Effects of high hydrostatic pressure on physico-chemical and structural properties of two pumpkin species

Elsevier Editorial System(tm) for Food Chemistry

Manuscript Draft

Manuscript Number: FOODCHEM-D-18-03671R1

Title: Effects of high hydrostatic pressure on physico-chemical and structural properties of two pumpkin species

Article Type: Research Article (max 7,500 words)

Keywords: antioxidant activity; enzymatic activity; high pressure processing; microstructure; pumpkin; Texture Profile Analysis.

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Abstract: The effects of high pressure treatments (200, 400, 600MPa for 5 min) and a thermal treatment (85°C for 5 min) were evaluated on cubes of two pumpkin species (Cucurbita maxima L. var. Delica and Cucurbita moschata Duchesne var. Butternut) up to 2 months of refrigerated storage. Increasing the pressure, small parenchyma cells from the pumpkin tissue exhibited collapses and separations, especially for Butternut. This species showed a lower hardness than Delica at time 0. For both species, 400MPa and thermal treatment were the most effective in the inactivation of pectinmethylesterase, which reactivated after 2 months, especially for Butternut. Colorimetric parameters decreased after all treatments. Antioxidant activity resulted affected by pressure, showing a significant increase during storage especially for the samples treated at 200MPa after 2 months, comparable to the thermal treated ones. Among the tested treatments, 400MPa may be considered as the best option for the quality retention during storage.

Cover Letter

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DI PARMA

DIPARTIMENTO DI SCIENZE
DEGLI ALIMENTI E DEL FARMACO

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To: Editor-in-Chief of Food Chemistry

Dear Editor,

I'm submitting the revised version of the paper entitled "*Effects of high hydrostatic pressure on physico-chemical and structural properties of two pumpkin species"*, by Maria Paciulli, *Massimiliano Rinaldi, Margherita Rodolfi, Tommaso Ganino, Michele Morbarigazzi and Emma Chiavaro FOODCHEM-D-18-03671* for the publication in Food Chemistry.

The total word count of the Manuscript (including tables and figure legends) is 6668, including also 42 references and 2 tables + 4 figures and it is prepared strictly according to the Journal format as provided in the instruction to authors.

We would like to thank the Editor to have considered the manuscript and the reviewers for their helpful suggestions. I'm attaching the new version of the paper, where changes are reported in red bold colour, and the detailed responses to the reviewers' comments.

I declare that all the co-authors have agreed for submission to Food Chemistry and the manuscript is not submitted or under consideration in any other journal. I also declare that there are any conflicts of interest.

I hope that you consider this paper. If there is anything else that you would like to know, please don't hesitate to get in touch with me.

Looking forward to hear you soon. Sincerely yours,

Parma, 03.09.2018

With my Best Regards

Dr. Massimiliano Rinaldi

***Response to Reviewers**

Reviewer #1: This work studies the effects of different high hydrostatic pressure treatments and a thermal treatment on the quality and the microstructure of pumpkin cubes from two different species immediately after the treatments and after one and two months of refrigerated storage. This is an interesting and original subject, which falls into the scope of the journal "Food Quemistry" and the results obtained are interesting too. However, there was a lack of clarity in the presentation of some results and the experimental design was not clear explained. The manuscript needs also a correction of the writing with a native English speaker.

Thank you. English has been edited by a commercial service. The editing declaration is attached.

The following issues should also be addressed:

Highlights:

Please, remove abbreviations…the highlights should stand alone.

Thank you. Abbreviations were removed and highlights rewritten.

Page 2 Abstract:

Lines 2-13: Please, clarify the abstract. It could be something like this:

The effects of high pressure treatments (200, 400, 600MPa for 5 min) and a thermal treatment (85°C for 5 min) were evaluated on cubes of two pumpkin species (Cucurbita maxima L. var. Delica and Cucurbita moschata Duchesne var. Butternut) up to 2 months of refrigerated storage. Increasing the pressure, small parenchyma cells from the pumpkin tissue exhibited collapses and separations, especially for Butternut. This species showed a lower hardness than Delica at time 0. For both species, 400MPa and thermal treatment were the most effective in the inactivation of pectinmethylesterase, which reactivated after 2 months, especially for Butternut. Colorimetric parameters decreased after all treatments. Antioxidant activity resulted affected by pressure, showing a significant increase during storage especially for the samples treated at 200MPa after 2 months, comparable to the thermal treated ones. Among the tested treatments, 400MPa may be considered as the best option for the quality retention during storage.

Line 17-19 Keywords: I suggest "pumpkin" instead of "Curcubita", "microstructure" instead of "microscopy" and "Texture Profile Analysis" instead of "texture"

Thank you. Keywords were modified as suggested

Page 3

Introduction

You should add the hypothesis of your work at the introduction. Please, see the authors guide of this journal. The inclusion of a hypothesis is mandatory. Then, you should respond to it at the discussion section.

Thank you. An hypothesis was added in the revised version of the manuscript

Line 26: Please, add a "s" in antioxidant

Thank you. Corrected

Line 27: Please, split the sentences and write: 10 mg/100g. In the case of vitamins, instead of about vitamins…

Thank you. Corrected

Line 31: I think you are referring to species in plural (the different species of pumpkins), so write "these" instead of "this" species.

Thank you. Corrected

Line 36 Please change the word psychotropic for psychrotrophic and add "and even pathogenic" in "due to psychrotrophic and even pathogenic bacteria,…

Thank you. Corrected

Line 40: Please, add "thermal" before pasteurization

Thank you. Corrected

Line 43: Please, write related instead of connected

Thank you. Corrected

Page 4

Line 56: Please, split the sentence: "…several factors. Besides, the texture is one of the most…" instead of "several factors with texture one of the most…"

Thank you. Corrected

Line 59: Please write something like this: "Regarding the publications about the effects of HPP on pumpkin products, this technology has been applied"…instead of "Regarding pumpkin treatment,…"

Thank you. Sentence was corrected

Lines 60-61: Add an "a" in "leading to a significantly higher …and complete the sentence like this: in comparison with the content of these compounds in thermal treated samples.

Thank you. Corrected as suggested

Line 66: Please, change the abbreviation HHP to HPP

Thank you. Abbreviation was corrected

Line 67: Please add the treatment holding times and process temperature of the study of Zhou et al. (2014)

Thank you. Treatment conditions studied by Zhou et al. (2014) are already reported at line 69.

Line 68: please change the sentence to something like this: had greater retention of colour, vitamin C, antioxidant capacity and total phenols than untreated samples but were equal to slices treated with a mild thermal treatment.

Thank you. The sentence was corrected as suggested

Page 5

Line 70: Please, add "the" before effects on

Thank you. the word was added

Line 71: Use capital letter after the dot: "In addition, …"

Thank you. Capital letter was corrected

Line 72: Write pumpkin species instead of vegetable species because there are many works analyzing the effect of HPP on different varieties or species of other fruits and vegetables

Thank you. sentence was corrected

Line 74: Please write …two distinct species and to explain some of them by the microstructural response of the tissue.

Thank you. sentence was corrected

Materials and methods

Line 81: Add species of pumpkins

Thank you. Species of pumpkin were already presented as *moschata* and *maxima*

Line 87: Explain the abbreviations of the vacuum bags: PA/PE and the permeability to gases and water

Thank you. Abbreviation was explained and technical data of plastic film were added

Page 6

Line 117: Explain the abbreviation of TPA (Texture Profile Analysis)

Thank you. Abbreviation was explained

Page 7

Lines 128-129: What do you mean with "selected avoiding cut zones"? The samples are cubes…

Thank you. The statement was not clear and was corrected: actually we avoided edges.

Line 130: You can add "Chroma or saturation" and "Hue angle or tone"

Thank you. Words were added

Page 8

I think you should add another determination (ABTS/ORAC/FRAP) in order to give information about the antioxidant capacity of the samples. Please, try to add it or remove this determination.

Thank you. we agree with Reviewer that only one antioxidant determination is a limit about the global changes on antioxidant molecules, but we think to have performed a good number of samples (2 species, 5 treatments and 2 times) with a multidisciplinary approach. For this reason, we would leave our data on antioxidant without adding another additional test.

I think you should consign at materials and methods section, the experimental design utilized for your study.

Thank you. A brief sentence about experimental design was added

Page 9

Results and discussion

It would be better if the scale is similar for the two species and for all the treatments. This is the only way to objectively compare the effects of the treatments. It is way too difficult to compare the micrographs when they are in a different size.

Thank you. We agree with Reviewer that the same scale would be better for comparing micrographs but the choice of different scales was made for better focusing on different structural effects. The use of same scale could be limiting in highlighting structural changes involving structures with different dimensions. For this reason, we would like to leave the proposed pictures.

Line 183: I think that the vascular bundles are visible in the picture 1C instead of picture 1A. In fact, they are marked in picture 1 C. I think you should move the sentence to the paragraph describing picture 1 C or you should mark the vascular bundles in picture 1A

Thank you. The sentence regarding vascular bundles was moved to the paragraph referred to figure 1C.

Page 10

Line 205: I think it would be better if you change the sentence: "Another effect…" to "Other effects of the HPP treatment are: a decrease in starch inclusions and an increase in gelatinized starch, visible in the micrographs of the tissues stained with IKI solution."

Thank you. Sentence was rewritten as suggested

Line 216: Please, write the word "authors" without capital letter and write HPP instead of HHP

Thank you. Words were corrected as suggested

Line 219: Was the starch gelatinization greater at 600 MPa or at 400 MPa (Picture 1 F)?

Thank you. Actually, gelatinization resulted directly related to pressure and 600 MPa gave higher gelatinization compared to 400 MPa. Sentence was updated.

Lines 229-230: I think you should add "and plasmolysis" in the sentence "…concerned only cell separations and plasmolysis".

Thank you. Sentence was corrected as suggested

Line 235: Please change the word interested for involved or affected

Thank you. Word was changed as suggested

Page 12

Line 245: Please change the word interested for affected

Thank you. Word was changed as suggested

Line 249: Please, change the title of the section 3.2 to Texture Profile Analysis of pumpkin cubes because texture alone is a sensory property that cannot be measured with an instrument.

Thank you. Title was changed as suggested

Please, revise the English of this section and along all the manuscript! There are many grammatical errors.

E.g. in line 266: "Balasubramaniam & Rastogi (2009) observed a decreasing in hardness,…" I think you should write "a decrease"

Thank you. The word was corrected

Page 13

Line 271, 276 and 283: I think you are talking about table 1 and you mentioned table 2.

