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Variations of polyphenols, sugars, carotenoids, and volatile constituents in pumpkin (*Cucurbita moschata)* during high pressure processing: a kinetic study

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ABSTRACT

High pressure processing (HPP) is an attractive technology for the preservation of natural bioactive components of vegetables. Pumpkins are good source of nutrients and bioactive compounds with health promoting properties. In this study pumpkin were treated at six different pressures (100 to 600MPa/20°C/3 min) at constant temperature and time. Polyphenols, carotenoids, sugars, and volatile content were evaluated with specific techniques for each class of compound. Polyphenol were extracted both with solvent and also obtained by squeezing the residual material. Results showed that HPP at medium pressures (200-400 MPa) resulted in higher number of extractable polyphenols. Total amount of sugars in HPP-treated samples was overall declining with increasing pressure, especially at 600 MPa. The total amount of carotenoids was higher in samples treated at lower pressures (100-300 MPa) and in the one at 600MPa compared to untreated ones. Regarding volatile compounds, significant changes were observed for some aldehydes that increase after HPP application. The study results revealed that treatment with intermediate pressure could ensure a higher amount of "availability" of polyphenols, carotenoids, volatiles, and total sugars in pumpkin samples.

Keywords: Pumpkin, Bioactive compounds, High pressure processing, HS-SPME–GC–MS

1. Introduction

Epidemiological studies have revealed that fruits and vegetables consumption can exert protective effects against several risk factors of chronic diseases. This can be in part related to their content of micronutrients, dietary fibre, and phytochemicals (Mokhtar et al., 2021; Wang et al., 2021). In addition, plant foods can also have a relevant role due to the presence of antioxidant and antimicrobial compounds that explain the activity against lipid peroxidation and counteract foodborne bacteria (Kourkoutas and Proestos, 2020). In this contest, pumpkin (*Cucurbita* L.) is a vegetal that has good nutritional properties and that can be a source of phytochemicals with health-promoting features. Pumpkins are now grown everywhere in the World in both temperate and tropical regions. It belongs to the *Cucurbitaceae* family, which contains around 800 species and 130 genera (Perez Gutierrez et al., 2016). They are primarily found in Europe, North and South America, and some Asian locations (India, China) (Yadav et al., 2010). As from all types of pumpkin varieties, Italy is the second largest producer (0.6Mtons) in the European Union (FOSTAT, 2019). With some cultivars, nearly all organs of the pumpkin plant (fruits, flowers, leaves, roots, shoots, and seeds) are edible, and this plant is largely consumed worldwide (Kwiri et al., 2014; Kulczynski et al., 2019) simply after baking, or processed into puree, marinades, juices, jams, pickles, infant food, dried items, and other food products (Nawirska et al., 2009). *Cucurbits* are classified as a functional food since they have a wide range of therapeutic benefits (Adams et al., 2011; Różyło et al., 2014; AlJahani et al., 2017). Pumpkin can play a role in the diet as source of carotenoids and polyphenols, as well as many essential compounds (minerals, vitamins, amino acids) (Men et al., 2021). These constituents exhibit a wide range of bioactivities for example, antioxidant, anti-inflammatory, anticancer, anti-diabetes properties. (Murkovic et al., 2002; Jiang and Du, 2011; Zhang et al., 2012; Bouamar et al., 2017; Rinaldi et al., 2021).

Carotenoids in *Cucurbita* fruits, primarily β-carotene (>80%), lutein, α-carotene, lycopene, (Seo et al., 2005) as well as zeaxanthin, result in yellow to dark orange colours (Adebayo et al., 2013; Provesi et al., 2015). Few carotenoids, such as β-carotene and β-cryptoxanthin, are important for ocular health because their activity as vitamin A precursors (provitamin A activity) (Chiu et al., 2019). According with several studies, carotenoids constituents and concentrations in different species and varieties of pumpkins can be influenced to the varieties itself or growing conditions (Kreck et al., 2006; Bergantin et al., 2018). Previous studies revealed that cooking, storage conditions, chemical structure, and characteristics of the vegetable matrix can have a positive and negative influence on the isomerization and/or degradation of carotenoids (Miglio et al., 2008).

Regarding polyphenols, previous studies reported that protocatechuic, chlorogenic, salicylic, p-hydroxybenoic, p-cumaric, syringic acids, eriodictyol-7-neohesperidoside, and hesperidin are the most common phenolic present in *Cucurbita* fruits (Zdunić et al., 2016; Kostecka-Gugała et al., 2020). Polyphenol-rich foods, particularly flavonoids, have been demonstrated to alter NO (nitric oxide) endothelial production and improve endothelial function in humans (Tangney et al., 2013). Furthermore, pumpkins have a pleasant feature due to organic acids and total soluble sugars, primarily fructose, glucose, and sucrose (Zhou et al., 2017). In this regard, preservation processes could play a key role in alterations in furan formation from sugars and amino acids in processed pumpkin (Limacher et al., 2008). Nevertheless, volatile components impart distinct flavours, in particular alcohol and aldehyde were most major compounds present in fresh pumpkin (Leffingwell et al., 2015), pressure processed pumpkin puree (García-Parra et al., 2020) moreover, hexanal, (E)-2-hexenal, and 3-hexen-1-ol have been reported as important volatile compounds for the flavour of freshly cooked pumpkins (Maarse, 2017).

High pressure processing (HPP) is an innovative technology which has already made a rapid transition from the laboratory to extensive commercial applications. In this context, HPP in the range 100-1000MPa for short time has been effectively used in wide range of food products on an industrial scale (Chauhan, 2019). This processes, can destroy organism, inactivate enzymes, stop detrimental effects, and extend shelf life of the treated product (Huang et al., 2020). Until now, some researchers have been carried out to determine the benefits of HPP in preserving the nutritional and sensory qualities of various fruits and vegetables (Patras, et al., 2009; Barba et al., 2013; Yu, Y et al., 2013; Vázquez-Gutiérrez et al., 2013; Alvarez-Jubete et al., 2014; García-Parra, J et al., 2016). In this contest, HPP has been demonstrated to be efficient in sustaining bioactive chemical levels and antioxidant activity in several fruits and vegetables (McInerney et al., 2007; Sánchez et al., 2014; Kostecka-Gugała et al., 2020), as well as reducing or significantly increasing volatile compound concentrations (Sampedro et al., 2009; Wongfhun et al., 2010; Viljanen et al., 2011; Chen et al., 2015; García-Parra, J et al., 2016).

Up to our knowledge, no research has been published on the changes in the carotenoids, polyphenols, volatile profile, and sugar content of pumpkin after HPP at different pressure. As a result, the goal of this study was to evaluate how different pressures at constant time and temperature (100 to 600 MPa at 20°C/3min, respectively) affected the pumpkin (*Cucurbita moschata*, Var. Violina) polyphenols, sugars, carotenoids, and volatiles compounds amount.

2. Materials and methods

2.1 Materials

Pumpkin Cv. Violina rugosa, botanically classified as *Cucurbita moschata*, a butternut squash at commercial maturity (average weight 2.5 to 3.5 kg) were obtained from a local company (Il Nuovo Fresco S.R.L., Montecchio Emilia, RE, Italy). All of them were of good quality well

mature, healthy, and free of serious mechanical damages. All pumpkins were stored at ambient temperature before preparation of sample. Pumpkins were washed with tap water, manually peeled, then cut into small cubes (1.5 cm side). To obtain homogenous sample, all pumpkin samples were cut, mix together uniformly then divided into 7 equal portions in order to get seven different samples: untreated (Raw) and for high pressure at six different pressures (from 100 to 600MPa). In final, all divided pumpkin samples were packed in high density polyethylene bags under vacuum by using a packaging (Lavezzini Univac, Fiorenzuola d'Arda (PC), Italy) machine. Raw sample was stored at 4°C and the rest of six vacuum packed samples were used for HPP treatment.

