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High hydrostatic pressure processing of pumpkin: Identification and quantification of the volatiles profile

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Summary

The aim of this study was to evaluate the volatiles profile of a pumpkin purée processed by high hydrostatic pressure (HHP) at various pressure values and holding times, specifically, 400 MPa and 600 MPa for 200 s, 400 s and 600 s. The content of volatiles was evaluated by the method of solid-phase microextraction with gas chromatography-mass spectrometry (SPME GC-MS). A total of 42 volatile compounds were identified. Major compounds were alcohols and aldehydes, while minor compounds were ketones, esters, aromatic compounds and terpenes. Significant changes were found after processing in all chemical groups except for ketones and esters. The effect of the increment of the volume of pressure was higher than the increment of holding time. Most volatile compounds detected have their origin in lipid oxidation reactions, few compounds were formed by carotenoids degradation, while none volatile compound was formed by Maillard reaction. Most compounds isolated in pumpkin purée were formed by lipid oxidation reactions, especially by the activity of oxidative enzymes such as lipoxygenase. The control of the development of these reactions could be essential to preserve the original aroma of this fruit.

Keywords

hydrostatic high pressure; volatile compound; pumpkin; solid phase microextraction

High hydrostatic pressure (HHP) is already established as a successful food technology, which is used for the manufacture of many different types of food products such as beverages or fish-, meat- and vegetables-based ready meals. The use of HHP as a technology for preservation of foods is an effective food decontamination process that improves the balance between safety and quality characteristics of the food products [1].

In literature, there are already some studies showing interesting results and conclusions about how HHP modifies bioactive compounds in pumpkins [2–4]. However, there are no studies analysing volatile compounds. HHP is assumed to not alter substantially the fresh odour of the fruits and vegetables, since small aromatic compounds are not directly affected by pressure [5]. GONZÁLEZ-CEBRINO et al. [6] reported that the volatiles profile of a plum purée was well preserved by HHP. However, HHP can also alter indirectly the content of some volatile compounds by enhancing or retarding enzymatic or chemical reactions, which subsequently results in undesired changes in the overall volatiles profile [7].

Pumpkin pulp is a good source of antioxidant compounds such as carotenoids, principally β-carotene and α-carotene, or phenolic compounds [2]. These antioxidants are degraded at high temperatures [8], so the application of alternative technologies to preserve health-promoting compounds of pumpkins is being investigated. In this respect, the study of changes in the volatile compounds could serve as a method to disclose development of unknown and unexpected compounds in pumpkin purée after processing [9, 10]. A previous study [11] about the effect of high pressure thermal processing (300–900 MPa at 60–80 °C) reported that lipid oxidation, Maillard reaction and carotenoids degradation were the main routes of formation of volatile com-

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pounds after processing. The analysis of volatile compounds was a useful tool to evaluate global changes in the aroma of processed pumpkin purée. Therefore, the aim of this study was to evaluate the global effect of the application of various HHP conditions on the volatiles profile of a pumpkin purée in order to provide a global view of the main chemical changes of volatile compounds after this type of processing.

MATERIAL AND METHODS

Production of the pumpkin purée

Pumpkins *Cucurbita moschata* cv. Butternut were obtained from a local vegetable plant in Santa Amalia, Spain and stored at 4 °C before the manufacture of the puree for one week. Pumpkins were peeled and then they were cut into small pieces (~50–100 g) and blended using a domestic food processor (Thermomix Vorwerk, Wuppertal, Germany) for approximately 2 min until obtaining a homogenous purée. This process was repeated several times (approximately 1 kg each time) until enough purée was obtained for all analyses planned to do. Immediately, 200 g of raw purée were vacuum-heat-sealed using a vacuum chamber machine VM-18 (Orved, Musile di Piave, Italy) in polyethylene foil (permeability 9.3 ml O_2 per 1 m^2 per 24 h at 0 °C) bags. Three replicate samples of the purée were prepared for each combination of pressure intensity and holding time.

High hydrostatic pressure treatment

HHP was applied in a semi-industrial discontinuous hydrostatic unit Hiperbaric Wave 6000/55 (Hiperbaric, Burgos, Spain), which uses tap water as the pressure-transmitting medium. Pumpkin purée was pressurized at various combinations of two pressure values and three holding times (400 MPa and 600 MPa for 200 s, 400 s and 600 s). Processing conditions were chosen according to results of previous studies carried out on pumpkin [4, 6]. Pumpkin flesh is very sensitive to microbial spoilage due to its pH (approximately pH 7), which is optimal for the growth of microorganisms. For that reason, strong microbial inactivation is required to ensure an adequate shelf-life of the product. All treatments assayed significantly reduced the most important microorganisms of pumpkin purée [4]. Come-up times to target pressures were 174 s and 230 s, respectively. When the treatment finished, the decompression (come-down time) took approximately 1 s. The initial temperature of the water in the high-pressure vessel was set at 10 °C. Both pressurized and unprocessed (control) purée samples were immediately stored at –80 °C. For the volatiles profile assay, 18 HHP-treated and 3 unprocessed samples were analysed ($n = 21$).

