LWT - Food Science and Technology 149 (2021) 111858

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

# Effect of high-pressure processing to improve the safety and quality of an *Quercus* acorn beverage

Rita Sardão [a,b], Renata A. Amaral [a], Elisabete M.C. Alexandre [a,b,**], Jorge A. Saraiva [a], Manuela Pintado [b,*]

[a] LAQV-REQUIMTE, Chemistry Department, University of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal
[b] Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005, Porto, Portugal

ARTICLE INFO

*Keywords:*
High-pressure processing
*Quercus* acorn
Beverage
Microbiological safety
Nutritional quality

ABSTRACT

The development of an acorn beverage can be an opportunity to increase the intake of bioactive compounds and add value to *Quercus* acorn. However, unprocessed plant-based beverages have a very short shelf-life and thermal treatments affect their overall quality. High-pressure processing (HPP) emerges as a possible solution to better retain nutritional properties and sensorial quality and extend shelf-life. This research assessed the impact of HPP (450/600 MPa, 5/12.5/20 min) and thermal processing (TP, 85 °C, 30 min) on an acorn beverage during 63 days of refrigerated storage (4 °C). From all processing conditions, HPP at 450 MPa for 5 min showed to be suitable to assure microbiological safety (mesophiles, psychrophiles, yeasts and moulds, and Enterobacteriaceae) during 63 days and to better preserve total soluble solids and colour. The antioxidant activity (ABTS/DPPH) was similar to higher for heat-treated samples, being gallic acid the main phenolic compound found in the beverage. Total carbohydrates, lipids, proteins, minerals, and fatty acids contents were similar for HPP and TP. This beverage revealed low thrombogenicity and atherogenicity indices, and potassium as the main mineral. Concluding, HPP extended the shelf-life of the beverage and improved the physicochemical quality, revealed by panellists preference, as revealed by sensorial analysis.

## 1. Introduction

The acorn nut from *Quercus* trees grows in temperate areas like the Mediterranean, being very abundant in Europe, where, in many regions, the acorn nut is left behind in the fields without any use (Rigo & Caudullo, 2016, pp. 152–153). Unfortunately, acorns are far from being widely used in the human diet, although they have good potential as an alternative functional food. Custódio et al. (2013) indicated that extracts from *Q. ilex* and *Q. suber* acorns are endowed with biocompounds that relieve symptoms of neurological disorders because they exhibit antioxidant and inhibitory activities and have the potential to prevent oxidative stress-induced cell damage. Custódio et al. (2015) reported the antioxidant potential and inhibitory activity of *Q. suber* leaf and acorn extracts on key enzymes relevant for hyperglycaemia and neurodegenerative diseases prevention. Ahmadi et al. (2019) and Tadayoni et al. (2015) reported that acorn polysaccharides have prebiotic properties since enhance the growth of the probiotic *Lactobacillus plantarum*. These features and the absence of gluten (Vinha et al., 2016) make acorn an outstanding starch source for the development of gluten-free healthy foods.

Plant-based beverages are excellent sources of nutrients and bioactive compounds. However, many of these beverages face technical issues related to conventional thermal methods, such as pasteurization and UHT treatment. These methods can cause losses of heat-labile nutrients and antioxidant activity, colour changes, and adverse effects on sensory attributes (Fernandez et al., 2018; Xu et al., 2018). High-pressure processing (HPP) is the most successful non-thermal food preservation technology already implemented in the food industries, responding to many of the problems associated with conventional thermal processing (Awsi et al., 2017). Pressures used for pasteurizing foods (300–600 MPa) have little or no impact on the covalent bonds (Ahn et al., 2017, pp. 231–263; Woldemariam & Emire, 2019), preserving better sensory

* Corresponding author..
** Corresponding author. Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005, Porto, Portugal.
*E-mail addresses:* elisabete.alexandre@ua.pt (E.M.C. Alexandre), mpintado@porto.ucp.pt (M. Pintado).

https://doi.org/10.1016/j.lwt.2021.111858
Received 31 December 2020; Received in revised form 14 May 2021; Accepted 1 June 2021
Available online 23 June 2021

properties, nutrients, and bioactive compounds, in contrast to the usual effects of thermal treatments. Molecular compression can only affect the weaker bonds and forces (hydrogen bonds, electrostatic interactions, and van der Waals forces). This changes the microbial membrane structures (Awsi et al., 2017; Sousa et al., 2016), resulting in microbial inactivation and, consequently, products' safety and shelf-life extension. Thus, a successful application of HPP to produce plant-based beverages guarantees microbiological safety and retains quality and natural freshness. HHP also can improve the bioaccessibility and bioavailability of nutritional and antioxidant compounds and reduce the allergenic character of some plant-based beverages (Munekata et al., 2020).

One of the main goals of this work was reducing food losses, promoting more sustainable production of acorns and their valorization by producing added-value products and using the surplus from the fields. For this, an acorn beverage was produced and the potential of HPP (450/600 MPa, 5/12.5/20 min), compared to thermal treatment (TP, 85 °C for 30 min), was evaluated during storage at 4 °C for 63 days to assure microbiological stability (mesophiles, psychrophiles, yeasts and moulds, and Enterobacteriaceae), physicochemical stability (pH, total soluble solids (TSS), colour, phenolic compounds, antioxidant activity, carbohydrates, protein, lipids, total fatty acids, minerals, and 5-hydroxymethylfurfural formation), and sensorial quality.

## 2. Materials and methods

### 2.1. Acorn beverage preparation

The acorns (*Q. ilex*) from Montemor-o-Novo, Portugal, were stored at 4 °C until processing, being then peeled, washed, and then soaked in water (10 g of acorn per 100 mL) overnight at room temperature. The leaching water was then removed, and the acorns were homogenized with water (200 mL per 10 g of acorns) using a food mixer and filtered through a linen cloth.

### 2.2. Beverage processing and storage

Beverage aliquots (50 mL) were placed in low permeability polyamide-polyethylene bags, which were heat sealed. After, the bags were subjected to pressure treatments of 450 or 600 MPa for 5, 12.5, and 20 min, at room temperature, using a pilot-scale HP equipment (Model 55, Hyperbaric, Burgos, Spain) with a pressure vessel of 55 L. These treatment conditions were selected based on literature (Fernandez et al., 2018; Xu et al., 2018) and previous exploratory experiments. Pressure build-up took place at a compression rate of approximately 250 MPa/-min while the decompression occurred within 2–3 s. The inlet water temperature used as the pressurizing fluid was comprised between 15 and 20 °C, and adiabatic heating/cooling caused an increase/decrease in temperature of approximately 3–5 °C for each 100 MPa applied (Pinto et al., 2020). For thermal treatment (TP), the samples were heated in a water bath until 85 °C was achieved, remaining at this temperature (±2 °C) for 30 min (Westerik et al., 2016) and then were immediately chilled in a water/ice mixture. The TP conditions were selected based on regular milk pasteurization (Westerik et al., 2016). All samples were stored in the dark at 4 °C and for each sampling time (0, 7, 14, 21, 35, 49, and 63 days of storage), one bag was opened aseptically to perform microbiological analyses and, afterwards, pH, TSS, and colour measurements. For the quantification of the remaining parameters, samples were stored at −80 °C until the analyses were carried out.

### 2.3. Microbiological analyses

Decimal dilutions were prepared with sterile 0.1% peptone water. Total aerobic mesophiles and total aerobic psychrophiles were enumerated in Plate Count Agar incubated at 30 ± 1 °C during 72 h and 7 ± 1 °C for 5 days, respectively. Yeasts and moulds were enumerated in Rose-Bengal Agar (supplemented with chloramphenicol) incubated at 25 ± 1 °C for 72 h. Enterobacteriaceae were enumerated on Violet Red Bile Glucose Agar incubated at 37 °C for 24 h and *Staphylococcus aureus* were enumerated on mannitol incubated at 37 °C for 24–48 h. The enumerations were performed by the spread plate technique, except for Enterobacteriaceae, for which the pour plate technique was used. The results were expressed as the decimal logarithm of colony-forming units (CFU) per mL of beverage. The detection and quantification limits (LoD and LoQ) associated with the surface plating technique were 1.60 log CFU/mL and 2.60 log CFU/mL, respectively, while for the pour plate technique the limits associated with were 0 log CFU/mL and 1.18 log CFU/mL, respectively. When microbiological counts exceeded the acceptable microbial limit of 6.00 log CFU/mL, the maximum load considered in this study (Codina-Torrella et al., 2018), the beverage was deemed to be expired.

### 2.4. Physicochemical analyses

#### 2.4.1. Hydrolyzable tannins content

Hydrolyzable tannins content (HTC) was evaluated as described by Saffarzadeh-Matin and Khosrowshahi (2017), with modifications. Previously, 5 mL of sample were centrifuged (Universal 320 R; Hettich, Tuttlingen, Germany) at 2370 g for 10 min. The sample supernatant (500 μL) was afterwards mixed with 2.5 mL of $KIO_3$ (25 g/L), and after 8 min, the absorbance was measured at 550 nm on a UV-VIS spectrophotometer (1240 UV–visible spectrophotometer (Shimadzu, Kyoto, Japan). A calibration curve (0.06–2.00 mg/mL) was set up using tannic acid, and HTC was expressed as mg of tannic acid equivalents in mL of beverage. The following Equation (1) was considered to evaluate the tannins leaching rate:

$$\text{Loss of tannins by leaching}\ (\%) = \frac{\text{HTC (leaching water)}}{\text{HTC (leaching water)} + \text{HTC (acorn beverage)}} \times 100 \quad (1)$$

where HTC (leaching water) is the concentration in water removed after soaking acorns and HTC (acorn beverage) is the concentration in the acorn beverage.

