

# **THESES OF THE DOCTORAL DISSERTATION**

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**Budapest**

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HUNGARIAN UNIVERSITY OF  
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**DIFFERENT SEQUENCE EFFECT OF COMBINED MINIMAL  
PROCESSING TECHNOLOGIES ON STRAWBERRY PUREE**

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The candidate has fulfilled all the conditions prescribed by the doctoral school of Hungarian University of Agriculture and Life Sciences, the comments and suggestion at the thesis workshop were taken into consideration when revising the thesis, so the dissertation can be submitted to a public debate.

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## ***1. INTRODUCTION AND OBJECTIVES***

In the field of food preservation, an increasing proportion of alternative food processing technologies are being used to effectively preserve the original freshness and nutritional value of food, with regard to the increasingly health-conscious lifestyle of society. The preservation of strawberry is no exception of this trend.

Strawberries are one of the most popular berries grown worldwide, accounting for 60-65% of all berries. It contains outstanding amounts of beneficial antioxidant compounds such as flavonoids, including anthocyanins (perlargonidine-3-glucoside and cyanidin-3-glucoside), which are antioxidants and protect our body's health from the dangerous free radicals caused by various stress effects, helping our immune system to function properly. Unfortunately, these are very sensitive compounds, they easily lose their stability during processing and storage due to various environmental factors (for instance high temperature, UV radiation, presence of oxygen).

Minimal processing technologies have low effect on the quality properties of foods, efficiently preserving their original fresh taste, aroma, texture and initial nutrient and vitamin content, and avoiding the use of additives or preservatives. These technologies include the High Hydrostatic Pressure (HHP) treatment, of which research results are very promising.

Among other minimal and non-thermal technologies, high hydrostatic pressure treatment (400-600 MPa, chilled or ambient temperature) is one of the most successfully adopted technologies in the food industry, because its benefits have met consumer's health-conscious expectations and have already enabled the development of many innovative food products. It plays a significant role in the preservation of foods containing bioactive components. However, in addition to safe food production, to preserve the organoleptic characteristics of freshness and the bioactive components of the products as much as possible during the storage, attempts are often carried out with the combination with other minimal processing technologies.

One successful attempt is to use HHP treatment in combination with mild heat treatment, more effectively preserving the above mentioned positive features during storage as well.

In the previous studies, the combined treatments and experiments were mostly performed simultaneously, so the pressure treatment and the mild heat treatment were carried out together in the same laboratory equipment. However, the feasibility of industrial application of combined treatment is currently unresolved. This is due to the problem of scale and mechanical

design, intensive amortisation of valves and sealings, and the problem of inhomogeneous temperature distribution in the treatment chamber and issue with proper monitoring of temperature.

Accordingly, during my dissertation, I aimed to investigate the possibility of the combined application of HHP treatment and mild heat treatment in the case of strawberry purees in such way that the treatments are carried out separately in space and time. Goal of this study was to examine how and to what extent the sensory, nutritional, and physicochemical parameters of the strawberry puree change due to the combined treatments, and whether the sequence of the treatments plays a role in it, and if yes, in which sequences to what extent.

## **2. MATERIAL AND METHODS**

For my experiments, I used commercially purchased quick-frozen strawberries, from which I made puree after thawing. After vacuum packaging, the samples were pressure treated at 300 and 600 MPa for 5 minutes and/or heat treated at 55 and 75 °C for 10 minutes. For each applied treatment parameter, I prepared single pressure-treated samples, single heat-treated samples, and samples with combined treatments in different sequences (the order of the sample's mark also indicates the order of the treatments, for instance: in the case of 55 °C / 300 MPa the heat treatment was performed first and then the pressure treatment). In my research, I also performed a 14-day long storage experiment by storing the samples at  $2 \pm 2$  °C and  $15 \pm 2$  °C, respectively to monitor changes during storage.

## **3. RESULTS**

From a microbiological point of view, both single and combined treatments ensured the food safety criteria and quality of the strawberry puree during 14 days of storage at both lower (2 °C) and higher (15 °C) storage temperatures. Both single and combined treatments at higher temperatures (75 °C) were more effective in reducing the initial colony forming unit (CFU) number of microorganisms. The yeast and molds counts (YMC) remained below the detection limit until the end of storage for all samples, except for the 55 °C single heat treatment, which indicates that the lower-level treatments were slightly less able to eliminate bacteria and yeast and molds.