Solid-phase microextraction

The technique of headspace solid-phase microextraction (HS-SPME) was used for the extraction of volatile compounds. Fifteen grams of purée were placed in a 50 ml vial with 3 g of NaCl added to facilitate the release of volatile compounds. Samples were homogenized and equilibrated at 40 °C for 5 min in a thermostatically controlled water bath under agitation at low velocity (~1.7 Hz). Next, the volatile compounds in the headspace of the vial were extracted during 40 min by means of a 1 cm SPME fibre coated with a 50/30 μm film thickness of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) StableFlex (Supelco, Bellefonte, Pennsylvania, USA). The fibre was transferred to the injection port of the gas chromatograph where volatile compounds were thermally desorbed at 250 °C for 2 min.

Analysis of volatiles by GC-MS

The volatile compounds were analysed on a CP-3800GC gas chromatograph coupled to a Saturn 2200 MS (both Varian Medical Systems, Palo Alto, California, USA). Compounds were separated using a capillary column HP-5 (50 m × 0.32 mm × 1.05 μm; Agilent Technologies, Santa Clara, California, USA). The carrier gas was He (purity: 99.999999 %) at a constant flow of 1 ml·min^{-1}. The oven temperature was set from 35 °C for 5 min, increasing by 2 °C·min^{-1} to 175 °C and then increasing by 25 °C·min^{-1} to 300 °C. The GC–MS transfer line was heated at 280 °C. The mass detector operated in electronic impact (EI) mode of 70 eV and source temperature of 230 °C with a speed of 0.7 scans per second over a mass range of m/z 30–400 in a 1 s cycle in a full scan mode [8]. The volatile compounds were tentatively identified by comparing their mass spectra with published spectra database NIST v.05 (National Institute of Standards and Technology, Gaithersburg, Maryland, USA) and library Scanview 8.0 (Varian Medical Systems). In addition, when it was possible, confirmation was performed by matching the retention time and mass spectrum of commercially acquired standard solutions (Sigma-Aldrich, St. Louis, Missouri, USA).

Statistical analysis

The differences in the concentration of volatile compounds were analysed using one-way analysis of variance. Honestly-significant-difference

(HSD) Tukey's test ($p < 0.05$) was applied to compare the mean values for different HHP treatments. Another two-way ANOVA was calculated to evaluate the effect of the volume of pressure and holding time. Mean values and standard error of the mean (SEM) are reported. Statistics analysis was performed using the SPSS programme, version 17.0 (SPSS, Chicago, Illinois, USA).

RESULTS AND DISCUSSION

The two-way ANOVA (Tab. 1) was used to provide a general view of the effects of the variables analysed, namely, the volume of pressure and holding time. The results showed that most volatile compounds were modified after processing. Specifically, 25 volatile compounds were affected by the volume of pressure applied (400 MPa vs 600 MPa) while 7 compounds were affected by the holding time (200–600 s). These results mean that the number of volatile compounds affected by the volume of pressure was higher than the number of volatile compounds affected by the holding time or by the combination of both variables (pressure, time). Interactions between previous factors were significant only in two compounds (nonane and 5-methyl-3-heptanone). Other studies also reported that the relevance of pressure value was higher than that of holding time [5], which would be consistent with our results. In general, high value of pressure are normally applied in commercial applications (500–600 MPa) with holding times shorter than 10 min. The application of holding times shorter than 10 min allows the application of a maximum number of cycles of processing per hour, which diminishes the equipment depreciation costs. The range of pressure intensity and holding time applied in this study would be in line with conditions required at commercial level to preserve this product.

The pumpkin purées processed under various high pressure conditions were analysed to identify and semi-quantify the volatiles profile. Tab. 2 shows the relative abundance expressed as arbitrary area units (AAU × 10^5) isolated in the pumpkin purée (unprocessed and high pressure-processed purées). In total, 42 volatile compounds were obtained by SPME. The most important volatile compounds were alcohols and aldehydes, followed by ketones, esters, terpenes and other aromatic components (classified as "others"). Some authors studied the aroma profile of various pumpkin cultivars [3, 12, 13]. Similar to our study, Zhou et al. [3] reported that the content of alcohols was very high in various pumpkin cultivars

Tab. 1. Two-way ANOVA of volatile compounds in pumpkin purée.

Compounds	*P*-value		
	Pressure	Time	Interaction
Alcohols			
Ethyl alcohol	0.014 *	0.390	0.971
(*Z*)-3-hexen-1-ol	0.000 ***	0.180	0.294
(*E*)-2-hexen-1-ol	0.004 **	0.197	0.162
1-hexanol	0.000 ***	0.207	0.241
1-nonen-4-ol	0.135	0.965	0.601
2-octanol	0.002 **	0.007 **	0.156
2-nonyn-1-ol	0.000 ***	0.107	0.275
1-octen-3-ol	0.000 ***	0.280	0.841
Aldehydes			
2-Butenal	0.023 *	0.200	0.259
Hexanal	0.000 ***	0.798	0.956
(E)-2-hexenal	0.000 ***	0.418	0.231
Heptanal	0.000 ***	0.048 *	0.152
2,6-nonadienal	0.214	0.483	0.208
6-nonenal	0.001 ***	0.508	0.428
Undecanal	0.004 **	0.791	0.135
Ketones			
2-pentanone	0.104	0.050 *	0.240
2-heptanone	0.067	0.126	0.591
2,3-butanedione	0.000 ***	0.449	0.596
3-methylcyclohexanone	0.001 ***	0.554	0.488
5-methyl-3-heptanone	0.196	0.001 ***	0.043 *
2-octanone	0.633	0.341	0.749
5-nonanone	0.642	0.000 ***	0.072
Esters			
Ethyl acetate	0.019 *	0.641	0.316
Methyl valerate	0.004 **	0.432	0.826
Ethyl hexanoate	0.050 *	0.100	0.807
Hexyl acetate	0.117	0.010 **	0.848
Diethyl malonate	0.223	0.758	0.175
Ethyl octanoate	0.008 **	0.798	0.427
Methyl decanoate	0.816	0.477	0.120
Aromatic compounds and terpenes			
Benzaldehyde	0.004 **	0.736	0.710
Anisyl alcohol	0.000 ***	0.299	0.623
benzyl alcohol	0.338	0.002 **	0.110
Guaiacol	0.262	0.400	0.528
Phenylethyl alcohol	0.010 **	0.998	0.746
γ-Terpineol	0.056	0.675	0.669
Nerol	0.911	0.853	0.879
Others			
Nonane	0.000 ***	0.066	0.013 *
Acetaldehyde diethylacetal	0.000 ***	0.490	0.142
3-methylthio-1-propanol	0.637	0.849	0.409
Undecane	0.336	0.705	0.348
7-methyl-1-nonyne	0.001 ***	0.746	0.670
Octanoid acid	0.772	0.759	0.935