#### 2.4.2. pH value and total soluble solids

The pH value was measured at room temperature (20 ± 1 °C) using a potentiometer with a Crison 52–08 electrode (Crison Instruments, Barcelona, Spain). The TSS content was measured as °Brix at room temperature (20 ± 1 °C) using a portable refractometer (Palette PR-32α, Atago Co. LTD, Tokyo, Japan). One drop was placed on the refractometer glass prism, and the TSS obtained as Brix percentage (distilled water was used as a blank).

#### 2.4.3. Colour

The colour parameters (L*, a*, and b*) were evaluated by the CIE colour system using a Konica-Minolta CR-400 chromameter equipped with a D65 illuminant and a CIE observer. The total colour change (ΔE) was calculated as shown in Equation (2):

$$\Delta E = \left[(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2\right]^{\frac{1}{2}} \quad (2)$$

where ΔE is the total colour change variation between a sample and the control (untreated acorn beverage values are identified with the subscript "0").

#### 2.4.4. Total polyphenolic content

The total polyphenolic content (TPC) was measured following the Folin-Ciocalteu method (Coscueta et al., 2018), with modifications. Firstly, 2 mL of sample were centrifuged (Centrifuge Boeco M-240; Germany) at 22780 *g* for 10 min. Then the supernatant (20 μL) was mixed with 80 μL of Folin-Ciocalteu reagent (10% v/v), followed by the

addition of 100 μL of $Na_2CO_3$ (7.5% w/v). After shaking and incubating for 1 h in the dark at room temperature, the absorbance was measured at 765 nm on a Multidetection plate reader (Synergy H1, Vermont, USA). A calibration curve of gallic acid (0.0017–0.0213 mg/mL) was established and the results were expressed as mg of gallic acid equivalents per mL of beverage.

#### 2.4.5. Identification and quantification of individual polyphenolic compounds

The polyphenol profile was evaluated by HPLC-DAD as described by Oliveira et al. (2015), with modifications. The samples were filtered with a 0.45 μm membrane and injected into the Waters Alliance e2695 (Mildford MA, USA) separation module system interfaced with a photodiode array UV–Vis detector 2998 Waters. The separation was done in a C18 reverse-phase column (COSMOSIL 5C1 8-AR-II Packed Column - 4.6 mm I.D. × 250 mm; Dartford, UK), and the oven temperature was set at 25 °C. The mobile phase was composed of solvent A: water/acetonitrile/TFA (94.8:5:0.2) and solvent B: acetonitrile/TFA (99.8:0.2) and the elution gradient used was: 0–1 min (0% B); 1–30 min (21% B); 30–42 min (27% B); 45–55 min (58% B); and 55–60 min (0% B). The flow rate was 1 mL/min, and the injection volume was 20 μL. In the chromatograms of the acorn beverage, only two peaks were identified, which correspond to gallic and ellagic acid, detected at 280 and 360 nm, respectively. The calibration curves (7.8–250.0 μg/mL) were established, and the results were expressed as mg of gallic/ellagic acid per mL of beverage.

#### 2.4.6. Antioxidant activity – ABTS and DPPH

Previously, 2 mL of sample were centrifuged (Centrifuge Boeco M-240; Germany) at 22780 *g* for 10 min to perform the following assays. ABTS scavenging assay was performed as described by Gonçalves et al. (2009) with modifications. The $ABTS^{\bullet+}$ was generated by reacting an ABTS aqueous solution (7 mM) with $K_2S_2O_8$ (2.45 mM) in the ratio 1:1 (v/v) for 16 h in the dark at room temperature. $ABTS^{\bullet+}$ solution was filtered using a 0.45 μm membrane and adjusted to an absorbance of 0.700 ± 0.020 at 734 nm with ultra-pure water. Then, 15 μL of sample supernatant were mixed with 200 μL of $ABTS^{\bullet+}$ solution, followed by incubation for 5 min in the dark at 30 °C and measurement of the absorbance at 734 nm. A calibration curve of ascorbic acid (0.0088–0.0881 mg/mL) was prepared and the results were expressed as mg of ascorbic acid equivalents per mL of beverage.

$DPPH^{\bullet}$ free radical assay was performed as described by Jing et al. (2015) with modifications. The supernatant sample (25 μL) was mixed with 175 μL of 60 μM methanolic $DPPH^{\bullet}$ (with adjustment of absorbance to 0.600 ± 0.100 at 515 nm). After 30 min in the dark at room temperature, the absorbance was recorded at 515 nm. A calibration curve of Trolox (0.0075–0.0751 mg/mL) was prepared, and the results were expressed as mg of Trolox equivalents per mL of beverage.

Incubation and absorbance measurements for both methods were performed using a Multidetection plate reader (Synergy H1, Vermont, USA).

#### 2.4.7. Total content of carbohydrates, protein, and lipids

Total carbohydrate content was determined as described by Dubois et al. (1956), with modifications. The sample (2 mL) was mixed with 1 mL phenol solution (5% w/v) and 5 mL of $H_2SO_4$ (95%). The mixture was then stirred and left to cool to room temperature for about 10 min before the absorbance was measured at 490 nm. Quantification was performed using a calibration curve built with glucose (20–100 mg/mL), and the results were expressed in g of glucose per 100 mL of beverage.

Total protein was determined through the Kjeldahl method, following the Association of Official Analytical Chemists methods (AOAC, 2012), using 6.25 as a converting factor and expressed in g per 100 mL of beverage.

Total lipid content was determined by the gravimetric Soxhlet method using *n*-hexane as a solvent. Soxhlet extractions were performed using 50 mL of acorn beverage previously lyophilized. The samples were frozen at −80 °C and then desiccated under vacuum ($6.7 \times 10^{-2}$ mbar) for 7 days in a freeze-drier (Martin Christ, Osterode am Harz, Germany) at room temperature, and the condenser was cooled at −55 °C. The total lipid content was expressed in g per 100 mL of beverage.

#### 2.4.8. Identification and quantification of total fatty acids

Fatty acids (FA) analysis was performed as described by Pimentel et al. (2015). Briefly, 200 μL of tritridecanoin was added to 500 mg of lyophilized sample (as described in section 2.4.7.). Then, 2.26 mL of methanol, 800 μL of hexane, and 240 μL of sodium methoxide (5.4 M) were added. Samples were vortexed and incubated at 80 °C for 10 min. After cooling in ice, 1.25 mL of N, *N*-dimethylformamide and 1.25 mL of sulphuric acid/methanol (3 M) were added. Samples were vortexed and incubated at 60 °C for 30 min. Finally, after cooling, they were vortexed and centrifuged (1250 *g*, 5 min). The upper layer containing FA methyl esters (FAME) was collected and the samples were analyzed in a gas chromatograph HP6890A (Hewlett-Packard, Avondale, PA, USA), equipped with a flame ionization detector and a BPX60 capillary column (60 m × 0.25 mm x 0.25 μm; SGE Europe Ltd, Courtaboeuf, France).

Two nutritional quality indices, atherogenicity (AI) and thrombogenicity (TI), of acorn beverage were analyzed, being calculated as proposed by Ulbricht and Southgate (1991), using **Equations 3 and 4**, respectively:

$$AI = \frac{[C12:0 + 4 \times C14:0 + C16:0]}{[\sum MUFA + \sum PUFA]} \quad (3)$$

$$TI = \frac{[C14:0 + C16:0 + C18:0]}{\left[0.5 \times (\sum MUFA + \sum \omega 6) + 3 \times \sum \omega 3 + \frac{\sum \omega 3}{\sum \omega 6}\right]} \quad (4)$$

where MUFA is the monounsaturated fatty acids, PUFA is the polyunsaturated fatty acids, and ω6 and ω3 are omega-6 and omega-3 FA, respectively.

Other indicators like the ratios ω6/ω3, MUFA/PUFA, and the PUFA/saturated fatty acids (SFA) were also calculated.

#### 2.4.9. Identification and quantification of individual minerals

Minerals analysis was performed as described by Santos et al. (2015) with modifications. Briefly, 200 mL of each sample were mixed with 5 mL of $HNO_3$ (65%) and 2 mL of $H_2O_2$ (30% v/v) in a Teflon reaction vessel and heated in a Speedwave™ MWS–3 + (Berghof, Germany) microwave system. The digestion procedure was conducted in five steps: (i) 160 °C/5 min, (ii) 190 °C/10 min, (iii) 190 °C/10 min, (iv) 100 °C/2 min, and (v) 100 °C/2 min. The resulting clear solutions were then brought to 50 mL with ultrapure water for further analysis using an ICP-OES OptimaTM 7000 DV (PerkinElmer, USA) with a radial configuration. The calibration curves (K: 0–120 ppm; P: 0–50 ppm; Mg: 0–30 ppm; Mn: 0–0.5 ppm; Cu: 0–1.5 ppm; Fe: 0–3 ppm) were established, and the results were expressed in mg per 100 mL of beverage.

#### 2.4.10. Quantification of 5-hydroxymethylfurfural

The 5-hydroxymethylfurfural (5-HMF) was measured at Laboratório de Análises e Ensaios an Alimentos e Embalagens (CINATE) from Escola Superior de Biotecnologia – Universidade Católica Portuguesa (ESB-UCP). The samples were filtered with a 0.45 μm membrane and injected directly into the Beckman Coulter HLPC (California, United States), equipped with a Beckman UV detector (model 168) and a C18 Hypersil ODS column (250 mm × 4.6 mm, 5 μm particle size), with detection at 284 nm. The analysis was performed by the standard external method and the mobile phase used was $KH_2PO_4$ (0.01 mol/L)/methanol (85:15 v/v).