2.2 HPP treatment

The 6 samples were subjected to high pressure processing (HPP) at *Stazione Sperimentale Industria Conserve Alimentari* (SSICA) by using 30L Avure^{TM} vertical machine (Model -AV-S), come-up time of 200 MPa per minute. The treatments were conducted from 100 (P1) to 600 (P6) MPa for 3 min, using cold water (4–5°C) as pressure medium. After treatment, all samples were stored at 4°C, next day samples were used for analysis of polyphenols, sugars, carotenoids, and volatile components identification.

2.3 Polyphenols

***Sample preparation*:** Fresh pumpkin was cut in small pieces covered with liquid nitrogen and grinded. Powder was transferred to a round bottom flask and freeze dried. Dried fruit samples were then ground into fine powder. Lyophilized samples were weighted and extracted using a ratio pumpkin solvent of 400 mg-50 mL of a mixture of methanol/water 50%. The sample was

sonicated for 10 minutes. For the solvent extraction of polyphenols, the choice of mixed organic/water solvent was due to the hydrophilic nature of some of the polyphenols. Once extracted the samples were centrifuged at 13000 rpm for 15 minutes and liquids were used for LC analysis for obtaining the extractable polyphenols from pumpkin. To analyse polyphenols in squeezed liquid, 1g of material after the solvent extraction was squeezed using a syringe and the residue was then transferred to an Eppendorf and centrifuged at 13000 rpm for 15 minutes. The extracted liquids were then collected and placed in vial for LC analysis.

Polyphenols determination: For the analysis, an Agilent 1260 LC chromatograph equipped with diode array and connected to a Varian MS 500 Mass spectrometer equipped with Electrospray (ESI) ion source were used. UV spectra were acquired in the range 200-640 nm while mass spectrometer collect spectra in m/z range 100-2000 in negative ion mode. As stationary phase an Agilent Eclipse XDB C18 3.0 x 150 mm (3,5 micron) was used. As mobile phase, water 1% formic acid (A), Acetonitrile (B) and methanol (C) were used. Gradient start with 98% of A, stays 1 minute isocratic then at minute 5 go to 90% A, 8% B 2% C. Then at minute 20 go to 70% A, 28% B and 2% C and stay isocratic up to minute 25. Finally, at minute 30 composition go to 80% B and 20% C and stay isocratic up to minute 34. The flow rate was 0.4 µl/min. Injection volume was 10 µL and the temperature set at 30°C. Identification of compounds was obtained based on comparison with the literature and reference compounds, when available. For compound quantification, the following standard were used, lutein-7-O-glucoside and rutin was used for quantification of rutin and flavonoid derivatives, while laempferol-3-O-glucoside was used to quantify kaempferol derivatives. Solution at four different concentrations for each reference's compounds were prepared in the range 100-1.0 µg/mL building calibration curves that were used for quantitative purpose.

2.4 Sugar content

***Sample preparation*:** 1 g of dried sample was extracted with 10 mL of water in a heated bath (37˚C) for 30 minutes. Samples were centrifuged at 13000 rpm for 15 minutes and liquids were used for LC analysis.

Sugar determination: For the analysis of sugar, an HPLC system coupled to evaporative scattering detector (ELSD) was used. The chromatographic system was composed by an Agilent 1100 HPLC pump coupled to a SEDERE Sedex 60 LT ELSD. An Agilent HI-PLEX Ca^{2+} column was used as stationary phase, while water was used as mobile phase, maintained at isocratic conditions for 30 min at a flux of 0.6 mL/min, column oven was kept at 80°C. ELSD parameters were as follows: gain, 10 AU; drying gas pressure, 2.2 bar; evaporative gas temperature, 60˚C. Carbohydrate in pumpkin samples were quantified using calibration curves of glucose, fructose, and sucrose, built with standard solution in water of each sugar in concentration ranges of 5-500 µg/mL.

2.5 Carotenoids

***Sample preparation*:** 600 mg of dried sample was extracted with 20 ml of acetone. Solution was filtered and solvent were evaporated in a rotary evaporator to dryness. Dried residues were dissolved in 0.5ml of acetone for chromatographic analysis and transferred to vial.

Carotenoid determination: For the analysis of carotenoids, a Waters alliance HPLC equipped with a 2489 UV-Vis detector was used. After chromatographic column a "T" connection splitted the flow equally to UV-Vis detector and Mass spectrometer. As Mass spectrometer, a Varian MS-500 ion trap mass spectrometer equipped with ESI ion sources. UV-Vis spectra were acquired in the range of 425–450 nm. MS spectra were recorded using ESI in positive

mode in the range of m/z 200–800. A turbo data-dependent scanning (TDDS) instrument function was used to acquire mass fragmentation pathways of the main ionic species. A YMC Carotenoid column (5 µm 4,6 x 250 mm) was used as stationary phase. For the mobile phase solvents were methanol (A) and methyl tertbutyl ether (MTBE)/methanol (90/10) (B). Gradient start with 100% A and in 30 minutes up to 10 % A and 90% B, then, isocratic up to 32 minutes. The flow rate was 1.3 µl/min. Injection volume was 10 µL and the temperature set at 35˚C. Identification of compounds was obtained based on comparison with the literature and reference compounds, when available. As reference standard, β-carotene, lutein, and zeaxanthin were used to quantify each compound, while β-carotene was used as external standard for the other compounds. Calibration curves were built using solution of each standard carotenoids in the range 5-500 µg/mL.

2.6 Volatile profile

Volatiles in *Curcubita* samples were analysed combining headspace (HS) extraction and by GC-MS. The HS-GC-MS system consisted of a DANI HSS 1000 module connected to a Varian Saturn 2000 GC-EI-MS (ion trap), equipped with a HP-INNOVAX capillary column (30 m, i.d. 250 mm, 0.25 µm) as stationary phase. For volatiles extraction, 2 g of sample was placed in a 10 mL vial closed with a plastic twisted-off lid and sealed with PTFE silicone septum. The sample was kept at 70˚C for 45 min, and the extracted volatile fraction was transferred to the GC system by a transfer line at 120˚C. The separation of volatile constituents in GC was achieved using a temperature ramp, as follows: 40˚C for 5 min, then to 180˚C at 6˚C/min, then isocratic for 2 min. The total analysis time was 29 min. Carrier gas flow was 1 mL/min, and injector temperature was set at 120˚C. MS data were acquired in the *m/z* range 30-500. Compounds were identified based on their Kovats Index as well as by comparison of mass

spectra with database. For comparison purposes of the HPP processes data of each chromatogram were compared using the internal normalization and data were expressed as percentage (%) of each considered compound on the total amount of detected volatiles.

2.7 Statistical analysis

Means and standard deviations were calculated with SPSS (Version 26.0, SPSS Inc., Chicago, USA) statistical software. SPSS was used to verify significant differences between data by one-way-analysis of variance (ANOVA) followed by Tukey's post-hoc test at $p < 0.05$ to identify differences among the samples.

3. Result and discussion

The chemical analysis was focused on four different classes of constituents that are significant in pumpkin in order to observe how the HPP process influences the composition of the food. In particular, we considered the polyphenols and the carotenoids due to their antioxidant properties, the volatile due to possible role in food aroma, the sugars due to their abundant amount in this vegetable and due to their role in nutrition. Furthermore, the four considered classes of compounds presented different physico-chemical properties: polyphenols have moderate lipophilicity and have partial water solubility, carotenoids are lipophilic compounds that are not water soluble, volatiles are low molecular a polar compound with limited water solubility, while sugar are highly polar compounds with good water solubility.