Thank you. we made a typing mistake and we corrected it

Page 14

Line 299: It is not clear what you mean with "the other"

Thank you. the words were removed

Line 305-312: It is true that the activity of the enzymes could be affected by the decompartmentalization of the tissue. However, this cannot be measured with the determination of the enzyme activity because you are extracting the enzyme from its matrix and you destroy the tissue to do that. Then, you add the substrate to see the reaction. Another reason for the differences in the enzyme activity could be the differences in the extractability of the enzyme from the tissue after the treatment. Please, consider changing the explanation of the differences observed in PME activity.

Thank you. The explanation regarding PME activity was changed as suggested and a paragraph regarding the higher extractability was added.

Line 317: see the above comment

Page 16

Line 368: Please write were instead of was

Thank you. The word was corrected

Page 17

Line 371: Please, write textural instead of texture

Thank you. The word was corrected

Antioxidant Capacity: The effects of the treatments on antioxidants are not very clear. Besides, you only use one method to determine antioxidant capacity. It is weird to me that thermal treatment did not cause any significant decrease in antioxidant capacity and it is also estrange the increase in thermal treated Butternut pumpkin after 2 months of storage. I think you should consider adding another antioxidant determination or removing these results.

Thank you. Effects of thermal treatment on antioxidant molecules such as carotenoids and polyphenols in vegetable generally is a balance between thermal inactivation and thermal release from cell wall components. In several papers carotenoids and polyphenols content, and antioxidant capacity as consequence, are reported to increase after thermal treatment. For this reason and by considering that our thermal treatment was mild, the not significant effect of thermal treatment could be accepted. Probably, during storage the extractability of antioxidant compounds increased due to tissue damages and resulted antioxidant capacity increased. A sentence about each of above mentioned concept was added in the text.

The increase observed in thermal treated Butternut samples could be linked to tissue damages caused by thermal treatment in this sample demonstrated also by the dramatic hardness reduction that could have caused an higher extractability of antioxidant compounds related also to a residual PME activity. A sentence was added in the text.

Figures and tables

It is not clear what you mean with "n" and with "sample size". Generally "n" and sample size refers to the same, the amount of experimental units per treatment. I don´t understand why you have different "n" and sample size. Please, explain.

Thank you. “n” represents the number of replicates per samples per each time of analysis while “sample size” was calculated as n*time of analysis. However, as this information is considered not useful only “n” was leaved in the revised version.

Figures and tables should stand alone, so you have to explain the meaning of abbreviations. E. g. HPP200, HPP400 and HPP600

Thank you. An explanation for all abbreviations was added

Table 1: At the caption, you write "among the four types of samples" and you have five types of samples (raw, TT, HPP200, HPP400 and HPP600).

Thank you. Number of samples was corrected

Figure 3 and 4 are inverted. The title of figure 3 is PME residual activity and the graphic is about antioxidant capacity and vice versa for figure 4.

Thank you. Figure captions were corrected

Figure 4: Please, add at the title 2 months of refrigerated storage (4°C)

Thank you. Title was modified as suggested

Reviewer #3: The major flaws in this study are the statistical analysis and the visual representation of the data.

The main statistical analysis is that multiple analyses were performed and so ANOVA was performed (I presume but not specified in the methods but assume as least significant differences (LSD) were expressed). However LSD does not correct for multiple analyses thereby there is a significant increased risk of Type 1 statistical errors (false positives). To rectify this Dunnett post-hoc if wanting to compare everything to a control should be performed or Tukey post hoc if wanting to compare each test condition with each other MUST be performed to then be able to discuss statistical significance. Results/Discussion of limited worth until this is performed. This applies to Tables 1 & 2 as well as figures 3 and 4.

Thank you. Statistical analyses were re-performed by means of Tukey post-hoc ($p<0.05$). Statistical Method was update in the revised text. Actually, significant differences and homogeneous subgroups resulted the same already written and for this reason neither Tables nor Figures were modified.

I found the capital/lowercase letters within the same figure very difficult to inteprete.

Thank you. Actually, same letters but capital and lower case could be difficult to be interpreted but it represents a common way to show data in graphs and in figure legend an explanation of letters is given.

Figure 3 and 4 need better description in title/legend as this is often first thing researcher looks at and no indication what BL stands for. At first I kept thinking baseline (realise it is Blanching). Also think graph figures are wrong way around as looks like the DPPH graph is under the PME title and vice-versa.

Thank you. Legends of Figure 3 and 4 were corrected also by adding a better explanation of codes.

In DPPH figure there is a raw sample but there isn't in PME Figure there isn't, why? If there were then everything could be related to the Raw 0 month data. If wanting to study effects of treatment type (variable 1) and length of storage (variable 2) on dependent variable such as DPPH or PME activity then should do 2 way ANOVA and looks like can do this as there are equal number of samples in each group.

Thank you. For the PME activity we decided to express PME activity as % referred to RAW sample as in other papers; in this way it would be better to appreciate the extent of inactivation or activation. Actually, relative % PME activity at time 0 is referred to Raw samples at 0 day while at time 2 months is referred to raw at 2 months. This approach was due for avoiding the effects of sample preparation at time 0 and after 2 months. For this reason 2-way ANOVA is not needed for PME activity

Is there any quantification possible for Figures 1 and 2? At present only qualitative and were to be quantitative then could discuss and relate more easily to other figures and tables.

Thank you. Unfortunately, Micrographs are only quantitative data as despite eighty sections were analysed and observed, tissue variability and differences didn't allow us to obtain objective data. We were able only to report ranges of cell dimensions.

Reviewer #5: As far as I am concerned the manuscript is well written. The subject area of research is significant in knowledge development. The introduction is interesting and correct. The results and discussion of the research were presented very well.

Maybe you should see the publication: Effectiveness of the fountain-microwave drying method in some selected pumpkin cultivars - LWT - Food Science and Technology 77 (2017) 276-281

Thank you. The suggested reference was added in the revised version of the manuscript

In absrect is no space before MPa, as well as in l. 207, and before C in line 96 and 97

Thank you. Spaces before units were added

in l. 27 and 28 in the unit, there is no space needed after 100, same in line 157

Thank you. Spaces not needed were removed as suggested

in l. 32 and in reference the name is badly saved must be Sokół-Łętowska

Thank you. The surname was corrected as suggested

in l. 136 the unit is wrongly written

Thank you. Unit was corrected

Highlights

- Effects of high pressure on pumpkin cubes was compared with thermal treatment
- The effect of three different pressures was evaluated on two pumpkin species
- **After high pressure treatment parenchyma cells exhibited collapses and separations**
- **Treatment at 400MPa and at 85°C resulted the most effective on pectin methylesterase**
- **High pressure treatment decreased antioxidant activity that increased during storage**

Effects of high hydrostatic pressure on physico-chemical and structural properties of two pumpkin species

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Declarations of interest: none

Abstract

The effects of high pressure treatments (200, 400, 600MPa for 5 min) and a thermal treatment (85°C for 5 min) were evaluated on cubes of two pumpkin species (*Cucurbita maxima* L. var. Delica and *Cucurbita moschata* Duchesne var. Butternut) up to 2 months of refrigerated storage. Increasing the pressure, small parenchyma cells from the pumpkin tissue exhibited collapses and separations, especially for Butternut. This species showed a lower hardness than Delica at time 0. For both species, 400MPa and thermal treatment were the most effective in the inactivation of pectinmethylesterase, which reactivated after 2 months, especially for Butternut. Colorimetric parameters decreased after all treatments. Antioxidant activity resulted affected by pressure, showing a significant increase during storage especially for the samples treated at 200MPa after 2 months, comparable to the thermal treated ones. Among the tested treatments, 400MPa may be considered as the best option for the quality retention during storage.

Keywords: antioxidant activity; enzymatic activity; high pressure processing; **microstructure**; **pumpkin**; **Texture Profile Analysis**.

1. Introduction

Genus *Cucurbita* belongs to the Cucurbitaceae family, and it comprises several species, such as *Cucurbita pepo* L., *Cucurbita moschata* Duchesne ex Poiret, *Cucurbita maxima* Duchesne and *Cucurbita mixta* Pang., that are classified according to their morphological traits and fruit texture (Xanthopoulou, Nomikos, Fragopoulou & Antonopoulou, 2009). Pumpkin fruits are an important source of antioxidant and vitamins; the amount of those bioactive compounds varies according to species and cultivars, but it is generally valuable. Regarding antioxidant**s**, carotenoids content in pumpkin fruits varied from 2 to **10 mg/100g**. **In the case of vitamins** it is possible to count Vitamin C (9–10 mg/100 g), Vitamin E (1.03–1.**06 mg/100g**), but also other vitamins as B6, K, thiamine and riboflavin (Assous, Saad & Dyab, 2014). Moreover, pumpkins fruits are a valuable source of minerals such as potassium, phosphorus, magnesium, iron, and selenium (Assous et al., 2014). The fruits of **these** species are mainly processed to obtain juices, pickles, dried products (Nawirska, Figiel, **Kucharska, Sokòł-Łętowska & Biesiada,** 2009), frozen products or puree.

In the recent years, the demand of fresh-cut fruits and vegetables increased due to their convenience in term of easiness to consume, freshness, and for their contribution to human health benefits (Ragaert, Verbeke, Devlieghere, & Debevere, 2004). For these categories of fresh products, there is an increasing concern for microbial quality, **due to psychrotrophic and even pathogenic bacteria**, such as *Listeria monocytogenes*, which are able to grow at low temperature even under modified atmosphere packaging (Beuchat & Brackett, 1990). Traditional preservation techniques that guarantee the inactivation of foodborne pathogens imply the use of heat with well-known detriment of organoleptic and nutritional quality of vegetables. In this context, high pressure processing (HPP) is an innovative nonthermal treatment, alternative to **thermal** pasteurization of food products. HPP treatment can result in microbial destruction and product stabilization without affecting their sensory qualities (Basak & Ramaswamy, 1998); in fact, low-molecular-weight compounds, **related** with the sensory, nutritional and health-promoting aspects of foods are less or rarely affected by

HPP (Oey, Lille, Van Loey, & Hendrickx, 2008a; Huang, Lung, Yang & Wang, 2014). However, HPP is reported to have an impact on other quality attributes such as texture, colour and flavour that generally depends, not only on process conditions, but also on the type of plant tissue (Oey, Plancken, Loey & Hendrickx, 2008b).