### 2.5. Sensory analysis

The sensory analysis was based on the work performed by Monteiro et al. (2016). Sessions took place at ISO 8589:2007 compliant sensory testing facilities of ESB-UCP with sixty non-experienced participants. The sensorial descriptors evaluated included overall appreciation, appearance, flavour, and mouthfeel. Scoring scales were "Dislike extremely" (1 point), "Dislike very much" (2 points), "Dislike moderately" (3 points), "Dislike slightly" (4 points), "Neither like nor dislike" (5 points), "Like slightly" (6 points), "Like moderately" (7 points), "Like very much" (8 points), and "Like extremely" (9 points). Afterwards, the participants were asked about their preference between the two samples, indicating how much they like one sample over the other. In this issue, "Much worse" meant - 2 points, "Similar" meant 0 points, and "Much better" meant +2 points.

### 2.6. Statistical analysis

All the analyses were performed in triplicate from duplicated samples. Statistical analysis was performed with IBM SPSS® statistics for windows (2012) version 21.0. When normal distribution was observed, the One-Way Analysis of Variance (ANOVA) test was employed in association with Tukey's post hoc test to analyze statistical differences. The differences were considered statistically significant at a 5% confidence degree level ($p < 0.05$). The results were expressed as mean ± standard deviation (SD).

## 3. Results and discussion

### 3.1. Microbiological analyses

Table 1 shows the total counts of aerobic mesophiles, psychrophiles, yeasts and moulds, Enterobacteriaceae, and *Staphylococcus aureus* for untreated acorn beverage, throughout storage time (63 days) at 4 °C. Immediately after HPP or thermal processing, all microbial counts were lower than the detection limit, which was also verified during subsequent storage of the beverage until 63 days (data not shown). So, both processed beverages were safe for this storage period with no significant differences in the selected microbial groups. Furthermore, *Staphylococcus aureus* was not detected in any sample tested, including the control samples (untreated).

Concerning the untreated beverage, on day 7, the Enterobacteriaceae counts were above 6.00 log CFU/mL, the maximum load established, thus limiting the beverage shelf-life. The mesophiles, psychrophiles, and yeasts and moulds showed values between 4 and 5 log CFU/mL. As far as we are aware, this is the first work covering the shelf-life of an acorn beverage, but similar results were reported by Corrales et al. (2012) using a raw tiger nuts beverage, with these authors concluding that the shelf-life of this beverage was generally not higher than 2 or 3 days when stored at 4 °C.

HPP (450/600 MPa, 5/12.5/20 min) and TP (85 °C, 30 min) had a significant effect ($p < 0.05$) on the microbial counts, since the initial counts of mesophiles, psychrophiles, yeasts and moulds, and Enterobacteriaceae were reduced from 4.70 ± 0.09, 4.46 ± 0.08, 5.37 ± 0.10, and 5.58 ± 0.30 log CFU/mL, respectively, to undetectable levels after all treatments and over 63 days. Thus, from the tested conditions, the HPP at 450 MPa for 5 min showed to be enough to inactivate several microorganisms, improving the shelf-life of the beverage from less than a week to at least 63 days, with no differences being observed between HPP and TP. Similar results were obtained by Xu et al. (2018), when a clear Se-enriched kiwifruit juice was processed by HPP at 500 MPa for 10 min and by high-temperature short time (HTST) at 110 °C for 8.6 s. However, some studies have recognized HPP as more effective in delaying/slowing down microbial growth when compared to thermal treatment. For instance, Picouet et al. (2014) reported that in processed carrot juice (600 MPa, 5 min) after 29 days of storage at 5 °C, the total mesophiles and yeasts and moulds counts were, respectively, 3.0 ± 0.4 and 0.8 ± 1.3 for HPP samples and 5.7 ± 0.7 and 3.9 ± 0.6 for heated samples (80 °C, 7 min). Our study used a more intense heat treatment (85 °C, 30 min), and no differences were found between the two processing methods.

### 3.2. Physicochemical analyses

#### 3.2.1. Hydrolyzable tannins content

*Quercus ilex* acorns are rich in hydrolysable tannins (Cantos et al., 2003), which cause the sensation of astringency. When these phenolic compounds are consumed in excess, they can be associated with digestibility problems and reduced nutritional value of foods (Chung et al., 1998). Although *Q. ilex* acorns are known to be sweeter than other acorn varieties (Costas, 2013), a leaching step was performed to reduce these effects. The concentration of hydrolyzable tannins in the acorn beverage and in the leaching water was 0.64 ± 0.04 and 0.47 ± 0.04 mg of tannic acid equivalents per mL, respectively and thus, the level of tannin elimination by leaching was 43%. On the other hand, there is no interest in completely eliminating the tannins present in the acorn beverage because they also have health benefits, like antimutagenic, anticarcinogenic and antimicrobial properties (Chung et al., 1998).

#### 3.2.2. pH value and total soluble solids

Fig. 1 shows the pH and TSS values of acorn beverages. The initial pH (day 0) of acorn beverage was 5.09 ± 0.07, 5.89 ± 0.01, and 6.20 ± 0.04 for untreated, heat-treated, and HPP-treated samples, respectively. The acorn beverage processing significantly increased the pH values ($p < 0.05$), with the HPP-treated samples showing significantly higher values (6.20 ± 0.04) than the heat-treated samples (5.89 ± 0.01) and similar values between different HPP conditions (Fig. 1-A). This effect of HPP on pH increase is generally not supported by other studies for different fruits and/or vegetable-based beverages, where pH value is usually unaffected by HPP (Tsai et al., 2018; Wang et al., 2018). The difference between untreated and treated samples continued over the storage time, although the pH value of all treated samples decreased significantly ($p < 0.05$) with time. Nevertheless, from day 35 of storage until the end of storage, no significant differences ($p > 0.05$) were observed between TP and HPP-treated samples.

The TSS content (Fig. 1-B) was significantly higher ($p < 0.05$) for

**Table 1**
Evolution of total aerobic mesophiles, total aerobic psychrophiles, yeasts and moulds, Enterobacteriaceae, and *Staphylococcus aureus* counts during 63 days of storage at 4 °C of untreated (0.1 MPa) acorn beverage (for HPP and thermal processing, microbial counts were always below the detection limit). Results are expressed as the mean ± SD (n = 2).

| Storage time (days) | | 0 | 7 | 14 | 21 | 35 | 49 | 63 |
|---|---|---|---|---|---|---|---|---|
| LogCFU/mL ± SD | Mesophiles | 4.70 ± 0.09$^{c}$ | 4.12 ± 0.16$^{a}$ | 4.34 ± 0.16 $^{ab}$ | 4.87 ± 0.24$^{c}$ | 4.76 ± 0.06$^{c}$ | 4.60 ± 0.14 $^{bc}$ | 4.68 ± 0.04$^{c}$ |
| | Psychrophiles | 4.46 ± 0.08 $^{ab}$ | 4.96 ± 0.24$^{c}$ | 4.38 ± 0.15$^{a}$ | 4.64 ± 0.32 $^{abc}$ | 4.80 ± 0.12 $^{bc}$ | 4.31 ± 0.08$^{a}$ | 4.43 ± 0.08 $^{ab}$ |
| | Yeasts and Moulds | 5.37 ± 0.10$^{d}$ | 4.69 ± 0.02 $^{ab}$ | 4.52 ± 0.13$^{a}$ | 5.15 ± 0.15 $^{cd}$ | 4.89 ± 0.07 $^{bc}$ | 4.93 ± 0.22 $^{bc}$ | 5.11 ± 0.04 $^{cd}$ |
| | Enterobacteriaceae | 5.58 ± 0.30$^{a}$ | * | * | * | * | * | * |
| | Staphylococcus aureus | < LoD | < LoD | < LoD | < LoD | < LoD | < LoD | < LoD |

Results only for untreated beverage. LoD (Limit of detection). * Microbiological counts exceed the satisfactory microbial limit for their acceptance (6.00 Log CFU/mL). Different lowercase letters (abcd) in the same row indicate significant statistical differences ($p < 0.05$) between storage times. The day 0 refers to the day of processing.

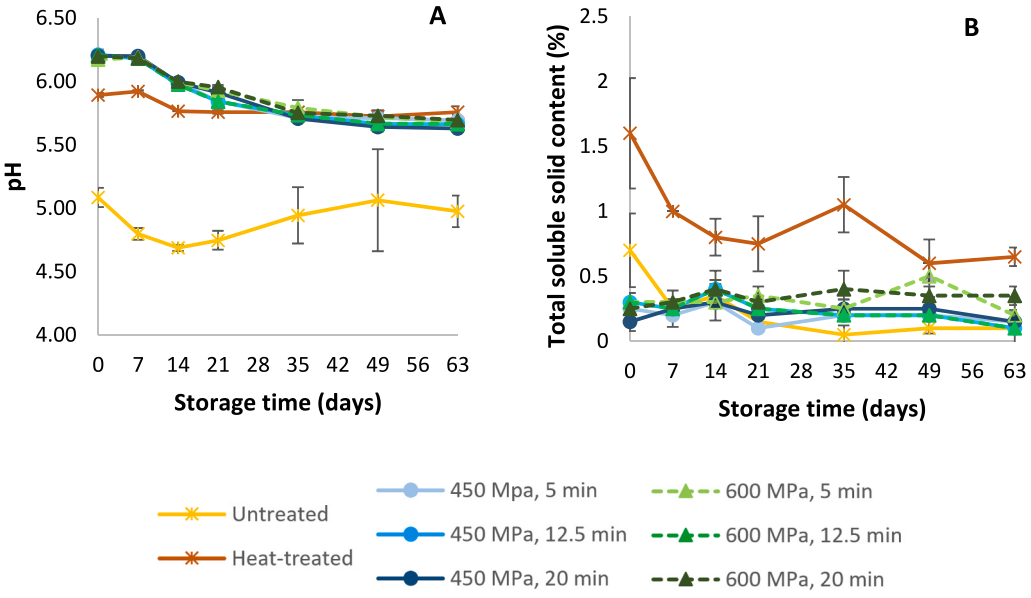


**Fig. 1.** Evolution of pH value (A) and total soluble solid content (B) during 63 days of storage at 4 °C in untreated (0.1 MPa), heat-treated (85 °C, 30 min), and HPP-treated (450/600 MPa, 5/12.5/20 min) acorn beverage samples. Results are expressed as the average ± SD (n = 2).

