

## Article

# Impact of High-Pressure Processing (HPP) on Selected Quality and Nutritional Parameters of Cauliflower (*Brassica oleracea* var. *Botrytis*)

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**Abstract:** In recent years, innovative food processing methods, such as high-pressure processing (HPP) treatment, have been shown to improve food quality. The purpose of this work was to determine the effects of high-pressure processing (HPP; 400 and 600 MPa for 2 or 5 min, 20 °C) of cauliflower. Microbial shelf-life (total aerobic count and spores), texture, color, drip loss, dry matter, antioxidative capacity, total phenolic content, and ascorbic acid were analyzed before and after processing, as well as during storage (4 °C) for up to 42 days. Among the different treatments, HPP at 600 MPa exhibited low microbial counts between days 14 and 28 of storage, while at 400 MPa already had high bacterial counts between days 7 and 14. HPP at both 400 and 600 MPa was the best method to maintain the color during storage. The texture of the cauliflower did not differ from the control during storage for HPP. For all samples, the dry matter content remained stable during storage, with few differences between treatments. The nutritional quality of high-pressure-processed cauliflower at 600 MPa for 2 min remained high until day 28. The overall results of this study demonstrate that HPP has the potential to preserve the quality of cauliflower.

**Keywords:** high-pressure processing; microbiology; cauliflower; texture



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## 1. Introduction

Cauliflower, also known as *Brassica oleracea* convar. *botrytis* var. *botrytis*, is a variety of cabbage within the Brassicaceae family. It consists of several flowerheads that are merged into the distinctive “head” of the cauliflower. The most common variant has a white color, but other variants with a purple or green color also appear. Cauliflower and other Brassicaceae vegetables are especially rich in bioactive compounds such as glucosinolates (GLSs—2–6 mmol K<sup>-1</sup> [1]), vitamin C (770 mg 100g<sup>-1</sup> [2]), and polyphenols (38.17 and 138.73 μM g<sup>-1</sup> DW [3]) and are known to present strong antioxidant properties [4].

Plant-based foods are often, before consumption, subjected to cooking or processing to increase their edibility and palatability. Processing also aims to prolong the shelf-life of greens while preserving the nutritional and sensory profile of the food to the highest degree, without putting a constraint on the microbial safety of the food [5]. Today's conventional thermal methods for preserving food can often lead to a reduction in its sensory and nutritional profile. Therefore, in recent years, there has been a wider interest in researching new processing methods such as high-pressure processing and microwave processing of foods, amongst others. The food industry is very active in technological innovation, and over the last two decades novel non-thermal processing technologies have been viewed as useful for microbial inactivation while maintaining the quality of fresh and processed fruits

and vegetables both in the form of solid cubes, slices or dices and in liquid form, such as juices or smoothies [6,7].

The first study on the use of HPP on food was described in 1899. However, it took several years to commercialize the HPP of foods, with Japan being the first country to use it in 1990 [8]. Several studies have proven that HPP can be effective at an industrial level, and the technology has gained more interest in recent years [9]. Using HPP can in some types of foods result in reduced use of preservatives, and it is therefore seen as commercially advantageous. It is expected that the use of HPP will further increase and lead to several innovations in the food industry [10]. HPP is a form of batch processing, which puts limitations on the volume being processed daily. Therefore, the type of food requires an increase in value to defend its use. However, for liquid and semi-liquid foods there is a possibility to apply continuous systems as well [11].

For plants, HPP can disturb cell permeability, resulting in the movement of water and other metabolites in the cells. How much the cell is disrupted is dependent on the pressure used, as well as the type of plant cell [5]. Small molecules that have no secondary, tertiary, and quaternary structures, such as amino acids, vitamins, and flavor and aroma compounds, remain unaffected by HPP [12]. Textural changes in vegetables during HPP are related to changes in the cell wall (enzymatic and non-enzymatic reactions) and the pressure itself. This is dependent on the structure of the cell wall and the space between the different cells [13]. When applying pressure to plant tissue, cell disruption can lead to different high molecular compounds leaking from different plant cells and interacting [14]. This can lead to softening and is also caused by loss of turgor pressure. The degree of cell disruption depends not only on the level of pressure applied but also on the plant cell type. HPP affects the organization of parenchyma cells. Plant cells disintegrate and intercellular spaces are no longer filled with gas (e.g., in a spinach leaf). A cavity develops after HP processing, and a firm texture and saturated appearance are noticeable after HP processing (e.g., cauliflower). Concerning HPP's effects on the texture of (solid) fruits and vegetables, hardness or firmness is mostly used as a parameter [5]. Nevertheless, compared to thermal processing, HPP results in minor changes in pectin depolymerization [15]. In general, HPP preserves the color of many types of foods, but some pressure-resistant enzymes and bacteria can lead to degradation of the color during storage and result in browning [16]. By comparison, it has also been shown that pressure applied at low and moderate temperatures can preserve pigments such as carotenoids and chlorophyll [17].

Pressures up to 600 MPa have been shown to inactivate vegetative pathogens, spoilage bacteria, yeast and molds [18]. On the other hand, spores can survive pressure treatments up to above 1000 MPa, and some enzymes are also pressure-resistant [19].

Often, combinations of HPP and heat are required to obtain inactivation of spores and enzymes. Pressure-resistant spore formers and enzymes are, however, less temperature-resistant when subjected to high pressure. It is generally recognized that high pressure yields increased food safety, but this is dependent on the food being investigated [20]. The food must contain certain amounts of water to achieve an effective reduction in microbial counts. Bacterial inactivation will also depend on the type of microbe, food composition, pH, and water activity of the food [20].