Although this novel technology is being increasingly investigated, the main targets of research regarding plant-based foods are purees or sauces (González-Cebrino, Durán, Delgado-Adámez, Contador & Ramírez, 2013; Medina-Meza, Barnaba, Villani & Barbosa-Cánovas, 2015) and juices (Queirós et al., 2014), while few studies have been focused on whole or minimally processed fruits and vegetables as pieces treated by HPP (Paciulli, Medina-Meza, Chiavaro & Barbosa-Cánovas, 2015; Pinela & Ferreira, 2017). The quality of processed fresh-cut fruits and vegetables represents a complex task and depends on several factors (Tripathi, Gupta, Mishra, Variyar & Sharma, 2014. **Besides, the texture is one of the most important** attributes in determining the overall quality of particulate products with well-defined shapes undergoing considerable softening during the thermal treatments (Basak & Ramaswamy, 1998).

Regarding the publications about the effects of HPP on pumpkin products, this technology has been applied with success to pumpkins puree at 400-600 MPa (Contador, González-Cebrino, García-Parra, Lozano & Ramírez, 2014) leading to **a** significantly higher carotenoid and phenolic content of the puree during storage **in comparison with the content of these compounds in thermal treated samples**. Similarly, González-Cebrino, Durán, Delgado-Adámez, Contador & Bernabé (2016) observed that HPP (400 to 600 MPa) did not affect largely the quality parameters and preserved the levels of bioactive compounds in pumpkin puree. Nevertheless, some Authors reported that HPP treatment did not achieve the complete inhibition of PPO, which could reduce the shelf-life of the products (Paciulli et al., 2016). The study of Zhou et al. (2014) reported that **HPP**-treated pumpkin slices (550 and 450 MPa), after processing and during storage, had greater retention of colour, vitamin C and antioxidant activity, and an elevated total phenols content **than**

untreated samples but were equal to slices treated with a mild thermal treatment (85 °C/ 5 min). However, samples of this study were blanched before pressurization and effects on **the** microbial and quality parameters could be influenced due to this pre-treatment. **In** addition, to the **Authors'** best knowledge no studies in the scientific literature deal with the analysis of different responses due to high pressure by different **pumpkin** species. **As different species of pumpkin are reported to present different characteristics and to respond in different ways to a technological treatment such as drying due to their intrinsic differences (Nawirska-Olszańska, Stępień and Biesiada, 2017), a different behaviour could be expectable also after high pressure processing with particular reference to microstructural effects.** Thus, the aim of this research was to characterize the textural colorimetric, enzymatic and antioxidant changes of pressure-treated pumpkin cubes belonging to two distinct species **and to explain some of them by the microstructural response of the tissue**.

2. Materials and Methods

2.1 Samples, preparation and storage

Fresh pumpkins belonging to two different species *Cucurbita maxima* L. var. Delica (DEL) and *Cucurbita moschata* Duchesne ex Poiret var. Butternut (BUT) at commercial maturity (average weight 5±1 kg) were purchased from a local market. The selected species together with *Cucurbita pepo* L., represent the most economically important species cultivated worldwide (Loy, 2004). In addition, C. maxima and C. moschata cultivars are those mainly used in the pumpkin canning industry (Ferriol & Picó, 2008).

The vegetables were brought to the laboratory and immediately stored at refrigerated temperatures (10 °C) till further processing. The whole pumpkin was washed under running tap water to remove adhered dust. It was then hand-peeled and cut, with a sharp knife, into small cubes of 1.5 cm × 1.5 cm × 1.5 cm. Samples were then vacuum sealed in vacuum bags **(PA/PE, Polyamide/ Polyethylene thickness 90 µm; water vapour permeability 2.6 $g_{water}m^{-2}24h^{-1}$ and oxygen permeability 50**

95 **$cm^3m^{-2}24h^{-1}bar^{-1}$)** by using a packaging machine (Lavezzini Univac, Fiorenzuola d'Arda (PC),
96 Italy). To obtain more homogeneous samples, each pumpkin specie was prepared in batches of 5 kg
97 and each batch was **then divided into five equal portions in order to obtain five different**
98 **samples: untreated (RAW), thermal treated (TT) and high pressure treated at three different**
99 **pressures (HPP).** One portion was kept raw (RAW); another was thermal treated at 85 °C for 5
100 min (TT) in a stirred water bath (JULABO Labortechnik GmbH, Seelbach, Germany), in
101 accordance to the procedure reported by Zhou et al. (2014). The remaining samples were subjected
102 to high pressure processing at the enterprise HPP Italia (Traversetolo, Italy) using a 300 L industrial
103 equipment (Avure Technologies Middletown, Ohio, USA model AV-30), operating with a come-up
104 time of 200 MPa per minute. The treatments were conducted at 200 (HPP200), 400 (HPP400) and
105 600 (HPP600) MPa for 5 min, using cold water (4-5°C) as pressure medium, to keep the
106 temperature of the system around 18-20°C, despite the temperature increasing due to pressurization.
107 After rapid chilling for the thermal treated samples, all samples were stored at 4 °C up to 2 months
108 and analysed after 1 day, 1 month and 2 months from the treatment.
109
110 *2.2 Histological analysis*
111 The samples were fixed in FAA solution (formalin: acetic acid: 60% ethanol solution, 2:1:17 v/v)
112 (Ruzin, 1999). After two weeks, they were dehydrated with gradual increasing alcohol
113 concentrations. The inclusion was made in a methacrylate resin (Technovit 7100, Heraeus Kulzer &
114 Co., Wehrheim, Germany) and the resulting blocks were sectioned at 3 μm thickness (transversal
115 cuts) with a semithin Leitz 1512 microtome (Leitz, Wetzlar, Germany). The sections were stained
116 with Toluidine Blue (TBO) solution (Ruzin, 1999) for the evaluation of the structure variation after
117 each treatment and IKI solution (potassium iodide; Ruzin, 1999) for the evaluation of the starch
118 inclusions. The sections were observed by means of an optical microscope Leica DM 4000 (Leica
119 Imaging Systems Ltd., Wetzlar, Germania) equipped with a digital camera Leica DMC2900 (Leica

120 Imaging Systems Ltd., Wetzlar, Germania). Eighty sections were obtained from three samples of
121 each treatment.
122
123 *2.3 Moisture content, textural and colorimetric analyses*
124 The moisture content (g/100g) of pumpkin samples was evaluated by means of gravimetric
125 technique following the official method (AOAC, 2002).
126 Texture of the all treatments (raw, TT and HPP) was analysed by **Texture Profile Analysis TPA**
127 test using a TA.XT2i Texture Analyzer equipped with a 35 mm diameter cylindrical aluminium
128 probe by means of a double compression with a speed of 1mm/s up to the 40% of the original
129 sample height. The textural parameters considered were: hardness (maximum peak force of the first
130 compression cycle, N), cohesiveness (ratio of positive force area during the second compression to
131 that during the first compression area, dimensionless), resilience (area during the withdrawal of the
132 penetration, divided by the area of the first penetration, dimensionless), and chewiness (product of
133 hardness x cohesiveness x springiness, N) (Bourne, 1978). Eight samples of each package at each
134 time of storage were analysed.
135 Colour determination was carried out using a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka
136 Japan) equipped with a standard illuminant D65. The assessments were carried out on the surface of
137 five different cubes, chosen as representative of the entire vegetable and selected avoiding **edges**.
138 L^* (lightness, black = 0, white = 100), a^* (redness >0, greenness <0), b^* (yellowness, $b^* > 0$, blue
139 <0), C (chroma **or saturation**, 0 at the centre of the colour sphere) and Hue° (Hue angle **or tone**,
140 red =0°, yellow =90°, green=180°, blue=270°) were quantified on each sample using a 10 degree
141 position of the standard observer. Ten samples of each package at each time of storage were
142 analysed.
143
144 *2.4 Pectin methylesterase (PME) activity assay*

PME residual activity was evaluated following the procedure reported by Vicente, Costa, Martínez, Chaves & Civello (2005), briefly 2 grams of fruit were ground with 6 ml of 1 M NaCl **and 8 g/l** PVPP. The suspension obtained was stirred for 4 h and then centrifuged at 10,000 × g for 30 min. The supernatant was collected, adjusted to pH 7.5 with 0.01 M NaOH and used for assaying the enzyme activity. The activity was assayed in a mixture containing 1200 µl of 0.5% (w/v) pectin, 300 µl of 0.01% bromothymol blue pH 7.5, 100 µl of water pH 7.5 and 200 µl of enzymatic extract. The mixture was incubated at 37 °C and the reduction of optical density at 620 nm was followed every 15 s. The results are expressed as percentage variation in comparison to the raw sample, using the values of the slope of a linear segment in the absorbance-time curve (Adams, Brown, Ledward, & Turner, 2003). Analyses were performed in triplicate.

2.5 DPPH free radical scavenging activity test

Antioxidant capacity was determined using DPPH assay (2,2-diphenyl-1-picrylhydrazyl free radical) following the procedure proposed by Zhou et al. (2014). The pumpkin pulp was centrifuged at 12,000 rpm for 15 min at 4 °C. Then, the supernatant was collected for further analysis. 0.2 mL of 10-fold diluted supernatant was mixed with 4.0 mL of a methanolic solution of DPPH (0.14 mmol/L). Analyses were performed in triplicate and the absorbance of the solution was measured at 517 nm after an incubation time of 70 minutes, in dark, at room temperature. The radical scavenging activity was calculated as follows: $I\% = [(Abs_0 - Abs_1)/Abs_0]*100$, were Abs_0 was the absorbance of the blank and Abs_1 was the absorbance of the sample.

For each antioxidant assay, a Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) aliquot was used to develop a 25–500 µmol/L standard curve. All data were then expressed as Trolox Equivalents (**µmol/100g** pumpkin pulp) and antioxidant activity referred to as Trolox Equivalents Antioxidant Capacity (TEAC) (Dini, Tenore & Dini, 2013).

TEAC value (Trolox Equivalent Antioxidant Capacity; mM Trolox/100g) of samples was obtained from the calibration curve calculated measuring the absorbance at 517 nm of Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) methanolic solutions at different concentrations.

Analyses were performed in triplicate.

2.6 Statistical analysis

Means and standard deviations were calculated with SPSS (Version 25.0, 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 samples.

3. Results and Discussion

3.1. Histological analysis

The histological analyses of both species were carried out at t0 and after two months (t2) of storage. The anatomical structure of the treated and untreated samples did not appear to be changed during storage. Thus, only the results of the observations made at t0 are reported and discussed; this consideration is applicable for all the treatments and for all the species.

3.1.1 Cucurbita maxima D. *cv Delica*

The internal parenchyma (mesocarp) of *Cucurbita maxima* D. cv Delica fruit is composed by isodiametric, thickened cells with few and small intercellular spaces. The cells of middle mesocarp are thin-walled and, in the cytoplasm, it is possible to observe an abundant quantity of starch inclusions (Fig. 1A, s). The cells present an elongated shape, characterized by an average minor diameter ranging from 30.0 to 136.2 μm and by an average mayor diameter ranging from 37.7 to 154.0 μm.