3.1 Polyphenols

The LC chromatogram recorded with diode array detector showed several peaks in the region between 12 and 21 minutes (**Figure 1**) with UV spectra supporting the presence of small phenolics and flavonoids (peaks in the range 16-19 minutes and 20-21 minutes, respectively). Identification of compounds was obtained comparing mass spectra and fragmentation of the observed ion species as well by comparison with reference compounds. Fourteen main derivatives were detected and are listed in **Chart 1**. From qualitative point of view, some of the most common phenolics as chlorogenic acid were not detectable, this may be related to cultivar type, ripening, or conservation.

Concerning the polyphenols for comparing the different HPP process parameter, we considered the amount of extracted polyphenol from 1 g of material and also, we measured the amount of squeezed liquid from the vegetal material in order to have values related to compounds that can be extracted with solvent or just with physical process. Results reported in **Table 1** and in

Figure 2 indicate that higher amount of polyphenol were obtained in the samples at intermediate pressure. The content of polyphenols was higher in P3 (205.02 mg/g) compared to P0 (198.29 mg/g) that was higher than P2 (179.68 mg/g) and P4 (165.63 mg/g) (**Table 1**). This indicate that HPP at medium pressure can have an influence on the polyphenol composition and on the extractable polyphenol from the matrix, in fact highest amounts are associated with P2, P3 and P4. This fact suggests that treatment with intermediate pressure could ensure a highest amount of "available" polyphenols when the food will be eaten. The reduced levels of polyphenols observed for higher pressures can be explained with alteration of vegetal tissues or with induction of enzymatic process of degradation of the polyphenols. Therefore, high levels of polyphenols would be associated to low polyphenol oxidase (PPO) activity and to a lighter colour of the processed pumpkin.

A further consideration can be done observing in the **Figure 2** the % of squeezed polyphenols for each sample. Also, in this case we can see that at P0 the squeezable amount of polyphenol was very similar to the one obtained at P6, while at intermediate pressures squeezed polyphenol quotes were higher ranging from 60-67%. These data suggest a positive influence of HPP treatment on pumpkin considering the available polyphenol contents. Previous literature reported an increase in polyphenol content of vegetables treated with HPP. For example, Barba et al., (2013) reported that HPP at 200MPa for 5–15 minutes significantly increased total phenolic content in blueberry juice. In another study it was found that HPP at 200MPa/10min retained higher amount of phenolic content (298.02 mg/l) in asparagus juice compared to 600MPa (278.67 mg/L) (Chen et al., 2015). Garcia-Parra et al., (2016) noticed that pumpkin purées following HPTP (high pressure thermal processing) at 600 MPa/70˚ C had the highest TPC (total phenolic content), with a 65% increase over the control purée. According to Zhou et al. (2014), HPP-treated pumpkin slices (550 and 450 MPa/10 min/room temperature) showed higher retention of total phenol content compared to control. All high pressure treated samples

had considerably greater phenolic contents than thermally processed samples ($p < 0.05$) (Patras et al., 2009). Regarding polyphenols, literature data support our results and HPP treatment thus indicating that this method can be valuable for preserving polyphenols in vegetal foods.

3.2 Sugar content

HPP treatment induced a higher yield in sugar extraction with water, suggesting that the application of high pressure can induce some modifications that allow an easier (**Table 2**). In the case of sugars due to the use of water solvent we did not divide the extractable amount into water and squeezed fractions. Total amount of sugars of the HPP-treated all pumpkin samples had a slight fluctuation, the overall trend is declining with high pressure especially at 600MPa (P6). This may be due to HPP-treated resulting in the more loss of the vegetable juice, then the content of sugars was cut down. From the P1 to P5, total amount of sugar is increased ranging from 3.3 to 5.0 g/100 g, while more amount of sugar was found at P4, respectively. In our study, results showed that treatment with intermediate pressure mainly 400MPa (P4) than P5 and P3, ensure a higher amount of extracted sugars more than treatments at elevated pressures.

3.3 Carotenoid determination

The representative LC-UV-APCI-MS chromatogram of *Cucurbita* (**Figure 3**) presents a series of peaks ascribable to carotenoid derivatives. Different classes of carotenoids were identified on the basis of literature, reference standard when available, *m/z* ion and MS/MS fragmentation pattern (**Table 3**). Peaks eluting in the range 5.4-7.8 minutes can be observed in UV and MS (**Figure 3** and **4**) and at mass detector present typical fragmentation pattern of epoxy-carotenoid. As example two peaks with retention times of 8.1 and 9.1minutes presenting *m/z* at 569 were assigned to lutein and zeaxanthin respectively on the basis of their spectra and

comparison with authentic standard (**Figure 3**). In the last part of chromatogram β-carotene and other isomer derivatives were detected (**Figure 4**).

Considering this class of compounds, we can observe a slightly different behaviour if compared with the results obtained with carotenoids and sugars. Due to the lipophilic nature of those compounds, we consider the total amount of carotenoids extracted with acetone after freeze-drying of the material, no squeezing process was in our opinion meaningful due to the non-solubility of this compounds in water. The total amount of carotenoids is higher in samples P1(384.17 μg/g), P2 (437.91 μg/g), P3 (339.93 μg/g) and P6 (373.25 μg/g) compared to untreated ones (351.26 μg/g). The most abundant derivative is α (102. 4 μg/g in P2) and β-carotene (163. 64 μg/g in P1 and 172.53 μg/g in P2) and the extracted amount of this compound appear to be strongly modified in the HPP treated sample with increase of nearly 3-fold compared to P0 (α-carotene 29.15 μg/g and β-carotene 55.05 μg/g) (**Figure 6**). This result may be explained due to possible conversion into β-carotene of other carotene derivatives that appear to be "reduced" after HPP such as 15-cis- β-carotene or to modification of structure of the pumpkin that allow a higher release of β-carotene to solvent. Lutein also is one of the most represented compounds, but from the P0 (66.68 μg/g) to P6 (56.60 μg/g) the extraction of this constituent in pumpkin it appears not to be significantly modified (**Table 3**). Although, some of this difference was neoxanthin (41.97 μg/g) observed at high quantity in P0 but at P6 was less around 27.66 μg/g whereas the same but little difference was found in the lutein extraction (**Figure 5**). Neoxanthin decrease was observed for all the HPP samples, suggesting a minor role of pressure on the extractability of such compound. Seo et al., (2005) extracted β-carotene as major carotenoid in pumpkin (>80%) with lesser amounts of lutein, lycopene, α-carotene, and *cis*-β-carotene and author stated that at moderate pressure best results were obtained even through certain studies discovered an increase in total carotenoid content after high-pressure treatment (Sanchez-Moreno et al., 2003). After high pressure treatments (400 or 600 MPa, 2 min), McInerney et

al. (2007) reported no influence on the amount of lutein and β-carotene in broccoli and lutein in green beans. Sanchez et al., (2014) reported that carotenoids were maintained by after HPP treatment (625 MPa, 5 min, 20°C) in carrot, red pepper, tomato, spinach, broccoli, green pepper vegetables. Patras et al., (2009) reported a significant reduction in carotenoid concentration in carrot and tomato puree at 400 MPa. When compared to unprocessed samples, a large significant increase (172 %) in carotenoids extracted occurred at 600 MPa. Our results show that pumpkin is a rich source of carotenoids, especially β-carotene, lutein and other derivatives, and these derivatives might be increased at moderate pressure ranging from 100 (P1) to 400MPa (P4), because at increased pressure oxidative chemical reaction were enhanced which is responsible for carotenoids degradation.