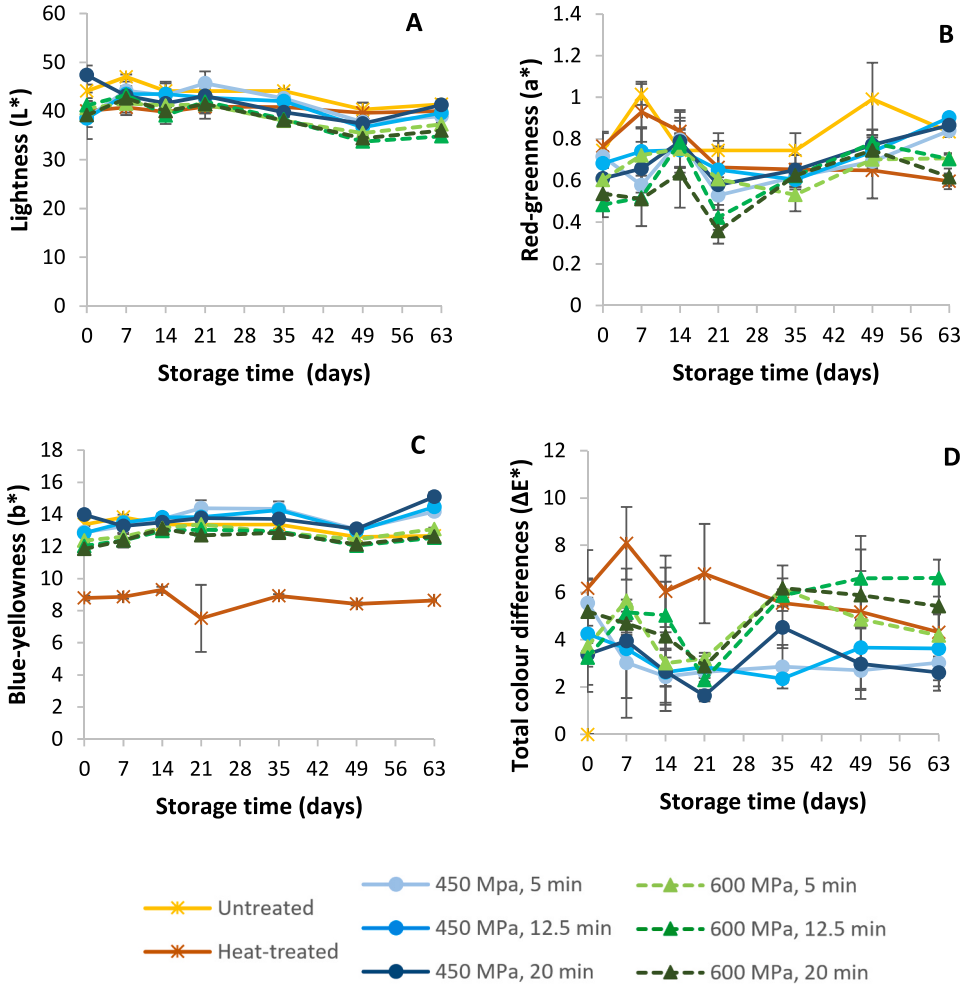


**Fig. 2.** Evolution of L* (A), a* (B), b* (C), and ΔE (D) during 63 days of storage at 4 °C in untreated (0.1 MPa), heat-treated (85 °C, 30 min), and HPP-treated (450/600 MPa, 5/12.5/20 min) acorn beverage samples. Results are expressed as the average ± SD (n = 2).

heat-treated samples (1.60 ± 0.42) compared to untreated and HPP-treated samples, probably due to the partial conversion of starch to soluble sugars (Wei et al., 2017). HPP did not influence the TSS content since no significant differences ($p > 0.05$) were observed between untreated (0.70 ± 0.28) and HPP-treated samples (0.15–0.30 ± 0.07). These results agree with those of a study reported by Huang et al. (2015) that used HPP (600 MPa/6 min) and thermal pasteurization to process sugarcane juice. Furthermore, our results also are alike the values reported by Jayachandran et al. (2015), who studied the effect of HPP (500 MPa, 20 min and 600 MPa, 15 min) on a litchi based mixed fruit beverage. However, in the previous work, untreated samples' TSS faced a significant decrease ($p < 0.05$) with the increase of storage time, which can be explained by microbiological fermentation of sugars (Rivas et al., 2006). Although the acorn has a low soluble sugar content since they are mostly in starch form (Costas, 2013).

### 3.2.3. Colour

The evolution of the colour parameters (L*, a*, b*, and ΔE) is presented in Fig. 2. Globally, the lightness (L*, Fig. 2-A) of acorn beverage was not significantly affected by the treatment ($p > 0.05$), but for some conditions (450 MPa, 20 min; 600 MPa, 12.5 and 20 min) decreased significantly ($p < 0.05$) from first (day 0) to the last day of storage (day 63). Regarding the red-greenness parameter (a*, Fig. 2-B), no significant changes were observed ($p > 0.05$) with the treatments or during the storage time, except at day 49, between untreated (1.0 ± 0.17) and processed samples (0.8 ± 0.06, the highest value in these samples). The blue-yellowness parameter (b*, Fig. 2-C) was the most affected parameter by the treatment, with heat-treated samples showing significantly lower values (between 8.4 ± 0.10 and 9.3 ± 0.20) than all the other samples ($p < 0.05$). a* and b* colour parameters had positive values, indicating that red and yellow were the primary contributions to the colour of that beverage.

Concerning the total colour variation (ΔE, Fig. 2-D), heat and HPP-treated samples were not significantly different ($p > 0.05$), except on day 21, where the heat-treated samples showed higher values (6.8 ± 2.1) than the HPP-treated samples (3.2 ± 0.2, the highest value in these samples), indicating that high pressure preserves better the beverage colour. In general, the colour differences were perceptible ($\Delta E > 2.0$) (Krapfenbauer et al., 2006) between the untreated and treated samples. The highest value of ΔE (8.1 ± 1.5) was obtained for the heat-treated beverage at day 7, while the samples treated at 450 MPa presented the lowest ΔE values. Although few samples showed significant differences ($p < 0.05$) in some colour parameters over the storage time, in general, no visible changes were observed for samples at the beginning and end of the of the storage time.

In a study with a Se-enriched kiwi fruit beverages, Xu et al. (2018) showed that heat-treated samples had a significant increase in ΔE value ($p < 0.05$), and all values were higher than 2.0 during the 42 days of storage. In its turn, HPP-treated samples showed no significant differences ($p > 0.05$) in ΔE value until the late storage stage, indicating that HPP had no noticeable effects on the overall colour quality in comparison to the thermal treatment. Similar outputs were reported with other vegetable beverages, like cucumber beverage (Liu et al., 2016).

### 3.2.4. Total polyphenolic content

Fig. 3 shows the TPC values obtained for acorn beverages, with the results showing that, immediately after HPP and TP, no significant effects ($p > 0.05$) were observed for TPC preservation. The highest TPC values were 0.0754 ± 0.0017, 0.0555 ± 0.0023, and 0.0629 ± 0.0045 mg of gallic acid equivalents per mL of the untreated, heat-treated, and HPP-treated acorn beverage samples, respectively. Throughout storage time, TPC values of heat-treated samples remained similar ($p > 0.05$). Differently, values for untreated and HPP-treated samples increased significantly ($p < 0.05$) from the beginning (day 0) to the end of the storage time (day 63). On day 63, the lowest TPC value was observed in heat-treated samples (0.0516 ± 0.0017), which is in accordance with the literature, where it has been reported that conventional heat-treatments result in lower TPC values. For instance, Ali et al. (2019) showed that TPC values of wheatgrass beverage were significantly decreased ($p < 0.05$) by 36.0% and 7.5% after thermal (75 °C, 15 s) and HHP (500 MPa, 60 s) treatments, respectively. Regarding the effect of storage time, some studies have shown that a longer storage time significantly promoted the degradation of phenolic compounds compared to the fresh product (Marszałek et al., 2019; Xu et al., 2018). Vieira et al. (2018) reported no significant changes ($p > 0.05$) between the TPC of fresh and processed orange juices (550 MPa, 70 s and 70 °C, 30 s) on the day of treatment. However, during storage, these authors observed that TPC of both processed orange juices decreased, being that behaviour more significant in heat-treated juices, for which a decrease of about 25% ($p < 0.05$) was observed after 36 days.