Significance level: * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$.

Tab. 2. Volatile compounds present in pumpkin purée after high hydrostatic pressure processing at various conditions.

Compounds	Arbitrary area units [$\times 10^{5}$]							*SEM*	*p*-value	Identification method	Origin
	Control	400 MPa			600 MPa						
		200 s	400 s	600 s	200 s	400 s	600 s				
Alcohols											
Ethyl alcohol	57^{b}	82^{ab}	86^{ab}	77^{ab}	101^{a}	103^{a}	92^{a}	4	0.009	RF	LO
(Z)-3-Hexen-1-ol	10496^{a}	7207^{b}	8257^{b}	7587^{b}	2375^{c}	2401^{c}	1563^{c}	740	0.000	RF	LO
(E)-2-Hexen-1-ol	139^{b}	938^{ab}	1841^{ab}	3056^{a}	0^{b}	822^{ab}	0^{b}	299	0.020	RF	LO
1-Hexanol	4731^{a}	2046^{ab}	1004^{b}	0^{b}	4898^{a}	3794^{a}	4832^{a}	466	0.000	RF	LO
1-Nonen-4-ol	151^{a}	68^{b}	41^{bc}	28^{bc}	21^{bc}	15^{c}	23^{bc}	11	0.000	TI	LO
2-Octanol	1026^{a}	476^{b}	173^{c}	124^{c}	121^{c}	24^{c}	23^{c}	78	0.000	RF	LO
2-Nonyn-1-ol	94^{a}	127^{a}	117^{a}	96^{a}	24^{b}	16^{b}	18^{b}	10	0.005	TI	LO
1-Octen-3-ol	260^{a}	152^{b}	148^{b}	119^{bc}	58^{cd}	42^{d}	33^{d}	18	0.000	TI	LO
Proportion	56.5%	42.6%	44.0%	43.2%	40.5%	37.2%	35.3%				
Aldehydes											
2-Butenal	132^{a}	66^{b}	41^{b}	25^{b}	81^{ab}	56^{b}	81^{ab}	8	0.002	TI	LO
Hexanal	7193^{a}	9536^{a}	9805^{a}	9494^{a}	5531^{b}	6189^{b}	5835^{b}	453	0.001	RF	LO
(E)-2-Hexenal	185^{a}	124^{b}	139^{b}	135^{b}	32^{c}	32^{c}	19^{c}	14	0.000	RF	LO
Heptanal	37^{c}	88^{c}	90^{c}	98^{c}	186^{b}	230^{ab}	255^{a}	18	0.000	RF	LO
2,6-Nonadienal	55	54	42	37	37	38	41	2	0.131	TI	LO
6-Nonenal	1314^{ab}	1129^{ab}	927^{b}	1084^{ab}	1353^{ab}	1428^{ab}	1531^{a}	56	0.018	TI	LO
Undecanal	68	113	122	80	56	55	75	7	0.047	RF	LO
Proportion	30.0%	42.7%	42.1%	42.6%	38.8%	41.3%	42.0%				
Ketones											
2-Pentanone	52^{ab}	63^{ab}	61^{ab}	44^{b}	59^{ab}	81^{a}	59^{ab}	3	0.075	TI	LO
2-Heptanone	61	55	62	64	59	82	79	3	0.189	TI	LO
2,3-Butanedione	116^{a}	87^{b}	93^{ab}	88^{b}	31^{c}	30^{c}	20^{c}	8	0.000	TI	LO
3-Methylcyclohexanone	31^{ab}	37^{ab}	54^{a}	49^{ab}	19^{ab}	19^{ab}	15^{b}	4	0.021	TI	LO
5-Methyl-3-heptanone	558^{a}	413^{ab}	427^{ab}	549^{a}	373^{b}	574^{a}	556^{a}	21	0.003	TI	LO
2-Octanone	458^{b}	632^{a}	654^{a}	616^{a}	667^{a}	652^{a}	615^{a}	17	0.001	RF	LO
5-Nonanone	39^{d}	113^{ab}	95^{b}	91^{bc}	126^{a}	92^{bc}	75^{c}	6	0.000	TI	LO
Proportion	4.4%	5.4%	5.5%	5.8%	7.1%	7.9%	7.6%				