Pinton, Bardsley, Marik, Boyer, and Strawn found that raw cauliflower was associated with recalls due to the potential for *L. monocytogenes* contamination [21]. This bacterium is ubiquitous in soil and therefore a potential risk, but several processing steps can reduce the presence of this microbe. Arroyo, Sanz, and Préstamo found that HPP of cauliflower (300/350/400 MPa, 30 min, 5 °C) gave no growth of total aerobic bacteria. HPP is a technology that can eliminate or inactivate vegetative microbes, but it needs to be tested on each specific product to evaluate its safety [22].

There are few studies on the effect of HPP on the quality of vegetables given processing in larger pieces. For this reason, we decided to undertake a study on the effect of HPP on the quality characteristics of cauliflower *Brassica oleracea* var. *botrytis*.

## 2. Materials and Methods

### 2.1. Cauliflower Raw Material

Pieces of frozen cauliflower (*Brassica oleracea* var. *botrytis* L.) were obtained from Fjordkjøkken AS. The cauliflower pieces, produced by Findus AS, had been steam-blached before freezing and were stored at  $-32\text{ }^{\circ}\text{C}$ . All cauliflower used in the experiments was from the same batch.

### 2.2. High-Pressure Processing

HPP was performed in a lab-scale high-pressure QFP 2L-700 unit (Avure Technologies Inc., Columbus, OH, USA). Samples were pressurized at 400 and 600 MPa for 2 and 5 min. When pressurizing, the come-up time was approximately 90 and 120 s for 400 and 600 MPa, respectively, and the pressure release was immediate. Processing was at ambient temperature. The duration of treatment (2 or 5 min) did not include the come-up time. Control samples were non-pressurized samples.

Before processing, frozen cauliflower samples were vacuum-packed (99.5%) in sous-vide bags and thawed in cold water. After HPP, the samples were cooled on ice. The samples were stored at  $4\text{ }^{\circ}\text{C}$  until further analysis for up to 42 days. All analyses were carried out with three technical replicates for each treatment unless stated otherwise. The experiments were performed twice, on different days.

### 2.3. Microbial Analysis

The microbial flora of processed cauliflower was determined after processing (day 0) and after refrigerated storage (days 7, 14, 28, 35, and 42). The cauliflower samples (10 g) were diluted 1:10 in 1% peptone water with 0.85% NaCl added and homogenized for 2 min using a stomacher. Total aerobic bacteria (TAB) quantification was performed via Plate Count Agar (PCA). The plates were incubated at  $37\text{ }^{\circ}\text{C}$  for 48 h. For detecting viable aerobic and anaerobic spores, 5 mL samples of the homogenized solution were heated at  $80\text{ }^{\circ}\text{C}$  for 10 min to inactivate vegetative cells. Thereafter, the samples were cooled and plated on PCA. The plates were incubated at  $37\text{ }^{\circ}\text{C}$  for 48 h. Anaerobic spores were incubated in anaerobic conditions. The presence of spores was checked at day 0 (control samples), and days 35 and 42. A mechanical spiral plater (Eddy Jet, IUL Instruments, Barcelona, Spain) was used for most enumerations, but some manual plating was performed for low dilutions. The detection level on PCA was  $2.3\text{ log cfu/g}$ . For plates with no colonies detected, the level was set to half of the detection limit (as described by Aaby, Grimsbo, Hovda, and Rode [23]). The results were calculated and given as  $\text{log cfu/g}$  sample. The microbial counts are the mean of three experiments with three technical replicates for each treatment.

### 2.4. Physical Parameter Measurements

#### 2.4.1. Dry Matter

The dry matter content of cauliflower was measured before and after processing, and during each day of sampling (days 0, 14, 28, 42). For measuring the dry matter content of the cauliflower, 5–8 g of non-processed (control) and processed samples were weighed in aluminum trays, then heated at  $105\text{ }^{\circ}\text{C}$  for 16–18 h according to the standard method (NMKL 23,1991). After heating, the samples were stored in a desiccator for 30 min before weighing. The formula used for measuring the water and dry matter content is the following (Equation (1)):

$$\text{Water (\%)} = \frac{\text{Wet weight (g)} - \text{Dry weight (g)}}{\text{Wet weight (g)}} \times 100 \quad (1)$$

#### 2.4.2. Drip Loss

The drip loss of cauliflower was measured before and after processing, and during each day of sampling (days 0, 7, 14, 28, 35, 42). Individual pieces (5–10 g) were used. The drip loss was calculated according to the formula below (Equation (2)):

$$\text{Drip loss (\%)} = \frac{\text{Weight at packaging (g)} - \text{Weight at sampling day (g)}}{\text{Weight at packaging (g)}} \times 100 \quad (2)$$

#### 2.4.3. Color Analysis

The color of both stems and florets of the cauliflower was determined before and after processing, and during each day of sampling (day 0, 14, 28, 42), using VeriVide's DigiEye system (VeriVide Ltd., Leicester, UK). This system was equipped with a DSLR camera (Nikon D90, Tokyo, Japan) and it captured an image of 4288 × 2848 pixels with a resolution of 96 dpi. Before each measurement, the camera was white balanced and calibrated with the color chart provided with the equipment. The  $L^*$ ,  $a^*$ , and  $b^*$  components were recorded at a D65 standard illuminant. Each piece of the sample was photographed in a light cabinet on a black background. The captured images were analyzed according to the CIELAB color scale using DigiEye 2.9 software, with the use of the "free form tool" because of the irregular shape of each piece. Data were obtained from 18 measurements on each sample. The  $L^* a^* b^*$  color space was used for determination of the color. Further, the total color difference (TCD) of the samples before (control) and after processing and storage were calculated as (Equation (3)) [24]:

$$\text{TCD} = \sqrt{[(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2]} \quad (3)$$

where  $L_0^*$ ,  $a_0^*$ , and  $b_0^*$  were the values for the untreated sample (control).