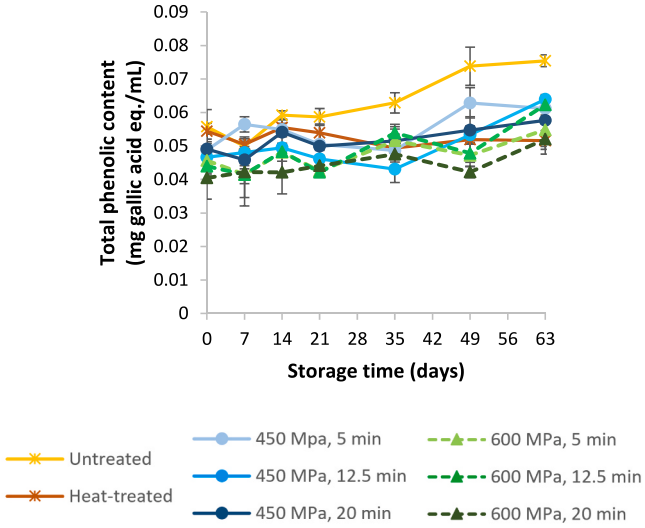


**Fig. 3.** Evolution of total phenolic content during 63 days of storage at 4 °C in untreated (0.1 MPa), heat-treated (85 °C, 30 min), and HPP-treated (450/600 MPa, 5/12.5/20 min) acorn beverage samples. Results are expressed as the average ± SD (n = 2).

### 3.2.5. Identification and quantification of individual polyphenolic compounds

Only gallic and ellagic acids were identified in the acorn beverage. At day 0, the gallic concentrations were 0.028 ± 0.004, 0.074 ± 0.014, and 0.064 ± 0.03 mg per mL for untreated, heat-treated, and HPP-treated samples (450 MPa, 5 min), respectively, while ellagic acid was detected only in trace amounts. With the heat treatment, the hydrolyzable tannins can hydrolyze to gallic acid, which is why heat-treated samples may have significantly higher concentrations (Coelho et al., 2018). For HPP-treated samples, the significantly higher concentrations of gallic acid compared with the untreated samples can be attributed to improved extraction due to tissue disruption by HPP (Patras et al., 2009).

### 3.2.6. Antioxidant activity – ABTS and DPPH

Fig. 4 summarizes the ABTS and DPPH results obtained for the acorn beverages. For the ABTS assay (Fig. 4-A), no significant differences ($p > 0.05$) were verified between the samples for day 0. On day 21, the antioxidant activity was significantly higher ($p < 0.05$) for untreated samples when compared with processed ones. However, for all the samples, differences between the beginning (day 0) and the end of the storage time (day 63) were not significant ($p > 0.05$). Trigo et al, (2020) obtained comparable results with the ABTS method for processed carrot juice fortified with a pomegranate extract obtained by high pressure (400 MPa for 30 min) after 28 days of storage.

When the DPPH method was used (Fig. 4-B), the heat-treated

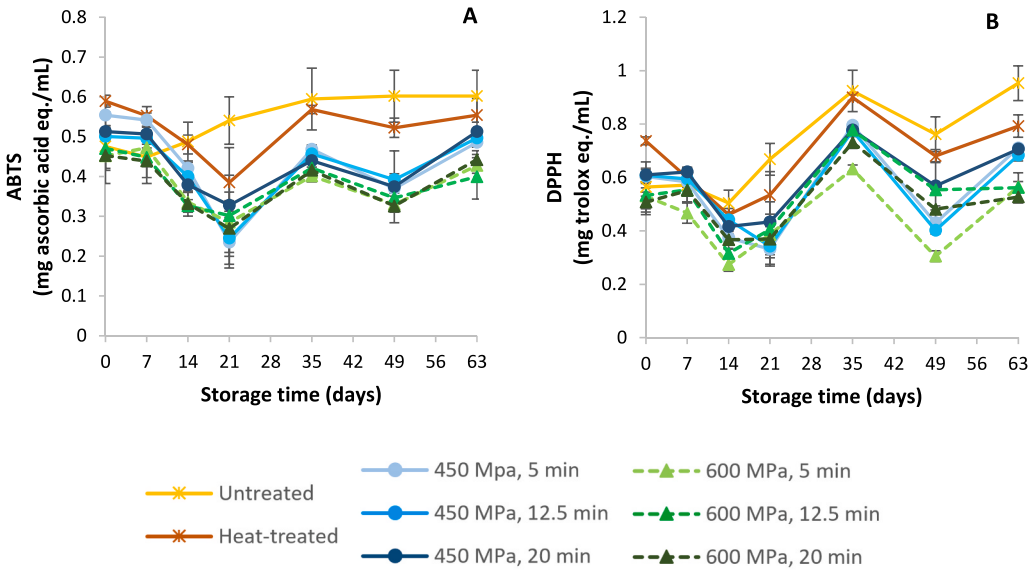


**Fig. 4.** Evolution of ABTS (A) and DPPH (B) values during 63 days of storage at 4 °C in untreated (0.1 MPa), heat-treated (85 °C, 30 min), and HPP-treated (450/600 MPa, 5/12.5/20 min) acorn beverage samples. Results are expressed as the average ± SD (n = 2).

samples presented significantly higher values ($p < 0.05$) on day 0, while untreated samples showed significantly higher values on 21 and 63 days of storage ($p < 0.05$) compared to the processed samples.

Although non-significant differences were observed, the antioxidant activity values obtained by both methods were generally higher for heat-treated samples than for HPP-treated samples, which may be due to the partial thermal hydrolysis of hydrolyzable tannins present in the acorn (Coelho et al., 2018) or due to the Maillard reactions (Manzocco et al., 2000). For HPP-treated samples, the results were similar between different pressurizations and processing times.

In the literature, several studies reported that HPP treatment produces better retention of antioxidant activity in fruits and juices than thermal treatment (Ali et al., 2019; Zhang et al., 2016). Ali et al. (2019) reported that antioxidant activity by DPPH assay of a wheatgrass beverage decreased significantly ($p < 0.05$) after thermal (75 °C, 15 s) and HPP (500 MPa, 60 s) treatments by 13.2% and 6.6%, respectively. However, thermal treatment can promote the partial thermal hydrolysis of hydrolyzable of tannins, with this increasing the antioxidant capacity. This hypothesis is supported by literature results, as for instance those of Terán-Hilares et al. (2017), who reported increased antioxidant capacity of tara (*Caesalpinia spinosa*) gallotannins due to thermal hydrolysis at 121 °C for 15 min. Regarding the impact of storage time, Changa et al. (2019) reported that the antioxidant capacity of pressurized white grape juice (300/600 MPa, 3 min) decreased throughout the storage time. However, the extent of these changes was significantly lower ($p < 0.05$) than that in heat-treated juice (90 °C, 60 s), indicating that HPP treatment can better retain the quality of grape juice.

### 3.2.7. Total content of carbohydrates, protein, and lipids

Table 2 results show that carbohydrates, proteins, and lipids, expressed in terms of total content after the thermal and HPP treatments had no significant variation ($p > 0.05$).

### 3.2.8. Identification and quantification of total fatty acids

Twenty-eight FA were identified in the untreated and HPP-treated (450 MPa, 5 min) samples. Table 3 shows the profile of the most relevant FA for pressurized and untreated samples stored during different times (0, 21 and 63 days). The most abundant FA found in acorn beverage were oleic (~62%) and linoleic (~16%) acids. In addition, a high amount of palmitic acid was found (~14%), whereas stearic acid was found in low amounts (~3%). The average amounts of these main FA in acorn beverage were very similar to those found in the literature (León-Camacho et al., 2004). Besides, additional 24 FA were detected in acorn beverage, but in amounts below 1% or in trace amounts (data not shown). In general, the lipid fraction of the acorn beverage showed high values of MUFA (63.52%) and PUFA (17.71%), which have been reported to reduce cholesterol levels.

The nutritional quality indices are also presented in Table 3, where can be seen that the lipid fraction of the untreated acorn beverage showed low AI and TI values, 0.17 ± 0.02 and 0.38 ± 0.05, respectively, indicated by Ulbricht and Southgate (1991) as desirable values. The PUFA/SFA ratio was 0.95 ± 0.02 (above 0.45), which follows general

**Table 2**
Total content of carbohydrates, protein, and lipids of untreated (0.1 MPa), heat-treated (85 °C, 30 min), and HPP-treated (450/600 MPa, 5/12.5/20 min) acorn beverage samples. Results are expressed as the mean ± SD (n = 2).

| | | Total content of | | |
|---|---|---|---|---|
| | | Carbohydrates (g of glucose/100 mL) | Protein (g/100 mL) | Lipids (g/100 mL) |
| Untreated (0.1 MPa) | | 3.42 ± 0.16 $^{ab}$ | 0.49 ± 0.16$^{a}$ | 0.37 ± 0.02 $^{ab}$ |
| Heat-treated (85 °C, 30 min) | | 2.45 ± 0.59$^{a}$ | 0.41 ± 0.12$^{a}$ | 0.26 ± 0.04$^{a}$ |
| HPP-treated | 450 MPa, 5 min | 3.76 ± 0.19$^{b}$ | n.d. | 0.40 ± 0.07$^{b}$ |
| | 450 MPa, 12.5 min | 3.87 ± 0.45$^{b}$ | n.d. | 0.39 ± 0.01 $^{ab}$ |
| | 450 MPa, 20 min | 3.29 ± 0.30 $^{ab}$ | 0.41 ± 0.11$^{a}$ | 0.41 ± 0.01$^{b}$ |
| | 600 MPa, 5 min | 3.15 ± 0.34 $^{ab}$ | n.d. | 0.41 ± 0.09$^{b}$ |
| | 600 MPa, 12.5 min | 3.05 ± 0.48 $^{ab}$ | n.d. | 0.44 ± 0.05$^{b}$ |
| | 600 MPa, 20 min | 3.39 ± 0.10 $^{ab}$ | 0.37 ± 0.06$^{a}$ | 0.39 ± 0.03 $^{ab}$ |

n.d. (not determined). Different lowercase letters (ab) in the same column indicate significant statistical differences ($p < 0.05$) between treatments.