Tab. 2. *continued*

Compounds	Arbitrary area units [×10^{5}]							*SEM*	*p*-value	Identification method	Origin
	Control	400 MPa			600 MPa						
		200 s	400 s	600 s	200 s	400 s	600 s				
Esters											
Ethyl acetate	95	70	38	17	97	267	268	33	0.178	RF	LO
Methyl valerate	63	69	66	69	86	76	83	2	0.036	RF	LO
Ethyl hexanoate	126^{b}	176^{ab}	149^{ab}	145^{ab}	189^{a}	177^{ab}	166^{ab}	6	0.026	TI	LO
Hexyl acetate	40^{b}	73^{a}	59^{ab}	53^{ab}	64^{ab}	51^{ab}	50^{ab}	3	0.009	TI	LO
Diethyl malonate	51^{a}	4^{b}	21^{b}	14^{b}	9^{b}	0^{b}	8^{b}	4	0.002	TI	LO
Ethyl octanoate	30^{b}	46^{ab}	43^{ab}	39^{ab}	59^{ab}	57^{ab}	70^{a}	4	0.033	TI	LO
Methyl decanoate	72	80	31	48	42	55	53	6	0.282	TI	LO
Proportion	1.6 %	2.0 %	1.5 %	1.5 %	2.9 %	3.5 %	3.7 %				
Aromatic compounds and terpenes											
Benzaldehyde	63^{a}	47^{ab}	52^{ab}	47^{ab}	36^{ab}	32^{b}	28^{b}	3	0.012	RF	LO
Anisyl alcohol	132^{c}	196^{b}	175^{b}	184^{b}	252^{a}	245^{a}	255^{a}	10	0.000	TI	LO
Benzyl alcohol	169^{bc}	236^{ab}	190^{abc}	207^{abc}	253^{a}	187^{abc}	157^{c}	9	0.005	RF	LO
Guaiacol	32	34	48	51	50	50	53	3	0.263	RF	CD
Phenylethyl alcohol	568^{a}	164^{b}	183^{b}	162^{b}	105^{b}	85^{b}	104^{b}	36	0.000	TI	OT
γ-Terpineol	29	36	33	34	40	40	46	2	0.209	TI	CD
Nerol	27	31	38	37	34	33	36	2	0.946	RF	CD
Proportion	3.4 %	2.9 %	2.7 %	2.8 %	4.1 %	3.5 %	3.6 %				
Others											
Nonane	57^{c}	172^{c}	128^{c}	147^{c}	316^{b}	437^{ab}	508^{a}	37	0.000	RF	LO
Acetaldehyde diethyl acetal	24^{c}	37^{b}	35^{bc}	34^{bc}	49^{ab}	60^{a}	58^{a}	3	0.000	TI	OT
3-Methylthio-1-propan-ol	600	614	628	555	592	551	601	15	0.854	TI	OT
Undecane	21^{b}	51^{a}	54^{a}	43^{ab}	55^{a}	50^{a}	55^{a}	3	0.006	RF	LO
7-Methyl-1-nonyne	443^{a}	238^{b}	224^{b}	201^{b}	147^{b}	134^{b}	149^{b}	23	0.000	TI	OT
Octanoic acid	99^{a}	64^{b}	57^{b}	61^{b}	59^{b}	56^{b}	61^{b}	4	0.009	RF	LO
Proportion	4.2 %	4.5 %	4.2 %	4.1 %	6.5 %	6.6 %	7.7 %				

Values represent proportion (percentage), i.e. sum of the arbitrary area unit of the volatile compounds of each chemical group divided by the sum of the total area of all compounds ×100. Different letters in superscript in the same row indicate significant statistical differences (Tukey's test, $p < 0.05$).
SEM – standard error of the mean. Identification method: RF – mass spectrum and retention time identical with a reference compound; TI – tentative identification by mass spectrum. Origin: most probable origin of compounds formed after processing according to literature: LO – lipid oxidation, CD – carotenoids degradation, OT – other.

such as *C. maxima*, *C. pepo* and *C. moschata*. In addition, both *C. maxima* and *C. moschata* cv Butternut showed similar aroma profiles regarding alcohols, aldehydes and ketones.

The highest percentages of volatile compounds (each compound respect to the total) identified in non-processed pumpkin purée were alcohols such as [*Z*]-3-hexen-1-ol (34.9 %), 1-hexanol (15.7 %) and 2-octanol (3.4 %) and aldehydes like hexanal (24.0 %) and 6-nonenal (4.3 %). The other aroma compounds were detected in low contents with less than 2 % of total AAU. According to the literature, C6–C10 aldehydes and alcohols including hexanal, [*Z*]-2-hexenal, 1-hexanol, [*E*]-2-hexene-1-ol, [*Z*]-3-hexene-1-ol and 2-octanol are responsible for the distinctive grassy, green aromas, whereas aldehydes like 6-nonenal give flower-like aromas [3, 14, 15]. Therefore, these two compound classes have a large influence on pumpkin flavour.