#### 2.4.4. Texture Measurement

The firmness of the cauliflower was measured before and after processing, and during each day of sampling (days 0, 14, 28, 42). A Texture Analyzer XT Plus (Stable Micro Systems Ltd., Godalming, UK), with a TA-42 knife probe with a 45° chisel blade, guillotine block, and slotted plate was used. The method applied was as described by Koskiniemi, Truong, McFeeters, and Simunovic with some modifications [25]. This type of test was used to quantify the force and the amount of work required to shear through the entire sample. A 50 kg load cell was used for all texture measurements. The pre-test speed was 3.00 mm/s, the test speed was 2.50 mm/s, and the post-test speed was 10.00 mm/s. The test mode was compression. The trigger force was set to 6 g, and the measured force was applied at 50% strain. Both height and weight were calibrated before analysis. All samples were tempered at room temperature for 1 h before analysis.

### 2.5. Chemical Analysis

#### 2.5.1. Antioxidation Potential Measured with DPPH

The antioxidant activity levels of the freeze-dried cauliflower were determined based on their 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, as described by Ahmed and Ali (2013), with some modifications. First, a methanolic solution of 0.1 mM DPPH was produced and stored cool in a dark bottle at 4 °C. Then, 0.5 g of freeze-dried powdered cauliflower was added to 15 mL tubes, and 10 mL of methanol was added to each. The mixture was homogenized (IKA T25, Ultra Turrax) for 1 min, followed by centrifugation at 5000 rpm for 10 min (Eppendorf centrifuge 5804). This supernatant was also used for phenolic content determination later.

Subsequently, 0.1 mL of the aliquot was added to a lab tube, and 4 mL of the DPPH solution was added. The absorbance of the DPPH reagent was determined after incubation for 30 min in darkness, using a spectrophotometer (Shimadzu UV-1800) set at 515 nm, by measuring the sample's decrease in absorbance against a negative control. The DPPH

radical scavenge activity results in decolorization and was calculated in terms of percentage reduction in DPPH according to the following equation (Equation (4)):

$$\text{Radical scavenging activity (\%)} = \frac{C - A}{C} \times 100 \quad (4)$$

where  $A$  is the difference in absorbance at 515 nm between control and sample, and  $C$  is the absorbance of the control. For control samples, 0.1 mL of methanol was used instead of the extract. Analyses were performed in duplicate on each sample, and the results are the mean of three repetitions of the experiment [26].

#### 2.5.2. Total Phenolic Content (TPC)

TPC of the vegetables was determined using the Folin–Ciocalteu method (Singleton and Rossi, 1965), with modifications from Ahmed and Ali [2]. The methanolic extracts from the DPPH experiment were used here as well. Briefly, 0.1 mL of the cauliflower extract was transferred to a 15 mL plastic tube, and 7.9 mL of deionized water was added. Afterward, 0.1 mL of Folin–Ciocalteu phenol reagent was added to each sample tube and left for 3 min. Then, 2 mL of 20% of sodium carbonate was added and vortexed. Samples were left for 60 min in darkness, and absorbance was measured at 760 nm. For control samples, 0.1 mL of methanol was used instead of the extract. Various concentrations of gallic acid standard solution were used to establish the standard calibration curve. The results were expressed as mg of gallic acid equivalents (GAE) per 100 g of dry weight (DW). Analyses were performed in duplicate on each sample, and the results are the mean of three repetitions of the experiment.

#### 2.5.3. Ascorbic Acid (AA)

AA of the extracts (20  $\mu$ L injection volume) was determined on an Agilent 1100 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA), with modifications to the method described by Castro et al. (2008). For the extraction of AA, 80 mg of powdered cauliflower samples was dissolved in 10 mL of 4% metaphosphoric acid in deionized water. This solution was homogenized for 2 min and rotated for 20 min in darkness. Afterwards, the mixture was centrifuged at 5000 rpm for 10 min (Eppendorf centrifuge 5804), then filtered using a filter syringe and transferred to a HPLC vial.

Separation was performed at room temperature on a monolithic column with mobile phase 0.2 M  $\text{KH}_2\text{PO}_4$  adjusted to pH 2.4. The retention time for AA was approximately 4.37 min, with a flow of 0.800 mL/min, and injection intervals were 7 min. The AA was detected at 254 nm and quantified by an external standard. Each sample was analyzed in triplicate. The identified AA (254 nm) was quantified based on peak area and compared with calibration curves obtained with the corresponding standards and then expressed as mg AA/100 g DW.

#### 2.6. Statistics

Statistical analysis was performed using Minitab<sup>®</sup> Statistical Software (version 19, Minitab Ltd., Coventry, UK). Significant differences ( $p < 0.05$ ) between samples throughout processing and storage (processing replicates;  $n = 3$ ) were determined with one-way analysis of variance (ANOVA) and *post-hoc* Tukey's multiple comparison test. General linear modeling ANOVA was performed to determine the significant effects of the experimental factors and their interactions.

### 3. Results

#### 3.1. Effect of HPP on Aerobic and Anaerobic Spores

Total aerobic bacteria (TAB) were determined in cauliflower before and after processing and storage (Table 1). Pressurization of cauliflower at 600 MPa for 2 and 5 min reduced TAB by 2.0 and 2.3 log cfu/g, respectively, on day 14. HPP at 400 MPa for 2 and 5 min yielded low counts until day 7, but high counts on day 14 (6.4 and 6.1 log cfu/g, respectively). The