3.4 Volatile profile

Intense changes were observed in the volatile constituents of the pumpkin by headspace sampling. The volatile flavour compounds in P0 (untreated) and HPP processed fresh pumpkin are shown in **Table 4**. Totally, 15 volatile compounds were identified in control and HPP treated pumpkin, respectively (**Figure 7**). The main volatile compounds in pumpkin, which included ethyl acetate, furan, 3-methyl (furan, 2-methyl), butanal, 2- methyl, butanal, 3-methyl, furan, 2-ethyl-, Pentanal, Hexanal, 1-penten-3-ol, 4, pentanal, 2-methyl-, furan, 2-pentyl-, 3-octanone, Acetoin, trans-2-(2-pentenyl) furan, 1-hexanal, benzaldehyde, and traces of others. These compounds have previously been identified as the major contributors to asparagus juice aroma treated (Chen et al., 2015). The development of theses volatile characteristics is primarily linked to the carotenoid -lipid degradation and maillard reaction, which oxidative chemical reactions seem to be favored by increased pressure (Sampedro et al., 2009; Kebede et al., 2014). For the most important volatile constituents that represents 65-97% of the total we can observe a decrease after HPP application. The most abundant compound the 3-methyl

butanal, known for fruity smell (http://www.thegoodscentscompany.com), represent the 97% of volatiles in P0 while it decreased after HPP application. Other minor constituents in P0 resulted in relative increase such as ethyl acetate (from 0,5% in P0 to 6,1% in P6) and 2-methyl butanal (from 0,1% in P0 to 2,7% in P6). Ethyl acetate is known for its ethereal fruity smell while 2-methyl butanal is associated with musty smell (http://www.thegoodscentscompany.com). As reported in **Table 4,** very significant changes were observed for some aldehydes that increase after HPP application. Viljanen et al., (2011) reported that at ambient temperature, higher pressure (800 MPa/20 °C) reduced the amounts of some aldehydes, ketones, and alcohols in tomato puree, whereas hexanal, heptanal, and octanal levels increased. Another study demonstrated an increase in the production of aliphatic aldehydes when pressure is high (Navarro et al., 2002). Garcia-Parra et al., (2020) examined that the alcohol was the most abundant volatile compound isolated from pumpkin puree by HPT, followed by aldehydes (14–28%), hydrocarbons (8–13%) and terpenes (7–10%).

The most distinctive compounds such as hexanal and 1-hexanol were found from the pumpkin variety used in this study. Similarly, Chun-li et al., (2015) reported same results for *Cucurbita moschata*, Duch. The principal component of the aroma of pumpkin purée was hexanol, which added fragrant characteristics associated to green vegetables. When the enzyme alcohol oxidoreductase reacts with hexanal in plant tissues, this chemical is produced (Wongfhun et al., 2010). The hexanal and 1-hexanol abundantly identified mainly in P3, P4 and P5 but at P4 showed highest average % (**Table 4**). 1-hexanol level in the P0 were statistically different to the HPP processed ones, which indicates that the pressure affects the activity of oxidoreductase enzyme, similar trend observed by García-Parra et al., (2020). 3-octanone and acetoin did not show big changes after HPP treatment. Whereas Chen et al., (2015) noticed that slightly increase in 3-octanone in green asparagus juice after HPP (200, 400, 600 MPa/10 and 20 min/room temperature) than control, most likely resulted from lipid oxidation. However, we

have to point out that the HPP treatments at 300 (P3) and 400MPa (P4), is the stage of formation and stabilization of volatile compounds, because it showed significantly ($p < 0.05$) higher concentration of all volatile fractions with respect to other pressure levels.

4. Conclusion

The high-pressure processing (HPP) is a valuable approach for treating foods because allows preservation without additives or heat. In this work we have considered pumpkin as model food and evaluated the effects of HPP process at different pressures on different chemical constituents. In particular, we evaluated the modifications on health-promoting constituents of this vegetable, namely the polyphenolic and carotenoid antioxidant. To assess the potential role of HPP on the polyphenolic levels, we performed the analysis on the extracted compounds with a methanol/water mixture but also on the liquid obtained by squeezed material. Carotenoids, on the other hand, should be extracted with more lipophilic solvent, thus we did not consider the "squeezed" amount that is negligible. Furthermore, to have information on the possible changes on other classes of food components, we measured sugar contents and volatile profile of pumpkin, offering a comprehensive chemical view of the possible changes due to HPP-treatment of pumpkin. The results showed that the levels of bioactive compounds decreased especially at higher pressure and ambient temperature, whereas moderate pressures ranging from 200 to 400 MPa increased and maintained the amount of availability of bio-active compounds. On the basis of our assessment, HPP at moderate pressure levels seems to be suitable for retaining stability and concentration of all bioactive components and sugar molecules.

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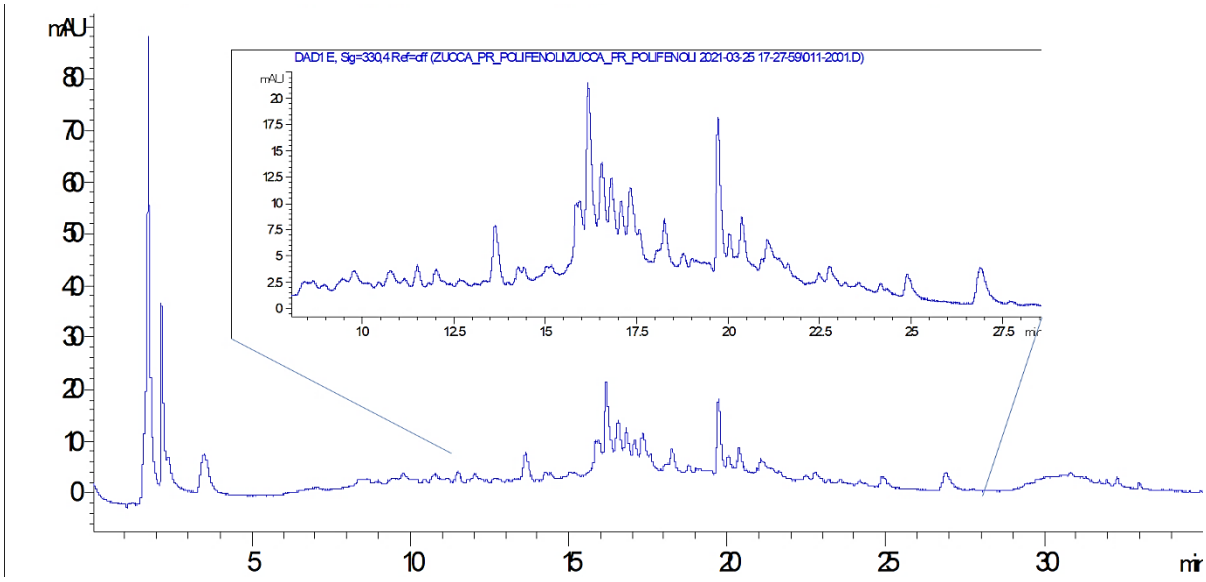
Captions for Figures and Tables

Figure 1: LC-DAD chromatogram of fresh *Curcubita Curcubita* sample processed by HPP (P5) at 330 nm, enlargement of the chromatogram time range from 9 to 29 minutes is highlighted.



	tr	[M-H]-	Compound
1	1,20	191,00	quinic acid
2	1,40	191,00	citric acid *
3	10,10	447,00	Lutein-7-O-glucoside *
4	10,90	447,00	kaempferol-3-O-glucoside*
5	12,30	447,00	kaempferol-7-O-glucoside *
6	10,95	609,00	rutin *
7	10,80	293,00	Unknown
8	10,25	175,00	isopropylmalic acid
9	13,10	221,00	Phtalic acid diethyl ester
10	16,50	593,00	kaempferol-3-O-rutinoside *

11	16,70	591,00	flavonoid derivative
12	17,00	593,00	kaempferol hexoside deoxyhexoside
13	17,30	698,00	flavonoid derivative
14	19,70	698,00	flavonoid derivative

Chart 1. Identified constituents of *Curcubita* samples by LC-DAD-MS analysis. The structures of compounds indicated with * were also confirmed by comparison with authentic standard

Table 1. Identified and quantified polyphenols (mg/g) (**Chart 1**) of *Curcubita* samples treated by HPP. P0-P6 are samples treated at different pressure with HPP and extracted with solvent. Samples P0S-P6S are squeezed liquid obtained by centrifugation of the samples treated at different pressures.