**Table 3**
Fatty acids profile along storage time (day 0, 21, and 63) of untreated (0.1 MPa) and HPP-treated (450 MPa/5 min) acorn beverage samples. Results are expressed as the average ± SD (n = 2).

| | | Untreated (0.1 MPa) | | | HPP-treated (450 MPa, 5 min) | | |
|---|---|---|---|---|---|---|---|
| Storage time (days) | | 0 | 21 | 63 | 0 | 21 | 63 |
| **Fatty acids (% in total FA)** | Palmitic (C16) | 14.23 ± 0.20 | 14.33 ± 0.22 | 14.19 ± 0.06 | 14.06 ± 0.08 | 14.23 ± 0.01 | 14.13 ± 0.14 |
| | Stearic (C18) | 3.25 ± 0.01 | 3.27 ± 0.01 | 3.27 ± 0.01 | 3.23 ± 0.02 | 3.28 ± 0.02 | 3.25 ± 0.03 |
| | Oleic (C18:1 c9) | 62.30 ± 0.20 | 62.20 ± 0.25 | 62.48 ± 0.04 | 62.43 ± 0.16 | 62.51 ± 0.02 | 62.63 ± 0.19 |
| | Linoleic (C18:2 c9c12) | 16.35 ± 0.06 | 16.16 ± 0.10 | 16.15 ± 0.02 | 16.30 ± 0.04 | 16.27 ± 0.02 | 16.30 ± 0.08 |
| | Gamma-linolenic, GLA (ɣ C18:3 c6c9c13) | 0.04 ± 0.01 | 0.04 ± 0.00 | 0.05 ± 0.01 | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.03 ± 0.01 |
| | Alpha-linolenic, ALA (α C18:3 c9c12c15) | 0.64 ± 0.02 | 0.62 ± 0.01 | 0.62 ± 0.00 | 0.63 ± 0.00 | 0.62 ± 0.00 | 0.62 ± 0.01 |
| | SFA | 18.61 ± 0.24 | 18.77 ± 0.24 | 18.60 ± 0.03 | 18.42 ± 0.14 | 18.62 ± 0.03 | 18.48 ± 0.19 |
| | MUFA | 63.52 ± 0.18 | 63.41 ± 0.26 | 63.64 ± 0.02 | 63.61 ± 0.11 | 63.64 ± 0.02 | 63.75 ± 0.14 |
| | PUFA | 17.71 ± 0.10 | 17.56 ± 0.03 | 17.51 ± 0.01 | 17.79 ± 0.15 | 17.61 ± 0.02 | 17.64 ± 0.08 |
| | Trans FA | 0.64 ± 0.03 | 0.70 ± 0.08 | 0.65 ± 0.00 | 0.78 ± 0.19 | 0.64 ± 0.00 | 0.64 ± 0.00 |
| | ω3 PUFA | 0.64 ± 0.02 | 0.62 ± 0.01 | 0.62 ± 0.00 | 0.63 ± 0.00 | 0.62 ± 0.00 | 0.62 ± 0.01 |
| | ω6 PUFA | 16.39 ± 0.05 | 16.20 ± 0.10 | 16.21 ± 0.01 | 16.33 ± 0.04 | 16.31 ± 0.02 | 16.34 ± 0.07 |
| **Quality parameter** | AI | 0.17 ± 0.02 | 0.17 ± 0.02 | 0.19 ± 0.01 | 0.18 ± 0.00 | 0.18 ± 0.00 | 0.18 ± 0.00 |
| | TI | 0.38 ± 0.05 | 0.38 ± 0.05 | 0.44 ± 0.03 | 0.42 ± 0.00 | 0.45 ± 0.04 | 0.42 ± 0.01 |
| | ω6/ω3 | 25.76 ± 0.67 | 26.20 ± 0.09 | 26.13 ± 0.11 | 25.85 ± 0.11 | 26.17 ± 0.04 | 26.19 ± 0.10 |
| | MUFA/PUFA | 3.59 ± 0.01 | 3.61 ± 0.02 | 3.63 ± 0.00 | 3.58 ± 0.04 | 3.61 ± 0.00 | 3.61 ± 0.01 |
| | PUFA/SFA | 0.95 ± 0.02 | 0.94 ± 0.01 | 0.94 ± 0.00 | 0.97 ± 0.02 | 0.95 ± 0.00 | 0.95 ± 0.01 |
| | (MUFA + PUFA)/SFA | 4.37 ± 0.07 | 4.31 ± 0.07 | 4.36 ± 0.01 | 4.42 ± 0.03 | 4.36 ± 0.01 | 4.40 ± 0.06 |

Results are expressed as the mean ± SD (n = 2). SFA (Saturated fatty acid). MUFA (Monounsaturated fatty acids). PUFA (Polyunsaturated fatty acids). ω3 (omega−3). ω6 (omega−6). AI (Index of atherogenicity). TI (Index of thrombogenicity). There were no significant differences ($p > 0.05$) between the nutritional quality indices of the untreated and HPP-treated samples and there were no significant differences with the storage time.

nutritional recommendations (British Department of Health, 1994). The MUFA/PUFA ratio was 3.59 ± 0.01, which provides better protection against heart diseases than a diet only rich in PUFA (Naydenova et al., 2014). In its turn, a low ω6/ω3 ratio (around 2) decreases the risk of cardiovascular diseases and some types of cancer (Simopoulos, 2008). Although this parameter is very high in acorn beverage (25.76 ± 0.67) and other acorn products (Silva et al., 2016), the lipid fraction corresponds only to 0.4%, so this index should not denote a risk. According to statistical analyses, it was concluded that there were no significant differences ($p > 0.05$) between the nutritional quality indices of the untreated and HPP-treated samples, and there were no significant differences with the storage time.

Fatty acids percentages and nutritional quality indices found in the present work were similar between the untreated and HPP-treated acorn beverage samples, which agrees with other studies with HPP application (Moltó-Puigmartí et al., 2011; Rodríguez-Alcalá et al., 2014). As far as we know, this is the first time that the AI and TI indices have been calculated for acorn beverage.

### 3.2.9. Identification and quantification of individual minerals

The main quantified mineral was potassium (13.880 mg/100 mL), confirming similar observations reported in the literature by Sekeroglu et al. (2017) for raw acorn. The remaining minerals, namely phosphorus, magnesium, manganese, copper, and iron, were found in minor amounts (0.019–1.811 mg/100 mL). Heavy metal concentrations, such as lead and nickel, were found in trace amounts. There were no significant differences ($p > 0.05$) between the mineral content of the untreated, heat-treated and HPP-treated samples.

### 3.2.10. Quantification of 5-hydroxymethylfurfural

The 5-hydroxymethylfurfural (5-HMF) was found to be below the quantification limit (LoQ < 0.5 mg/L) in the analyzed samples, showing that the processing conditions were at the level to form this compound, and browning did not occur due to its formation (data not showed).

## 3.3. Sensory analysis

Table 4 shows the sensory scores obtained by the heat-treated and HPP-treated (450 MPa, 5 min) samples. The samples were found to be similar ($p > 0.05$) in overall appreciation and flavour. On the other hand, regarding mouthfeel, the participants preferred ($p < 0.05$) heat-treated samples and concerning the appearance, they preferred HPP-treated. In the overall question about the preference between the two samples, the panel preferred the HPP-treated sample since the sum of the score was equal to 3 in favour of the HPP-treated sample. Nonetheless, the overall improvement of the acorn beverage recipe for better consumer acceptance is necessary.

**Table 4**
Heat-treated (85 °C, 30 min) and HPP-treated (450 MPa, 5 min) acorn beverage sensory scores by a non-trained panel.

| | Appearance | Flavour | Mouthfeel | Overall appreciation |
|---|---|---|---|---|
| Heat-treated (85 °C, 30 min) | 5.72 ± 1.83[a] | 3.61 ± 1.79[a] | 4.81 ± 2.3[b] | 3.86 ± 1.93[a] |
| HPP-treated (450 MPa, 5 min) | 5.90 ± 1.60[b] | 3.66 ± 1.42[a] | 4.02 ± 1.58[a] | 4.00 ± 1.62[a] |

Non-trained panel of 60 participants. Different lowercase letters (ab) in the same column indicate significant statistical differences ($p < 0.05$) between treatments.

## 4. Conclusions

From the tested conditions, HPP treatment at 450 MPa for 5 min showed to be enough to inactivate Enterobacteriaceae, mesophiles, psychrophiles, and moulds and yeasts over nine weeks of storage, like the thermal treatment. The HPP preserved TSS and colour better than the thermal treatment, but the heat-treated samples showed superior antioxidant activity (ABTS/DPPH) compared to HPP-treated samples, which can be due to the higher partial thermal hydrolysis of hydrolyzable tannins. Only two phenolic compounds were detected (gallic and ellagic acids), of which gallic acid was found in greater amount, while ellagic acid was found in trace amounts. Carbohydrates, lipids, and protein contents were similar between treatments. The acorn beverage proved to be a useful source of minerals, namely potassium, and has a good fatty acids profile, with very low values of AI and TI. In the sensory analysis, a preference for the HPP-treated acorn beverage was observed.

Although both treatments have shown equivalent results, HPP showed better colour retention, and consumers preferred this beverage. Regarding the processing time, it is possible to process a packaged sample by HPP in less time (5 min) than thermal processing (30 min) without over-processing the surface area of the product. These findings

showed that HPP is a potential non-thermal technology to be applied to acorn beverage pasteurization, increasing its shelf-life, and improving quality after treatment. The developed acorn beverage can be a thorough source of bioactive compounds, even for lactose and/or gluten intolerant consumers. It can be important as a starting point for the development of other supplemented beverages. To the best of our knowledge, this is the first study concerning the effect of HPP on an acorn beverage and further research is of interest to deepen the knowledge of its possible industrial implementation.