non-treated samples (control) showed bacterial counts of 5.0 log cfu/g on day 0. The HPP holding time did not significantly ( $p > 0.05$ ) change the bacterial counts on all days (except 400 MPa day 0), indicating that shorter processing times could be beneficial. Throughout storage, and especially at the end of the storage period, a larger deviation between the samples was found. Some individual 600 MPa samples had no detectable growth at day 42, whereas others had high counts. The pressure of 600 MPa had a significant ( $p > 0.05$ ) effect on TAB. The increase in TAB could be due to the growth of surviving cells, in addition to recovery of injured cells. The HPP samples had an acidic smell after day 28, indicating possible lactic acid bacteria present. Vegetables have a low buffering capacity, which makes anaerobic conditions favorable for lactic acid bacteria to grow [27]. Measurements of the pH during storage could also indicate spoilage. Ulmer et al. investigated the effects of pressure on *Lactobacillus plantarum* in beer, a possible spoilage bacterium, and found that at pressures of 600 MPa, all cells were killed during pressure and the fraction of barotolerant cells was at or below the detection level (120 cfu/mL). They concluded that the use of HPP was effective in preventing or delaying the growth of spoilage bacteria in beer, instead of using sterilization at high temperature [28]. The carbohydrate content of cauliflower could contribute to some protection from pressure in bacteria [29].

**Table 1.** Microbial counts, TAB (total aerobic bacteria [log cfu/g]) \*, in cauliflower before (control) and after HPP treatment. The samples were stored for up to 42 days in cold storage (4 °C).

Storage (Days)	Control		HPP		
	0.1 MPa	400 MPa		600 MPa	
	0 min	2 min	5 min	2 min	5 min
0	5.0 ± 0.2 <sup>A, a</sup>	3.3 ± 0.2 <sup>A, b</sup>	<2.3 <sup>A, c</sup>	2.4 ± 0.4 <sup>A, d</sup>	<2.3 <sup>A, d</sup>
7	7.2 ± 0.9 <sup>B, a</sup>	3.8 ± 0.3 <sup>B, c</sup>	3.2 ± 0.3 <sup>A, c</sup>	<2.3 <sup>A, d</sup>	2.3 ± 0.3 <sup>A, d</sup>
14	nm <sup>**</sup>	6.4 ± 0.5 <sup>C, b</sup>	6.0 ± 0.7 <sup>B, b</sup>	3.1 ± 1.0 <sup>A, c</sup>	2.8 ± 0.4 <sup>A, B, cd</sup>
28	nm	9.3 ± 0.5 <sup>D, a</sup>	9.1 ± 0.5 <sup>C, a</sup>	5.5 ± 1.7 <sup>B, b</sup>	5.0 ± 1.9 <sup>B, C, b</sup>
35	nm	nm	nm	6.6 ± 1.9 <sup>B, a</sup>	6.1 ± 2.7 <sup>C, D, a</sup>
42	nm	nm	nm	5.7 ± 2.8 <sup>B, a</sup>	7.5 ± 2.2 <sup>D, a</sup>

\* Each value in the table is the mean ± standard deviation (n = 9). The capital letters (A, B, C, and D) indicate a significant difference ( $p < 0.05$  multiple Tukey's adjusted analyses of variance) within the same treatment and days (same column). Small letters (a, b, c, and d) indicate a significant difference ( $p < 0.05$ ) between different treatments (same row). The detection level was 2.3 log cfu/g. Where no survival was detected, the values were set to 50% of the detection limit. \*\* Not measured.

Few studies have been conducted on the effects of HPP on the shelf life of cauliflower, and none during storage for 42 days. Among the few, Arroyo et al. (1999) looked at the effects of HPP of cauliflower (300, 350, 400 MPa/30 min/5 °C) on different microbes [30]. They found low counts (below the detection limit) of TAB at 400 MPa immediately after processing. However, they processed their cauliflower in homogenized pieces in peptone water. This is different from whole pieces, and concerning the long processing conditions, comparison of their results is difficult. Arroyo et al. performed HPP of tomato and salad greens (100–400 MPa/10 min/20 °C or 20 min/10 °C) [22]. The results showed that a pressure of 350 MPa was needed to reduce the amount of molds and most Gram-negative bacteria. At 400 MPa there were still some surviving Gram-positive bacteria, and pressurizing at 400 MPa had little effect on spores of Gram-positive bacteria.

Bacterial spores represent a hazard for the food industry when they germinate and grow in food, which can lead to food poisoning and spoilage [31]. For both aerobic and anaerobic spores, low growth was detected for all samples at all stages during the storage period (Table 2). No spores were detected at day 0 of the control sample. The presence of aerobic and anaerobic spores was also checked on day 35 and 42 for HPP samples. For HPP, no significant differences were found either within or between treatments and days ( $p > 0.05$ ), and most counts were close to the detection limit. Even low numbers of spores can pose a threat to food safety. With the right conditions, the spores present in cauliflower could germinate and cause food poisoning. It is therefore of utmost importance to apply more specific methods to detect viable spores after processing. Unfortunately,

bacterial spores are extremely resistant to commercially attainable pressure levels. As a result, low-acid shelf-stable products cannot be achieved by elevated pressure alone [32]. Pressures up to 1000 MPa have been suggested to inactivate spores sufficiently, but from an industrial point of view, this would demand higher energy output and more time to build up pressure [33]. To ensure low acid products, such as cauliflower, with a long shelf life, a combination of high pressure and elevated temperature could be necessary [20]. This combination of high pressure and high temperature was shown to be successful in reducing the bacterial spore load [34,35]. Nevertheless, the different food matrixes that have been tested do not include pieces of cauliflower, and it is well known that the food matrix can affect bacterial survival. The overall picture showed a low presence of bacterial spores after 42 days. The incubation temperature was low (4 °C), and this too could have a positive effect on the inhibition of growth. Arroyo et al. tested different pure cultures of microbes and vegetables (tomato and salad greens) under HPP (100–400 MPa/10 min 20 °C or 10 °C/20 min). They found that reductions in cultures of *Bacillus cereus* spores at 400 MPa (10 °C/20 min) gave less than 1 log cfu/mL reductions. Furthermore, Arroyo et al. found that strains of *B. cereus* spores (in pure suspension) were sensitive to 400 MPa ( $>10^2$  cfu mL).

