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THE EFFECT OF MINIMAL PROCESSING TECHNOLOGIES ON FRUIT PUREE PRODUCTS

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1. BACKGROUND OF THE WORK, OBJECTIVES

It is well known that a balanced diet is based on the regular consumption of fruits and vegetables. The vitamins, antioxidants, and minerals found in them are essential for maintaining our health. Although most fruits and vegetables are consumed fresh, due to their seasonal nature, most of them are not available all year round. Conscious consumers look for preservative- and additive-free products, which poses challenges for the food industry. Traditional preservation methods based on heat treatment (thermal) generally reduce the fresh character of fruits and do not have a favourable effect on the valuable components found in them. The growing demand for high-quality, fresh food induced the creation of a new trend in preservation: gentle technologies (also known as minimal processing). These are non-thermal processes that enable preservation without the addition of chemicals and without the loss of valuable nutritional components. An excellent example of this is High Hydrostatic Pressure processing (HHP), which achieves shelf-life by applying several hundred MPa of pressure while preserving the sensory and nutritional value of the product.

Since HHP alone is not always effective enough against pressure-resistant bacteria and spores, it is often applied in combination with other treatments. Combined treatment is based on hurdle technology, where several inhibitory factors are applied simultaneously, the effects of which are not only additive but promote microbiological safety in a synergistic manner. Thanks to technological development, these new products made with minimal processes are becoming more widely available, but further research is needed to produce products that are fully compliant from a food safety perspective.

In my thesis, I investigated the effect of different combinations of gentle heat treatment and high hydrostatic pressure treatment on fruit puree products. The starting point of my research work was a previous study **(Salamon et al., 2021**), in which combinations of 5-minute pressure treatments at 300, 450, and 600 MPa, as well as 10-minute heat treatments at 55, 65, and 75 °C, were applied to strawberry puree samples. We examined its colour, anthocyanin content, and sensory characteristics immediately after the treatments, as well as following two weeks of storage at 2 °C and 15 °C. Based on these results, the treatments used proved to be effective in terms of preserving the physico-chemical and sensory characteristics of the strawberry puree. Therefore, in my thesis, I approached the changes occurring due to treatments and storage primarily from a microbiological and food safety perspective instead of examining nutritional factors. Initially, I only changed one of the storage temperatures from the experimental settings used in the previous research: I stored the samples at 6 °C and 15 °C for two weeks instead of 2 °C and 15 °C. This change was justified by the fact that although according to the recommendations of the **WHO and FAO (2009**), the temperature of the refrigerator should be between 2–4 °C, several surveys report that the temperature of refrigerators in households exceeds even 6 °C (**Laguerre**

et al., 2002; Ovca et al., 2021). With the two chosen storage temperatures, I intended to model average cooling and incorrect storage conditions (e.g., a faulty refrigerator).

Furthermore, my goal was to use strawberry puree as a main ingredient in a product and to examine the effect of treatments and storage within this complex system on the general microbiological state of the product and on foodborne pathogenic bacteria. My aim was to select a treatment combination that represents the minimum dose necessary for the preservation of the product from a food safety and microbiological point of view, while influencing its original properties to the smallest extent possible. Additionally, my objective was to investigate, through both human sensory evaluation and instrumental measurements, what sensory changes the selected treatment combination causes in the examined product. The aim of my investigations was also to answer whether the order of treatments is significant, and if so, which sequence is more advantageous to apply?

2. MATERIALS AND METHODS

A portion of the experiments was conducted with strawberry (strawberry puree), then the strawberry was used as a raw material in a smoothie, which formed the subject of further experiments.

2.1. Experiments with strawberry

2.1.1. Sample preparation

To ensure the constant quality of the raw material, quick-frozen strawberries were used for the experiments. The thawing of the strawberries took place in their original packaging at room temperature. The thawed strawberries were pureed using a Robot Coupe C80A type pulping machine. The finished puree (pH 3,6) was filled into sterile bags in 25 g portions. To investigate the effect of the applied heat and pressure treatment on pathogenic bacteria, a portion of the samples was inoculated before sealing the bags with a mixture of *Listeria monocytogenes* CCM4699 and *Salmonella enterica* subsp. *enterica* serotype Hartford B.01310 (hereinafter referred to as *Salmonella* Hartford) so that their initial cell count was 10^6 CFU/ml. To determine the combined amount of aerobic and facultative anaerobic, mesophilic, and psychrotrophic microorganisms (hereinafter: total microbial count), the other part of the samples containing the original (uninoculated) strawberry puree was hermetically sealed immediately after filling.

2.1.2. HHP and heat treatment of strawberry puree samples

The treatment of the samples was carried out based on a 2n type factorial experimental design. Some of the samples underwent heat or pressure treatment applied alone, while another part was subjected to a combination of the two treatments applied in different orders. Henceforth, samples that were first heat-treated and then pressure-treated are called TP-treated, and those that were first pressure-treated and then heat-treated are called PT-treated. Several series of treatments were performed with the strawberry puree, the results of two of which are presented in detail.

In the first experiment, the heat treatments used were carried out at 55, 65, and 75 °C for 10 minutes, and the pressure treatments at 300, 450, and 600 MPa for 5 minutes. In the second series of experiments, a 5-minute heat treatment at 50, 55, and 60 °C and a 5-minute pressure treatment at 150, 200, and 250 MPa were applied. In both experimental series, 15 types of treatments were applied according to **Tables 1 and 2**, in 3 replicates. Heat treatments were performed in a water bath (Labor Műszeripari Művek LP507/1), and HHP treatments were carried out using a Resato FPU-100-2000 type equipment manufactured by Resato International B.V. (Assen, Netherlands) at room temperature. Resato PG fluid was used as the pressure transmitting medium. During the treatments, the

degree of adiabatic temperature rise was minimal. The samples were immediately cooled with ice water following the treatments.

Table 1. *Treatment settings applied during the 1. series of experiments*

No.	1. treatment	2. treatment
1(control)	-	-
2	55 °C	-
3	300 MPa	-
4	75 °C	-
5	600 MPa	-
6	55 °C	300 MPa
7	300 MPa	55 °C
8	75 °C	300 MPa
9	300 MPa	75 °C
10	55 °C	600 MPa
11	600 MPa	55 °C
12	75 °C	600 MPa
13	600 MPa	75 °C
14	65 °C	450 MPa
15	450 MPa	65 °C

Table 2. *Treatment settings applied during the 2. series of experiments*

No.	1. treatment	2. treatment
1(control)	-	-
2	50 °C	-
3	150 MPa	-
4	60 °C	-
5	250 MPa	-
6	50 °C	150 MPa
7	150 MPa	50 °C
8	60 °C	150 MPa
9	150 MPa	60 °C
10	50 °C	250 MPa
11	250 MPa	50 °C
12	60 °C	250 MPa
13	250 MPa	60 °C
14	55 °C	200 MPa
15	200 MPa	55 °C

2.1.3. Determination of Total Plate Count

To determine the total plate count (TPC), the pour-plate method was applied (MSZ EN ISO 4833-1:2014), with the modification that 0.5 ml of 5% triphenyl tetrazolium chloride (TTC) solution was added to 500 ml of plate count agar (PCA agar, Merck 105463) to facilitate counting. The plates were evaluated after three days of incubation at 30 °C under aerobic conditions. The detection limit was 1 CFU/ml. The analyses were performed in triplicate.