Of the compounds quantified in this study, 32 were affected by HHP conditions while 10 of them were not modified after processing. Among the former 32 compounds, 29 showed significant differences between HHP-treated and untreated samples. These differences indicated that any high pressure treatment could increase or decrease the content of some volatile compounds [2]. Compounds such as 6-nonenal, 2-pentanone or 3-methylcyclohexanone showed intermediate levels in control purée and they were increased or decreased depending on the combination of pressure and holding time applied (400 MPa and 600 MPa for 200 s, 400 s and 600 s), since some combinations increased the levels while others decreased them.

Alcohols

Volatile alcohols were the principal volatile compounds quantified in the pumpkin purée, representing 56.5 % of the total AAU in the non-processed purée. In our study, eight alcohols (ethyl alcohol, [*Z*]-3-hexen-1-ol, [*E*]-2-hexen-1-ol, 2-octanol, 1-octen-3-ol, 1-nonen-4-ol and 2-nonyn-1-ol) were separated and their levels were found to be modified by HHP. This fact may be important regarding the aroma profile as some studies reported that alcohols such as ethanol, 1-hexanol, [*Z*]-3-hexen-1-ol or 1-octen-3-ol were significantly involved in the aroma of pumpkin [3, 12].

Most alcohols isolated from pumpkin purée originate from lipid oxidation, which were produced by their corresponding aldehyde, i.e. hexanol is originated from the oxidation of hexanal, heptanol is formed from the oxidation of heptanal. In general, a decrease in the total AAU of various alcohols was observed after HHP. Alcohols such as [*Z*]-3-hexen-1-ol, 1-nonen-4-ol, 2-octanol or 1-octen-3-ol showed a significant decrease in purées processed by 400 MPa and 600 MPa compared to raw samples. The lowest levels of these alcohols were observed when purées were pressurized at the most intense processing conditions (600 MPa for 200 s, 400 s and 600 s). In the same line, a previous study in melon juices indicated that the content of some alcohols decreased after high pressure treatment (400 MPa and 500 MPa, 10 min, 45 °C) [16]. The modifications of these alcohols in processed purée showed that HHP could substantially alter the original aroma of pumpkin purée.

Other alcohols, like 1-hexanol, significantly decreased by processing at 400 MPa for 200 s and 400 s, disappearing at 400 MPa for 600 s, and were not modified at 600 MPa compared to unprocessed purée. González-Cebrino et al. [6] also reported that the levels of hexanol after HHP (400 MPa and 600 MPa for 1 s, 150 s and 300 s) were similar to unprocessed plum purées. By contrast, Xia et al. [17] applied high pressure treatments (100–500 MPa, 15 min, 20 °C) on pre-germinated brown rice, observing an increase of 1-hexanol. These results could indicate that the varied response of volatile fraction contents to pressure value was partially linked to the modification of enzyme activities by HHP [2, 12].

The levels of [*E*]-2-hexen-1-ol increased by HHP at 400 MPa (significantly during 600 s) and at 600 MPa for 400 s (disappearing at 600 MPa for 200 s and 600 s). As the origin of this compound could be in oxidation of [*E*]-2-hexanal, the differential activity of oxidative enzymes that could remain active at specific processing conditions might explain the variable effect of the treatments. This fact may be important regarding the aroma profile as [*E*]-2-Hexen-1-ol is involved in grassy odour notes [18].

Relative content of another minor alcohol ethanol increased by HHP treatment at 600 MPa. This was in line with observations of a previous study in melon, which showed that alcohols such as ethanol were significantly higher in samples treated by 400 MPa (10 min, 45 °C) compared with the untreated juices, indicating that the amounts of these components increased at elevated pressure [16]. Other alcohol 2-nonyn-1-ol had a higher relative content in samples treated at 400 MPa, while it significantly decreased at the highest pressures (600 MPa).

Aldehydes

Aldehydes are a group of chemical compounds, which contribute to the characteristic aroma of fresh foods, such as fruits and vegetables [19, 20].

Aliphatic aldehydes are mostly linked with degradation of unsaturated fatty acids, originating in lipid oxidation reactions. The fat content of pumpkin (1 g·kg^{-1}) is relatively high compared to other fruits, although the total fatty acids content is only 0.5 g·kg^{-1} [21]. Aldehydes represented 30 % of the total AAU of the control samples, increasing in case of various aldehydes during various high pressure treatments (Tab. 2). An increased formation of aliphatic aldehydes under high pressure was also previously reported in other studies [5, 22]. In our study, seven aldehydes were detected in pumpkin purée. Among them, five were modified by HHP (2-butenal, hexanal, [*E*]-2-hexenal, heptanal and 6-nonenal).

Hexanal is one the most relevant volatile constituents in various pumpkin cultivars [3, 15]. Purées treated at 400 MPa had higher hexanal levels than those treated at 600 MPa and the unprocessed pumpkin purée, although differences were not statistically different. Previous studies showed that processed purée contained more hexanal induced by high pressure treatment at various values [7, 9, 17, 23]. This effect could be due to the breakdown of fruits and vegetables, enhancing lipoxygenase (LOX) and hydroperoxide lyase (HPL) from the fatty acids oxidation pathway [10, 12, 24]. By contrast, when purées were pressurized at 600 MPa, a significant decrease in the relative content of hexanal was observed. Thus, when the pressure was subsequently increased, the contents of hexanal decreased due to possible reduction of enzyme activities (LOX and HPL) at high pressure levels. LAMBERT et al. [23] found that high intense processing conditions at 800 MPa for 20 min induced low levels of hexanal. In addition, other authors reported that the amount of lipid-derived compounds generated by LOX activity decreased after HHP (600 MPa, 10 min) due to enzyme inactivation [25].