**Table 2.** Microbial counts (total viable count (log cfu/g)) \* for aerobic (A) and anaerobic (B) spores in cauliflower before (control) and after HPP. The samples were stored for up to 42 days in cold storage (4 °C).

Storage (Days)	Control 0.1 Mpa	Aerobic Spores		Anaerobic Spores	
		HPP		HPP	
		600 MPa		600 MPa	
	0 min	2 min	5 min	2 min	5 min
0	<2.3	nm	nm	nm	nm
35	nm **	<2.3	2.4 ± 0.9	2.7 ± 1.1	2.6 ± 0.8
42	nm	2.4 ± 0.8	2.6 ± 1.1	2.5 ± 0.7	2.6 ± 1.14

\* Each value in the table is the mean ± standard deviation f (n = 9). The detection level was 2.3 log cfu/g. Where no survival was detected, the values were set to 50% of the detection limit. \*\* Not measured.

### 3.2. Effects of HPP on Physical Parameters of Cauliflower

The dry matter (DM) content of food consists of all its constituents excluding water. Table 3 represents the influence of different processing conditions on the percentage of dry matter content in pieces of cauliflower. The results showed that DM content was higher in control samples than in processed ones.

The DM content of raw cauliflower seems to vary among different studies. Florkiewicz, Socha, Filipiak-Florkiewicz, and Topolska [36] reported 8.42% DM for raw cauliflower, while Cebula, Kunicki, and Kalisz reported 6.57% DM. One reason for the variation could be that frozen pre-blanching cauliflower was used [37]. Kapusta-Duch et al. reported DM of 9.18% for raw purple cauliflower [38]. During frozen storage, they observed a significant increase in DM to 11.7%. Similarly, Gębczyński and Kmiecik reported 7.28 and 6.61% DM in raw and blanched cauliflower, respectively [39]. When the blanched sample was further frozen at −30 °C, a DM of 7.56% was observed. For HPP samples, Melse-Boonstra et al. reported 7.7% and 6.8% DM, for control and HPP (200 MPa, 5 min) cauliflower, respectively. No other studies were found on DM in HPP cauliflower [40]. Drip loss of control and HPP cauliflower is presented in Table 3. The lowest drip loss ( $p < 0.05$ ) was observed for 600MPa 5 min cauliflower. The drip loss was significantly higher for HPP cauliflower than it was for untreated samples. There was not a significant difference in drip loss between treatment times for 2 and 5 min within each pressure level. For each treatment, drip loss remained stable during storage. On the other hand, for some days the SD was high, for instance on day 28 (400 MPa, 2 min). The use of a high vacuum (99.5%) and pressurization might explain some of the high drip loss of HPP cauliflower. Trejo Araya et al., in HPP carrots (100–550 MPa, 2/10/30 min), reported that pressures cause instantaneous deformation of plant cells, resulting in turgor loss and loss of water [41]. The findings from Préstamo and

Arroyo described above could also describe the drip loss of HPP cauliflower. Clariana et al. analyzed moisture loss during compression, observing higher water losses in HPP swedes at 400 than at 600 MPa [42]. The water loss during cutting when measuring the texture of cauliflower could be interesting to investigate further.

**Table 3.** Dry matter content (%), drip loss (%) and firmness (kg) for pieces of cauliflower measured before (control) and after HPP (400 and 600 MPa). The samples were stored for up to 42 days in cold storage (4 °C).

Storage (Days)	Dry Matter (%)					Drip Loss (%)					Firmness (kg)				
	Control		HPP			Control		HPP			Control		HPP		
	0.1 MPa	400 MPa	600 MPa	2 min	5 min	0.1 MPa	400 MPa	600 MPa	2 min	5 min	0.1 MPa	400 MPa	600 MPa	2 min	5 min
0	6.9 ± 0.3 <sup>a</sup>	6.1 ± 0.7 <sup>A,a</sup>	6.6 ± 0.2 <sup>A,a</sup>	6.3 ± 0.6 <sup>A,a</sup>	5.8 ± 0.1 <sup>A,a</sup>	18.0 ± 4.8 <sup>A,b,c</sup>	31.7 ± 7.6 <sup>A,a</sup>	32.3 ± 6.0 <sup>A,a</sup>	27.2 ± 5.6 <sup>A,a,b</sup>	33.0 ± 7.4 <sup>A,a</sup>	15.9 ± 3.8 <sup>a</sup>	14.4 ± 3.8 <sup>A,a</sup>	14.5 ± 4.3 <sup>A,a</sup>	15.6 ± 4.5 <sup>A,a</sup>	12.5 ± 6.4 <sup>A,B,a</sup>
14	nm <sup>**</sup>	6.3 ± 0.4 <sup>A,b</sup>	6.8 ± 0.4 <sup>A,ab</sup>	6.3 ± 0.3 <sup>A,b</sup>	6.2 ± 0.3 <sup>A,b</sup>	17.9 ± 3.9 <sup>A,b</sup>	32.9 ± 9.4 <sup>A,a</sup>	28.5 ± 3.3 <sup>A,a</sup>	30.6 ± 6.2 <sup>A,a</sup>	29.9 ± 5.1 <sup>A,a</sup>	nm <sup>**</sup>	11.3 ± 5.3 <sup>A,a</sup>	12.4 ± 4.0 <sup>A,a</sup>	13.6 ± 3 <sup>A,a</sup>	10.4 ± 5.8 <sup>B,a</sup>
28	nm	6.5 ± 1.0 <sup>A,a</sup>	6.5 ± 0.8 <sup>A,a</sup>	6.2 ± 0.3 <sup>A,a</sup>	6.4 ± 0.2 <sup>A,a</sup>	nm <sup>**</sup>	27.4 ± 6.1 <sup>A,a,b</sup>	29.5 ± 5.8 <sup>A,a,b</sup>	34.3 ± 6.6 <sup>A,a</sup>	26.6 ± 4.0 <sup>A,a,b</sup>	nm	16.6 ± 3.9 <sup>A,a</sup>	14.3 ± 5.9 <sup>A,a</sup>	13.1 ± 5.3 <sup>A,a</sup>	15.7 ± 5 <sup>A,a</sup>
42	nm	6.7 ± 0.4 <sup>A,ab</sup>	6.6 ± 0.5 <sup>A,ab</sup>	6.3 ± 0.1 <sup>A,ab</sup>	6.1 ± 0.4 <sup>A,b</sup>	nm	32.3 ± 12.7 <sup>A,a</sup>	27.4 ± 8.2 <sup>A,a</sup>	29.9 ± 6.0 <sup>A,a</sup>	27.0 ± 8.3 <sup>A,a</sup>	nm	17.1 ± 3.7 <sup>A,a</sup>	17.3 ± 2.6 <sup>A,a</sup>	16.0 ± 3.3 <sup>A,a</sup>	12.6 ± 3 <sup>AB,a</sup>