In the first experimental series with strawberries, the total plate count of the samples was examined immediately after treatment (following cooling), as well as after two weeks of storage at 6 and 15 °C. In the second experimental series, in addition to the two aforementioned sampling times, total plate counts were also determined after one week of storage.

Samples examined immediately after treatment, following cooling, are hereafter referred to as "fresh samples."

2.1.4. Examination of the effects of treatments on pathogenic bacteria

The presence of *Salmonella* and the count of *Listeria monocytogenes* were determined according to standard methods (MSZ EN ISO 6579-1:2017, MSZ EN 11290-2:1998) in triplicate. The detection limit for both pathogens was 10 CFU/ml.

According to Regulation (EC) No 2073/2005, different limit values apply to foods that support the growth of *Listeria monocytogenes* and those that do not. To determine which category strawberry puree falls into and which detection method should be applied, a preliminary experiment was conducted (the results of which are not presented). It was established that strawberry puree does not support the growth of *Listeria monocytogenes*; therefore, a presence/absence test was not performed.

The counts of *Salmonella* and *Listeria monocytogenes* were determined after preparing a decimal dilution series using the spread-plate method: on XLD agar (OXOID HARLEQUIN™ *SALMONELLA* ABC MEDIUM, Oxoid PO0993A) for *Salmonella*, and on ChromoCult agar (ChromoCult® *Listeria* agar (base) acc. OTTAVIANI and AGOSTI, Merck 100427) for *Listeria monocytogenes*. Evaluation was performed after incubation at 37 °C under aerobic conditions for one day for *Salmonella* and two days for *Listeria*.

The count of pathogenic bacteria was examined in fresh samples, as well as on the 3rd, 7th, and 14th days of storage.

2.1.5. Data Analysis

For the statistical analysis of total plate count results, one-way ANOVA followed by Tukey's or Games–Howell post hoc tests were performed ($p<0.05$) using SPSS (version 27.0.1.0). The normality of the data was verified using Shapiro-Wilk or Kolmogorov–Smirnov tests, and homogeneity of variance was checked using the Levene's test. During evaluation, samples stored for the same duration under identical conditions were compared. Furthermore, Response Surface Methodology (RSM) was also applied during the evaluation of the results.

2.2. Experiments with smoothie

2.2.1. Sample preparation

The smoothie I made contained strawberry (37%), almond drink (26%), banana (24%), and avocado (13%). The strawberries were again obtained in a frozen state, while the other fruits were purchased fresh. The ingredients were blended into a homogeneous mixture (Robot Coupe Mini MP 160 V.V.), and the finished smoothie was filled into pressure-resistant, sterile bags in 25 g portions. Subsequently, in the manner previously described for strawberry (Chapter 4.1.1), a portion of the smoothie samples was inoculated before sealing the bags with a mixture of *Listeria monocytogenes* and *Salmonella* Hartford so that their initial cell count was 10^6 CFU/ml. The other part of the samples (original, uninoculated) was sealed immediately after filling.

2.2.2. HHP and heat treatment of smoothie samples

The heat and/or pressure treatment of the smoothie samples was performed as described for the strawberry (Chapter 4.1.2). In this case, heat treatment was carried out at 50, 55, and 60 °C for 5 minutes, and pressure treatment at 150, 200,

and 250 MPa for 5 minutes. A total of 15 different treatments were performed in three replicates, according to **Table 2**. The samples were again stored at 6 or 15 °C for two weeks. All batches were prepared under identical chilled conditions (< 10 °C) and adjusted to the same pH value (pH 4.5).

2.2.3. Determination of total microbial count and investigation of the effect of treatments on pathogenic bacteria

Regarding the determination of the total microbial count of the smoothie, as well as the count of *Salmonella* and *Listeria monocytogenes*, I proceeded as described for the strawberry (Chapters 4.1.3 and 4.1.4). Both the total microbial count and the pathogenic bacteria count were determined in three replicates. In a preliminary experiment, I also established for the smoothie that it does not support the growth of *Listeria monocytogenes* (results are not presented), so a presence/absence test was not necessary. The detection limit was 1 CFU/ml for the total microbial count and 10 CFU/ml for the two pathogens. Sampling for the total microbial count was performed on fresh samples and after 7 and 14 days of storage. The number of pathogenic bacteria was determined in fresh samples and on storage days 3, 7, and 14.

2.2.4. Sensory evaluation

During the sensory evaluation, the assessors qualified both the fresh samples and those stored for 14 days. To ensure the fresh and stored samples were comparable, the smoothie samples were prepared in two separate batches. The first batch consisted of samples stored at 6 °C for 14 days, while the second batch consisted of fresh samples prepared on the day of evaluation. Sensory evaluation was performed by 20 untrained assessors from the Institute of Food Science and Technology at the Hungarian University of Agriculture and Life Sciences, whose task was to rank the samples according to the following criteria:

- from least brown to most brown;
- from least fruity aroma to most fruity aroma;
- from least dense texture to most dense texture;
- from least fruity taste to most fruity taste;
- from least liked to most liked.

To determine if there were significant differences between samples, the largest differences between rank sums were compared with the critical values of differences between rank sums. If the difference was significant, multiple comparisons had to be performed to determine which pairwise comparison resulted in a significant difference. Samples are considered significantly different if the difference between their rank sums is equal to or exceeds a specified critical value (**Christensen et al., 2006**).

2.2.5. Investigation of rheological properties

Rheological properties were evaluated according to the method of **Hidas et al.** (**2023**) for fresh and 14-day stored samples at 20 °C, in three replicates. Measurements were carried out using a concentric cylinder geometry system on a rotating MCR 92 rheometer (Anton Paar, Ltd., Les Ulis, France). The device is operated using RheoCompass software (version 1.21.852, Anton Paar, Ltd.). Shear stress and apparent viscosity were measured at 3-second intervals in the logarithmically increasing and decreasing shear rate range between 10 and 1000 s^{-1}. The evaluation was performed by comparing the decelerating sections of the flow curves (shear rate-shear stress) and viscosity curves (shear rate-shear stress-apparent viscosity). To further characterize the rheological behaviour of the samples, I applied the Herschel-Bulkley model – which I previously determined to be the best-fitting model – using Excel Solver (least squares method). To judge the goodness of fit, the coefficient of determination (R^2>0.95) was used.

2.2.6. Colour measurement

Colour characteristics were quantified using a Konica Minolta CR-400 (Konica Minolta, INC., Tokyo, Japan) tristimulus colourimeter in 5 replicates. The instrument determines lightness (L*), red-green (a*), and yellow-blue (b*) colour coordinates. From these coordinates, the ΔE∗ colour difference, chroma value (C*), and hue angle (h°) can also be calculated.

2.2.7. Electronic nose analysis

The investigation was conducted at the Adexgo Kft. site in Herceghalom using a Heracles Neo 300 electronic nose (Alpha M.O.S., Toulouse, France) according to the methodology published by **Yakubu et al.** (**2022**). 1 gram aliquots of each sample were weighed into 20 ml headspace vials in 8 replicates and sealed with magnetic caps equipped with UltraClean™ polytetrafluoroethylene/silicon septa. Retention times of chromatograms recorded on the flame ionization detectors (FID) of the two gas chromatography columns (MXT-5; MXT-1701, Restek, Bellefonte, PA, USA) of the electronic nose were converted into retention indices (RI) (**Roszkos et al., 2021**). AlphaSoft (ver 16) software (Alpha M.O.S., Toulouse, France) was used to operate the electronic nose and record data.