	P0	**P0S**	**P1**	**P1S**	**P2**	**P2S**	**P3**	**P3S**	**P4**	**P4S**	**P5**	**P5S**	**P6**	**P6S**
1	3.93±0.09 ^{A}	0.46±0.05$^{\#}$	2.92±0.08^{B}	0.58±0.05$^{\#}$	8.31±0.10^{C}	3.29±0.09*	11.95±0.09^{D}	2.66±0.08 §	7.06±0.06^{C}	4.82±0.10$^{\&}$	9.51±0.10^{c}	4.55±0.08$^{\&}$	7.24±0.09^{C}	4.25±0.09$^{\&}$
2	7.25±0.09^{A}	4.98±0.07$^{\#}$	6.10±0.08^{B}	4.62±0.05$^{\#}$	11.36±0.11^{C}	4.50±0.05$^{\#}$	16.39±0.12^{D}	4.15±0.05$^{\#}$	7.91±0.08^{A}	5.60±0.05§	13.04±0.09^{C}	4.90±0.05$^{\#}$	8.12±0.09^{D}	3.27±0.05$^{\&}$
3	2.87±0.05^{A}	3.66±0.05$^{\#}$	4.11±0.06^{B}	5.78±0.05§	3.07±0.05^{A}	5.96±0.09§	1.22±0.05 ^{C}	1.25±0.05§	0.77±0.07^{D}	1.14±0.09*****	0.97±0.05^{D}	1.65±0.05*****	0.79±0.05^{D}	0.91±0.05$^{\&}$
4	1.99±0.07^{A}	1.37±0.08$^{\#}$	2.17±0.09^{A}	2.33±0.09§	2.29±0.07^{B}	2.40±0.05§	1.20±0.05 ^{c}	0.88±0.05*	0.75±0.03^{D}	0.81±0.05*	0.96±0.05^{c}	0.70±0.05*	0.77 ±0.03^{D}	0.51±0.05$^{\&}$
5	1.04±0.05^{A}	1.11±0.05^{A}	2.32±0.05 ^{B}	1.19±0.05 $^{\#}$	3.08±0.05 ^{C}	1.22±0.05$^{\#}$	1.40±0.05^{A}	0.74±0.05*****	0.79±0.05*	0.67±0.05*	1.11±0.05^{D}	0.47±0.05 $^{\&}$	0.81±0.05*****	0.44 ±0.05$^{\&}$
6	1.37±0.05^{A}	0.47±0.04$^{\#}$	2.56±0.09^{B}	0.47±0.03 $^{\#}$	0.81±0.05^{c}	0.47±0.03$^{\#}$	0.79±0.06^{C}	0.45±0.04$^{\#}$	0.52±0.05$^{\#}$	0.43±0.05^{D}	0.63±0.04§	0.45±0.05^{D}	0.53±0.05$^{\#}$	0.41±0.05^{D}
7	2.14±0.05^{A}	0.77±0.05$^{\#}$	2.42±0.09^{B}	3.24±0.07$^{\#}$	2.19±0.08^{a}	3.41±0.09$^{\#}$	1.53±0.06^{C}	2.31±0.06^{B}	0.57±0.04*****	2.22±0.05^{A}	1.22±0.05§	1.94±0.03^{C}	0.59±0.04*	1.80±0.07^{C}
8	8.53±0.09^{A}	0.84±0.05$^{\#}$	4.27±0.09^{B}	3.02±0.04§	6.06±0.08^{C}	4.46±0.09*****	1.99±0.05^{D}	3.88±0.08$^{\&}$	2.80±0.07^{e}	9.42±0.09$^{\$}$	1.58±0.06^{f}	9.14±0.09$^{\$}$	2.87±0.07§	2.38±0.07§
9	78.65±0.11^{A}	45.44±0.11$^{\#}$	48.39±0.13^{B}	38.58±0.09§	58.84±0.09^{c}	50.84±0.09^{D}	84.25±0.11^{E}	58.49±0.05^{C}	48.34±0.05^{B}	51.91±0.15^{D}	67.02±0.11^{F}	52.80±0.13^{D}	49.60±0.15^{B}	38.58±0.11§
10	4.08±0.05 ^{A}	2.04±0.05$^{\#}$	5.52±0.15^{B}	2.15±0.05$^{\#}$	3.67±0.05 ^{C}	3.18±0.05 §	4.00±0.05 ^{A}	5.11±0.09*	2.62±0.05^{D}	5.67±0.11*	3.17±0.06^{c}	4.76±0.05$^{\&}$	2.69±0.05^{D}	4.36±0.08$^{\&}$
11	76.08±0.11 ^{A}	18.84±0.09$^{\#}$	68.33±0.11^{B}	21.16±0.09$^{\#}$	67.37±0.11^{B}	35.73±0.09§	69.23±0.12^{B}	43.20±0.09*	91.55±0.13^{c}	4.59±0.04$^{\&}$	55.07±0.06^{D}	3.79±0.05*	53.93±0.09^{c}	3.29±0.07*
12	8.79 ±0.05^{A}	0.82±0.03$^{\#}$	7.69±0.09^{B}	3.73±0.08 §	11.36±0.09^{C}	4.50±0.06*	9.47±0.05^{D}	7.64±0.05^{B}	0.58±0.05^{E}	7.38±0.11^{F}	0.53±0.05^{G}	5.60±0.08^{H}	0.59±0.05 ^{E}	4.71±0.15*****
13	0.85±0.06 ^{A}	0.30±0.05^{B}	0.75±0.15^{A}	0.30±0.05^{B}	0.65±0.05^{C}	0.31±0.05^{B}	0.80±0.05^{A}	3.20±0.05^{C}	0.67±0.09^{A}	2.93±0.05^{D}	0.64±0.05^{A}	2.58±0.05^{E}	0.68±0.05 ^{A}	1.80±0.05^{F}
14	0.73±0.05^{A}	0.32±0.05^{B}	0.66±0.05^{A}	0.33±0.05^{B}	0.61±0.05^{A}	0.34±0.05^{B}	0.79±0.05^{A}	3.34±0.05^{C}	0.69±0.04^{A}	3.11±0.11^{C}	0.63±0.05^{A}	2.76±0.08^{D}	0.71±0.05^{A}	2.15±0.05^{E}
Total	**198.29 ^{A}**	**81.43 ^{B}**	**158.19^{C}**	**87.46^{D}**	**179.68^{E}**	**120.61^{F}**	**205.02^{G}**	**137.30^{H}**	**165.63^{C}**	**140.70^{H}**	**156.09^{C}**	**96.07^{D}**	**129.93^{F}**	**69.84^{I}**

Compound confirmed by comparison with reference standard. Different letters (A, B, C D, E, F, G, H, I) indicate statistical significance ($p<0.05$) for samples treated at different pressures. Different symbols (§, & * and #) indicate statistical significance ($p<0.05$) for squeezed samples

Figure 2. Total polyphenols measured in *Curcubita* samples treated by HPP, the graph in the left part of the figure represent total polyphenol content obtained by extraction with solvent (methanol/water 50%) in mg/g. The graph on the right report the amount of polyphenols obtained by squeezing and is expressed as % of squeezed polyphenols compared to the total amount.