After the thermal treatment (Fig. 1B), the parenchyma cells showed a different degree of plasmolysis and became more separated presenting wide intercellular spaces (Fig. 1B). Several Authors observed that cells separation did not involve any visible change in cell walls (Van Marle et al., 1997; Lecain, Ng, Parker, Smith & Waldron, 1999; Sila, Doungla, Smout, Van Loey & Hendrickx, 2006; Paciulli, Ganino, Pellegrini, Rinaldi, Zaupa, Fabbri & Chiavaro 2015). They also hypothesized that cells separation was due to a breakage of chemical bonds between the pectic components of the middle lamellae of adjacent cells and/or to a hydrolysis of some other components of the cell wall such as pectin, hemicelluloses and cellulose. In our study, the separation of cells after thermal treatment might be ascribed to a decrease of the strength of cell-cell interactions in the middle lamella, adjacent to the intercellular spaces.

Regarding HPP200 samples, the tissue showed no microstructure modifications in comparison to

raw ones (Fig. 1C): the cells appeared turgid, with evident starch inclusions (Fig. 1C, s) and with a little degree of starch gelatinization (Fig. 1D) while the intercellular spaces appeared similar to those of the untreated samples. **Immersed in the parenchyma tissue, vascular bundles are visible**.

After the 400 HPP treatment, parenchyma did not show evident modifications in comparison to raw samples: main changes are well-defined in the parenchymatic little cells (Fig 1E). In these cells, the membrane appeared destructured and detached from the cell wall (Fig. 1E) with formation of intercellular spaces. Similarly, in the study of Trejo Araya et al. (2007) when pressures of 100-200 MPa were applied to carrots, very slight tissues changes were observed, while under pressures of 300 and 400 MPa, more extended modifications were evidenced at the expenses of cell walls with reduction of cell to cell contact, presumably as a result of middle lamella breakdown. **Other effects of the HPP treatment are: a decrease in starch inclusions and an increase in gelatinized starch, visible in the micrographs of the tissues stained with IKI** (Fig. 1F).

The most severe treatment (**600 MPa**), did not involve excessive microstructure damages in pumpkin samples if compared to the other treatments. The main structural changes, appeared in the parenchymatic small cells, in which, the detachment of the cell membrane from the cell wall is highlighted (Fig. 1G) and a significant decrease in the quantity of starch granules is observed. This reduction could be due to the starch gelatinization caused by high pressures (Fig. 1H) (Stute et al., 1996; Alvarez, Fuentes & Canet, 2015). Alvarez et al. (2015) reported that the degree of gelatinization depends on the intensity of the pressure and the treatment time. However, Stute et al. (1996) emphasized that the **HPP**-gelatinization, in the presence of an excess of water (i.e., vegetables characterized by high water content), is positively correlated to the applied pressure. The same Authors identified the pressure range in which **HPP** gelatinization of starches can be achieved, and this pressure seems to be between 400 to 900 MPa depending on starch origin. Our study confirmed the **presence of starch gelatinization in Delica samples after HPP treatment**

already at 200 MPa (Fig. 1D) and, to a greater extent, in the samples treated with a pressure of 400 and with a further increase in samples treated at 600 MPa (Fig. 1H).

3.1.2 Cucurbita moschata Duchesne ex Poiret var. Butternut

The internal parenchyma (mesocarp) of cv Butternut fruits consisted in isodiametric, thickened cells with few and small intercellular spaces (Fig. 2A). The cells of the middle mesocarp are thin-walled, but unlike cv Delica, it is not possible to observe starch inclusions (Fig. 2A). The mesocarp cells are characterized, on average, by minor diameters, ranging from 32.2 to 154.7 μm and by major diameters varying from 46.6 to 203.3 μm. Immersed in the parenchyma tissue, vascular bundles surrounded by small parenchymatic cells are observed (Fig. 2A).

After the thermal treatment (Fig. 2B), Butternut parenchymatic tissue, showed different degrees of plasmolysis. The damages observed in the parenchymatic tissues concerned only cell **separations and plasmolysis** (Fig. 2B) and this effect was more evident than in Delica samples (Fig. 1B). Cells appeared detached, as a result of the heating treatment, that promotes the separation of the pectic bonds at median lamella level (Van Marle et al., 1997; Lecain et al., 1999; Sila et al., 2006; Paciulli et al., 2015).

After the 200 MPa treatment, it is possible to identify damages to the parenchymal cells (Fig. 2C). In this case, however, the damages are not uniform throughout the mesocarp, but they **involved** only the small cells in the surround of the vascular bundles. These small cells, after the treatment, appeared severely modified and they collapsed by losing their structure (Fig. 2D) with intercellular spaces (Fig. 2D), as consequence.

The increase of the treatment intensity at 400 MPa, caused damages to all parenchyma tissues: clear changes are visible in the smallest cells surrounding the vascular bundles (Fig. 2E). These cells do not appeared linked to each other and, as a consequence, intercellular spaces are present (Fig. 2E).

When the samples are exposed to 600 MPa treatment, deep changes of the structure occurred (Fig. 2F). The tissue appears disorganized and composed by shapeless cells that have lost their turgidity

and more intercellular spaces emerged (Fig. 2F). The bigger parenchymatic cells appear without evident signs of cell wall breakage. Instead, the damages **affected** only the small cells surrounding the vascular bundles; the result is a peculiar continuous structural damage that involve all the small cells widespread in the pumpkin parenchyma (Fig. 2F).

3.2. Texture Profile Analysis of pumpkin cubes

Textural parameters of Delica pumpkin cubes are reported in Table 1. At time 0, RAW samples presented the highest values of hardness while the TT the lowest ones, as expected, as thermal treatments generally cause a loss of firmness in pumpkin (Assous et al., 2014) The loss of firmness in the TT samples is explained from the histological observations that showed cells detachment as effect of the thermal treatment on the components of the middle lamella. High pressure-treated HPP200 samples, showed the hardness values more similar to RAW if compared to HPP400 and HPP600 samples, confirming the good retention of structure, as observed in the histological analysis. During refrigerated storage, RAW and TT samples presented substantially stable hardness values, while all HPP samples on the contrary presented a significant decrease at time 2 months, probably due to the residual activity of PME that was released by damaged cells and continued the breakage of the cell walls' structure. This effect was more evident for HPP600 samples, probably due to the highest structural degradation observed just after the treatment (Figure 1 and 2), and resulting at the end of the storage softer than the TT samples. The obtained results are partially in agreement with Zhou et al. (2014) who reported a decrease in hardness values of pumpkin slices after HPP treatment, both after the treatment and during 2 months of refrigerated storage, but with a better retention of hardness for samples treated at the highest pressure (550 vs. 450 MPa). Kingsly, Balasubramaniam & Rastogi (2009) observed a **decrease**, even if not significant, in hardness values of pineapple slices with increasing applied pressure. More recently, Denoya et al. (2017) reported that hardness was significantly affected by the pressure level for minimally processed peaches.

281 Cohesiveness values of all the treated samples resulted significantly lower compared to RAW,
282 being the TT ones the lowest **(Table 1).** Cohesiveness indicates how well the product withstands a
283 second deformation relative to its resistance under the first deformation. Thus, all the treated
284 samples are less able to resist to the second deformation due to the structural damages (Figure 1).
285 Among treated samples, the HPP200 ones presented the highest value of cohesiveness thanks to the
286 limited tissue's damages (Figure 1C), as discussed above for the hardness values. During storage
287 **(Table 1),** cohesiveness values tended to decrease with the exception of TT that remained stable.
288 Probably, in RAW and in lowest pressure HPP samples, enzymes were not inactivated and caused
289 the degradation of cell walls while, in addition, observed gelatinised starch could have played a
290 significant role in product aging. Finally, in accordance with Kingsly et al. (2009), springiness is
291 reduced after high-pressure processing with no differences between the tested pressures (**Table 1),**
292 showing, during storage, the same trend observed for cohesiveness.
293 Butternut pumpkin presented lower values of hardness in comparison to Delica, for all the samples
294 (**Table 1).** A similar trend to that described for Delica was observed for Butternut cubes, with few
295 differences. After high pressure processing, the decrease of mean hardness in Butternut samples
296 was higher than in Delica samples (48% vs. 22%), demonstrating a weaker structure of the
297 Butternut specie to stress caused by high pressures. On the contrary, hardness decrease during
298 storage, was less pronounced for Butternut specie. The observed differences could be explained by
299 the structural changes in the two studied species (Figure 1 and 2): Butternut presented more evident
300 mechanical damages at the expense of parenchymal cells compared to Delica. Regarding
301 cohesiveness and springiness, Butternut presented significantly lower values compared to Delica,
302 but these values were stable during storage for HPP400 and HPP600 samples, confirming that the
303 main changes in Butternut structure happened just after the HPP treatment with few variations in
304 the time.
305

306 *3.3 PME activity*

307 Residual activity values of PME in pumpkin cubes from the two species at time 0 and 2 months are
308 reported in Figure 3. The effects of high pressure on enzymes are difficult to be predicted as the
309 obtained results will be the sum of activation, and inactivation, due to conformational changes
310 (Hendrickx, Ludikhuyze, Van den Broeck & Weemaes, 1998). BL samples presented very low
311 PME residual activity (about 15-20 %) for both species as expected due to thermal denaturation of
312 the protein. Generally, these values are maintained also after 2 months of storage, confirming the
313 irreversible inactivation of PME. Regarding high pressure treated samples at time 0 (Figure 3) a
314 similar trend was observed for both species: the lowest PME residual activity was observed for
315 samples treated at 400 MPa, while the highest one for those treated at 600 MPa. **The activation**
316 **and inactivation of enzymes induced by high pressure, depend on conformational changes but**
317 **also on pressure-induced decompartmentalization (Butz, Koller, Tauscher & Wolf, 1994). In**
318 **intact tissues, enzymes and substrate are often separated by compartmentalization, which can**
319 **be destroyed once low pressure is applied (Butz et al., 1994). Moreover, observed pressure-**
320 **induced membrane damages (Figure 1 and 2) could have caused a higher extraction of**
321 **enzyme during the analysis with a greater observed activity, as consequence. Tissue damages**
322 **resulted dependent on the pressure level as in Figure 1 and 2 leading to an expected highest**
323 **extraction in samples treated at 600 MPa.**

324 At time 2 months, Delica samples did not shown significant variation in residual PME activity for
325 all treated samples with the except of HPP600, while Butternut presented great and significant
326 increase of residual PME activity for samples treated with high pressure (Figure 3), probably due to
327 the structural changes observed after HPP treatments (Figure 2) which could have increased the
328 extraction of enzyme and the contact between enzyme and substrate.