2.2.8. Electronic tongue analysis

Electronic tongue measurements were performed at the MATE Department of Food Measurement Technology and Automation using an Alpha Astree type electronic tongue (Alpha M.O.S., Toulouse, France). The instrument uses an Ag/AgCl reference electrode and chemically modified field-effect transistor sensors (AHS, PKS, CTS, NMS, CPS, ANS, SCS) developed for liquid analysis. To prepare the samples, 10 g of sample was placed in a 100 ml volumetric flask and filled to the mark with distilled water. The samples were then filtered through a metal filter first, then through filter paper. Before the actual analysis of the smoothie samples, the instrument was conditioned in two steps recommended by

the manufacturer. In the first step, a 0.01 mol/dm^3 HCl solution was used, and in the second step, an equal-part mixture of the examined smoothie samples was used for conditioning to reduce sensor drift. Signal collection was performed for 120 seconds for each randomly examined sample. Each sample was measured nine times with the electronic tongue, resulting in a total of 54 readings for the examined smoothie samples.

2.2.9. Investigation of bioactive components

The anthocyanin content of the samples was determined using the pH differential method (**Giusti and Wrolstad, 2001**). The method is based on the colour change of monomeric anthocyanin components. The coloured form appears at pH 1.0, and the colourless form at pH 4.5; the change is reversible. The absorbance of the samples was determined at 520 and 700 nm using a Hitachi U-2900 type spectrophotometer. The concentration of anthocyanin pigments was expressed as cyanidin-3-glucoside equivalents.

The antioxidant capacity value of the samples was determined using the FRAP (Ferric Reducing Ability of Plasma) method (**Benzie and Strain, 1996**). The essence of the method is that antioxidant compounds reduce Fe^{3+} ions to Fe^{2+} ions, which form a blue ferro-tripyridyl-triazine complex with tripyridyl-triazine (TPTZ). The complex thus formed can be measured photometrically at 593 nm. The FRAP value is obtained by comparing the absorbances measured at 593 nm of the sample to be measured and a sample with a known Fe^{2+} concentration.

Total polyphenol content determination was performed using Folin-Ciocalteau reagent based on the method of **Singleton and Rossi** (**1965**). Total polyphenol content was determined based on a calibration curve prepared from gallic acid at 760 nm.

2.2.10. Data analysis and chemometrics

Results obtained during the investigation of total microbial count and pathogenic bacteria were analysed using one-way ANOVA or two-way ANOVA models, followed by pairwise comparisons with Tukey's or Games–Howell post hoc tests ($p < 0.05$) using SPSS (version 27.0.1.0). Data normality was checked with Shapiro-Wilk or Kolmogorov–Smirnov tests, and homogeneity of variance with the Levene test. During evaluation, I first examined the temporal change in microbial counts during the two-week storage at 6 °C and 15 °C. Subsequently, the results were analysed grouped by storage time and then by storage temperatures. In many cases, the number of pathogenic bacteria remained below the detection limit; consequently, statistical evaluations were only performed on samples with quantifiable results. Response Surface Methodology (RSM) was also applied during the evaluation of the results, which, for the reasons mentioned above (pathogens were often not detectable), was only possible for total microbial counts.

Colour and rheological measurement results were also analyzed using one-way ANOVA models, followed by Tukey's pairwise comparison (colour) or Games-Howell test (rheology) ($p < 0.05$) using SPSS (version 27.0.1.0). Data normality was checked with Shapiro-Wilk or Kolmogorov–Smirnov tests, and homogeneity of variance with the Levene test. For both parameters, samples were compared using two approaches: one comparing fresh and stored samples treated in the same way, and the other comparing three fresh and three stored samples (control; 60 °C-250 MPa; 250 MPa-60 °C).

In the case of the electronic tongue, the stabilized and optimally sensitive signals of the various sensors—i.e., data from the last 10 seconds of the recorded signals—were averaged and used for further statistical analysis. These raw sensor signals were first visualized to detect potential sensor errors, drifts, and outliers. Differences detected by the electronic tongue sensors in the chemical fingerprints of the examined samples were compared using Euclidean distances (**Ito et al., 2013, Legin et al., 2009**).

For drift correction of sensor signals, the "Additive correction relative to all samples" method described by **Kovacs et al.** (**2020a**) was applied to avoid potential baseline differences and sensor signal drift. Chemometric evaluation of sensor signals was performed using Principal Component Analysis (PCA). Calculations and visualization were performed using R-project (ver. 4.2.3) (**R Core Team, 2023**).

Multivariate data describing the odour profiles of the samples from electronic nose measurements were analysed using AlphaSoft (ver. 16) software. Chromatograms were converted into a signal sequence of virtual sensors based on identified chromatogram peaks and areas under the curves (**Kovacs et al., 2020b**). Data processing and evaluation of multivariate data were performed as described by **Yakubu et al.** (**2022**). The most characteristic virtual sensors were selected during data evaluation. Specific volatile compounds were assigned to the appropriate retention index associated with the virtual sensor using AlphaSoft software AroChemBase v7.

To evaluate the results of bioactive components, a paired-sample T-test ($p < 0.05$) using Excel and a one-way ANOVA model followed by Tukey's post hoc test ($p < 0.05$) using SPSS (version 27.0.1.0) were applied. Data normality was checked with Shapiro-Wilk or Kolmogorov–Smirnov tests, and homogeneity of variance with the Levene test.

3. RESULTS AND DISCUSSION

3.1. Results of experiments with strawberry

3.1.1. Results of the experimental series performed in the higher pressure and temperature range

3.1.1.1. Changes in total microbial counts due to treatments and storage

Based on the results of the experiments conducted with strawberry puree in the pressure range of 300–600 MPa and the temperature range of 55–75 °C, all treatments were able to maintain the total microbial count at an acceptable level during the two-week storage at 6 °C. Moreover, with the exception of the 55 °C heat treatment and the 300 MPa pressure treatment applied alone, all treatments resulted in a product of adequate quality even at higher storage temperatures, based on the 4/1998 (XI.11.) EüM decree (limit value: 10^3 CFU/ml). Except for the sample heat-treated at 55 °C, all treated samples differed significantly from the control. Among the combined treatments, no distinction could be made as to which order (TP or PT) was more advantageous.

3.1.1.2. Investigation of total microbial count changes using response surface analysis

In the experiment conducted in the 300–600 MPa pressure range and 55–75 °C temperature range, there was no significant difference between the samples treated in TP (temperature-pressure) and PT (pressure-temperature) order for either the fresh or the 14-day stored samples. Regardless of the treatment order, a linear relationship could be observed between the total microbial count and the independent variables (pressure and temperature). As pressure and temperature increased, the total microbial counts decreased.

3.1.1.3. Investigation of the effect of treatments on pathogenic bacteria

During the experiments, all treatments proved effective against *Salmonella* Hartford and *Listeria monocytogenes*, as they could only be detected in the fresh control sample. It should be noted that, beyond the effect of the treatments, the acidic pH of the strawberry (pH 3.6) also played a significant role in the results (hurdle theory). Based on the results, the applied treatments were successful from a microbiological point of view, and furthermore, based on previous investigations, regarding colour, sensory properties, and anthocyanin content (**Salamon et al., 2021**). Since my goal was to find a treatment combination that is minimally necessary for shelf-life extension and microbiological food safety while having the least possible impact on valuable nutritional components, I decided to reduce the treatment parameters. I repeated the previously presented experiment, but this time I only examined the microbiological effect of the treatments. After examining several treatment series, I finally reached the 50–55–60 °C and 150–200–250 MPa range, which was at the limit below which treatment parameters cannot be reduced for microbiological food safety reasons.

