussion
3.1. Antimicrobial effects
The effects of HPP on the microbial inactivation of broccoli puree were compared to control and conventional processing methods. The microbiological assays showed that the inoculated *Listeria Inoccua* were reduced greater than 6 log, after the HPP and thermal processing at all intensity levels to less than 10 cfu/ml (Table 1). In spite of the impressive safety records associated with conventional thermal processing, it has the tendency to induce permanent changes to the nutritional and sensory attributes of foods (Awuah, Ramaswamy, & Economides, 2007). This result highlights the major advantage of HPP over conventional thermal processing. Different from thermal processing, HPP allows instant pressure transmission in food, thereby achieving reduced product damage. Moreover, this process leads to inactivation of microorganisms. Similarly, (Jung, Lee, Kim, Lee, & Ahn, 2012) observed microbial inactivation (more than 5 log cfu/g) in carrots and spinaches treated at 500 MPa after 5 min.

3.2. Colour Parameters
The instrumental colour parameters showing differences among control (non-treated), thermal and pressurised broccoli puree samples are presented in Table 2. Low-intensity pasteurization by high pressure resulted in an increase in L* values in comparison with the control samples ($p > 0.05$). This can be partly related to HPP's formation of a transculant structure which affect the extent of internally scattered light and the distribution of surface reflectance (Oey, Van der Plancken, Van Loey, & Hendrickx, 2008).

The increase in L* values following HP light pasteurization, obtained in the present study is in agreement with previously published studies of vegetables such as pressurised spinach puree (Wang et al., 2013), cucumber juice (Zhao et al., 2013) and vegetable beverage (Barba, Esteve, & Frigola, 2010). However, the L* values of the samples treated with both light and severe thermal processing, were significantly lower than those of the control ($p > 0.05$). It was probably due to the thermal degradation resulting in non-enzymatic browning (Wang et al., 2013). Hue angel was used to express the degree of colour change induced by processing (HPP and thermal) in broccoli puree. All thermal processed samples showed lower hue angel compared to the control and pressurized samples. (Decrease in hue angle indicates a change from green to yellow). No a* value changes were observed between control and pressurized samples at 600 MPa (10 °C) ($p > 0.05$). All thermal treatments and high pressure severe pasteurization resulted in a decrease in a* values in comparison with the control samples. Better colour retention in broccoli purees treated by Low-intensity pasteurization by high pressure is probably due to better extraction of chlorophylls from the vegetable matrix. Discoloration due to thermal treatments can result from several reactions, including membrane leakage, cell and chlorophyll breakdown, and Millard reaction (Ibarz, Pagan, & Garza, 2000; Tian, Woolf, Bowen, & Ferguson, 1996).

3.3 Water Vapour Transmission Rate (WVTR)
Thermal Severe and light pasteurization caused a significant increase in WVTR of both PET/PA/LLDPE and BOPP/LLDPE packaging materials ($p < 0.05$) (Table 1). Changes in WVTR seem to be less significant than O_2 permeability. Due to the structure of BOPP, LLDPE and other polyolefins having tertiary carbons, they might be more vulnerable to oxidative connections than linear polymers. Severe and light HP treatments did not cause significant changes in WVTR of packaging pouches and confirm that the permeability of structures are less affected by HP processing.

(Caner et al., 2004), also studied the water vapor permeability of different packaging films and did not report significant changes in water permeability properties of PET/SiOx/LDPE; whereas a significant increase (up to 150%) was observed in the permeability of oxygen, carbon dioxide, and water vapor of Metalized-PET/EVA/LLDPE.

Other researchers investigated the effect of HPP on EVOH based packaging materials and stated that the WVPR of the films was increased after HPP (Galotto et al., 2010; Halim, Pascall, Lee, & Finnigan, 2009). It may be related to the fact that this polymer absorbs water due to OH bonding and cannot be a good water vapour barrier during and after most of the processes like HP and thermal processing.