Other abundant aldehyde in unprocessed purée was 6-nonenal. This compound showed a slight decrease when the purées were treated at 400 MPa but maintained the same or showed increased levels when purées were pressurized at 600 MPa. The compound contributes to the cucumber-like, green odour [26] and preservation of this compound after processing is interesting due to the fact that it impairs pleasant flavours. PEI et al. [16] showed that HHP (400 MPa and 500 MPa, 10 min, 45 °C) in melon juices enhanced the content of this aldehyde, increasing the fragrant odour of the processed samples. Conversely, another study in cucumbers showed that the content of 6-nonenal was not significantly changed after HHP (500 MPa, 5 min) [26].

The levels of minor aldehyde heptanal gradually increased at the highest pressure values and holding times, reaching the highest levels of this compound at 600 MPa for 600 s. VERVOORT et al. [1] observed that heptanal was present in low content in untreated carrot and was formed at HHP due to the increase of the oxidation of unsaturated fatty acid. Previous studies did not find changes in the heptanal content of fruits and vegetables as a result of HHP [2, 17] or even were not detected after high pressure treatment [16].

Other aldehydes 2-butenal and [*E*]-2-hexenal decreased after HHP. Relative content of 2-butenal significantly decreased at 400 MPa (200 s, 400 s and 600 s) and 600 MPa (only for 400 s), while [*E*]-2-hexenal had the lowest levels at 600 MPa. [*E*]-2-Hexenal is another important product of oxidative degradation of linoleic and linolenic acids, which can be reduced to hexanal [27]. By contrast to our results, GONZÁLEZ-CEBRINO et al. [6] observed high levels of [*E*]-2-hexenal in plum purée processed at 600 MPa and 150 s.

The increase in the aldehyde levels in processed pumpkin purée could contribute to enhancing a green and floral flavour after HHP, influencing significantly the aroma profile of the pumpkin purée.

Ketones

Ketones are volatiles related to fruity, floral and herbaceous notes in plants, contributing to the flavour and odour in various varieties of pumpkin [3, 12]. Regarding their origin, most ketones detected in this study probably originated from lipid oxidation. Ketones accounted the 4.4 % of the total volatiles compounds isolated in the headspace of the raw samples Principal ketones identified in raw pumpkin purée were 2,3-butaneidone, 5-methyl-3-heptanone and 2-octanone, while 2-pentanone, 2-heptanone, 3-mathylcyclohexanone and 5-nonanone were found in lower content. HHP treatment increased the percentage of ketones to 5.4–7.6 % of total volatiles compounds isolated in the headspace of the processed pumpkin purée.

In pumpkin purée, the levels of 2-octanone and 5-nonanone increased in samples treated with 400 MPa and 600 MPa, while the levels were statistically different in comparison to unprocessed purée. Previous studies in plum reported that ketones like 2-nonanone increased after processing at 400 MPa for 1 s [6]. Conversely, 2,3-butanedione showed a significant decrease in the processed purées, being at the lowest level after treatment at 600 MPa. Nevertheless, XIA et al. [17] found that 2,3-butanedione had the highest content in samples treated at 300–500 MPa.

The levels of 2-pentanone, 3-methylcyclohexanone and 5-methyl-3-heptanone after HHP treatment were similar as those in unprocessed purées, only 5-methyl-3-heptanone was reduced after treatment at 600 MPa for 200 s. Similarly, PEI et al. [16] observed no significant differences in the levels of ketones (2,2,6-trimethyl-3-butanedione and (5E)-6,10-dimethylundeca-5,9-dien-2-one) in melon juice between juices processed at 400 MPa and 500 MPa and untreated juice.

Esters

Esters represent the main compounds responsible for the fruity aroma [2, 16], so their preservation is important to maintain fresh-like aromas in processed vegetables. Alcohol acyltransferase is mainly involved in the formation of volatile esters in fresh fruits, transferring the acetyl moiety from acetyl CoA, formed via β-oxidation of fatty acids, to an alcohol substrate [28]. In our study, 7 esters were separated, although their levels were quite low (1.6 %, proportion of esters respect to the total volatiles compounds) in raw pumpkin (control samples). This was in accordance with previous studies where most of the volatile compounds present were alcohols or aldehydes [3, 12].

In our study, HHP preserved or increased the levels of esters at 400 MPa (1.5–2% proportion of esters respect to the total volatiles compounds in samples treated at 400 MPa) and 600 MPa (2.9–3.7 % proportion of esters respect to the total volatiles compounds in samples treated at 600 MPa). In the same line, a previous study on strawberry coulis found that pressure treatment resulted in an increase in the content of esters [23]. Among the different esters detected in the pumpkin purée, ethyl hexanoate, hexyl acetate and ethyl octanoate increased after HHP treatment, whose levels were the highest in samples treated at 600 MPa (200 s), 400 MPa (200 s) and 600 MPa (600 s). These esters provide fruity, floral and herbal notes, so HHP treatment could increase the pleasant flavour in pumpkin purée. By contrast, GONZALEZ-CEBRINO et al. [6] found a decrease in the levels of hexyl acetate after HHP treatment in plum purée.