Subsequently, I focused on the investigation of this specific pressure range below 300 MPa.

3.1.2. Results of the experiment performed in the reduced pressure and temperature range

3.1.2.1. Changes in total microbial counts due to treatments and storage

Based on the results of the experiment conducted in the 150–250 MPa pressure range and 50–60 °C temperature range, following 14-day storage at 6 °C, the total microbial count of all samples proved to be adequate based on the 4/1998 (XI.11.) EüM decree, with the exception of the two lowest-level treatments (50 °C and 150 MPa). However, following storage at the higher temperature of 15 °C, only those samples met the limit value prescribed by the decree where combined treatment was applied, and only in cases where the two lowest treatment levels (50 °C and 150 MPa) were not combined.

In the case of fresh samples, with the exception of the 50 °C, the 150 MPa, and the combination of these two, all treated samples differed significantly from the control; the applied treatments reduced the total microbial count of the strawberry puree by approximately 1 order of magnitude.

During the two-week storage at 15 °C, the differences between samples treated in different ways became increasingly significant. However, among the combined treatments, I could not distinguish between treatment orders during either the 15 °C or the 6 °C storage.

3.1.2.2. Investigation of total microbial count changes using response surface analysis

The response surfaces fitted to the total microbial count results of the experiment performed in the 150–250 MPa pressure range and 50–60 °C temperature range showed a similar trend to the response surfaces fitted to the results of the experiment performed in the higher pressure and temperature range (the relationship between variables was linear), except for the PT- and TP-treated samples stored for 7 days at 6 °C. Total microbial counts decreased with increasing pressure and temperature. For the TP-treated samples stored for 7 days at 6 °C, the relationship between total microbial counts and the independent variables is not entirely linear, but the linear model dominates, whereas for the samples treated in PT order, a curved response surface was obtained.

3.1.2.3. Investigation of the effect of treatments on pathogenic bacteria

During the investigation of the effect of reduced treatment parameters on pathogenic bacteria, growth was observed for *Salmonella* at both 6 °C and 15 °C in the control and in the sample treated at 150 MPa. *Listeria monocytogenes* was detectable this time from the control stored at 15 °C during the examined storage period.

3.2. Results of experiments with the smoothie

3.2.1. Effect of treatments on the total microbial count of the smoothie

3.2.1.1. Temporal changes in total microbial counts during two-week storage

The total microbial count of samples stored at 6 °C increased during the two-week storage in the control and in cases where only heat or only pressure treatment was applied. In these instances, the total microbial count increased by as much as 2.5–3 orders of magnitude compared to the initial cell count by the end of storage. In contrast, for samples where combined treatments were applied, the total microbial counts remained nearly constant. All combined treatments resulted in samples of adequate quality according to the 4/1998 (XI.11.) EüM decree, as the total microbial count of the samples stored for two weeks remained below 10^3 CFU/ml.

During the two-week storage at 15 °C, the total microbial count of the samples clearly showed an increase. However, for samples where the highest temperature (60 °C) or its combination with pressure treatment was applied, as well as for the combination of medium temperature and pressure values (55 °C and 200 MPa), the cell counts measured after one week were roughly equivalent to the values on day 0. In the two cases where 60 °C was combined with the highest pressure (250 MPa), the extent of the increase in total microbial count after two weeks was smaller than for other treatments. For samples treated at 250 MPa–60 °C and 60 °C–250 MPa, I observed an increase of only 2–2.5 orders of magnitude compared to the initial total microbial counts. For the 60 °C, 200 MPa–55 °C, and 55 °C–200 MPa treated samples, the increase was 4–4.5 orders of magnitude, while for the other samples, it reached 6–7.5 orders; these samples spoiled.

None of the samples stored at 15 °C for two weeks met the requirements of the 4/1998 (XI.11.) EüM decree. However, if stored for only 7 days, the combination of 250 MPa pressure with 50 °C or 60 °C heat treatment, as well as the 200 MPa pressure with 55 °C heat treatment, resulted in an adequate product even at higher storage temperature.

3.2.1.3. Evaluation of total microbial counts grouped by storage time

Evaluating the total microbial counts grouped by storage time, I established that as time progressed, differences between treated samples became increasingly significant, indicated by the increased number of post-hoc groups on day 14. Storage temperature had a significant effect on all samples stored for 14 days, whereas for 7-day samples, it was significant only in certain cases. The combinations of 60 °C/250 MPa and 55 °C/200 MPa resulted in the lowest total microbial counts at both low and high storage temperatures. No distinction could be made between combined treatments regarding the order of application.

3.2.1.4. Evaluation of total microbial counts grouped by storage temperature

Analysis of samples grouped by storage temperature showed that at higher storage temperatures, differences between samples became increasingly significant. Storage time had a significant effect on all samples stored at 15 °C, while for those

at 6 °C, only the single treatments fell into significantly different groups at the two different storage temperatures. At the lower storage temperature, there was almost no statistical difference between single and combined treatments on sampling day 7; however, by the end of storage, all combined treatments yielded better results than single ones. At both storage temperatures, only the 60 °C/250 MPa and 55 °C/200 MPa combinations showed significantly better results than the other treatments by the end of the storage period. There was no significant difference between combined treatments regarding the order of treatments.

3.2.1.5. Investigation of total microbial count changes using response surface analysis

For fresh samples, those stored for 7 days at 15 °C, and those stored for 14 days at 6 °C, there was no significant difference between TP and PT treated samples. In both cases, a linear relationship was observed between the total microbial count and the independent variables. Microbial counts decreased with increasing pressure and temperature regardless of the order. The change in temperature had a more dominant effect, while the pressure change was less pronounced.

For samples stored for 7 days at 6 °C, a significant difference was observed based on the treatment sequence. In the case of TP treatment, there was no interaction between pressure and temperature; counts decreased with pressure, while temperature changes had almost no effect. For PT-treated samples, the fitted surface was curved, indicating a non-linear relationship. For samples stored for 14 days at 15 °C, similar parabolic response surfaces were obtained regardless of the treatment order, meaning the interaction between the independent variables (pressure and temperature) was significant in both cases.

Based on the results, I concluded that pressure treatment below 300 MPa combined with mild heat treatment can extend the shelf-life of the fruit smoothie, even at higher storage temperatures. Using 250 MPa and 60 °C, the total microbial count remained below 10^3 CFU/ml even after two weeks at 6 °C, and did not exceed 10^4 CFU/ml even after two weeks at 15 °C.

3.2.2. Investigation of the effect of treatments on pathogenic bacteria

3.2.2.1. Effectiveness of treatments on the presence/absence of Salmonella

Determination of *Salmonella* counts was only possible for the control and the 50 °C, 150 MPa, 50 °C-150 MPa, and 150 MPa-50 °C treated samples. However, the test microbe was detectable by presence/absence tests in the fresh and 3-day (15 °C) stored 250 MPa treated samples, as well as in the fresh 50 °C-250 MPa, 55 °C-200 MPa, and 200 MPa-55 °C treated samples.

For the 250 MPa treated sample, a difference was observed based on storage temperature: the result was negative at 6 °C but positive at 15 °C after 3 days. For the 50 °C + 250 MPa combination, the presence/absence results for fresh samples differed by order: the "heat then pressure" sample was positive, while the "pressure then heat" sample was negative.