3.4. Differential scanning calorimetry (DSC)

The DSC heating curves of the BOPP/LLDPE and PET/PA/LLDPE systems are presented in Figures 1 and 2. The LLDPE, BOPP, PET, PA endothermic melting peak of control samples were at 114.24°C, 158.46°C, 249.3 ± 0.35°C and 229.60 °C, respectively. The PET/PA/LLDPE shows three peaks corresponding to the melting points of three polymers and, the BOPP/LLDPE shows two peaks. BOPP, PET, PA and LLDPE polymers in BOPP/LLDPE and PET/PA/LLDPE had almost identical melting temperatures. However, after thermal light and severe pasteurisation treatments, a slight but not statistically significant decrease ($p < 0.05$) in the melting point of PA phases in PET/PA/LLDPE and significant decrease in the Tg of BOPP/LLDPE ($p < 0.05$) was observed. On the other hand, no difference in Tg values was observed between the untreated, HPP, and thermally treated PET/PA.LLDPE packages. Alterations, including changed configuration of the amorphous regions could be the reason for decrease in Tg values. Additionally, thermal severe pasteurization caused the significant changes in the shape of the heating curves in both PET/PA/LLDPE and BOPP/LLDPE packaging materials. The difference in the shape of the heating curves could be caused by a change in the structure (e.g. Tg value) of polymers as a result of thermal treatments. PET/PA/LLDPE shows two small endothermic peaks before melting peaks of LLDPE and after melting peaks of PET (Fig 2), while there are no intermediate peaks between those of BOPP and LLDPE in the BOPP/LLDPE packaging films. This indicates that there is probable formation of cocrystallites of the lower melting fraction of LLDPE and the higher melting fraction of PET. Furthermore, In the case of BOPP/LLDPE and PET/PA/LLDPE, Heating curves corresponding to the high pressure treated samples are a bit more regular and arranged, which may indicate an improvement of their crystalline structure. There has been a limited number of studies about comparing pressure and heat treatment effect on thermal properties of packaging materials. (Galotto et al., 2010) reported that there was a slight decrease in Tm and DH of PE/EVOH/PE associated with EVOH after processing at 400 MPa and 20, 60 °C for 30 min. Combined pressure-heat treatment caused a similar change in the shape of the melting peaks of EVA in both Nylon/EVOH/EVA and Nylon/EVA packaging materials and Polyethylene in MetPET/PE polymers. (Mensitieri et al., 2013) mentioned that when there are different layers inside the package, the behaviour with pressure could be more complex than a simple layer, since there is also the effect promoted by other layers. Contrary to the results reported above, (Sansone, 2008) reported that HP promoted an upward shift on Tm of packaging materials. LDPE and PLA were suitable for HP pasteurization (low temperature) while during HP/HT sterilization, PLA is subjected to a glass to rubber transition and this appeared to be a critical factor for scalping.

4. Conclusion

The application of pressure and temperature in mild and severe pasteurization treatments of foods may alter structural, functional and mechanical properties of multilayer polymer films. Under the practical point of view, PET/PA/LLDPE and BOPP/LLDPE can withstand mild and severe HP pasteurization conditions. A variation of 7-14% may be detected in functional and structural properties of films compared to their starting value. Moreover, an improvement in tensile strength and heating curve arrangements were observed in both packaging materials after HP processing, which is due to an improvement in crystalline morphology. Thermal process damaged packaging materials and therefore causes a drastic increase in OTR and WVTR compared to untreated and HP packaging materials. Also, DSC curves implied that there was a change in the structure of packaging while employing thermal processing. This effect may change amorphous and crystalline domains in polymer films which promotes rubbery to glassy state transition induced by heating.

Using appropriate packaging material is another important element infood processing.The packaging material should be flexible enough to resist compression forces and temperatures while maintaining physical integrity and suitable barrier and mechanical properties. Our research demonstrated that PET/PA/LLDPE and BOPP/LLDPE were more compatible with HP light and severe pasteurizing compared to thermal pasteurization.

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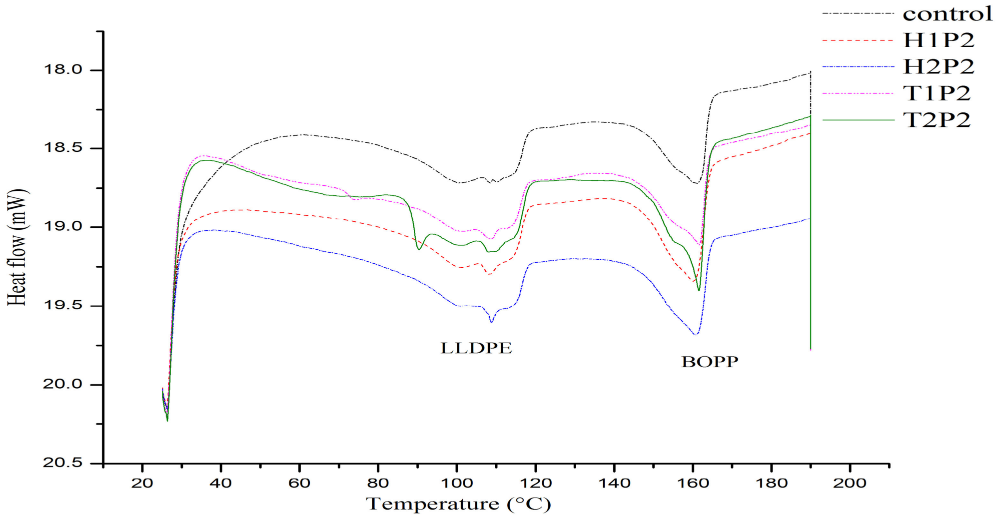

Fig 1. DSC thermogram of control, HP light pasteurization (H1P2), HP severe pasteurization (H2P2), thermal light pasteurization (T1P2) and thermal severe pasteurization (T2P2) of BOPP/LLDPE polymer films.