329

330 *3.4. Colour of pumpkin cubes*

331 Colour changes (*L**, *a** and *b**) of the two studied pumpkin species, as affected by treatments and
332 time of storage, are presented in Table 2. After all the treatments, a reduction ($p<0.05$) of *L**, *a** and
333 *b** was observed, for both the pumpkin species in comparison to untreated sample (RAW), in
334 accordance with Zhou et al. (2014). The reduction of *L**, *a** and *b** on pumpkin, after thermal
335 treatments, was already reported by other **Authors** (Gonçalves, Pinheiro, Abreu, Brandão & Silva,
336 2007; Zhou et al., 2014) and associated to non-enzymatic browning reaction with the result of
337 darker samples and generally a loss of redness, yellowness and vivid characteristics due also to
338 oxidation and isomerization of β-carotene (Camorani et al.,2015). Moreover, the starch
339 gelatinization was reported to influence the lightness (*L**) reduction in blanched potatoes, because
340 of the clarity-like characteristic of gelatinized starch (Pimpaporn, Devahastin & Chiewchan, 2007).
341 The starch gelatinization was also seen in all the HPP treated samples, thus the assertion may be
342 extended also to these samples. For both Delica and Butternut species, no significant differences
343 were observed between BL and HPP samples, being however HPP200 the more affected ones,
344 showing for Butternut the lowest values of *b** (30.8 ± 3.0) and for Delica the lowest values of *L**
345 (49.4 ± 0.9), among all. During storage, the colour of Butternut samples resulted more stable
346 compared with Delica samples, that instead, presented significant changes in colorimetric
347 parameters mainly for HPP200 treatment. The treatment at 200 MPa for 5 min may have probably
348 produced simultaneously important damages to cells with a release of compounds and an
349 incomplete inactivation of oxidative enzymes and microorganisms, which can result in undesired
350 chemical reactions (both enzymatic and non-enzymatic) during storage (Oey et al., 2008a). From
351 colorimetric data, also ΔE values were calculated (data not shown) and values higher than 12 were
352 obtained for all samples, leading to a great colour difference from RAW ones as reported by Limbo
353 & Piergiovanni (2006).
354
355 *3.5. Antioxidant activity*

356 Results of antioxidant capacity of Delica specie are reported in Figure 4. The obtained data are in
357 accordance with Zhou, Mi, Hu & Zhu (2017) who reported a significant higher Trolox equivalent
358 value for *Cucurbita maxima* L. (2.61 ± 0.04 mmol/100g) compared to *Cucurbita moschata*
359 Duchesne ex Poiret (1.92 ± 0.01 mmol/100g). **For both species, thermal treatment didn't cause**
360 **significant variations of the DPPH values in accordance to results reported by Dini et al.**
361 **(2013) that observed an increase in DPPH value in pumpkin after several cooking treatments.**
362 On the contrary, the high-pressure process significantly reduced the antioxidant activity at time 0
363 with the lowest values observed for 600 MPa treated samples, especially for Delica, which showed
364 a 93.8% reduction in comparison to RAW. On the other hand, HPP600 Butternut showed only a
365 48.3% reduction (Fig.4, panel A and B). During shelf life, the antioxidant capacity of the RAW
366 samples significantly decreased for both species, probably due to the degradation of antioxidants
367 (Tripathi et al., 2014). TT showed an opposite trend between Delica and Butternut specie (Figure
368 4): the former presented a significant reduction of antioxidant capacity after two months, while the
369 latter an increase. This fact could be explained by the different structural changes that could have
370 influenced the extraction of active compounds from the pumpkin fles**h. In fact, thermal treatment**
371 **caused a higher extent of cell detachment in Butternut (Figure 2) samples compared to Delica**
372 **ones (Figure 1) with a dramatic reduction in hardness values (Table 1), that linked to a**
373 **residual enzymatic activity could have allowed a more efficient extraction of antioxidant**
374 **molecules**. On the contrary, all HPP samples, with the exception of HPP200 of Butternut, showed a
375 significant increase of antioxidant activity for both species, and especially for Delica, probably
376 thanks to the increased release of active compounds from damaged pumpkin tissues.
377
378 **4. Conclusions**
379 The effects of high hydrostatic pressure treatments at 200, 400 and 600 MPa for 5 min on diced
380 pumpkins belonging to two species (*Cucurbita maxima* L. cv. Delica and *Cucurbita moschata*

381 Duchesne ex Poiret cv. Butternut) **were** evaluated and compared to traditional mild thermal
382 treatments, showing advantages and disadvantages depending on the treatment intensity and the
383 pumpkin specie.
384 In general, observing the structure, both histological and **textural** analyses showed that high
385 pressure processing was able to better retain the raw product characteristics, in comparison to
386 thermal treatment. The high pressures affected the pumpkin structure proportionally to the intensity
387 of the treatment. In particular, the Butternut variety suffered more than Delica the effect of high
388 pressure, although during the shelf life it resulted more stable, especially for 400 and 600 MPa
389 samples, while further modifications were observed for the 200 MPa samples, being this treatment
390 probably too mild to inactivate the enzymes. As a matter of fact, the evaluation of the residual PME
391 activity showed that the most effective HPP treatment, for both the varieties, other than thermal
392 treated, was HPP400, even if, after two months of storage, a recovery of the enzymatic activity was
393 observed for the pressurized samples, especially for Butternut ones, even to values higher than those
394 of raw. The effects of the high pressures on colour was very similar to those of thermal treatment,
395 both for Delica and Butternut, with a general discoloration in comparison to the raw samples. The
396 treatment at 200 MPa at t0 seemed to be the most detrimental on colour, even if during shelf-life a
397 recover of the colorimetric parameters was observed for both the varieties, being however Butternut
398 the less stable. The antioxidant activity did not result modified by thermal treatment, while the
399 HPP600 resulted the most damaging treatment for both the cultivars. Increased antioxidant
400 activities were observed after 2 months of storage for both the cultivars, resulting even higher for
401 the Delica HPP treated samples than those of raw and thermal treated ones.
402 In conclusion, 400 MPa could be considered the right compromise, taking into account the too weak
403 effect of HPP200 on enzymes and the too intense effect of HPP600 on structure and antioxidant
404 activity. The Delica samples responded better to the effect of high pressures, probably due to the
405 compactness of its structure; a different effect was observed also for thermal treatment but with less

406 evident differences. Finally, high pressure treatments could represent a valid alternative to thermal
407 treatment for Delica variety considering the lower impact on the structure and the retention or
408 improvement of the antioxidant properties
409

410 **References**

411 Adams, J.B., Brown, H. M., Ledward, D. A., & Turner, R. (2003). Heat-inactivated peroxidases and
412 the role of calcium abstraction as a cause of their enhanced lipid oxidation activity: potential effects
413 on the flavour quality of heatprocessed vegetables. *Food Chemistry*, 80, 499-510.
414 Alvarez M.D., Fuentes R., & Canet W. (2015). Effects of Pressure, Temperature, Treatment Time,
415 and Storage on Rheological, Textural, and Structural Properties of Heat-Induced Chickpea Gels.
416 *Foods* 4:80-114.
417 AOAC Official methods of analysis (16th ed.), Association of Official Analytical
418 Chemists, Arlington, VA (2002).
419 Assous, M. T. M., Saad, E. S., & Dyab, A. S. (2014). Enhancement of quality attributes of canned
420 pumpkin and pineapple. *Annals of Agricultural Sciences*, 59(1), 9-15.
421 Basak, S., & Ramaswamy, H. S. (1998). Effect of high pressure processing on the texture of
422 selected fruits and vegetables. *Journal of Texture Studies*, 29(5), 587-601.
423 Beuchat, L.R., & Brackett, R.E. (1990). Survival and growth of Listeria monocytogenes on lettuce
424 as influenced by shredding, chlorine treatment modified atmosphere packaging and temperature.
425 *Journal of Food Science*, 55, 755–758.
426 Bourne, M.C. (1978) Texture profile analysis. *Food Technology*, 32, 62-66.
427 Butz, P., Koller, W. D., Tauscher, B., & Wolf, S. (1994). Ultra-high pressure processing of onions:
428 chemical and sensory changes. *LWT-Food Science and Technology*, 27(5), 463-467.
429 Camorani, P., Chiavaro, E., Cristofolini, L., Paciulli, M., Zaupa, M., Visconti, A., Fogliano V. &
430 Pellegrini, N. (2015). Raman spectroscopy application in frozen carrot cooked in different ways and
431 the relationship with carotenoids. *Journal of the Science of Food and Agriculture*, 95(11), 2185-
432 2191.
433 Contador, R., González-Cebrino, F., García-Parra, J., Lozano, M., & Ramírez, R. (2014). Effect of
434 hydrostatic high pressure and thermal treatments on two types of pumpkin purée and changes

435 during refrigerated storage. *Journal of Food Processing and Preservation*, 38(2), 704-712.
436 Denoya, G. I., Vaudagna, S. R., Chamorro, V. C., Godoy, M. F., Budde, C. O., & Polenta, G. A.
437 (2017). Suitability of different varieties of peaches for producing minimally processed peaches
438 preserved by high hydrostatic pressure and selection of process parameters. *LWT-Food Science and*
439 *Technology*, 78, 367-372.
440 Dini, I., Tenore, G. C., & Dini, A. (2013). Effect of industrial and domestic processing on
441 antioxidant properties of pumpkin pulp. *LWT — Food Science and Technology*, 53(1), 382–385.
442 Ferriol M., Picó B. (2008) Pumpkin and Winter Squash. In: Prohens J., Nuez F. (eds) Vegetables I.
443 Handbook of Plant Breeding, vol 1. Springer, New York, NY.
444 Gonçalves, E. M., Pinheiro, J., Abreu, M., Brandão, T. R. S., & Silva, C. L. (2007). Modelling the
445 kinetics of peroxidase inactivation, colour and texture changes of pumpkin (*Cucurbita maxima* L.)
446 during blanching. *Journal of Food Engineering*, 81(4), 693-701.
447 González-Cebrino, F., Durán, R., Delgado-Adámez, J., Contador, R., & Bernabé, R. R. (2016).
448 Impact of high pressure processing on color, bioactive compounds, polyphenol oxidase activity, and
449 microbiological attributes of pumpkin purée. *Food Science and Technology International*, 22(3),
450 235–245.
451 González-Cebrino, F., Durán, R., Delgado-Adámez, J., Contador, R., & Ramírez, R. (2013).
452 Changes after high-pressure processing on physicochemical parameters, bioactive compounds, and
453 polyphenol oxidase activity of red flesh and peel plum purée. *Innovative Food Science and*
454 *Emerging Technologies*, 20, 34–41.
455 Hendrickx, M., Ludikhuyze, L., Van den Broeck, I., & Weemaes, C. (1998). Effects of high
456 pressure on enzymes related to food quality. *Trends in Food Science & Technology*, 9(5), 197-203.
457 Huang, H. W., Lung, H. M., Yang, B. B., & Wang, C. Y. (2014). Responses of microorganisms to
458 high hydrostatic pressure processing. *Food Control*, 40, 250-259.