The capital letters (A and B) indicate a significant difference ( $p < 0.05$ , multiple Tukey's adjusted analysis of variance) within the same treatment and days (same column). Small letters (a, b, and c) indicate a significant difference ( $p < 0.05$ , multiple Tukey's adjusted analysis of variance) between different treatments (same row). \*\* Not measured.

As all samples in all experiments were processed in hermetically sealed bags, the content of DM and DL add up to the same total for each process, i.e., the DM lost from the cauliflower must be lost to the DL. According to Table 3 there was no correlation between DM and DL, or any possible correlation was overshadowed by the high variation between the parallel samples. Consequently, there is a large variation in the number of solids dissolved in the DL.

The importance of the loss in DM depends on how the product is used by the end consumer. If the DL is used in cooking the amount of DL is not important for the nutritional aspect, but if the DL is poured away the contents of the DL are important. Low DM content should then correlate with a loss of nutrients and the highest loss would be expected to be found for samples processed at 600 MPa for 5 min.

The total color difference (TCD) data for both florets and stems of cauliflower are summarized in Table 4 and Figure 1. All HPP florets had significantly higher ( $p < 0.05$ )  $b^*$ -values during storage compared to stems, indicating more yellow colors. Overall, samples processed at both 400 and 600 MPa and for both 2 and 5 min gave low changes in TCD during storage. On day 0, the stems of HPP samples were below or close to a TCD of 3, indicating low changes from the control sample. There was no significant interaction found between pressure and time during HPP on the TCD of both stems and florets ( $p > 0.05$ ). For stems subjected to 400 MPa (2 min), only day 42 was significantly different from days 0 and 14 ( $p < 0.05$ ). The high TCD value for stems of the samples until day 42 was due to low  $L^*$ -values (76.8 to 71.5) and increased  $a^*$ -values (−0.9. to +0.7), indicating darkening and less green color in the sample. Miglio et al. (2008) and Oey et al. (2008) explained that differences in color during HPP could be related to textural changes, where the loss of firmness could change the surface reflecting properties and light penetration, yielding



changes in lightness ( $L^*$ -value) [5,43]. Microbial deterioration during storage could also affect color measurements, such as sliminess [44]. Alvarez-Jubete et al. found an inverse correlation between texture values and changes in TCD of HPP white cabbage (400/600 MPa, 20/40 °C) [45]. This could also explain the changes in TCD of HPP cauliflower. Trejo Araya et al. found that the color of HPP carrots (600 MPa/2 min) was well preserved during storage (4 °C) for up to 14 days [44]. These findings agree with those of Vervoort et al., who reported a TCD of 2.52 for HPP carrots (600 MPa, 10 °C, 10 min) [32].

**Table 4.** The color difference (TCD) \* for stems (A) and florets (B) of cauliflower as measured after HPP (400 and 600 MPa) treatment. The samples were stored for up to 42 days in cold storage (4 °C).

A. Stem					
Storage (Days)	Control	HPP			
	0.1 MPa	400 MPa		600 MPa	
	0 min	2 min	5 min	2 min	5 min
0	-	2.9 ± 1.7 A, a	3.3 ± 1.3 A, a, b	3.2 ± 1.5 A, a, b	3.2 ± 1.4 A, a, b
14	nm **	2.8 ± 1.2 A, a	3.2 ± 1.3 A, a, b	3.7 ± 1.4 A, a, b, c	3.9 ± 1.9 A, c, b
28	nm	3.5 ± 1.7 A, B, a	3.7 ± 1.1 A, a	3.4 ± 1.5 A, a	3.7 ± 1.5 A, a
42	nm	4.2 ± 2.2 B, a	3.9 ± 1.4 A, a	3.4 ± 1.6 A, a	4.0 ± 1.5 A, a