These results can be explained as follows: HHP causes significant damage to the cell walls, membranes, and cytoplasm of *Salmonella* (**Yang et al., 2012; Wang et al., 2013**). This treatment also leads to partial protein degradation, impairing the bacteria's ability to recover from subsequent stress (**Wang et al., 2013**). Initial HHP treatment makes the cells more sensitive to subsequent mild heat (**Ogihara, 2009**). If HHP is followed by heat, the damaged cells cannot withstand the additional stress, leading to effective inactivation. Conversely, if mild heat is applied first, it does not cause sufficient damage to prevent regeneration during the subsequent HHP treatment.

3.2.2.2. Temporal changes in Salmonella count during two-week storage

Salmonella counts exhibited a decreasing trend during the two-week storage at 6 °C. The *Salmonella* counts of the treated samples remained below those of the control throughout the storage period, regardless of whether single or combined treatments were applied. Among the procedures, combined treatments proved to be the most effective. Between the individual heat and pressure treatments applied alone, pressure treatment demonstrated greater efficacy.

The *Salmonella* counts for samples treated at 200 MPa-55 °C, 60 °C-150 MPa, 150 MPa-60 °C, 50 °C-250 MPa, 250 MPa-50 °C, 60 °C-250 MPa, 250 MPa-60 °C, 55 °C-200 MPa, and 200 MPa-55 °C remained below the detection limit (analysed sample volume: 1 ml); however, for the samples treated at 200 MPa-55 °C, 55 °C-200 MPa, and 50 °C-250 MPa, the pathogen was detectable using the presence-absence test (from a 25 ml sample).

For those samples where the *Salmonella* count was not below the detection limit, an initial increasing trend was observed during the two-week storage at 15 °C, followed by a decrease after the third day, with the exception of the sample treated at 50 °C-150 MPa. The initial increase was considerably lower in the combined-treated samples than in those receiving only heat or only pressure treatment. In the case of the 50 °C-150 MPa treated sample, the *Salmonella* count remained nearly constant until the 7th day. While the *Salmonella* counts of the combined-treated samples remained below the control throughout the entire storage period, the counts in samples subjected only to heat or only to pressure treatment reached the level of the control after one week of storage.

3.2.2.4. Evaluation of Salmonella counts grouped by storage time

Evaluating the results by time showed that combined treatments yielded the lowest *Salmonella* counts across all sampling days and temperatures. Regarding the sequence, on the 7th day at higher temperature, PT treatment was significantly more effective than TP treatment.

3.2.2.5. Salmonella counts grouped by storage temperature

At higher temperatures, counts were initially high and then decreased over time, whereas they remained relatively constant at lower temperatures. PT and TP

combinations were the most effective, with the PT sequence specifically showing higher effectiveness on the 3rd (low temp) and 7th (high temp) days.

3.2.2.6. Temporal changes in Listeria monocytogenes count during two-week storage

In the case of *Listeria monocytogenes*, countable results were obtained only for the control samples and those treated at 50 °C, 150 MPa, 50 °C-150 MPa, 150 MPa-50 °C, and 250 MPa; for all other samples, *Listeria* counts remained below the detection limit.

The *Listeria monocytogenes* counts showed a decreasing trend during the two-week storage at both 6 °C and 15 °C. At both storage temperatures, the 250 MPa treatment was the most effective among the samples where the *Listeria monocytogenes* count was not below the detection limit, resulting in a greater reduction even compared to the combined treatments using lower pressure values. Compared to the control, the *Listeria monocytogenes* count was initially 2 orders of magnitude lower, and by the end of storage, 4 orders of magnitude lower in the sample where 250 MPa pressure treatment was applied; in contrast, for the 50 °C, 150 MPa, 50 °C-150 MPa, and 150 MPa-50 °C treatments, only a 0.5–1 order of magnitude reduction was observed. The achieved reduction is comparable to the results of **Scolari et al.** (**2018**), who achieved the highest cell lethality (6.0 log CFU/ml) by combining 300 MPa pressure treatment with 50 °C heat treatment. Furthermore, it is consistent with the results of **Zacconi et al.** (**2015**) (5.16 log cell reduction), who treated fruit smoothies at a pressure of 229 MPa and a temperature of 45 °C.

At lower storage temperatures, among the combined treatments, the one where pressure treatment was applied first followed by heat treatment was more successful; the same finding applies to higher storage temperatures, but only until the 3rd day, after which the heat and pressure treatments applied in a different order yielded similar results.

Overall, it can be stated that there was no significant difference between the effects of the 50 °C heat treatment and the 150 MPa pressure treatment—applied either alone or in combination—on *Listeria monocytogenes*. It can also be observed that storage temperature did not have a significant effect in the case of *Listeria*, which is due to its psychrotrophic nature.

The effectiveness of HHP treatment below 300 MPa for the inactivation of *Listeria* in fruit juices, purées, or smoothies has been investigated by only a limited number of studies, whether involving pressure treatment alone or in combination with mild heat treatment. **Buzrul et al.** (**2008**) applied a pressure treatment of 300 MPa at 20 °C for 5 minutes for the inactivation of Escherichia coli O157:H7 and *Listeria innocua*. With the same treatment, they achieved a 4-log reduction in kiwi juice, whereas only a 1-log reduction was achieved in pineapple juice. **Barba et al.** (**2014**) achieved at least a 5-log reduction in the

number of L. *monocytogenes* bacteria in a buffered fruit extract when it was exposed to pressures greater than 300 MPa for longer than 5 minutes.

3.2.2.7. Listeria monocytogenes counts grouped by storage time

Storage temperature had no significant effect on calculable *Listeria* counts; samples were grouped similarly by one-way ANOVA at both temperatures. The 250 MPa treatment was the most effective and remained stable throughout. On day 3, the PT sequence outperformed the TP sequence.

3.2.2.8. Listeria monocytogenes counts grouped by storage temperature

Summarizing the analysis of the samples grouped by storage temperature, the 15 °C storage temperature proved to be a stable environment for *Listeria monocytogenes*, as the *Listeria* count did not significantly deteriorate or improve under any of the treatments during the two-week storage period. Among the analysed treatments, the 250 MPa treatment was the most effective at both lower and higher storage temperatures. Although the PT (pressure-then-heat) treatment numerically performed better among the combined treatments (PT – group b, TP – group bc), from a statistical perspective, it cannot be stated that applying pressure treatment before heat treatment yielded significantly better results at either lower or higher storage temperatures. With the exception of the 150 MPa-50 °C and 150 MPa treatments at 15 °C, no distinction could be made between the single treatments (except for 250 MPa) and the combined treatments either.

Based on the results of the investigations, it can be concluded that *Salmonella* showed increased sensitivity to the applied treatments compared to *Listeria monocytogenes*. This is similar to the study by **Xu et al.** (**2009**), who reached the same conclusion when investigating *S. enterica* and *L. monocytogenes* bacteria in deionized water, orange juice, and tomato juice after 300 MPa treatment.

Based on the microbiological tests performed with the smoothie samples, the combination of 250 MPa pressure treatment and 60 °C mild heat treatment, in addition to maintaining a low total viable count, proved to be appropriate from a microbiological food safety perspective. The two investigated pathogenic microorganisms (*Salmonella* and *Listeria monocytogenes*) were not detectable in any case, regardless of the order of the treatments; therefore, for further investigations (sensory evaluation, viscosity measurement, colour measurement, total monomeric anthocyanin content, antioxidant capacity, total polyphenol content), I selected and applied the combination of these two treatments during the preparation of the product, which was subsequently stored only at 6 °C.