## CRediT author contribution statement

Manuela Pintado contributed to the conception and design of the study, as well as with the supervision of all work. Renata A. Amaral contributed to HPP samples processing. Jorge A. Saraiva supervised the experiments related to high-pressure processing. Elisabete M.C. Alexandre supervised all the work, provided support in the design, contributed to the analysis and interpretation of the results, and contributed to samples processing. Rita Sardão was responsible for performing the experiments, analyzing and interpreting the obtained data and writing the original draft. All authors contributed to reviewing the manuscript.

## Declaration of competing interest

The authors declare no competing financial interests.

## Acknowledgements

Thanks are due to the Catholic University of Portugal for the financial support of the CBQF associate laboratory under the FCT project UID/Multi/50016/2019 and the project Multibiorefinery funded by Programa Operacional Competitividade e Internacionalização (POCI-01-0145-FEDER-016403) and by Programa Operacional Regional de Lisboa (LISBOA-01-0145-FEDER-016403) and by FCT (SAICTPAC/0040/2015). Also, to the University of Aveiro and FCT/MCT for the financial support for LAQV-REQUIMTE Research Unit (FCT UIDB/50006/2020) through national funds and, where applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement, and for financing the PhD grant of Renata A. Amaral (SFRH/BD/146009/2019). This work was also supported by NewFood project Food Technologies Valorization co-financed by FEDER through Norte 2020 programme (NORTE 01 0246 FEDER 000043).

## References

Ahmadi, S., Sheikh-Zeinoddin, M., Soleimanian-Zad, S., Alihosseini, F., & Yadav, H. (2019). Effects of different drying methods on the physicochemical properties and antioxidant activities of isolated acorn polysaccharides. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 100*, 1–9. https://doi.org/10.1016/j.lwt.2018.10.027. July 2018.

Ahn, D. U., Mendonça, A. F., & Feng, X. (2017). The storage and preservation of meat: II-nonthermal technologies. *Lawrie's meat science* (8th ed.). https://doi.org/10.1016/B978-0-08-100694-8.00008-X

Ali, N., Popović, V., Koutchma, T., Warriner, K., & Zhu, Y. (2019). Effect of thermal, high hydrostatic pressure, and ultraviolet-C processing on the microbial inactivation, vitamins, chlorophyll, antioxidants, enzyme activity, and color of wheatgrass juice. *Journal of Food Process Engineering, 43*(1), e13036. https://doi.org/10.1111/jfpe.13036.

AOAC. (2012). *Association of official analytical chemists. Official methods of analysis –AOAC International. 19thed. Maryland, USA*. AOAC International.

Awsi, J., Monika, S., Sa, S., & Tsering, N. (2017). Non-thermal processing in food applications: A review. *International Journal of Food Sciences & Nutrition, 2*(6), 171–180. https://www.researchgate.net/publication/322537988.

British Department of Health. (1994). Nutritional aspects of cardiovascular disease. In *Report on health and social subjects no, 46*. London: HMSO.

Cantos, E., Espín, J. C., López-Bote, C., Hoz, L. D. L. A., Ordóñez, J. A., & Tomás-Barberán, F. A. (2003). Phenolic compounds and fatty acids from acorns (Quercus spp.), the main dietary constituent of free-ranged iberian pigs. *Journal of Agricultural and Food Chemistry, 51*(21), 6248–6255. https://doi.org/10.1021/jf030216v.

Changa, Y.-H., Wub, S.-J., Chenc, B.-Y., Huangb, H.-W., & Wang, C.-Y. (2017). Effect of high pressure processing and thermal pasteurization on overall quality parameters of white grape juice. *Journal of the Science of Food and Agriculture, 97*(10), 3166–3172. https://doi.org/10.1002/jsfa.8160.

Chung, K. T., Wong, T. Y., Wei, C. I., Huang, Y. W., & Lin, Y. (1998). Tannins and human health: A review. *Critical Reviews in Food Science and Nutrition, 38*(6), 421–464. https://doi.org/10.1080/10408699891274273.

Codina-Torrella, I., Guamis, B., Zamora, A., Quevedo, J. M., & Trujillo, A. J. (2018). Microbiological stabilization of tiger nuts' milk beverage using ultra-high pressure homogenization. A preliminary study on microbial shelf-life extension. *Food Microbiology, 69*, 143–150. https://doi.org/10.1016/j.fm.2017.08.002

Coelho, M., Silva, S., Rodríguez-Alcalá, L. M., Oliveira, A., Costa, E. M., Borges, A., Martins, C., Rodrigues, A. S., & Pintado, M. M. E. (2018). Quercus based coffee-like beverage: Effect of roasting process and functional characterization. *Journal of Food Measurement and Characterization, 12*(1), 471–479. https://doi.org/10.1007/s11694-017-9660-9

Corrales, M., Souza, P. M. De, Stahl, M. R., & Fernández, A. (2012). Effects of the decontamination of a fresh tiger nuts' milk beverage (horchata) with short wave ultraviolet treatments (UV-C) on quality attributes. *Innovative Food Science & Emerging Technologies, 13*, 163–168. https://doi.org/10.1016/j.ifset.2011.07.015

Coscueta, E. R., Malpiedi, L. P., & Nerli, B. B. (2018). Micellar systems of aliphatic alcohol ethoxylates as a sustainable alternative to extract soybean isoflavones. *Food Chemistry, 264*, 135–141. https://doi.org/10.1016/j.foodchem.2018.05.015

Costas, C. (2013). *Manual de Cocina Bellotera para la Era Post Petrolera*. CreateSpace Independent Publishing Platform.

Custódio, L., Patarra, J., Alberício, F., Neng, N. R., Nogueira, J. M. F., & Romano, A. (2013). Extracts from Quercus sp. acorns exhibit in vitro neuroprotective features through inhibition of cholinesterase and protection of the human dopaminergic cell line SH-SY5Y from hydrogen peroxide-induced cytotoxicity. *Industrial Crops and Products, 45*, 114–120. https://doi.org/10.1016/j.indcrop.2012.12.011

Custódio, L., Patarra, J., Alberício, F., Neng, N. R., Nogueira, J. M. F., & Romano, A. (2015). Phenolic composition, antioxidant potential and in vitro inhibitory activity of leaves and acorns of Quercus suber on key enzymes relevant for hyperglycemia and Alzheimer's disease. *Industrial Crops and Products, 64*, 45–51. https://doi.org/10.1016/j.indcrop.2014.11.001

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*(3), 350–356. https://doi.org/10.1021/ac60111a017

Fernandez, M. V., Denoya, G. I., Agüero, M. V., Jagus, R. J., & Vaudagna, S. R. (2018). Optimization of high pressure processing parameters to preserve quality attributes of a mixed fruit and vegetable smoothie. *Innovative Food Science & Emerging Technologies, 47*, 170–179. https://doi.org/10.1016/j.ifset.2018.02.011

Gonçalves, B., Falco, V., Moutinho-Pereira, J., Balcelar, E., Peixoto, F., & Correia, C. (2009). Effects of elevated CO2 on grapevine (Vitis vinifera L.): Volatile composition, phenolic content, and in vitro antioxidant activity of red wine. *Journal of Agricultural and Food Chemistry, 57*(1), 265–273. https://doi.org/10.1021/jf8020199

Huang, H. W., Chang, Y. H., & Wang, C. Y. (2015). High pressure pasteurization of sugarcane juice: Evaluation of microbiological shelf life and quality evolution during refrigerated storage. *Food and Bioprocess Technology, 8*, 2483–2494. https://doi.org/10.1007/s11947-015-1600-2

Jayachandran, L. E., Chakraborty, S., & Rao, P. S. (2015). Effect of high pressure processing on physicochemical properties and bioactive compounds in litchi based mixed fruit beverage. *Innovative Food Science & Emerging Technologies, 28*, 1–9. https://doi.org/10.1016/j.ifset.2015.01.002

Jing, L., Ma, H., Fan, P., Gao, R., & Jia, Z. (2015). Antioxidant potential, total phenolic and total flavonoid contents of Rhododendron anthopogonoides and its protective effect on hypoxia-induced injury in PC12 cells. *BMC Complementary and Alternative Medicine, 15*, 287. https://doi.org/10.1186/s12906-015-0820-3

Krapfenbauer, G., Kinner, M., Gossinger, M., Schonlechnet, R., & Berghofer, E. (2006). Effect of thermal treatment on the quality of cloudy apple juice. *Journal of Agricultural and Food Chemistry, 54*, 5453–5460. https://doi.org/10.1021/jf0606858

León-Camacho, M., Viera-Alcaide, I., & Vicario, I. M. (2004). Acorn (Quercus spp.) fruit lipids: Saponifiable and unsaponifiable fractions: A detailed study. *Journal of the American Oil Chemists Society, 81*, 447–453. https://doi.org/10.1007/s11746-004-0921–0928

Liu, F., Zhang, X., Zhao, L., Wang, Y., & Liao, X. (2016). Potential of high-pressure processing and high-temperature/short-time thermal processing on microbial, physicochemical and sensory assurance of clear cucumber juice. *Innovative Food Science & Emerging Technologies, 34*, 51–58. https://doi.org/10.1016/j.ifset.2015.12.030

Manzocco, L., Calligaris, S., Mastrocola, D., Nicoli, M. C., & Lerici, C. R. (2000). Review of non-enzymatic browning and antioxidant capacity in processed foods. *Trends in Food Science & Technology, 11*(9–10), 340–346. https://doi.org/10.1016/S0924-2244(01)00014-0