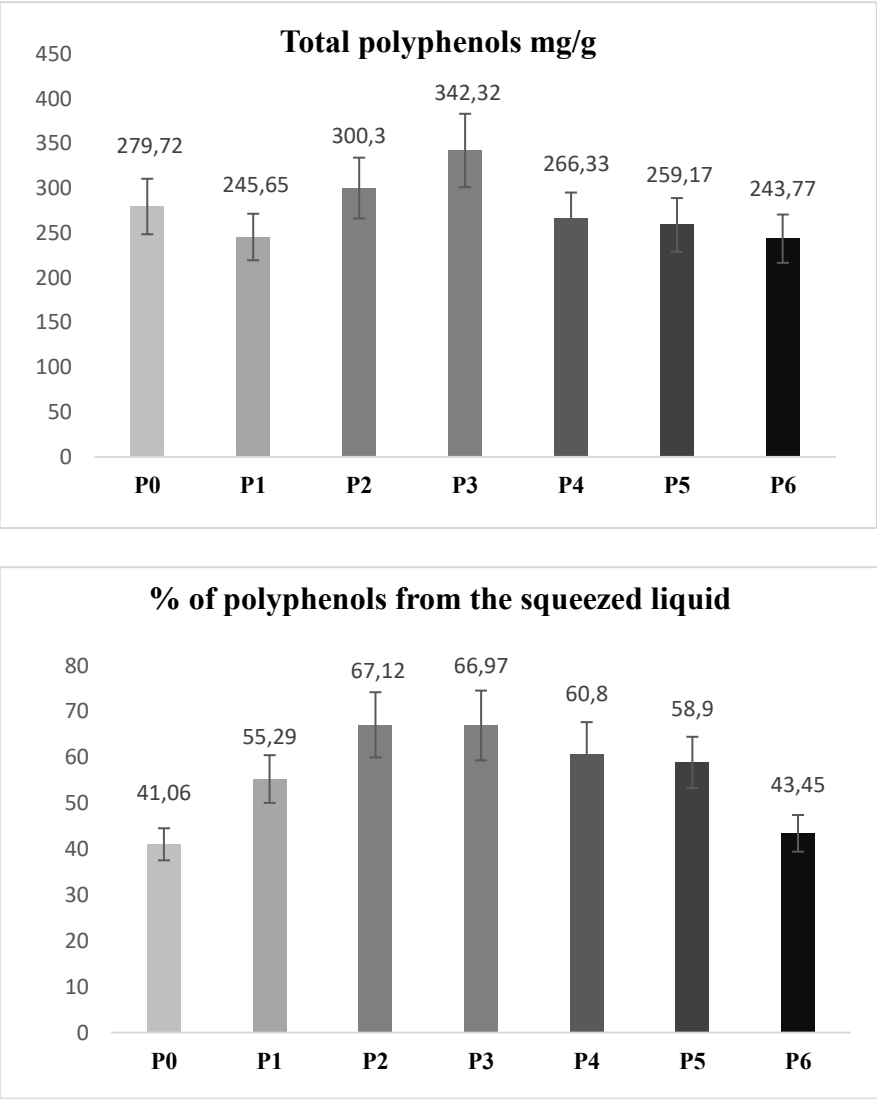


Table 2: Quantification of sugar (mg/g) in *Curcubita* samples.

Compound	**P0 g/100g**	**P1 g/100g**	**P2 g/100g**	**P3 g/100g**	**P4 g/100g**	**P5 g/100g**	**P6 g/100g**
Sucrose	0.560±0.083	0.570±0.077	0.499±0.061	0.586±0.087	0.590±0.051	1.104±0.099	0.950±0.085
Glucose	0.764±0.071	0.287±0.033	0.917±0.087	1.029±0.101	1.008±0.096	1.271±0.100	0.298±0.041
Fructose	0.413±0.031	0.499±0.034	1.428±0.099	1.551±0.104	1.569±0.124	1.863±0.127	0.271±0.041
Other carbohydrates	0.552±0.049	1.894±0.103	1.226±0.099	1.631±0.103	2.268±0.135	0.756±0.091	0.972±0.047
Total amount	2.290^{A}	3.249^{B}	4.070^{C}	4.796^{D}	5.434^{E}	4.995^{D}	2.491^{A}

Figure 3: Exemplificative LC-UV-Vis chromatogram of HPP treated *Curcubita* samples at 425 nm, the main constituents are highlighted in the figure. Peaks indicated with * were quantified using authentic standards.

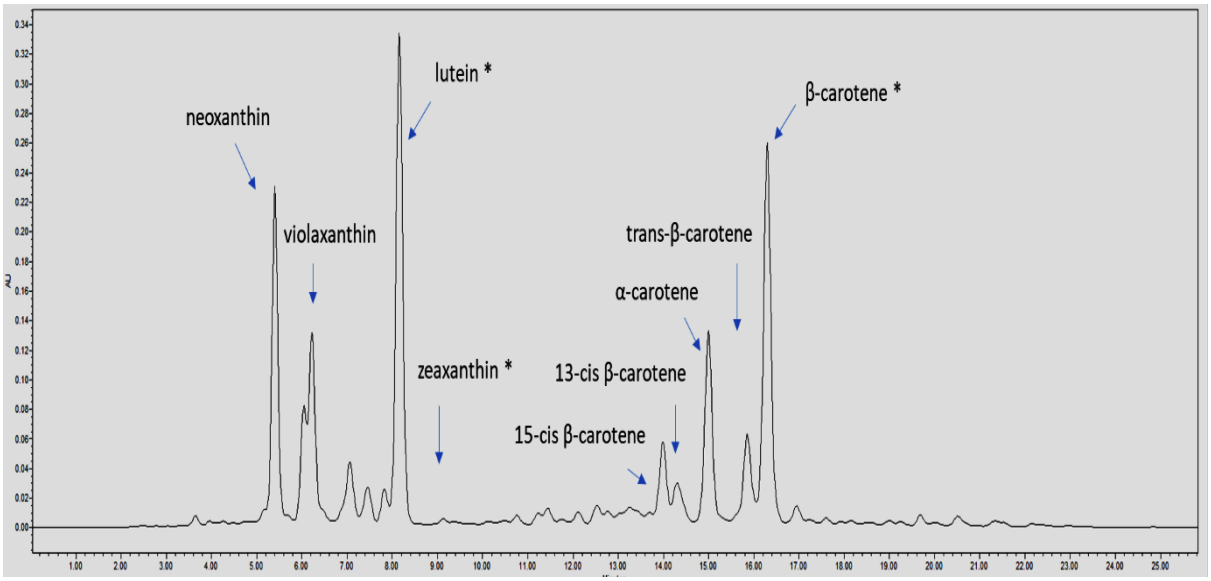


Figure 4: LC-ESI-MS chromatogram of *Curcubita* sample with main peak highlighted.