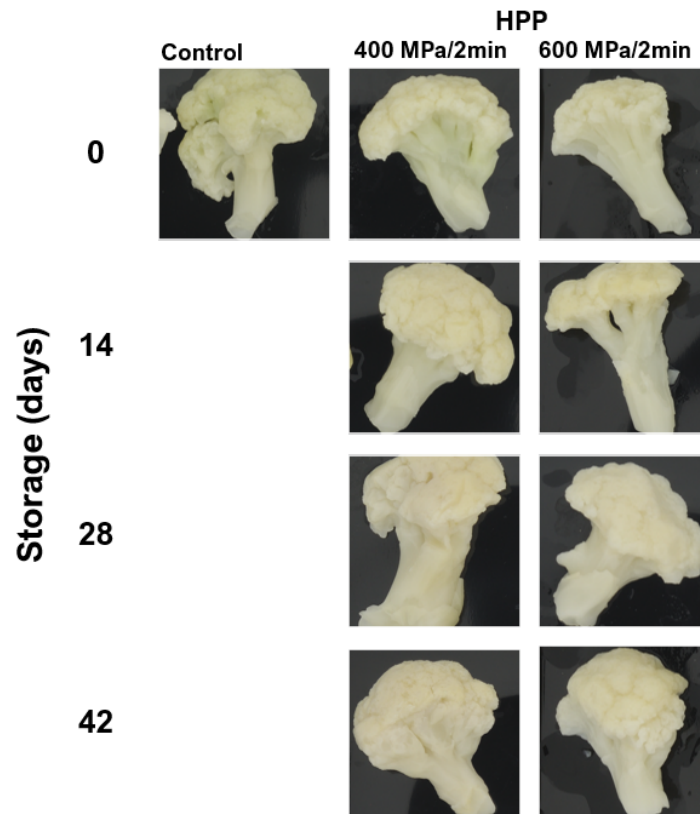
B. Floret					
Storage (Days)	Control	HPP			
	0.1 MPa	400 MPa		600 MPa	
	0 min	2 min	5 min	2 min	5 min
0	-	4.0 ± 2.4 A, a	4.7 ± 2.9 A, a, b	4.2 ± 1.9 A, a	4.2 ± 2.3 A, a
14	nm	4.3 ± 1.9 A, a	5.1 ± 2.1 A, a, b	4.2 ± 2.0 A, a	4.5 ± 2.1 A, B, a, b
28	nm	4.5 ± 2.2 A, a	4.7 ± 2.3 A, a	4.7 ± 2.0 A, a	5.7 ± 2.5 B, a
42	nm	4.8 ± 2.5 A, a	5.1 ± 2.2 A, a	4.6 ± 2.1 A, a	5.0 ± 2.0 A, B, a

\* Each color value (TCD) in the table is the mean ± standard deviation from processing parallel samples ( $n = 3$ ). The capital letters (A and B) indicate significant differences ( $p < 0.05$ , multiple Tukey's adjusted analysis of variance) within the same treatment and storage time (same column). Small letters (a, b, and c) indicate significant differences ( $p < 0.05$ , multiple Tukey's adjusted analysis of variance) between different treatments (same row). \*\* Not measured.

Changes in the texture of the cauliflower were measured using a shear force test. As can be seen from Table 3, none of the HPP procedures resulted in a significant difference ( $p < 0.05$ ) from the control sample at day 0. The only exception from this was 600 MPa, 5 min, at day 14 ( $p > 0.05$ ). During storage, the texture remained firm for HPP samples. The overall picture of the HPP samples shows that the texture remains intact both during storage and between the different conditions used. The duration of the HPP did not result in a significant difference between the HPP samples ( $p > 0.05$ ).

Consequently, from an industrial point of view, the use of 2 min instead of 5 min could be beneficial. On a general basis, HPP of vegetables has been found to yield low changes in pectin solubilization and depolymerization [32], but this is dependent on the type of vegetable. For instance, Arroyo et al. observed that tomato loosened the skin but retained its firm texture during HPP from 300–400 MPa (10 °C, 20 min), while the flavor and color remained unchanged [22]. Alvarez-Jubete et al. found that the texture of white cabbage significantly increased in firmness after HPP at 600 and 400 MPa at 20 °C, compared to the non-treated samples [45]. They suggested that HPP could liberate pectin methylesterase because of cell rupture. In turn, the de-esterified pectin could form a gel network that could give increased firmness in vegetables. This could also explain the HPP preservation of the texture in the cauliflower samples. Clariana et al. observed a similar trend, where swedes subjected to 600 MPa (20 °C, 5 min) yielded significantly firmer textures than those subjected to 400 MPa during compression [42]. They explained this via a possible inactivation of polygalacturonase (PG) at pressures above 500 MPa. When the same authors measured the cutting force, swedes at 600 MPa resulted in a similar texture as the control,

while at 400 MPa the texture increased further in firmness. For carrots, Trejo Araya et al. found slightly higher cutting forces for HPP (600 MPa, 2 min) samples until day 14 of cold storage, compared to non-treated carrots [44]. This trend was also observed for HPP cauliflower during day 42 of storage. More recently, Hu et al. (2020) subjected frozen pumpkin ( $-20\text{ }^{\circ}\text{C}$ ) to pressure (100–600 MPa/2 min) and found a significant reduction in the texture attribute “hardness” [46]. At 400 and 600 MPa, they reported a decrease in hardness of approximately 42% compared to the control. During storage for seven days, the authors observed a reduction in hardness of 54% (600 MPa) and 36% (400 MPa). These findings were not in agreement with the cauliflower samples and could be a result of different plant structures.



**Figure 1.** Visual comparison of selected pieces of cauliflower after HPP treatment of 400 MPa/2 min until day 42.

The high firmness of cauliflower during HPP could be explained by the finding that HPP of cauliflower (400 MPa/30 min/ $5\text{ }^{\circ}\text{C}$ ) resulted in cavity formation and a firm and soaked texture and appearance. This was explained by loss of turgor pressure within the cell and the presence of fluid in intercellular spaces that were previously filled with gas. Arroyo et al. conducted a sensory evaluation of HPP cauliflower (400 and 350 MPa / $5\text{ }^{\circ}\text{C}$ /30 min) and reported the texture as “firm” [22].

### 3.3. Effect of HPP on the Nutrient Composition of Cauliflower

Only the HPP cauliflower treated at 600 MPa for 2 min was chosen to be further analyzed chemically (until day 28). The choice of 600 MPa and 2 min was selected due to lower bacterial counts (compared to the other HPP parameters), and because 5 min did not differ significantly from 2 min in the abovementioned quality parameters (Table 5).