Based on the examination of the pH of strawberry puree samples, the pH of the firstly mild heat-treated strawberry purees shows a more intense decrease than the firstly pressure-treated variants. The change is most intense in the cases where the mild heat treatments were performed at 55 °C. Based on the fluctuation of the data, it seems that the combined treatments are able to

provide a more stable pH to the strawberry puree than the single treatments, especially if one of the higher-level treatments (600 MPa or 75 °C) was used in the combined treatment. Storage for 14 days at 2 and 15 °C refrigerated temperature did not cause significant changes in the pH values of the strawberry puree.

There were no significant changes in the colour of the strawberry puree due to the different single and combined treatments, however, after 14 days of storage, the values of the colour components already showed significant changes. The values of the a\* red and b\* yellow colour components decreased, and the samples lost from their red and yellow colour intensities. The values of the L\* colour component showed an increase, so the colour of the samples became lighter. According to the storage temperatures, it is not possible to clearly identify in which cases the changes were more intense. In almost all cases, the combined treatments represented more stable values than single pressure- or heat-treated samples, and there were fewer outliers, thus it is possible that combined treatments may be attributed a kind of beneficial effect, which may make the colour retention after treatment and storage more favourable and stable. Regarding the sequence of treatments, it can be observed in the case of L\* results of the first heat-treated and then pressure-treated samples appear to be in a wider data range than results of those samples treated in the reversed order. Therefore, the firstly applied pressure treatment can be beneficial in this case.

Based on the examination of the colour difference values, according to the pairwise comparison of the same level treatments but in a different order, I also experienced smaller changes in the colour in the cases where pressure treatment was firstly applied. If the combined treatment contains one of the higher level of treatments (75 °C or 600 MPa), the colour of the strawberry puree can be better preserved, which is probably due to the more efficient enzyme inactivation and the formation of fewer brown colour compounds during storage. In addition, changes at lower temperatures (2 °C) are also less intensive based on pairwise comparisons.

The results of the bioactive components (anthocyanin content, polyphenol content, antioxidant capacity) of the strawberry puree samples suggest that the values decreased only slightly due to the treatments, significant decreases occurred due to the 14-day storage. In cases of the single pressure or heat treatments, the extent of the degradations was more intense than in case of the combined treatments. Based on these findings the combined treatments are more advantageous in preserving the bioactive components. Based on other studies, the activity of polyphenol oxidase (PPO), peroxidase (POD) and  $\beta$ -glucosidase is responsible for the degradation of these bioactive components in strawberries. In addition, storage at a lower temperature (2 °C) was also found to be more positive in preventing changes than higher storage temperature (15 °C).

No clear conclusions could be established regarding the levels and sequences of combined treatments.

Based on the results of the 2<sup>n</sup> factorial experimental design, it can be stated that during the combined usage of pressure and heat treatment, the results do not vary based on first-order kinetics, particularly the results of the centre points differ from the expected values.

Based on the results of the sensory evaluation test between the combined treatments with different sequences (300 MPa / 55 °C – 55 °C / 300 MPa and 600 MPa / 75 °C – 75 °C / 600 MPa) no mathematically verifiable significant sensory differences were observed. However, after 14 days of storage at 15 °C, sensory difference was found by the panellists in case of the low-level (300 MPa / 55 °C - 55 °C / 300 MPa) combined treatments in different sequence, presumably due to less efficient enzyme inactivation, whereas at the higher-level treatments (600 MPa / 75 °C - 75 °C / 600 MPa) there was still no difference in the sequence of treatments. Samples first pressure treated and then heat treated (300 MPa / 55 °C) were characterized as follows: "denser and sweeter", "more intense strawberry flavour", "stronger flavour", "fresh and more intense aroma and taste", "less aftertaste", "slightly brighter colour". Comparing the characteristics with the previous test results, the first pressure-treated sample (300 MPa / 55 °C) had a slightly higher L\* value, which may have contributed to the reviewers perceiving the sample as brighter.