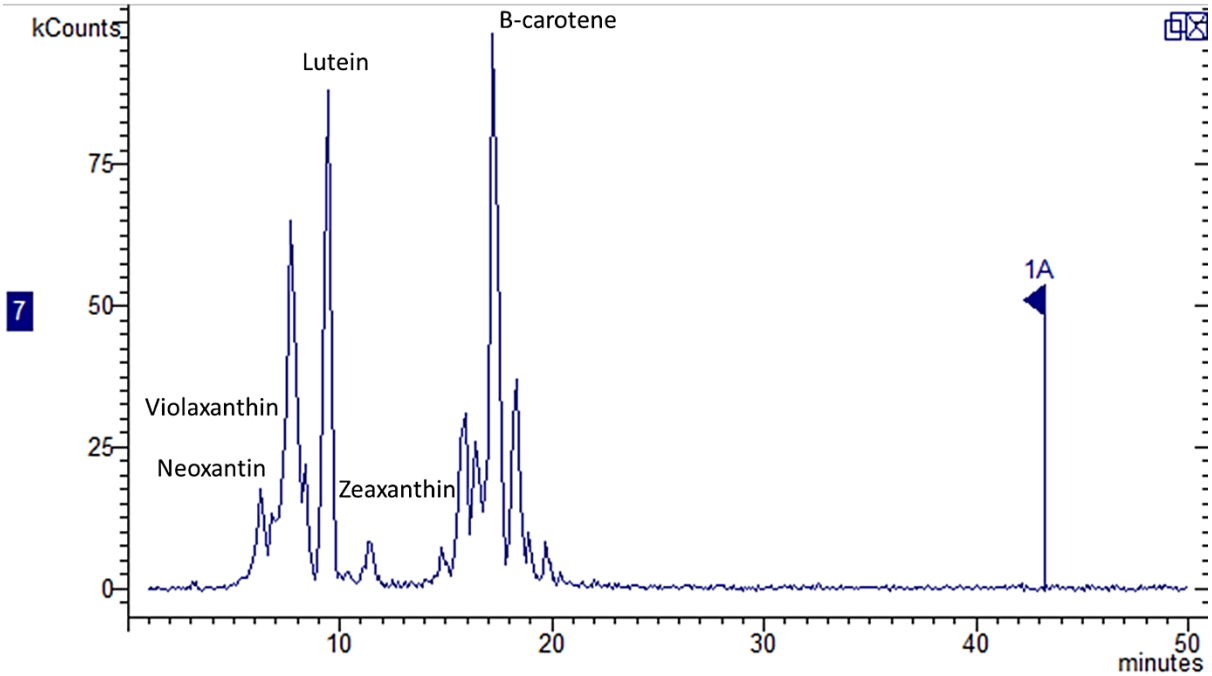


Figure 5: Positive ion ESI mass spectra (*m/z* 569) and MS/MS fragments of lutein

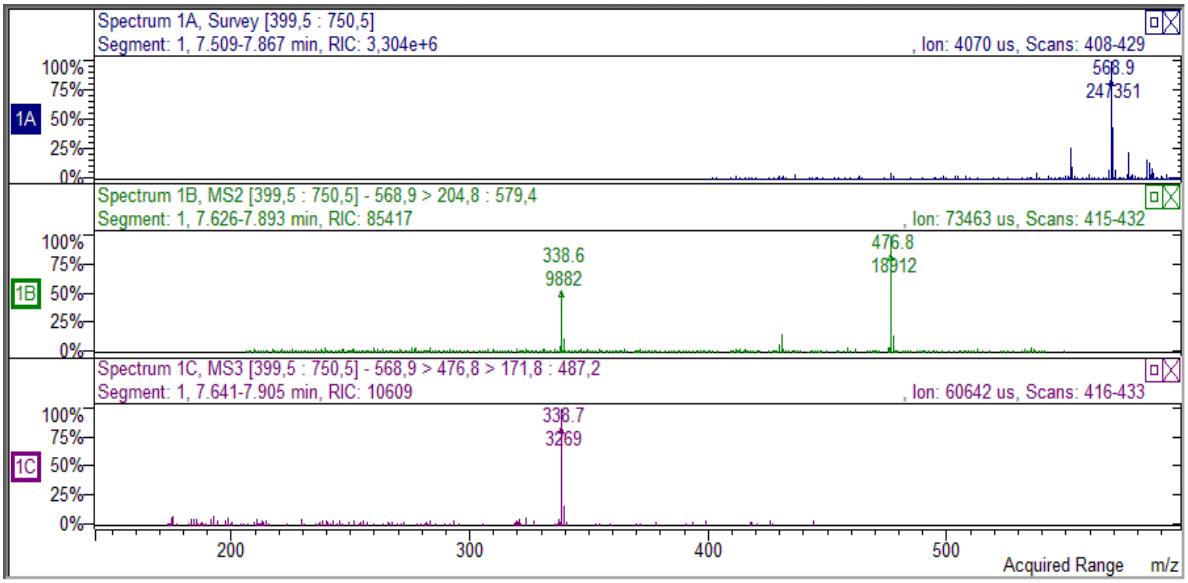


Figure 6: Positive ion ESI mass spectra (*m/z* 537) and MS/MS fragments of β-carotene.

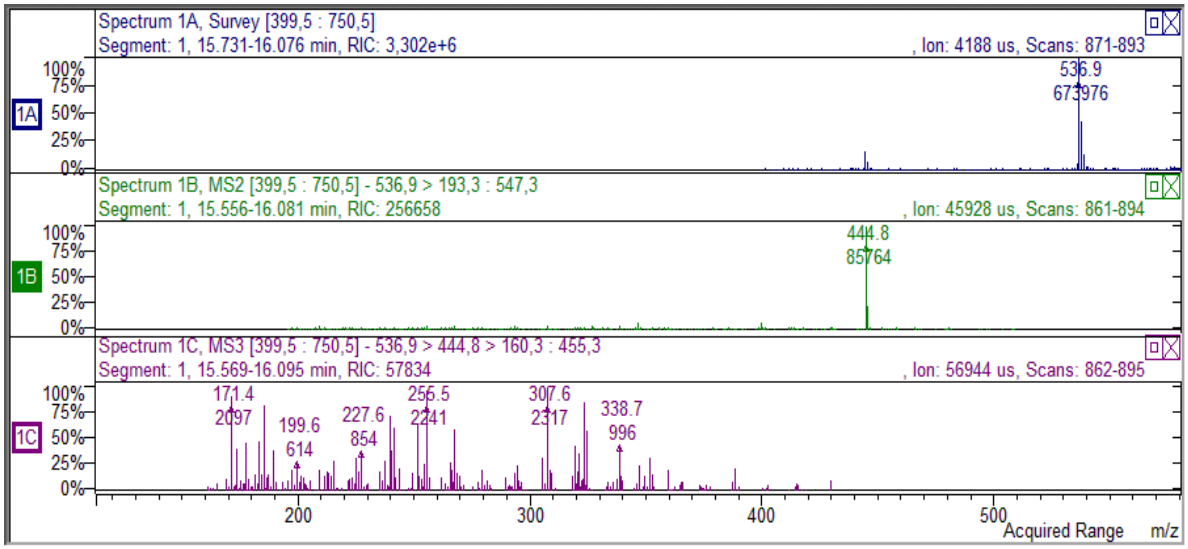


Table 3. Identified and quantified carotenoids (µg/g) of fresh and HPP processed *Curcubita*

				P0	P1	P2	P3	P4	P5	P6
Compound	**retention time**	**$[M+H]^+$**	**fragments**		**µg/g**	**µg/g**	**µg/g**	**µg/g**	**µg/g**	**µg/g**
Neoxanthin	5.4	601	547 529 509 491	41.97±0.25^a	28.37±0.30^b	11.05±0.15^c	16.26±0.11^d	13.01±0.09^c	16.97±0.11^d	27.66±0.21^b
Violaxanthin	6.2			35.79±0.42^a	30.94±0.36^b	32.14±0.35^b	23.15±0.29^c	29.30±0.30^b	28.85±0.21^b	40.92±0.33^d
Epoxy-carotenoid type	7.1			9.05±0.11^a	7.10±0.49^b	14.80±1.09^c	6.29±0.78^a	12.04±1.02^c	10.09±1.11^d	8.94±0.95^a
Epoxy-carotenoid type	7.5			4.36±0.51^a	3.24±0.33^b	7.17±0.45^c	3.03±0.35^d	4.62±0.55^a	3.93±0.51^a	5.58±0.49^e
Epoxy-carotenoid type	7.8			2.22±0.25^a	1.04±0.12^b	1.14±0.15^b	1.93±0.21^c	1.81±0.15^c	1.29±0.17^d	1.27±0.15^d
lutein *	8.2	569	476 430 338	66.68±1.19^a	48.35±0.65^b	52.06±1.45^b	49.33±1.98^b	59.81±2.11^c	69.11±2.15^d	56.60±1.27^c
zeaxanthin *	9.1	569	476 338 270 206	0.50±0.10^a	0.21±0.10^b	0.26±0.11^b	0.32±0.14^b	0.36±0.12^b	0.33±0.14^b	0.23±0.11^b
15-cis β-carotene	13.9	537	444 338 307 255 171	89.80±01.35^a	10.03±0.99^b	18.14±0.99^b	6.40±0.55^c	24.52±1.00^d	17.49±0.84^b	23.17±0.85^d
13-cis β-carotene	14.3	537	444 338 307 255 171	4.18±0.65^a	4.24±0.35^a	10.51±0.97^b	2.81±0.31^c	6.58±0.55^d	4.34±0.50^a	2.78±0.19^c
α-carotene	15.0	537	481 444 413 388 321 183	29.15±0.31^a	74.93±0.95^b	102.24±1.11^c	64.62±0.71^d	59.25±0.61^e	54.63±0.58^e	78.25±0.83^b
trans-β-carotene	15.8	537	444 338 307 255 171	10.49±1.10^a	8.84±0.99^b	12.77±1.05^a	6.36±0.71^c	18.90±1.01^d	10.75±1.09^a	20.35±1.21^d
β-carotene *	16.3	537	444 338 307 255 171	55.05±0.61^a	163.64±1.73^b	172.53±1.74^c	157.32±1.68^d	95.37±1.10^e	92.38±0.99^e	104.83±1.05^f
carotene type	17.0	537		2.02±0.22^a	3.25±0.25^b	3.09±0.25^b	2.12±0.21^a	3.42±0.29^b	3.54±0.39^b	2.69±0.31^b
			Total	**351.26^a**	**384.17^b**	**437.91^c**	**339.93^d**	**329.00^e**	**313.70^e**	**373.25^b**