3.3. Sensory and physical-chemical results

3.3.1. Sensory evaluation results

During the sensory evaluation of the samples selected based on the microbiological results, the evaluators found that the control sample stored for two weeks showed the highest thickness, while the samples treated at 60 °C-250

MPa and 250 MPa-60 °C were the thinnest. The control sample stored for two weeks differed significantly from the other samples, except for the sample treated at 250 MPa-60 °C and stored for two weeks. Fresh control samples showed slightly higher thickness than the treated samples, indicating that the treatments resulted in a decrease in the thickness of the samples. The 60 °C-250 MPa treatment appears to be more effective in preserving the original texture of the smoothie during the two-week storage period, but it did not statistically differ from its counterpart treated in the reverse order.

The fresh sample treated at 250 MPa-60 °C was the least brownish in colour, while the sample treated in the same way and stored for two weeks appeared to be the brownest. The fresh sample that received heat treatment after pressure treatment browned the least. Although the fresh control sample was browner, it cannot be concluded that the treatment resulted in lightening; rather, it can be assumed that the combined treatments slowed down the browning process. By the time the test samples were presented to the evaluators, the fresh control sample had browned slightly. This difference between the samples is noteworthy, as it completely disappeared by the end of the 14-day storage, and the evaluators judged them to be equally brown.

The evaluators found that the sample treated at 250 MPa-60 °C had the most fruity aroma, while it was least perceptible in the sample treated at 60 °C-250 MPa and stored for two weeks. Despite storage, the control sample preserved its favourable aroma characteristics and achieved essentially the same score as the fresh samples. For the fresh samples, it appears that the heat treatment applied after the pressure treatment resulted in a more intense fruity character.

The evaluators perceived the fresh control sample as having the most fruity taste and the sample treated at 60 °C-250 MPa and stored for 14 days as the least fruity. There was a significant difference in taste between the fresh and stored samples for both treated and untreated samples. The progression of the rankings clearly shows that the storage conditions had a decisive effect on the taste of the samples, as opposed to the presence or type of the preservation method used. Regardless of the type of combined treatment, the evaluators perceived the stored samples as less fruity in taste than the fresh samples.

In terms of overall preference, the evaluators ranked the fresh control first and its counterpart stored for two weeks last. Again, a significant difference was observed between the fresh and stored samples. As expected, based on the rankings, the evaluators preferred the freshly prepared samples.

Keenan et al. (**2012a**) investigated the sensory properties of apple, strawberry, banana, and orange smoothies treated with HHP and mild heat treatment. They found that the freshness characteristics of the products—including fresh colour, fresh aroma, fresh taste, and pink colour—gradually decreased during storage. The degradation of taste is attributed to the decomposition or oxidation of ester and aldehyde compounds, presumably caused by residual enzyme activity.

3.3.2. Viscosity measurement results

It is observable that a non-linear relationship exists between shear rate and shear stress, as well as between shear rate and apparent viscosity, which confirms the non-Newtonian rheological behaviour of the samples. The convex profile of the flow curves and the flow behaviour index ($0<n<1$) values indicate that the smoothie samples exhibited pseudoplastic flow behaviour. With increasing shear rate, this pseudoplastic behaviour is characterized by increasing shear stress values and decreasing apparent viscosity values, as molecular interactions weaken (**Figura et al., 2007**). The pseudoplastic flow behaviour of smoothie samples has been observed by several researchers (**Keenan et al., 2012b, Ozcan et al., 2011**).

It is important to note that all investigated samples possessed a yield stress, indicating that a minimum shear stress is required to initiate flow (**Figura et al., 2007**). Furthermore, for all stored samples, the fluctuation in apparent viscosity observed in the low shear rate range (10–73.6 s−1) suggests texture inhomogeneity.

The yield stress value was the only parameter in the Herschel–Bulkley model capable of statistically differentiating the samples. For this parameter, a significant difference was observed in all cases between fresh and stored samples. Following storage, lower shear stress was required to induce flow. For fresh samples, the applied mild preservation methods had no significant effect on the yield stress of the smoothie. However, after storage, a significant difference was observed between the control sample and the sample treated with heat followed by pressure treatment.

A similar trend was observed for apparent viscosity values at lower (100 s^{-1}) and higher (1000 s^{-1}) shear rates. The sole difference was that, after storage, both treated samples significantly differed from the control. It can be concluded that for fresh samples, the effect of treatment on apparent viscosity was not detectable at the investigated shear rates; however, storage clearly reduced the viscosity of all samples. After storage, the sample treated at 60 °C-250 MPa most closely resembled the fresh samples, while the control sample exhibited the greatest decrease in viscosity. This result stands in contrast to the findings of the sensory evaluation, where panellists rated the control sample as the thickest, which is likely attributable to the presence of inhomogeneous particles within the sample.

The direction of changes in the consistency index followed the trends observed for apparent viscosity, though these differences were not significant in any case. **Wang et al.** (**2014**) also reported that the consistency index does not necessarily reflect changes in apparent viscosity. Regarding the order of application in combined treatments, no significant differences were found based on yield stress, apparent viscosity, or consistency index.

3.3.3. Evaluation of colour properties

Overall, the colour of the samples underwent minimal changes as a result of treatment and storage. The freshly prepared smoothie sample exhibited a pink hue. Following treatment, the L∗ and b∗ values of the fresh (non-stored) samples showed a slight increase, while the a∗ values decreased slightly (indicating that the samples became somewhat lighter, yellower, and less reddish in tone). Regarding the samples stored for 14 days, a slight decrease in L∗ values and a minor increase in a∗ values were observed when comparing control samples to treated ones. **Terefe et al.** (**2009**) observed a similar trend in L∗ values during the 4–6 week storage of strawberries. The a∗ values of the treated samples showed reductions of 10.18% and 14.80% compared to the control. The decrease in the red colour of the samples can be attributed to residual enzyme activity, which causes the enzymatic browning of phenolic compounds. **Škegro et al.** (**2021**) reported a similar trend regarding the a∗ parameter in fruit smoothies treated at pressures of 350 and 450 MPa.

In general, the sequence of treatments did not exert a significant effect on the colour of the samples. The same conclusion was reached in our previous study on strawberry puree samples, where higher processing parameters were applied during the combination of HHP and heat treatment (**Salamon et al., 2021**).

Comparison between the fresh samples and those stored for 14 days revealed significant differences in nearly all cases, with the exception of the L∗ factor. The only exceptions were the b∗ and C∗ values of the fresh versus stored control samples, and the chroma values of the fresh versus stored samples subjected to the 250 MPa−60 °C treatment.

Comparison of the fresh samples showed no significant differences in L∗ and chroma values. For all other parameters, however, the control sample differed significantly from the treated samples.

When comparing samples stored for 14 days, no significant differences were detectable between samples regarding L∗ and b∗ factors. However, in all other cases, the control sample differed significantly from the treated samples. Furthermore, the two samples treated in different sequences also exhibited differences, particularly regarding the a∗ value and the hue angle. For the a∗ factor, the sample treated in the PT sequence resembled the fresh control more closely, while for the hue angle, the sample treated in the TP sequence showed greater similarity to the fresh control.

The colour comparison of the samples was also performed based on the measured colour factors and the total colour difference (ΔE∗). The ΔE∗ values were calculated relative to the control sample and to each other. The ΔE∗ values indicated that the treatments did not induce significant changes in the colour of the samples. Even the largest difference was categorized only as "noticeable." Changes were noticeable between the two control samples, between the control

and treated samples, and as a result of storage. No noticeable difference was observed between the fresh (non-stored) samples treated in different sequences, which supports our previous finding that the order of treatment does not influence colour change.