459 Kingsly, A. R. P., Balasubramaniam, V. M., & Rastogi, N. K. (2009). Effect of high- pressure
460 processing on texture and drying behavior of pineapple. *Journal of Food Process Engineering*,
461 32(3), 369-381.
462 Lecain, S., Ng, A., Parker, M. L., Smith, A. C., & Waldron, K. W. (1999). Modification of cell-wall
463 polymers of onion waste—Part I. Effect of pressure-cooking. *Carbohydrate Polymers*, 38(1), 59-67.
464 Limbo, S., & Piergiovanni, L. (2006). Shelf life of minimally processed potatoes: Part 1. Effects of
465 high oxygen partial pressures in combination with ascorbic and citric acids on enzymatic browning.
466 *Postharvest Biology and Technology*, 39(3), 254-264.
467 Loy, J. B. (2004). Morpho-physiological aspects of productivity and quality in squash and
468 pumpkins (Cucurbita spp.). *Critical reviews in plant sciences*, 23(4), 337-363.
469 Medina-Meza, I. G., Barnaba, C., Villani, F., & Barbosa-Cánovas, G. V. (2015). Effects of thermal
470 and high pressure treatments in color and chemical attributes of an oil-based spinach sauce. *LWT-*
471 *Food Science and Technology*, 60(1), 86-94.
472 Nawirska, A., Figiel, A., Kucharska, A.Z., **Sokòl-Łętowska, A.,** Biesiada, A., 2009. Drying kinetics
473 and quality parameters of pumpkin slices dehydrated using different methods. *Journal of Food*
474 *Engineering*, 94 (1), 12–14.
475 **Nawirska-Olszańska, A., Stępień, B., & Biesiada, A. (2017). Effectiveness of the fountain-**
476 **microwave drying method in some selected pumpkin cultivars. LWT-Food Science and**
477 **Technology, 77, 276-281.**
478 Oey, I., Lille, M., Van Loey, A., & Hendrickx, M. (2008a). Effect of high-pressure processing on
479 colour, texture and flavour of fruit-and vegetable-based food products: a review. *Trends in Food*
480 *Science & Technology*, 19(6), 320-328.
481 Oey, I., Plancken, I. V., Loey, A. V., & Hendrickx, M. (2008b). Does high pressure processing
482 influence nutritional aspects of plant based food systems? *Trends in Food Science and Technology*.
483 19, 300–308.

484 Queirós, R.P., Santos, M.D., Fidalgo, L.G., Mota, M.J., Lopes, R.P., Inácio, R.S., Delgadillo, I., &
485 Saraiva, J.A. (2014). Hyperbaric storage of melon juice at and above room temperature and
486 comparison with storage at atmospheric pressure and refrigeration. *Food Chemistry,* 147, 209–214.
487 Paciulli, M., Ganino, T., Pellegrini, N., Rinaldi, M., Zaupa, M., Fabbri, A., & Chiavaro, E. (2015).
488 Impact of the industrial freezing process on selected vegetables—Part I. Structure, texture and
489 antioxidant capacity. *Food Research International*, 74, 329-337.
490 Paciulli, M., Medina-Meza, I. G., Chiavaro, E., & Barbosa-Cánovas, G. V. (2016). Impact of
491 thermal and high pressure processing on quality parameters of beetroot (*Beta vulgaris* L.). *LWT-*
492 *Food Science and Technology*,68, 98-104.
493 Pimpaporn, P., Devahastin, S., & Chiewchan, N. (2007). Effects of combined pretreatments on
494 drying kinetics and quality of potato chips undergoing low-pressure superheated steam drying.
495 *Journal of Food Engineering*, 81(2), 318-329.
496 Pinela, J., & Ferreira, I. C. (2017). Nonthermal physical technologies to decontaminate and extend
497 the shelf-life of fruits and vegetables: Trends aiming at quality and safety. *Critical Reviews In Food*
498 *Science And Nutrition*, 57(10), 2095-2111.
499 Ragaert, P., Verbeke, W., Devlieghere, F., & Debevere, J. (2004). Consumer perception and choice
500 of minimally processed vegetables and packaged fruits. Food Quality and Preference, 15(3), 259-
501 270.
502 Ruzin, S. *Plant Microtechnique And Microscopy*. Oxford University Press, Oxford (1999).
503 Sila, D. N., Doungla, E., Smout, C., Van Loey, A., & Hendrickx, M. (2006). Pectin fraction
504 interconversions: insight into understanding texture evolution of thermally processed
505 carrots. *Journal of Agricultural and Food Chemistry*, 54(22), 8471-8479.
506 Stute R., Klinger R.V., Boguslawski S., Eshtiaghi M.N., & Knorr D. (1996). Effects of high
507 pressures treatment on starches. *Starch*, 48:399–408.

508 Trejo Araya, X. I., Hendrickx, M., Verlinden, B. E., Van Buggenhout, S., Smale, N. J., Stewart, C.,
509 & Mawson, A. J. (2007). Understanding texture changes of high pressure processed fresh carrots: A
510 microstructural and biochemical approach. Journal of food engineering, 80(3), 873-884.
511 Tripathi, J., Gupta, S., Mishra, P. K., Variyar, P. S., & Sharma, A. (2014). Optimization of radiation
512 dose and quality parameters for development of ready-to-cook (RTC) pumpkin cubes using a
513 statistical approach. *Innovative Food Science & Emerging Technologies*, 26, 248-256.
514 van Marle, J. T., Stolle-Smits, T., Donkers, J., van Dijk, C., Voragen, A. G., & Recourt, K. (1997).
515 Chemical and microscopic characterization of potato (Solanum tuberosum L.) cell walls during
516 cooking. *Journal of Agricultural and Food Chemistry*, 45(1), 50-58.
517 Vicente, A. R., Costa, M. L., Martínez, G. A., Chaves, A. R., & Civello, P. M. (2005). Effect of
518 heat treatments on cell wall degradation and softening in strawberry fruit. *Postharvest Biology and*
519 *Technology*, 38(3), 213-222.
520 Xanthopoulou M.N., Nomikos T., Fragopoulou E. & Antonopoulou S. (2009). Antioxidant and
521 lipoxygenase inhibitory activities of pumpkin seed extracts. *Food Research International*, 42(5-6),
522 641-646.
523 Zhou, C. L., Liu, W., Zhao, J., Yuan, C., Song, Y., Chen, D., Yuan-Ying, N., & Li, Q. H. (2014).
524 The effect of high hydrostatic pressure on the microbiological quality and physical–chemical
525 characteristics of Pumpkin (*Cucurbita maxima* Duch.) during refrigerated storage. *Innovative Food*
526 *Science & Emerging Technologies*, 21, 24-34.
527 Zhou, C. L., Mi, L., Hu, X. Y., & Zhu, B. H. (2017). Evaluation of three pumpkin species:
528 correlation with physicochemical, antioxidant properties and classification using SPME-GC–MS
529 and E-nose methods. Journal of Food Science and Technology, 54(10), 3118-3131.
530

531 **Declarations of interest: none**

532

Figure captions

Figure 1. Transverse sections of *Cucurbita maxima* L. var. Delica samples stained with Toluidine Blue (A, B, C, E and G) or potassium iodide (IKI - D, F and H): A. raw; B. blanched; C. HPP200; D. HPP200; E. HPP400; F. HPP400; G. HPP600; H. HPP600

Legend: cd=cell detachment; d=cell damage; s = starch granules; sg=gelatinized starch granules; vb=vascular bundles;.

Figure 2. Transverse sections of *Cucurbita moschata* Duchesne ex Poiret var. Butternut samples stained with Toluidine Blue: A. raw/untreated; B. blanched; C. HPP200; D. detail of cell damages after HPP 200MPa treatment; E. HPP400; F. HPP600

Legend: cd=cell detachment; d=cell damage; p= plasmolysis; sm= small parenchymatic cells of mesocarp; vb=vascular bundles.

Figure 3. PME residual activity expressed as % referred to RAW of Delica (A) and Butternut (B) at 0 and 2 months. Error bars represent ± 1 SD[a].

[a] n=3. for each sample at each storage time. Means followed by different capital or lowercase letters significantly differ ($p < 0.05$) among the four types of samples at the same storage time. Means in column followed by stars significantly differ ($p < 0.05$) among different times for the same sample.

Figure 4. Antioxidant capacity of Delica (panel A) and Butternut (panel B) samples at 0 and 2 months[a].

[a] n=3. for each sample at each storage time. Means followed by different capital or lowercase letters significantly differ ($p < 0.05$) among the four types of samples at the same storage time. Means in column followed by stars significantly differ ($p < 0.05$) among different times for the same sample.