The levels of diethyl malonate decreased significantly during HHP treatment. Conversely, ethyl acetate, methyl valerate and methyl decanoate were not modified by HHP processing. Previously, some authors reported that some esters decreased with increasing pressure (400–600 MPa) [6, 16]. The most possible way of reduction of esters was hydrolysis into corresponding alcohols and acids [29].

Aromatic compounds, terpenes and other compounds

Aromatic compounds were probably formed by lipid oxidation reaction while terpenoids have their origin in carotenoids degradation. These compounds play a key role in the aroma of fruits and vegetables [14], which might be responsible for a wide range of aromas in pumpkin [3, 12].

The levels of terpenes (guaiacol, nerol and γ-terpineol) after HHP treatment were similar to those in unprocessed purées. These results were consistent with previous studies, where certain bioactive compounds' content (carotenoids and total phenols) was preserved in pumpkin purée after application of HHP at similar conditions as in our study [2, 11, 30]. Furthermore, GONZÁLEZ-CEBRINO et al. [2] reported that HHP treatment (400 MPa, 500 MPa and 600 MPa for 200 s, 400 s and 600 s) applied was insufficient to reduce the residual polyphenol oxidase (PPO) activity. In this regard, levels of terpenoids might be maintained in this study due to the fact that carotenoids content and PPO activity were not affected by HHP.

Various aromatic compounds detected in the raw pumpkin purée showed significant differences between unprocessed purée and some of the processed purées. Benzylalcohol and benzaldehyde are implicated in the floral scent [17], when benzaldehyde is formed from benzyl alcohol by oxidation catalysed by dehydrogenases [31]; so, the reduction of these compounds may reduce pleasant notes in the purée. In this study, the levels of benzylalcohol remained unchanged after processing and slightly increased at 600 MPa for 200 s. In addition, the levels of benzaldehyde were not modified at various treatment conditions but significantly decreased at the highest pressures and holding times (600 MPa for 400 s and 600 s). In line with our results, GONZÁLEZ-CEBRINO et al., [6] reported in plum purée a decrease in the levels of benzaldehyde after HHP treatment at 600 MPa, while purées processed at 400 MPa showed higher content compared to the unprocessed purée [7, 17].

Phenylethyl alcohol is a volatile compound identified in various fruits such as strawberry, melon, tomato and grape varieties, which imparts fresh and sweet-floral characters [18]. The levels of phenylethyl alcohol decreased during the HHP treatment and showed lower values in samples treated at 600 MPa compared to the unprocessed purée. Conversely, anisyl alcohol was significantly increased when samples were treated at the most intense processing conditions (600 MPa). TAKAHASHI et al. [32] identified anisyl alcohol for the first time in vanilla beans, which was one of the most important compounds involved in the floral and anise-like odour. Therefore, the preservation of this compound may add pleasant odour notes.

Other compounds separated were nonane, acetaldehyde diethyl acetal, 3-methylthio-1-propan-ol, undecane, 7-methyl-1-nonyne and octanoic acid, which were classified in the group of other compounds, representing 4.2 % of the total AAU in the unprocessed purée. Only 3-methylthio-1-propan-ol was not modified after HHP. This compound originating from methionine is a volatile, which is also present in wines [33]. Nonane and undecane, probably formed by lipid oxidation, were previously found in raw pumpkin and brown rice [17]. In our study, nonane and undecane significantly increased when the purée was HHP-treated at 400 MPa (both compounds) or 600 MPa (only nonane). In the same line, acetaldehyde diethyl acetal increased when purées were HHP-treated at 400 MPa (200 s) and 600 MPa. In contrast, a significant decrease in the levels of 7-methyl-1-nonane and octanoic acid were found in the processed purées in samples treated at 400 MPa and 600 MPa. Octanoic acid is formed by lipid oxidation and it is a compound, which contributes to the aroma of various plum varieties [14, 18]. The origin of acetaldehyde diethyl acetal and 7-methyl-1-nonyne is not clear in the literature.

General changes after high hydrostatic pressure processing

Global changes in HHP-treated pumpkin purée are summarized in Fig. 1, which shows the sum of total area units of volatile compounds at various processing conditions. Significant changes ($p < 0.05$) were found after processing in all chemical groups except for ketones and esters. Major compounds were alcohols and aldehydes, while minor compounds were ketones, esters, aromatic compounds and terpenes. Alcohols were the most abundant chemical group in control purée, while the lowest levels were found after HHP treatment. All processed purées showed important reduction of alcohols, however, these were more intense at 600 MPa than at 400 MPa. Aldehydes were the second most abundant group of volatile compounds and they slightly increased at 400 MPa while slightly decreased or remained unchanged at 600 MPa. Changes in the levels of aldehydes and alcohols could be explained by the oxidation of aldehydes into alcohols.

Most volatile compounds have their origin in lipid oxidation reactions, while a few compounds are formed by carotenoids degradation (Tab. 2). Carotenoids of pumpkin were well-preserved after HHP treatment, which is consistent with changes in volatile compounds [4, 6]. Thus, HPP could be an efficient tool to preserve the nutritional quality of the pumpkin. On the other hand, non-volatile compounds were formed by the Maillard reaction. In studies of García-Parra et al. [9] and Kebede et al. [10] on the formation of volatile compounds in processed pumpkin, Maillard reaction-derived compounds were found in samples processed by conventional sterilization or HHP treatment. This fact agrees with no application of heating or thermal treatment in the current study, in contrast to the conditions of the previous ones [9, 10].