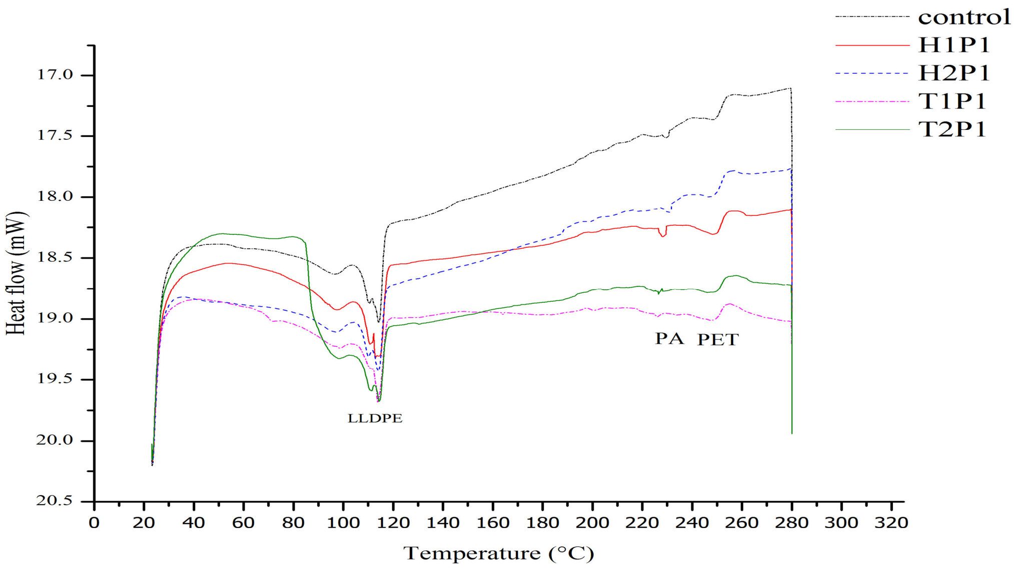

Fig 2. DSC thermogram of control, HP light pasteurization (H1P1), HP severe pasteurization (H2P1), thermal light pasteurization (T1P1) and thermal severe pasteurization (T2P1) of PET/PA/LLDPE polymer films.

Table 1. Inactivation of Listeria innoccua by HPP and thermal treatments. TSAYE indicates a non-selective medium (Tryptone Soya Agar + 0.6% yeast extract) and OCLA indicates a selective medium (Oxoid Chromogenic Listeria Agar).

Process conditions	OCLA counts (cfu[1]/g)	TSAYE counts (cfu/g)
Suspension used for inoculating	1.46×10^{10}	1.46×10^{10}
Control	1.37×10^{8}	1.64×10^{8}
Thermal light pasteurization	n.d.[2]	n.d.
Thermal severe pasteurization	n.d.	n.d.
HPP light pasteurization	n.d.	n.d.
HPP severe pasteurization	n.d.	n.d.

[1]cfu: colony forming units
[2]n.d.: not detected or lower than detection limit which were 10 cfu/g

Table 2. Effect of processing methods on instrumental colour parameters of broccoli puree

	Colour parameters		
Process conditions	-*a** value	*l** value	$\tan^{-1}$ b/a
Control	10.41	34.13	130.1
Thermal light pasteurization	8.23	29.31	125.8
Thermal severe pasteurization	5.82	25.83	120.5
HPP light pasteurization	10.45	41.15	129.7
HPP severe pasteurization	8.15	33.94	128.5

Table 3. Oxygen transmission rate (OTR) and Water vapor transmission rate (WVTR) for packaging materials before and after thermal and HP treatments. For thermal treatments, T1 indicates thermal light pasteurisation and T2 the severe pasteurisation. For HP treatments, P1 and P2 indicate HP light and severe pasteurisation, respectively.

	OTR (cc/m^2 –day)					WVTR (g/m^2 -day)				
Packaging material	Control	H1	H2	T1	T2	Control	H1	H2	T1	T2
PET/PA/LLLDPE	7.785^{a}	7.796^{a}	8.401^{ab}	9.801^{b}	11.995^{c}	0.080^{a}	0.088^{a}	0.087^{a}	0.10^{b}	0.159^{b}
BOPP/LLDPE	102.12^{a}	102.56^{a}	107.23^{a}	123.93^{b}	148.62^{c}	1.101^{a}	1.165^{a}	1.103^{a}	1.36^{b}	1.485^{b}

Means with different letters within a column are significantly different ($P < 0.05$).