3.3.4. Electronic nose measurements

The advantage of utilizing virtual sensors lies in their ability to identify volatile compounds associated with specific sensors, specifically by determining the retention indices (RI) of chromatographic peaks. This facilitates the identification of the sample's aromatic compounds from international or proprietary fixed databases, a capability that is seldom possible with other sensor-based electronic noses. Based on the chromatograms of representative samples from each group and the RI of the peaks, acetaldehyde, ethanol, propanal, hexanal, and 1-hexanol were the most dominant volatile substances, all of which are indicative of a fruity aroma. The presence of these identified components can be explained by the following processes: fatty acids, typically linoleic and linolenic acid, are converted into volatile aldehydes such as hexanal via oxidative degradation by lipoxygenase or hydroperoxide lyase (**Ozcan et al., 2011**). These aldehydes are subsequently converted into alcohols by alcohol dehydrogenases (**Mitchell and Jelenkovic, 1995**).

Principal Component Analysis (PCA) of the multivariate data obtained from the electronic nose measurements revealed a dominant olfactory difference between fresh and stored samples. The odour variance of the stored samples was higher. The control and treated samples differed from one another in both fresh and stored states. The overlap between the two treated groups indicates a similarity in their aromatic profiles; however, it is noteworthy that samples treated at 60 °C-250 MPa differed most significantly from the control groups, whether fresh or stored. Discriminant Factor Analysis (DFA) exhibited the same pattern, although the separation between groups was more pronounced due to the supervised nature of the approach. For sensor selection, the automatic sensor selection function of the Alpha Soft software was employed. When Discriminant Factor Analysis was performed using the selected sensors, the results indicated that certain volatile compounds were lost during storage. The most significant reductions were observed in components that are not dominant in the formation of fruity aromas, such as benzaldehyde, although ethanol and ethyl hexanoate also decreased. Octanol levels showed an increase in all stored samples. Propanal and acetaldehyde concentrations increased during storage in the control and 60 °C-250 MPa treated groups.

3.3.5. Electronic tongue measurements

Due to the instability of the electronic tongue sensors, the first three replicate measurements of the samples were excluded from further analysis based on the evaluation of the raw sensor signals. It was observed that the majority of the

sensors exhibited significant differences between at least some of the investigated samples.

Based on the Euclidean distances calculated between the raw signals of the electronic tongue sensors for the investigated smoothie samples, the largest distances were observed between the groups of fresh and stored samples. This indicates the formation of two main clusters based on the chemical profiles of the smoothie samples. The groups of treated fresh samples were at a shorter distance from the fresh control than the samples stored for 14 days.

According to the results of the Principal Component Analysis (PCA) performed on the drift-corrected data from the six selected sensors, the first two principal components accounted for approximately 94% of the total variance, resulting in a clear separation of the six sample types. The most prominent separation was observed along PC1 between the fresh and stored sample groups. PC2 clearly distinguishes the control group from the treated sample groups. Furthermore, separation was observable between the different treatment types as well (PC2–PC3). The role of the six selected electronic tongue sensors in the formation of the first three principal components confirms the previously mentioned separation of patterns.

3.4. Comparison of human sensory evaluation and instrumental analysis results

Comparing the results of human sensory evaluation and instrumentally measured sensory characteristics, the results of instrumental analyses matched the human sensory evaluation with only a few exceptions. One such difference occurred in texture measurement, where judges found the 14-day stored 60 °C–250 MPa treated sample to be the most similar to the control, while instrumental measurements showed that the fresh control and its fresh counterpart treated in the reverse order differed the least in rheological properties. This discrepancy is likely due to inhomogeneous particles in the sample. Interestingly, while both the panellists' opinions and viscosity measurements indicated that the 14-day stored control was the least similar to the fresh control, the judges perceived it as the densest, while instrumental measurements proved it to be the thinnest. Neither the judges nor the instrumental measurements could find a significant difference between the various combined treatments.

During the investigation of colour, judges considered the 14-day stored control and the 14-day stored 60 °C–250 MPa treated sample most similar to the fresh control. Statistically, no distinction could be made between these two and the samples treated at 250 MPa–60 °C; thus, colour measurement and human sensory results did not differ significantly. Both human and instrumental measurements identified the 250 MPa–60 °C treated sample as the most different from the control.

In the investigation of fruity aroma, both the judges and the electronic nose analysis found the 250 MPa–60 °C treated sample to be the most similar to the original, and the 60 °C–250 MPa treated sample the most different from the fresh control. Although the judges perceived no difference between the two fresh or the two stored samples treated in different orders, instrumental analysis detected a difference between the stored TP and PT sequences.

Regarding flavour, the sample most similar to the fresh control was the 250 MPa–60 °C treated one in both cases. While judges found the 14-day stored 60 °C–250 MPa sample most different from the control, electronic tongue measurements identified the 14-day stored control as the most divergent. However, as the judges' rankings showed no significant difference between the 14-day stored samples, there was no major contradiction between instrumental and human results. While judges could not distinguish samples by treatment order, the instrumental analysis was able to differentiate between the stored samples.

3.5. Bioactive components of the smoothie

Both the applied treatments and the storage had a significant effect on the anthocyanin content of the smoothie samples. For fresh samples, treated samples differed significantly from the control, and differences were detectable based on treatment order; the TP-treated sample was more similar to the control. Over time, the TP-treated sample preserved its anthocyanin content best, though overall, anthocyanin levels decreased significantly during storage.

Storage had no significant effect on the total polyphenol content (TPC), but treatments significantly influenced this bioactive component. The treatments caused a decrease in TPC compared to the control, and similar to anthocyanins, the TP sequence proved more favourable for both fresh and stored samples. This difference in treatment order was statistically detectable at both sampling points.

Antioxidant capacity values showed a significant decrease due to treatments. In this case, the PT sequence resulted in higher antioxidant capacity. When comparing the three fresh and three stored samples, significant differences were observed in all cases. Storage also had a significant impact, with values decreasing significantly over time.

Overall, treatments had a significant effect on the quantity of bioactive components, as did storage (with the exception of TPC). I was able to distinguish between treatments based on their order for all investigated parameters. For anthocyanin and total polyphenol content, the TP sequence was more advantageous, while for antioxidant capacity, the PT sequence was preferable.

Although few studies focus on the effect of the order of HHP and mild heat combinations, these seemingly contradictory phenomena can be explained as follows: heat treatment is much more effective at inactivating polyphenol oxidase (PPO) and peroxidase (POD) enzymes, while HHP often only achieves partial inactivation. Heat or HHP combined with mild heat can result in more stable

polyphenol levels because the initial thermal effect eliminates oxidative enzymes, leaving nothing to degrade them during storage. Furthermore, the shorter heat exposure combined with HHP may reduce the degradation of heat-sensitive compounds while still inactivating enzymes (**Terefe et al., 2010; Salazar-Orbea et al., 2021**).