Table 1. Textural parameters of analysed pumpkin dices[a]

DELICA Samples	Time (months)	Hardness (N)	Cohesivness	Springiness (mm)
RAW	0	353.3±27.3**a** x	0.60±0.04**a** x	68.9±4.2**a** x
	1	329.1±52.0A x	0.49±0.11A xy	61.2±6.0A xy
	2	331.7±56.2*a* x	0.39±0.06*a* y	52.1±2.7*a* y
TT	0	169.2±32.8**d** x	0.17±0.04**d** x	37.8±3.2**c** x
	1	177.3±46.7B x	0.19±0.02C x	40.8±2.0B x
	2	178.8±44.8*bc* x	0.18±0.04*bc* x	45.7±7.2*a* x
HPP200	0	316.9±44.4**ab** x	0.43±0.06**b** x	49.7±4.4**b** x
	1	328.7±69.6A x	0.37±0.10AB xy	39.1±2.7B y
	2	212.0±26.2*b* y	0.22±0.04*b* y	35.3±0.9*b* y
HPP400	0	274.0±48.8**bc** x	0.41±0.04**bc** x	48.5±2.6**b** x
	1	248.3±66.2AB x	0.26±0.03BC y	38.9±4.0B y
	2	125.3±22.4*cd* y	0.14±0.01*c* z	30.7±0.6*b* z
HPP600	0	229.3±27.3**cd** x	0.35±0.04**c** x	45.0±4.2**b** x
	1	217.3±43.8AB x	0.26±0.05BC y	38.3±3.8B y
	2	62.9±12.5*d* y	0.14±0.01*c* z	30.3±3.4*b* z

BUTTERNUT Samples	Time (months)	Hardness (N)	Cohesivness	Springiness (mm)
RAW	T0	291.6±47.8**a** x	0.45±0.06**a** x	50.6±7.3**a** x
	T1	297.3±60.9A x	0.38±0.09A xy	44.3±4.8A xy
	T2	251.3±48.6*a* x	0.25±0.02*a* y	38.4±6.0*a* y
TT	T0	98.5±19.5**c** x	0.14±0.04**c** x	40.9±7.7**b** x
	T1	96.0±15.0B x	0.08±0.03B y	30.5±6.3B xy
	T2	107.2±15.7*b* x	0.11±0.05*b* xy	28.2±4.5*b* y
HPP200	T0	166.7±8.6**b** x	0.19±0.04**b** x	38.3±6.4**b** x
	T1	126.9±16.4B y	0.15±0.01B x	28.7±1.4B y

	T2	95.9±9.2*b* z	0.15±0.01*b* x	28.1±1.2*b* y
HPP400	T0	127.6±18.4**bc** x	0.17±0.04**c** x	35.4±6.1**b** x
	T1	112.6±15.0B x	0.14±0.02B x	31.0±2.1B x
	T2	125.3±19.5*b* x	0.17±0.02*b* x	28.5±1.7*b* x
HPP600	T0	157.5±11.0**b** x	0.16±0.02**c** x	33.7±6.4**b** x
	T1	111.8±40.5B x	0.14±0.01B x	30.8±1.7B x
	T2	119.8±14.6*b* x	0.18±0.02*ab* x	32.4±9.1*b* x

[a] n=40. [a,b,c] Means followed by different bold, capital and italic letters significantly differ ($p < 0.05$) among the **five** types of samples at the same storage time. [x,y,z] Means in column followed by different letters significantly differ ($p < 0.05$) among different times for the same sample.

[**a,b,c**] bold letters: significant differences at time 0 between treatments; [A,B,C] capital letters: significant differences at time 1 between treatments; [*a,b,c*] italic letters: significant differences at time 2 between treatments; [x,y,z] significant differences between times for each sample.

HPP200, HPP400, HPP600: high pressure treated at 200, 400 and 600 MPa for 5 min. TT: 85 °C for 5 min.

Table 2. Colorimetric parameters of analysed pumpkin species[a].

Samples	Time (months)	*Delica*			*Butternut*		
		L*	***a****	***b****	***L****	***a****	***b****
RAW	0	64.1±2.9**a** y	24.7±2.3**a** x	53.6±5.5**a** x	65.5±0.4**a** x	32.1±1.0**a** x	51.0±1.3**a** x
	1	69.8±2.2A x	22.4±0.6A x	43.9±3.5A y	53.8±3.1A z	25.3±3.9A y	47.7±4.3A y
	2	70.5±1.3*a* x	24.5±1.9*a* x	51.1±1.5*a* x	59.8±0.9*a* y	30.6±0.8*a* x	48.6±3.3*a* x
TT	0	56.7±1.5**b** x	18.5±1.8**c** xy	42.8±5.8**b** x	51.6±1.9**b** y	20.9±3.1**b** x	37.9±5.2**b** x
	1	56.0±1.1BC x	20.8±1.3A x	36.8±4.3A x	51.6±0.5B y	17.4±1.9B x	35.7±2.5C x
	2	55.4±2.3*b* x	17.1±1.4*b* y	40.0±5.5*b* x	54.8±0.7*b* x	19.6±4.5*b* x	36.0±10.0*b* x
HPP200	0	49.4±0.9**c** z	18.9±1.7**bc** x	34.8±5.4**b** y	49.3±2.0**b** y	23.1±1.4**b** x	30.8±3.0**c** x
	1	59.7±4.6B x	18.3±3.1A x	40.8±4.8A xy	52.6±1.5AB xy	20.7±3.2B x	33.1±6.4C x
	2	55.0±0.8*b* y	19.6±0.8*b* x	42.8±3.7*ab* x	53.4±2.4*b* x	21.9±2.5*b* x	36.5±2.9*b* x
HPP400	0	54.7±4.3**b** x	20.2±1.5**ab** x	38.4±6.8**b** y	52.2±1.9**b** x	24.0±3.4**b** x	34.3±3.7**bc** x
	1	54.3±2.6C x	19.5±3.2A x	40.9±2.4A y	51.8±2.4B x	20.9±2.7B x	41.8±5.2B x
	2	57.3±3.7*b* x	19.0±1.0*b* x	49.7±4.9*a* x	53.6±3.8*b* x	19.2±2.8*b* x	37.2±6.9*ab* x
HPP600	0	56.9±4.3**b** x	22.2±1.5**ab** x	42.4±3.8**b** x	51.9±0.9**b** x	20.3±1.3**b** x	33.4±3.6**bc** y
	1	56.3±1.6BC x	21.1±1.4A x	43.4±3.9A x	51.5±2.1B x	20.1±3.7B x	40.7±3.9B x
	2	58.6±1.4*b* x	17.5±1.5*b* y	45.1±6.1*ab* x	51.4±1.3*b* x	20.4±1.2*b* x	35.3±4.6*b* xy

[a] n=10. Means followed by different letter differed significantly ($p < 0.05$). **[a,b,c]** bold letters: significant differences at time 0 between treatments; [A,B,C] capital letters: significant differences at time 1 between treatments; [*a,b,c*] italic letters: significant differences at time 2 between treatments; [x,y,z] significant differences between times for each sample.

HPP200, HPP400, HPP600: high pressure treated at 200, 400 and 600 MPa for 5 min. TT: 85 °C for 5 min.

Figure 1

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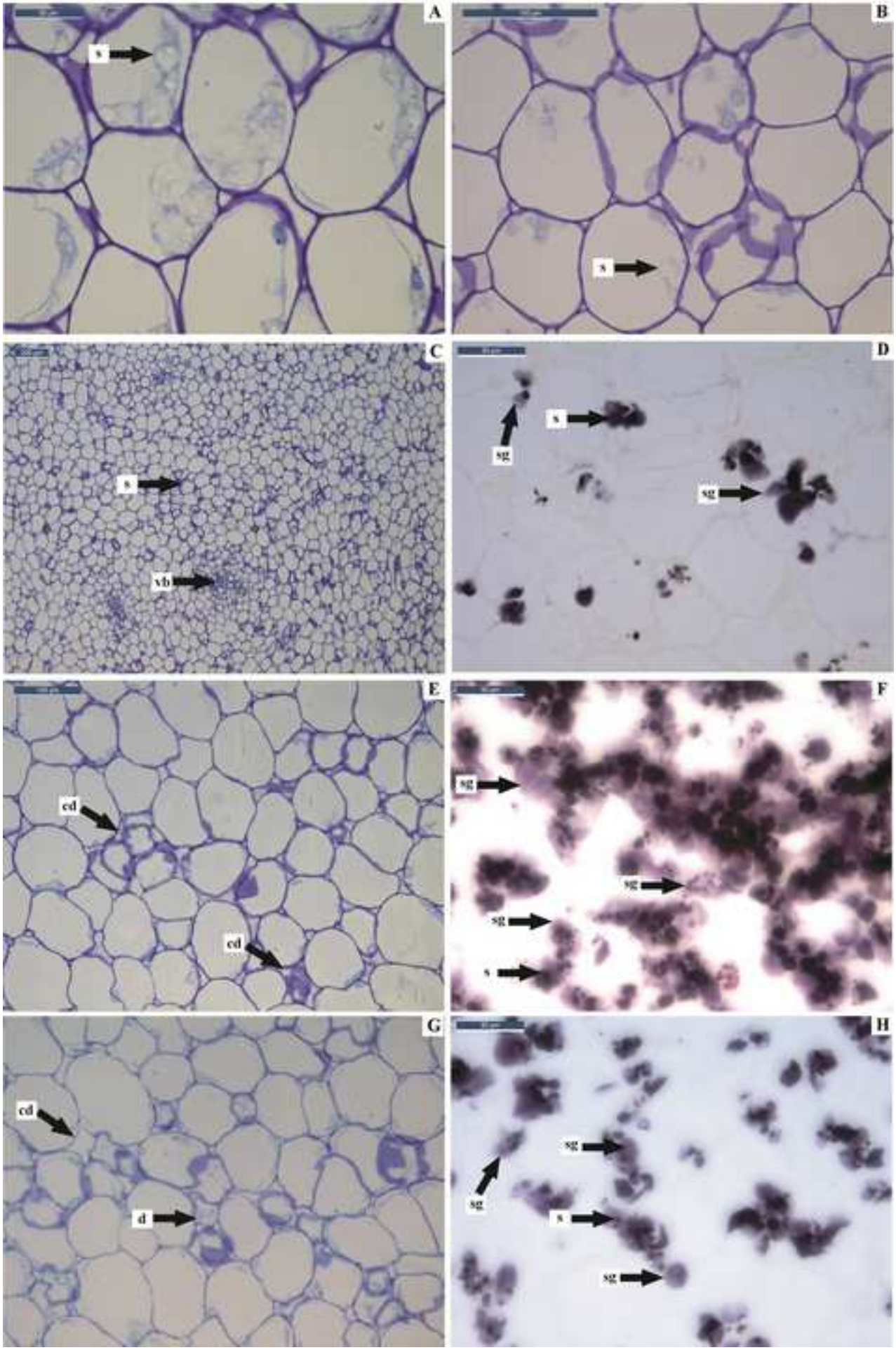