* Compounds were quantified with corresponding reference standard; other constituents were quantified using β-carotene as reference compound. Different letters (a, b. c, d, e) in the same row indicate a significant difference ($p < 0.05$),

Figure 7: HS-GC-MS chromatogram of *Curcubita* sample treated with HPP, peak number indicate identified constituents (See table 4 for peak identification).

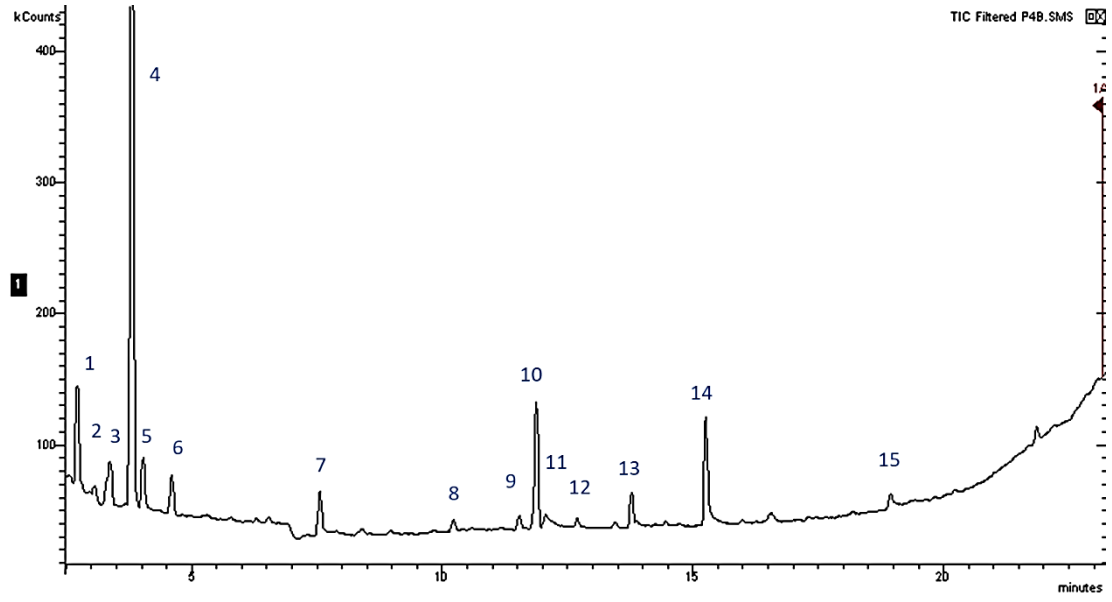


Table 4: Effect of HPP treatments on concentrations of different volatile compound identified in fresh pumpkin by HS-GC-MS (data are expressed as relative percentage of compound after internal normalization).

				P0	P1	P2	P3	P4	P5	P6
n	**Rt**	**compounds**	**RI**	**Average %**	**Average %**	**Average %**	**Average %**	**Average %**	**Average %**	**Average %**
1	2.7	**Ethyl acetate**	849.0	0.489±0.017^{a}	n.d	1.009±0.263^{a}	6.274±1.538^{b}	3.805±1.791^{c}	3.245±1.028^{c}	6.112±3.496^{b}
2	3.1	**Furan. 3-methyl (furan. 2-methyl)**	889.8	1.823±1.916^{a}	3.641±2.102^{b}	7.604±0.859^{c}	0.826±0.196^{a}	0.873±0.400^{a}	1.548±0.119^{a}	1.323±0.624^{a}
3	3.4	**Butanal. 2-methyl-**	910.0	0.102±0.034^{a}	0.387±0.192^{a}	0.686±0.218^{a}	4.305±1.173^{b}	2.403±0.944^{c}	2.818±0.229^{c}	2.738±1.177^{c}
4	3.8	**Butanal. 3-methyl-**	930.0	93.994±1.677^{a}	89.637±1.919^{a}	74.301±3.055^{b}	55.406±1.131^{c}	54.957±9.355^{c}	57.277±14.658^{c}	65.036±8.123^{c}
5	4.1	**Furan. 2-ethyl-**	945.0	0.399±0.100^{a}	0.279±0.162^{a}	0.171±0.120^{b}	5.038±2.150^{c}	3.657±2.915^{d}	2.121±0.386^{d}	1.817±0.122^{d}
6	4.6	**Pentanal**	970.0	0.821±0.496^{a}	3.320±0.707	9.239±2.474	2.591±0.589	1.936±0.203	1.926±0.422	1.697±0.651
7	7.6	**Hexanal**	1082.8	0.228±0.167	0.196±0.163	0.229±0.024	4.534±2.000	13.712±18.133	12.300±9.862	6.185±3.988
8	10.3	**1-penten-3-ol**	1173.3	0.169±0.076	0.151±0.081	0.381±0.137	0.927±0.053	1.180±0.034	1.091±0.381	0.816±0.020
9	11.6	**4-pentenal. 2-methyl-**	1218.5	0.185±0.028	0.306±0.100	1.361±0.147	1.191±0.208	0.667±0.043	0.183±0.025	0.307±0.020
10	11.8	**Furan. 2-pentyl-**	1225.9	0.139±0.045	0.507±0.602	1.435±0.437	7.918±2.319	5.745±2.717	10.437±4.135	6.816±5.097
11	12.7	**3-octanone**	1259.3	0.100±0.048	0.118±0.040	0.194±0.029	0.695±0.234	0.787±0.190	0.832±0.013	0.658±0.127
12	13.4	**Acetoin**	1285.2	0.175±0.058	0.161±0.115	1.546±0.200	0.689±0.398	0.321±0.051	0.242±0.043	0.232±0.070
13	13.8	**Trans-2-(2-pentenyl) furan**	1300.0	0.074±0.012	0.115±0.163	0.504±0.216	1.640±0.798	1.074±0.202	1.685±0.626	1.497±0.818
14	15.2	**1-hexanol**	1358.3	0.811±0.153	0.770±0.003	0.908±0.139	6.878±2.627	8.031±5.198	2.713±0.408	2.535±0.937
15	18.9	**Benzaldehyde**	1523.8	0.490±0.448	0.410±0.446	0.434±0.110	1.087±0.328	0.853±0.073	1.582±0.295	2.232±1.062

Data are expressed as mean (±standard deviation); *n.d.: Not Detectable. n: peak number. Rt: retention time (minutes). RI: Kovats retention index
Different letters (a.b.c.d) in the same row indicate a significant difference ($p<0.05$) no letter indicate no significant differences.