Phenolic compounds in plant cells are frequently present in bound forms. HHP disrupts plant cell walls and cell membranes, increasing their permeability, which facilitates the release and extraction of bound polyphenols from the cellular matrix (**Navarro-Baez et al., 2022; Zhao et al., 2017**). This phenomenon explains why, in many cases (**Hu et al., 2020; Yasunaga et al., 2018**), higher total phenolic content (TPC) is measured after HHP treatment compared to control samples. According to most studies (**Patras et al., 2009; Keenan et al., 2012c**), HHP treatment preserves the phenolic compounds and antioxidant capacity of fruits and vegetables more effectively than heat treatment, which often leads to a reduction through the oxidation and degradation of heat-sensitive molecules. The underlying mechanisms are associated with numerous factors, including pressure parameters, the food matrix, storage conditions, packaging, additives, dissolved oxygen, residual enzymatic activity, and interactions between phenolic compounds and other constituents (**Zhao et al., 2017**).

4. CONCLUSIONS AND RECOMMENDATIONS

Based on the results for both the strawberry puree and the developed smoothie, it can be established that mild high hydrostatic pressure (HHP) treatment or mild heat treatment applied alone is insufficient to produce a microbiologically acceptable product. However, the combination of these two treatments resulted in a significant improvement in product stability. My results indicate that by combining pressure treatment below 300 MPa with mild heat treatment, the shelf-life of the fruit smoothie can be extended even at higher-than-average storage temperatures. Using a combination of 250 MPa and 60 °C, the total microbial count of the product remained below 10^4 CFU/ml even after two weeks of storage at 15 °C. The applied treatment combination effectively inactivated the two investigated pathogenic bacteria (*Salmonella* and *Listeria monocytogenes*) and proved beneficial in preserving valuable nutritional components and sensory properties.

Based on the microbiological, sensory, and chemical analysis, the shelf-life of the developed smoothie (treated with 250 MPa and 60 °C) can be determined as 14 days when stored at 6 °C, and it remains stable for up to 7 days even at 15 °C. To define the exact shelf-life, further studies may be required, such as testing for other potential pathogens (e.g., pathogenic *E. coli* strains), determining yeast and mold counts, and monitoring pH, soluble solids (Brix°), and Vitamin C content with more frequent sampling.

From an industrial perspective, applying lower pressure combined with other technologies—such as mild heat—is a viable alternative for preserving acidic products like fruit smoothies, thereby reducing technological costs. Future research should focus on a comparative cost-benefit analysis of combined technology versus exclusive HHP treatment. Furthermore, synergies between mild heat alternatives, such as Pulsed Electric Fields (PEF) or the addition of natural inhibitors (e.g., ascorbic acid, citric acid), should be investigated regarding complex quality parameters and economic efficiency.

Overall, storage had the greatest impact on the investigated parameters, although treatments also caused changes compared to untreated samples. In combined treatments, the order of application played the smallest role, though differences were observed in specific cases. Instrumental sensory analysis confirmed the human panel results, providing a reliable, objective tool for quality assessment. Future research should also extend to the effects of these treatments on enzyme activity and test the methodology on other berry-based (raspberry, blueberry, blackberry) smoothies.

5. NEW SCIENTIFIC RESULTS (THESES)

1. I have demonstrated that for strawberry puree (pH 3.6), treatments below the industrial minimum of 300 MPa yield a safe product if combined with heat, provided the pressure reaches or exceeds 200 MPa (5 min, ambient temperature) and the heat treatment reaches 55 °C (5 min). The total microbial count of the product treated this way does not exceed 10^3 CFU/ml, and the presence of *Salmonella* Hartford and *Listeria monocytogenes* is not detectable even after 14 days of storage at 15 °C.
2. I have demonstrated that for the smoothie product (pH 4.5; strawberry 37%, banana 26%, almond drink 24%, avocado 13%; initial *Salmonella* Hartford count: 10^6 CFU/ml), treatments below 300 MPa yield a safe product if combined, provided the pressure reaches or exceeds 250 MPa (5 min, ambient temperature) and the heat treatment reaches 50 °C (5 min). *Salmonella* was not detectable immediately after treatment or during 14 days of storage at 15 °C, provided that the pressure treatment preceded the heat treatment (PT sequence).
3. I have demonstrated that for the smoothie product (pH 4.5; initial *Listeria monocytogenes* count: 10^6 CFU/ml), the number of *L. monocytogenes* can be effectively reduced below CFU/ml if the pressure reaches or exceeds 200 MPa (5 min, ambient temperature) and the heat treatment reaches 55 °C (5 min). In the product treated this way, the number of *Listeria monocytogenes* remained below the detection limit immediately after treatment and throughout the 14-day storage at 15 °C.
4. I have demonstrated that for the smoothie product (pH 4.5; strawberry 37%, banana 26%, almond drink 24%, avocado 13%), if the pressure level of the combined treatment does not exceed 250 MPa and the temperature does not exceed 60 °C, no significant deterioration of sensory properties occurs.
5. I have demonstrated that the order of treatments affected the bioactive components of the smoothie (pH 4.5; strawberry 37%, banana 26%, almond drink 24%, avocado 13%) during 14-day refrigerated storage (6 °C). For anthocyanin and total polyphenol content, the TP sequence (60 °C heat followed by 250 MPa pressure) was more effective at preservation. Conversely, for antioxidant capacity, the PT sequence (250 MPa pressure followed by 60 °C heat) proved to be more successful.

6. PUBLICATIONS RELEVANT TO THE DISSERTATION

Journal Articles:

Zakariás, F., Taczman-Brückner, A., Kiskó, G. and Dalmadi, I. (2026). Enhancing food safety in fruit smoothies: Efficacy of high hydrostatic pressure and mild heat against *Salmonella* and *Listeria monocytogenes*. *Progress in Agricultural Engineering Sciences.* (published online ahead of print 2026), 446.2025.00270, Article 446.2025.00270, Elérhető: AKJournals https://doi.org/10.1556/446.2025.00270

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Zakariás, F., Bajramović, B., Almoheb, Z., Dalmadi, I. and Kiskó, G. (2023). Investigation of injured cells due to minimal processing. *BiosysFoodEng 5th International Conference on Biosystems and Food Engineering Book of Proceeding*. Budapest, 2023. június.09. Elérhető: https://biosysfoodeng.hu/2023/USB/posters/E527.pdf (poster)

Zakariás, F., Dalmadi, I. és Kiskó, G. (2023). Kíméletes hőkezelés és nagy hidrosztatikus nyomás egyedi és kombinált kezeléseinek hatása gyümölcs pürék mikrobiológiai biztonságára és minőségére az ajánlások összefüggésében [Effect of single and combined treatments of mild heat treatment and high hydrostatic pressure on the microbiological safety and quality of fruit purées in the context of recommendations]. *Nagykőrösi Tartósítóipari Konferencia [Nagykőrös Canning Industry Conference]*. Nagykőrös, 09. May 2023. (presentation)

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Zakariás, F. (2022). Preservation of fruits by minimal process and its microbial effect. *MTA Élelmiszer-mikrobiológiai és Élelmiszer-biztonsági Munkabizottsági ülése* [*Meeting of the MTA (Hungarian Academy of Sciences) Working Committee on Food Microbiology and Food Safety*]. Budapest, 12. May 2022. (presentation)

Zakariás, F. (2021). The effect of combined treatment on pathogenic bacteria on different berries. *MiCEnt 2021: Integrative Biology Symposium Microbiology, Enteric Nervous System, Central Nervous System*, 30. June 2021.30. (presentation)

Leopold, A. and Zakariás, F. (2017). Kombinált tartósító eljárások hatása szamócapürék színére [Effect of combined preservation methods on the color of strawberry purées], XXII. Bolyai Konferencia [*XXII. Bolyai Conference*], Budapest, 18. May 2017. (poster)

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