Figure 2

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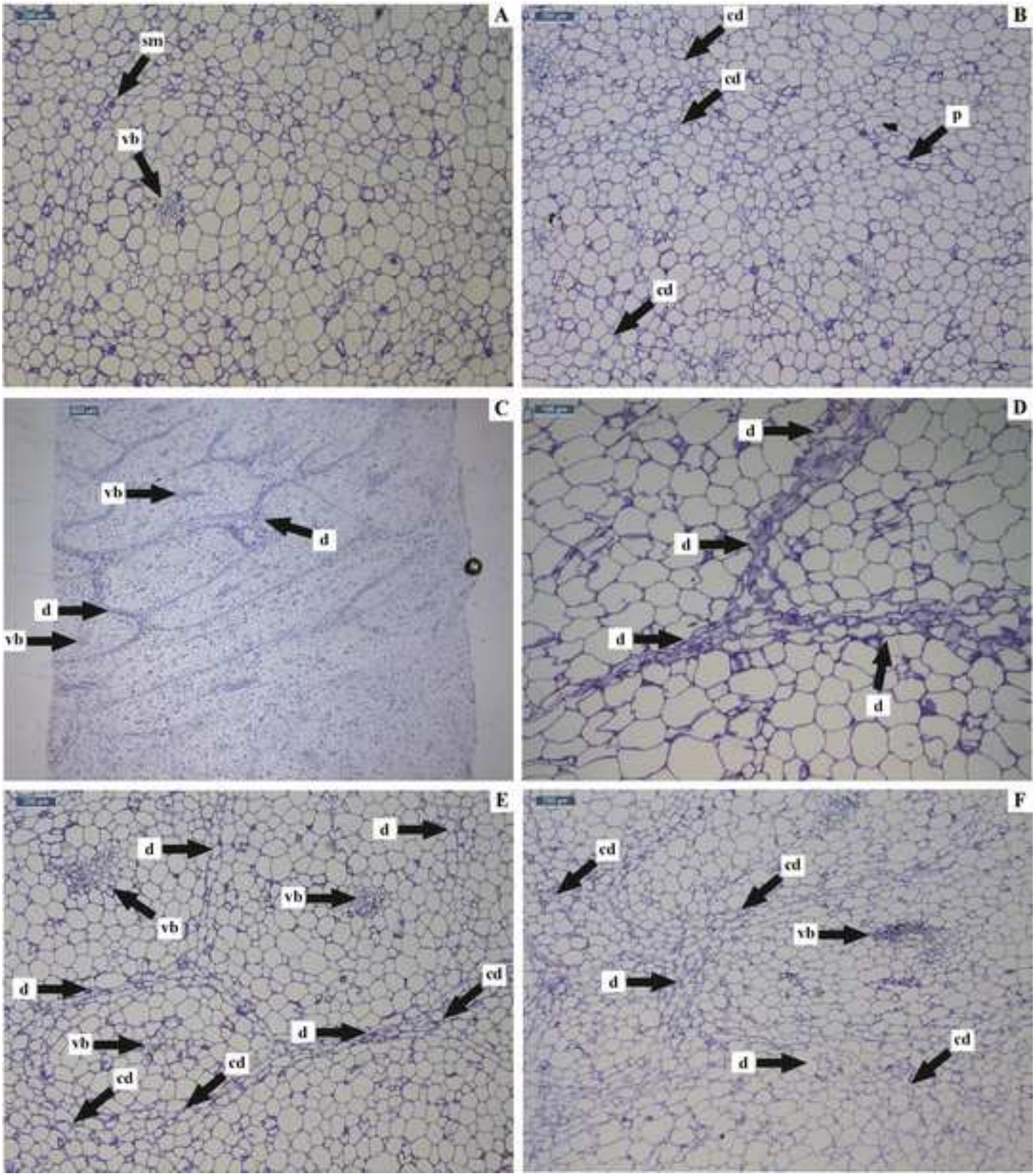

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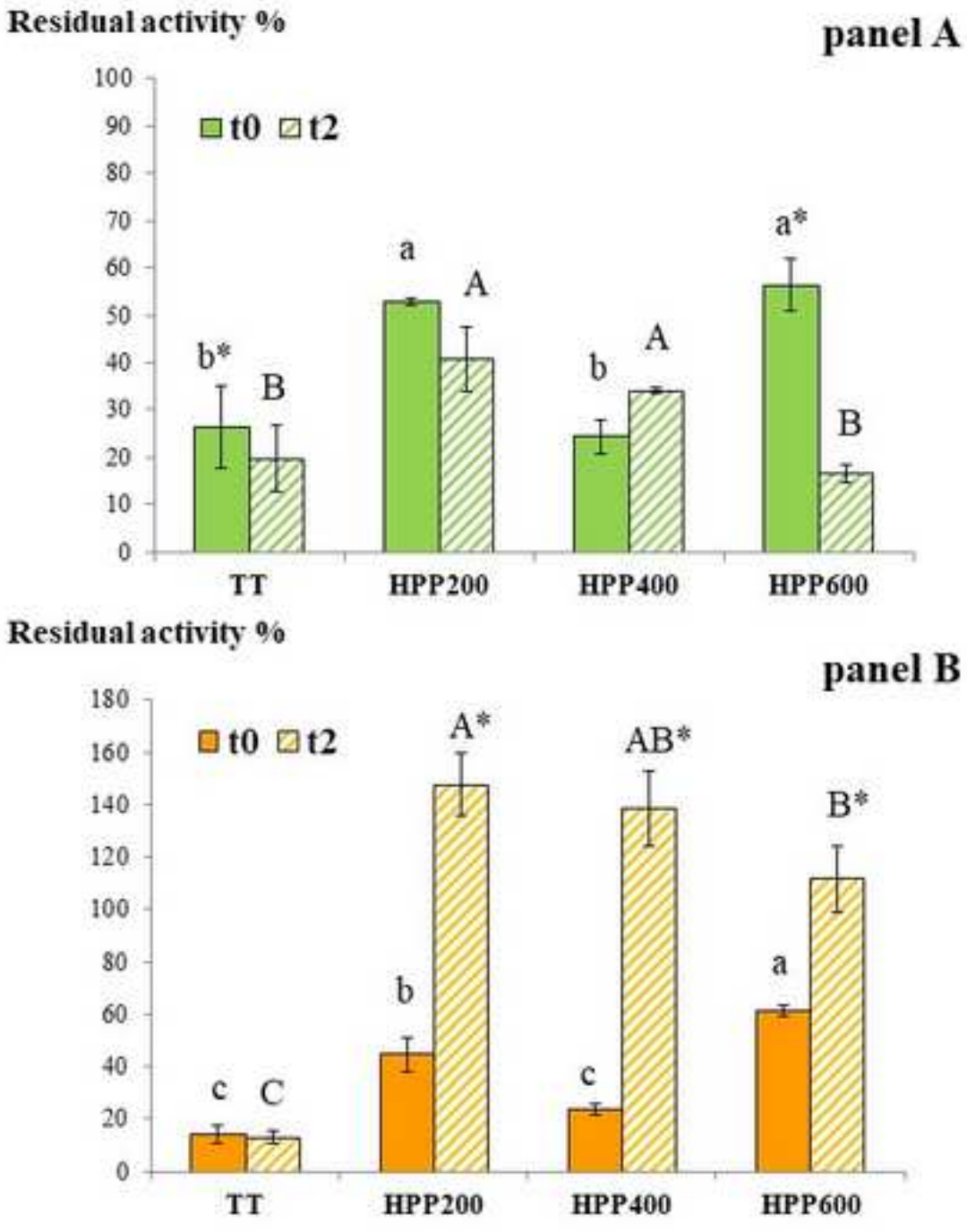

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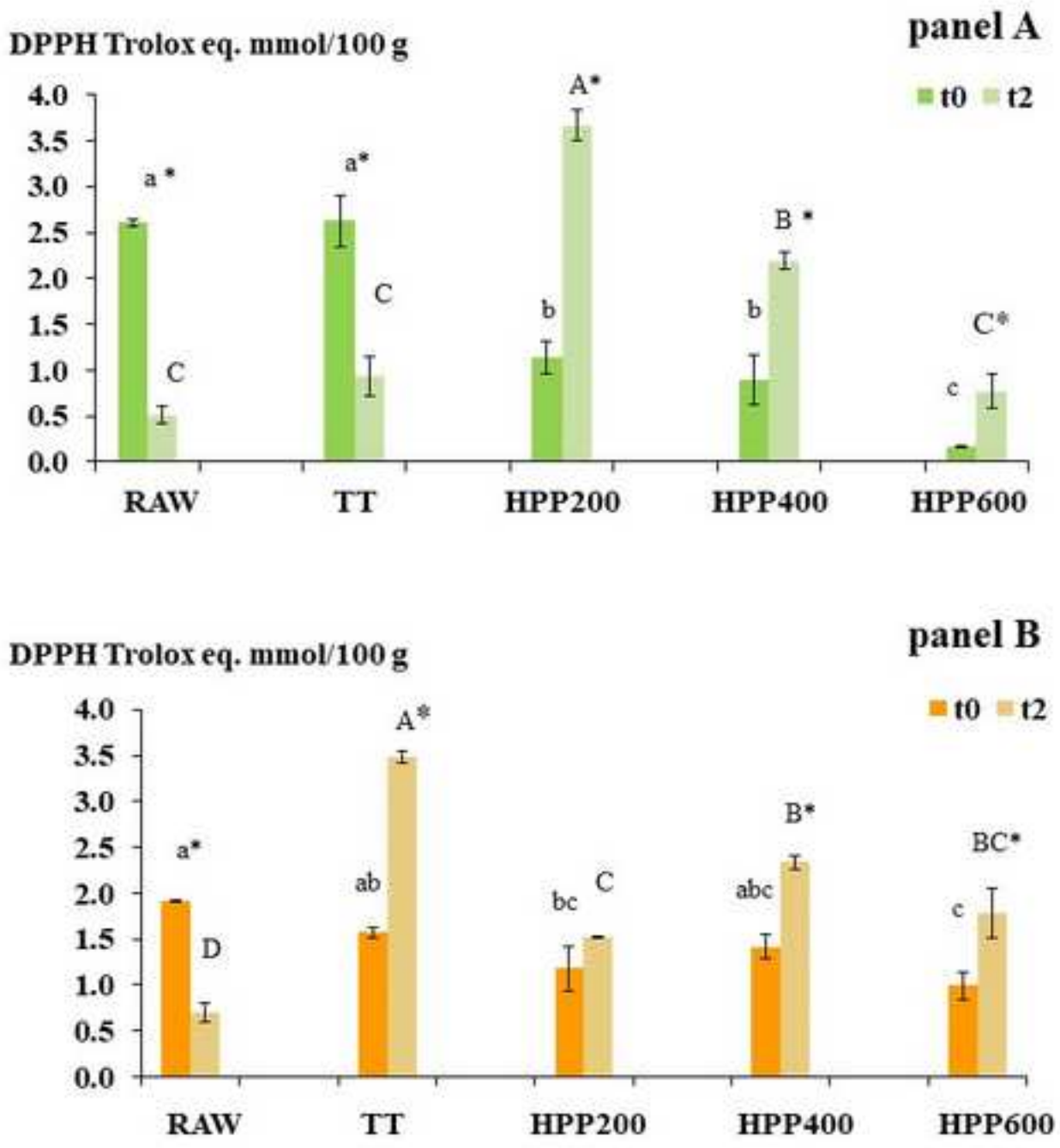

Graphical abstract

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