The measurement results of the volatile components are in line with the obtained conclusions, according to which the measured results after the treatments were basically not separated by the discriminant analysis, so the order of the treatments and the levels of the treatments were basically indistinguishable. Significant differences were observed after 14 days of storage at 15 °C, the groups were significantly separated in the discriminatory space compared to the groups without storage and also based on treatment levels. There is no significant difference between the 600 MPa / 75 °C and 75 °C / 600 MPa groups, however, at lower treatment levels, the 300 MPa / 55 °C sample group differs from the 55 °C / 300 MPa samples, so here the sequence of treatments had a significant effect on the volatile components of strawberry puree.

Analysing the changes in the rheology of strawberry puree at higher treatment levels (75 °C / 600 MPa - 600 MPa / 75 °C), the samples showed higher resistance against the deformation force after the treatments and even after 14 days of storage, which means a stronger structure formed, more gel-like structure characterized the samples with higher apparent viscosity values. Based on the flow curves, storage at 15 °C showed lower shear stress values than the results of samples at day 0 or stored at 2 °C. The shear stress of the first heat-treated samples was also

lower than the first pressure-treated samples, the structure of the samples showed less resistance against the deformation force. The texture of these samples visibly became more dilute, more loose in structure, especially at the lower treatment level (55 °C / 300 MPa), which correlate with the results reported in the sensory evaluations. The use of HHP as kind of a pre-treatment thus allows PME-mediated pectin changes, which may prove useful to counteract tissue softening due to heat treatment. Based on the evaluation of MFA, we can conclude that 14-day storage played a crucial role in the complex changes, to which the bioactive and volatile components changes are most closely related, and we can observe also observe similar directions in the changes of a\* and b\* colour components. Thereafter, the changes in the results are mostly attributable to the storage temperature, which are more intense in case of 15 °C storage than 2 °C. The directions and extent of these changes are mostly attributed to changes in the rheology of strawberry puree and also related to the L\* colour parameter changes.



#### ***4. CONCLUSIONS AND RECOMMENDATIONS***

The cultivation, processing and use of strawberries are on a growing trend worldwide, with a growing role of minimal processing technologies, which are often used in combination to ensure the original freshness of the products during storage.

In my dissertation I studied the application of high hydrostatic pressure treatment in combination with mild heat treatment in the case of strawberry puree preservation. As the simultaneous implementation of the combined treatments under industrial conditions has not yet been resolved, my fundamental goal was to explore the differences between the different treatments performed in different sequences. The pressure (300 and 600 MPa) and heat-treated (55 and 75 °C) strawberry puree samples proved to be safe in case both of the individual and combined treatments for 14 days of refrigerated storage even at 2 and 15 °C. Based on the changes in colour and bioactive components, the combined treatments, especially at the higher treatment level (600 MPa / 75 °C), proved to be more favourable in maintaining the above-mentioned quality parameters of strawberry puree both after treatment and during storage. The majority of the changes occur during storage, which according to other studies are mainly due to the activity of endogenous enzymes. Storage at a lower temperature (2 °C) proves to be more favourable in preventing changes than storage at a higher temperature (15 °C). Based on the results of the 2<sup>n</sup> factorial experimental design, it can be concluded that by increasing the treatment levels during the combined application of pressure treatment and mild heat treatment, the changes do not follow first-order kinetics. Based on sensory evaluations and examination of volatile components, no significant changes were observed after the 14 day storage at 15 °C by application of higher levels (600 MPa / 75 °C) of treatment parameters, however, low levels (300 MPa / 55 °C) of treatment parameters have been shown to be less effective, differences between the samples were found regarding sequence of the treatments, the first applied pressure treatment proved to be more favourable. Similarly, in terms of the rheology of strawberry puree, the first pressure-treated samples had more favourable rheological properties than the samples treated in the reverse order of combined treatment, and the use of a lower storage temperature proved to be more beneficial.

Thus, higher level of pressure and heat treatment parameter is recommended for the industrial application of combined treatments, which must be validated in all cases by comprehensive kinetic studies. It is recommended to choose lower storage temperature. Regarding the sequence of treatments, it is recommended to perform the pressure treatment first, before the heat treatment for a more favourable final result.