**Table 5.** DPPH antioxidant activity (%), total phenolic content (TPC) expressed as mg gallic acid equivalents (GAE)/100 g DW and content of ascorbic acid (AA) (mg/100g DW) of cauliflower measured before (control) and after HPP treatment.

Storage (Days)	DPPH Antioxidant Activity (%)		TPC as mg Gallic Acid Equivalents (GAE)/100 g		AA (mg/100g DW)	
	Control	HPP	Control	HPP	Control	HPP
	0.1 MPa	600 MPa	0.1 MPa	600 MPa	0.1 MPa	600 MPa
	0 min	2 min	0 min	2 min	0 min	2 min
0	72.1 ± 2.5 <sup>A</sup>	52.4 ± 6.9 <sup>C, b</sup>	579.6 ± 19.4 <sup>A</sup>	506.6 ± 55.6 <sup>B, a</sup>	890.9 ± 53.6 <sup>A, B</sup>	862.1 ± 45.6 <sup>B, a</sup>
14	nm <sup>**</sup>	46.5 ± 4.6 <sup>c</sup>	nm <sup>**</sup>	477.8 ± 47.9 <sup>a</sup>	Nm <sup>**</sup>	730.7 ± 22.2 <sup>b</sup>
28	nm	68.3 ± 4.3 <sup>a</sup>	nm	510.8 ± 24.6 <sup>a</sup>	nm	790.4 ± 95.0 <sup>a, b</sup>

The capital letters (A, B, and C) indicate significant differences ( $p < 0.05$ , multiple Tukey's adjusted analysis of variance) within the same treatment and days (same column). Small letters (a, b, and c) indicate significant differences ( $p < 0.05$ , multiple Tukey's adjusted analysis of variance) among different treatments and days (same row). \*\* Not measured.

No studies on the effects of HPP on antioxidant activity (AOC) in cauliflower were found. For other vegetables, the effects of HPP on AOC have been shown to vary. Alvarez-Jubete et al. subjected white cabbage to HPP (200/400/600 MPa, 20/40 °C), and found that 600 MPa gave significantly higher retention of AOC with almost four times higher activity compared to 200 and 400 MPa [45]. Clariana et al. (2011) also found the same trend, where swedes pressurized at 600 MPa were insignificantly higher than the control and had 2.6 times higher AOC than at 400 MPa. This could indicate that lower pressure levels would not be as beneficial for cauliflower, although this would have to be tested first [42].

Other studies on HPP's effects on TPC in vegetables show that in most cases, pressure increases the concentration of phenolic compounds. For instance, Alvarez-Jubete et al. (2014) found that among HPP treatments for white cabbage, 600 MPa yielded the highest retention of TPC (393 mg GAE/100 g DW) compared to lower pressures. Clariana et al. also observed a similar trend in swedes, where 600 MPa gave the highest retention of TPC (399 mg GAE/100 g DW) compared to lower pressures [42,45]. These findings indicate that higher pressures for cauliflower could be beneficial in nutrient retention.

Ascorbic acid (AA) is regarded as one of the most heat-sensitive vitamins and is therefore used as a key indicator of the effects of processing on vitamins (Davey et al., 2000). The results in Table 5 represent the influence of different processing conditions on the content of AA (mg/100g dry weight (DW)) in pieces of cauliflower. The results reveal that HPP had a negative impact on ( $p < 0.05$ ) AA content. The treated samples resulted in a 40% reduction in AA compared to the control. HPP is a mild processing condition that does not significantly affect low-molecular-weight molecules, such as AA, because of the low compressibility of covalent bonds [31]. On the other hand, some degradation of AA could result due to remaining active enzymes that were not inactivated by the 600 MPa pressure.

The effects of HPP on AA in vegetables and fruits were shown to vary. Furthermore, Alvarez-Jubete et al. found a significant reduction in AA in white cabbage after HPP (600/400 MPa, 20 °C, 5 min) compared to a non-treated control. Their control had approximately 840 mg AA/100 g DW, while 400 MPa yielded under 100 mg/100 g DW, and 600 MPa yielded approximately 620 mg/100 g DW [45]. A similar trend was also observed in Clariana et al., who subjected swede to the same HPP conditions as the mentioned study above, with 81% retention of AA at 600 MPa (664 mg/100 g DW), while 400 MPa gave the lowest retention of AA (257 mg/100 g DW) [42]. They hypothesized that the high retention at 600 MPa could be a result of higher inactivation of the POD enzyme.

#### 4. Conclusions

The impact of HPP on the quality of cauliflower was investigated. During storage, pressures at 600 MPa for 2 min resulted in minor changes in texture, color, drip loss, dry matter, and nutritional value compared to the control. Regarding bacterial counts, 600 MPa

resulted in better preservation compared to 400 MPa, with moderate bacterial counts until day 28 of storage. From day 28 to day 42, high bacterial counts were observed (up to 7.5 log cfu/g) for 600 MPa. Low bacterial counts for both aerobic and anaerobic spores were detected for all treatments. Since the holding time of pressure treatment did not significantly impact the physical quality, a processing time of 2 min can be advised.

To obtain better preservation and quality, a combination of HPP with other treatments, such as heat, should be investigated. As heat treatment influences sensory parameters, analysis of taste, texture, and color of the processed cauliflower should be carried out. In addition, it would be interesting to investigate the processed cauliflower after reheating, as it would in an RTE meal.

The microbial flora also needs to be further investigated, where different target pathogens could be inoculated in the cauliflower. Since nutritional analysis was only performed for the 600 MPa–2 min samples, it would have been of interest to perform nutritional analysis to evaluate and compare with other pressure and time combinations.

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