Marszałek, K., Szczepańska, J., Starzonek, S., Woźniak, Ł., Trych, U., Skąpska, S., Rzoska, S., Saraiva, J. A., Lorenzo, J. M., & Barba, F. J. (2019). Enzyme inactivation and evaluation of physicochemical properties, sugar and phenolic profile changes in cloudy apple juices after high pressure processing, and subsequent refrigerated storage. *Journal of Food Process Engineering, 42*(4), e13034. https://doi.org/10.1111/jfpe.13034

Moltó-Puigmartí, C., Permanyer, M., Castellote, A. I., & López-Sabater, M. C. (2011). Effects of pasteurization and high-pressure processing on vitamin C, tocopherols and fatty acids in mature human milk. *Food Chemistry, 124*(3), 697–702. https://doi.org/10.1016/j.foodchem.2010.05.079

Monteiro, M. J. P., Costa, A. I. A., Fliedel, G., Cissé, M., Bechoff, A., Pallet, D., Tomlins, K., & Pintado, M. M. E. (2016). Chemical-sensory properties and consumer preference of Hibiscus beverages produced by improved industrial processes. *Food Chemistry, 225*, 202–212. https://doi.org/10.1016/j.foodchem.2016.11.127

Munekata, P. E. S., Domínguez, R., Budaraju, S., Roselló-Soto, E., Barba, F. J., Mallikarjunan, K., Roohinejad, S., & Lorenzo, J. M. (2020). Effect of innovative food processing technologies on the physicochemical and nutritional properties and quality of non-dairy plant-based beverages. *Foods, 9*(3), 288. https://doi.org/10.3390/foods9030288

Naydenova, N., Kaishev, I., Iliev, T., & Mihaylova, G. (2014). Fatty acids profile, atherogenic and thrombogenic health indices of white brined cheese made from buffalo milk. *Agricultural Science and Technology, 6*(3), 352–355. http://agriscitech.eu/wp-content/uploads/2014/09/024.pdf.

Oliveira, C. M., Barros, A. S., Ferreira, A. C. S., & Silva, A. M. S. (2015). Influence of the temperature and oxygen exposure in red port wine: A kinetic approach. *Food Research International, 75*, 337–347. https://doi.org/10.1016/j.foodres.2015.06.024

Patras, A., Brunton, N. P., Da Pieve, S., & Butler, F. (2009). Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. *Innovative Food Science & Emerging Technologies, 10*(3), 308–313. https://doi.org/10.1016/j.ifset.2008.12.004

Picouet, P. A., Sárraga, C., Cofán, S., Belletti, N., & Guàrdia, M. D. (2014). Effects of thermal and high-pressure treatments on the carotene content, microbiological safety and sensory properties of acidified and of non-acidified carrot juice. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 62*(1), 920–926. https://doi.org/10.1016/j.lwt.2014.07.027

Pimentel, L., Fontes, A. L., Gomes, A. M., & Rodrı, L. M. (2015). Considerations about the in situ derivatization and fractionation of EFA and NEFA in biological and food samples. *Methods (Duluth), 2*, 475–484. https://doi.org/10.1016/j.mex.2015.11.006

Pinto, C. A., Moreira, S. A., Fidalgo, L. G., Inácio, R. S., Barba, F. J., & Saraiva, J. A. (2020). Effects of high-pressure processing on fungi spores: Factors affecting spore germination and inactivation and impact on ultrastructure. *Comprehensive Reviews in Food Science and Food Safety, 19*(2), 553–573. https://doi.org/10.1111/1541-4337.12534

Rigo, D. De, & Caudullo, G. (2016). Quercus ilex in Europe: Distribution, habitat, usage and threats. *European atlas of forest tree species*.

Rivas, A., Rodrigo, D., Martínez, A., Barbosa-Cánovas, G. V., & Rodrigo, M. (2006). Effect of PEF and heat pasteurization on the physical - chemical characteristics of blended orange and carrot juice. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 39*(10), 1163–1170. https://doi.org/10.1016/j.lwt.2005.07.002

Rodríguez-Alcalá, L. M., Alonso, L., & Fontecha, J. (2014). Stability of fatty acid composition after thermal, high pressure, and microwave processing of cow milk as affected by polyunsaturated fatty acid concentration. *Journal of Dairy Science, 97*(12), 7307–7315. https://doi.org/10.3168/jds.2013-7849

Saffarzadeh-Matin, S., & Khosrowshahi, F. M. (2017). Phenolic compounds extraction from Iranian pomegranate (Punica granatum) industrial waste applicable to pilot plant scale. *Industrial Crops and Products, 108*(July), 583–597. https://doi.org/10.1016/j.indcrop.2017.07.022

Santos, C. S., Roriz, M., Carvalho, S. M. P., & Vasconcelos, M. W. (2015). Iron partitioning at an early growth stage impacts iron deficiency responses in soybean plants ( Glycine max L .). *Frontiers of Plant Science, 6*, 325. https://doi.org/10.3389/fpls.2015.00325

Sekeroglu, N., Ozkutlu, F., & Kilic, E. (2017). Mineral composition of acorn coffees. *Indian Journal of Pharmaceutical Education and Research, 51*(3), S504–S507. https://doi.org/10.5530/ijper.51.3s.75

Silva, S., Costa, E. M., Borges, A., Carvalho, A. P., Monteiro, M. J., & Pintado, M. M. E. (2016). Nutritional characterization of acorn flour (a traditional component of the Mediterranean gastronomical folklore). *Journal of Food Measurement and Characterization, 10*, 584–588. https://doi.org/10.1007/s11694-016-9340-1

Simopoulos, A. P. (2008). The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Experimental Biology and Medicine, 233*(6), 674–688. https://doi.org/10.3181/0711-MR-311

Sousa, S. G., Delgadillo, I., & Saraiva, J. A. (2016). Human milk composition and preservation: Evaluation of high-pressure processing as a nonthermal pasteurization technology. *Critical Reviews in Food Science and Nutrition, 56*(6), 1043–1060. https://doi.org/10.1080/10408398.2012.753402

Tadayoni, M., Sheikh-Zeinoddin, M., & Soleimanian-Zad, S. (2015). Isolation of bioactive polysaccharide from acorn and evaluation of its functional properties. *International Journal of Biological Macromolecules, 72*, 179–184. https://doi.org/10.1016/j.ijbiomac.2014.08.015

Terán-Hilares, R., Chirinos, R., Pedreschi, R., & Campos, D. (2017). Enhanced antioxidant properties of tara (Caesalpinia spinosa) gallotannins by thermal hydrolysis and its synergistic effects with α-tocopherol, ascorbyl palmitate, and citric acid on sacha inchi (Plukenetia volubilis) oil. *Journal of Food Process Engineering, 41*(1), e12613. https://doi.org/10.1111/jfpe.12613

Trigo, J. P., Alexandre, E. M. C., Oliveira, A., Saraiva, J. A., & Pintado, M. (2020). Fortification of carrot juice with a high-pressure-obtained pomegranate peel extract: Chemical, safety and sensorial aspects. *International Journal of Food Science and Technology, 55*(4), 1599–1605. https://doi.org/10.1111/ijfs.14386

Tsai, M., Cheng, M., Chen, B., & Wang, C. (2018). Effect of high-pressure processing on immunoreactivity, microbial and physicochemical properties of hazelnut milk. *International Journal of Food Science and Technology, 53*(7), 1672–1680. https://doi.org/10.1111/ijfs.13751

Ulbricht, T. L. V., & Southgate, D. A. T. (1991). Coronary heart disease: Seven dietary factors. *The Lancet, 338*(8773), 985–992. https://doi.org/10.1016/0140-6736(91)91846-m

Vieira, F. N., Lourenço, S., Fidalgo, L. G., Santos, S. A. O., Silvestre, A. J. D., Jerónimo, E., & Saraiva, J. A. (2018). Long-term effect on bioactive components and antioxidant activity of thermal and high-pressure pasteurization of orange juice. *Molecules, 23*(10), 2706. https://doi.org/10.3390/molecules23102706

Vinha, Barreira, Costa, & Oliveira. (2016). *Comprehensive Reviews in Food Science and Food Safety, 15*, 947–981. https://doi.org/10.1111/1541-4337.12220

Wang, H., Chen, B., & Wang, C. (2018). Comparison of high pressure and high temperature short time processing on quality of carambola juice during cold storage. *Journal of Food Science & Technology, 55*(12), 1716–1725. https://doi.org/10.1007/s13197-018-3084-3

Wei, S., Lu, G., & Cao, H. (2017). Effects of cooking methods on starch and sugar composition of sweetpotato storage roots. *PloS One, 12*(8), e0182604. https://doi.org/10.1371/journal.pone.0182604

Westerik, N., Wacoo, A. P., Sybesma, W., & Kort, R. (2016). Novel production protocol for small-scale manufacture of probiotic fermented foods. *Journal of Visualized Experiments, 115*, e54365. https://doi.org/10.3791/54365

Woldemariam, H. W., & Emire, S. A. (2019). High pressure processing of foods for microbial and mycotoxins control: Current trends and future prospects. *Cogent Food & Agriculture, 5*(1). https://doi.org/10.1080/23311932.2019.1622184

Xu, X., Deng, J., Luo, D., Bao, Y., Liao, X., Gao, H., & Wu, J. (2018). Comparative study of high hydrostatic pressure and high temperature short time processing on quality of clear and cloudy Se-enriched kiwifruit juices. *Innovative Food Science & Emerging Technologies, 49*, 1–12. https://doi.org/10.1016/j.ifset.2018.07.010

Zhang, Y., Liu, X. C., Wang, Y., Zhao, F., Sun, Z., & Liao, X. (2016). Quality comparison of carrot juices processed by high-pressure processing and high-temperature short-time processing. *Innovative Food Science & Emerging Technologies, 33*, 135–144. https://doi.org/10.1016/j.ifset.2015.10.012