Alcohols and aldehydes are the major compounds of pumpkin purée. The oxidation of fatty acids provides aldehydes and these are subsequently oxidized to alcohols. Alcohols could also

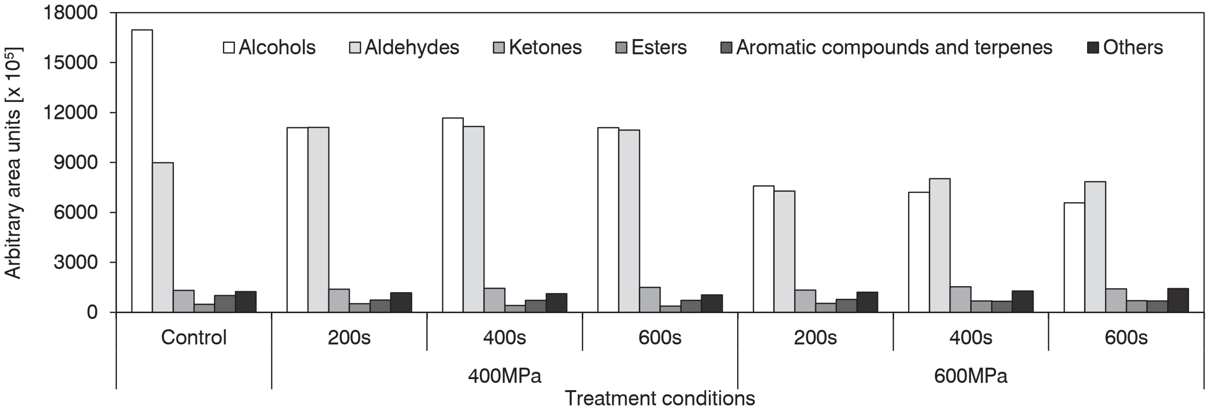

Fig. 1. Volatile compounds from the chemical groups separated from pumpkin purée after high hydrostatic pressure processing at various conditions.

Sum of the total of arbitrary area units of each chemical group per treatment is presented.

be oxidized to form ketones and they also react with acids to form esters. Aldehydes are formed from two major reaction pathways: (i) enzymatic by the lipoxygenase pathway; and (ii) thermally-induced autoxidation [34]. Aldehydes, in turn, are also oxidized to form alcohols. Both aldehydes and alcohols are the major chemical groups of volatile compounds in pumpkin purée. Therefore, according to the most probable origin of aldehydes, the effect of processing on the inactivation of lipoxygenase and other oxidative enzymes could play a key role in the levels of these compounds formed by the enzymatic lipid oxidation pathway. However, measurement of the activity this enzyme would be required to test this hypothesis. The inactivation of these oxidative enzymes was mentioned to be crucial for preservation of the quality of other processed vegetables [35]. According to that review, lipoxygenase can be thermally inactivated above 60 °C with a resulting improvement in the shelf life of foods. However, heating also increases non-enzymatic oxidation and thus may reduce the benefits of HHP treatment.

The application of high pressure thermal processing (300–900 MPa, 60–80 °C, 1 min) to pumpkin purée [9] reduced the levels of some lipid-derived compounds. One hypothesis about this fact was discussed in that study. It was suggested that the treatment applied was insufficient to inactivate the oxidative enzymes such as lipoxygenase or peroxidase, and those enzymes had continued their activity in the control sample during the exposure of SPME fibre to the headspace of the sample (30 min). At similar conditions of processing by HHP as in the current study, the activity of polyphenoloxidase was not reduced in pumpkin purée [2]; however, the activity of lipoxygenase was not evaluated. The activity of lipoxygenase could explain the high levels of lipid-derived compounds in control purée. One possible explanation to the higher levels at 400 MPa than at 600 MPa determined could be that lipoxygenase could be more intensively inactivated at the highest value of pressure. On the other hand, recently PACIULLI et al. [36] studied the effects of HHP treatment (200–600 MPa for 5 min) on diced pumpkins of two species (*Cucurbita maxima* L. cv. Delica and *Cucurbita moschata* Duchesne *ex* Poiret *cv*. Butternut). They concluded that to preserve the textural properties and the antioxidant activity of the diced pumpkin, 400 MPa was optimal. In our study, pumpkin was studied in the form of a purée and thus, structural changes were not important and the best processing conditions would be those that provided the most effective microbial and enzyme inactivation.

CONCLUSIONS

Alcohols and aldehydes were the families of compounds most abundantly separated in the headspace of pumpkin purée. HHP treatment significantly affected the compounds that may be responsible for the aroma profile of the pumpkin purée. The effect of the increase in pressure was higher than that of the holding time. Most volatile compounds separated are known to have their origin in lipid oxidation reactions, a few compounds are known to be formed by carotenoids degradation, and none by the Maillard reaction. Consequently, the application of HHP treatment would preserve bioactive compounds content of pumpkin, like carotenoids, and would avoid the formation of Maillard reaction compounds, which indicates an advantage of this technology. However, the control of the lipid enzyme oxidation reactions could be important to avoid the development of undesirable reactions. Therefore, application of a blanching treatment would be desirable prior to high pressure treatment to avoid the formation of lipid oxidation compounds.

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