## **5. NEW SCIENTIFIC RESULTS**

1. I have established that the combined applications of high hydrostatic pressure treatments at 300 or 600 MPa for 5 minutes at ambient temperature and mild heat treatments at 55 or 75 °C for 10 minutes adequately ensure the shelf life of strawberry purees for 14 days at both of 2 and 15 °C storage temperature.
2. I have concluded that in contrast to the single application of pressure (300 or 600 MPa, 5 min, ambient temperature) or heat treatment (55 or 75 °C, 10 min) when their combination is used, the colour of strawberry puree and the amount of its bioactive components (total anthocyanin content, total phenol content, antioxidant capacity) can be better preserved both after treatments and during storage, especially in those cases where the combination treatment contains one of the higher level treatment (75 °C or 600 MPa ).
3. Based on the results of the 2<sup>n</sup> factorial experimental design, I have established that during the combined application of pressure treatment (300 or 600 MPa, 5 min, ambient temperature) and heat treatment (55 or 75 °C, 10 min) by increasing the level of the treatment parameters, changes in the colour and bioactive component (total anthocyanin content, total phenol content, antioxidant capacity) of strawberry puree are not followed by first-order kinetics.
4. Based on the sensory evaluations results and the changes in the volatile components, I have concluded that after the combined treatments of strawberry puree, regarding the sequence of the treatments there is no difference between the low- (300 MPa / 55 °C - 55 °C / 300 MPa) and high-levels (600 MPa / 75° C - 75 °C / 600 MPa) comparison. After 14 days of storage at 15 °C, there is still no difference between the high-level treatments (600 MPa / 75 °C - 75 °C / 600 MPa), but at low-level treatments (300 MPa / 55 °C - 55 °C / 300 MPa) samples can be distinguished in terms of the sequence of the combined treatments.
5. I have established that in terms of the sequence of combined treatments, changes in the colour, sensory and rheological properties of strawberry puree were more beneficial if pressure treatment (300 or 600 MPa, 5 min, ambient temperature) is applied at first than heat treatment (55 or 75 °C, 10 min).

## 6. PUBLICATIONS RELATED TO THE THESIS

### *IF or Q1-Q4 publications in foreign language:*

Salamon, B., Zakariás, F., Csehi, B., Kiskó, G., Dalmadi, I., 2021. Different sequence of high-hydrostatic pressure and mild-heat treatment on the colour and sensory characteristics of strawberry puree. *Acta Alimentaria* 50, 93–101. <https://doi.org/10.1556/066.2020.00165>

Salamon, B., Farkas, V., Kenesei, G., Dalmadi, I., 2017. Effect of added sugar and ascorbic acid on the anthocyanin content of high pressure processed strawberry juices during storage. *J. Phys.: Conf. Ser.* 950, 042005. <https://doi.org/10.1088/1742-6596/950/4/042005>

### *Publications in Hungarian language:*

Dalmadi, I., Salamon, B., Jónás, G., Kenesei, GY., Farkas, V., Balla, CS., 2014. Különböző mennyiségű cukorral és aszkorbinsavval kiegészített szamócalevek antocianin tartalmának változása a nagy hidrosztatikus nyomású kezelés során, *ÉLELMISZER - TUDOMÁNY TECHNOLÓGIA LXVIII*: 2 pp. 9-15., 7 p.

Salamon, B., 2011. Különböző hőmérsékleteken tárolt, nagy hidrosztatikus nyomással kezelt szamócapürék színváltozásának vizsgálata, *ÉLELMISZER - TUDOMÁNY TECHNOLÓGIA LXV*: 1 pp. 14-17., 4 p.

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- Salamon, B., Gere, A., Csehi, B., Dalmadi, I., 2018. Effect of high hydrostatic pressure and mild heat treatment applied in different sequence on sensory attributes of strawberry puree, in: 56th EHPRG Meeting Abstracts. European High Pressure Research Group (56th), Aveiro, Portugal, p. P112.
- Salamon, B., Tóth, A., Palotás, P., Südi, G., Csehi, B., Németh, C., Friedrich, L., 2016. Effect of high hydrostatic pressure (HHP) processing on organoleptic properties and shelf life of fish salad with mayonnaise. *Acta Alimentaria* 45, 558–564.  
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