Effect of added sugar and ascorbic acid on the anthocyanin content of high pressure processed strawberry juices during storage

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Abstract. Berries have high nutritional value and can be processed in many kinds of ways. Their pigments (anthocyanins, flavonoids, carotenoids) have antioxidant properties, effectively neutralize the health-damaging free radicals. High hydrostatic pressure (HHP) technology is a minimal processing technique which is a promising alternative solution instead of traditional preservation technologies. Low molecular weight materials such as colour pigments are well preserved by application of HHP. However, the effect can be influenced by the composition of the treated food matrix. The available scientific information related to the impact of sugar and ascorbic acid content on the preservation of anthocyanins in the samples is controversial. Thus, the aim of our study was to determine the effect of HHP treatment parameters (pressure, treatment time) on the preservation of the anthocyanin content of strawberry juice supplemented by different amounts of sugar and ascorbic acid. 2^n type factorial experimental design was used to evaluate the effect of four factors (refraction index, ascorbic acid, pressure, treatment time) on the residual content of total anthocyanins immediately after HHP treatment and after 21 days storage at room temperature.

1. Introduction

The berries, such as strawberries, contain prominent amounts of natural and beneficial antioxidant compounds. These help the immune system in proper functioning and are designed to protect the health of the body from dangerous free radicals as results of different stress effects [1]. Unfortunately, these compounds are very sensitive and easily lose their stability during processing and storage as an effect of various environmental factors (e.g. high temperature, pH, UV radiation). In addition, the composition of the berry itself and the composition of the product made of it also affect the stability of anthocyanins. Other research results regarding the effects of ascorbic acid and sugar content on the anthocyanin content are controversial. Number of researchers reported the negative impact of ascorbic acid on anthocyanins [2-6], while others showed its protective effect [7] and there are also studies where researchers were unable to demonstrate any significant effect of ascorbic acid on anthocyanins [8].

Similar discrepancies are observed in case of saccharides. While some studies have demonstrated that the presence of saccharides increased the pigment loss [9-11], other sources identified anthocyanin-stabilizing effect of the higher sugar content [12-15]. However, according to Malien-Aubert the anthocyanin content is not influenced by the addition of saccharide [16]. In our previous study we examined the effect of addition of five different kinds of sugar to strawberry purées and it was found that in terms of coloration, glucose was the most protective after treatments and also during storage [17].
Consequently, the conventional processing of strawberries may cause degradation of the anthocyanins, since the industry applies large quantities of sugar and a small amount of ascorbic acid for preservation in numerous cases. Therefore, in this area it would be worth to use new types of mild food processing techniques. High hydrostatic pressure (HHP) treatment is a preservative method where liquid or solid foods are exposed to hydrostatic pressure from 100-800 MPa. The advantage of HHP treatment is that, according to the Pascal’s principle, the hydrostatic pressure is transmitted instantaneously and uniformly (isostatically) throughout the whole product, because the food, that is packed in flexible packaging material, preferably free of air, is immersed into a pressure transmitting fluid. Thus the effect of pressure treatment does not depend on the size or shape of the food in contrast to other preservation methods [18]. Phenols, like anthocyanins, prove to be quite resistant to HHP treatments [19]. Salamon found that the 400 MPa and 600 MPa treated strawberry purées had well preserved colour stability for 3-4 weeks, even at 20 °C storage as well, and it did not influence the consumer’s perception [20]. During this period they could have been safely presented in the commercial market. Based on these our research goal was to determine the effect of different pressure levels and different treatment times on anthocyanin content of the sugar and/or the ascorbic acid supplemented strawberry juices.

2. Material and methods

2.1. Sample preparation, treatment and storage

Frozen strawberries were used in our experiments. Strawberries were thawed at room temperature then purée was prepared by an automatic sieve. Then the purée was centrifuged twice for 20 minutes at 1000 rpm and 4 °C and the supernatant was used for the treatments because in this case the residual oxygen in the fibres cannot affect the anthocyanin content. The obtained fiber-free juice had pH value of 3.6; and refraction index was 8.5 °Brix. Subsequently, each sample was supplemented with glucose up to 10, 15 and 20 ºBrix, and with ascorbic acid to 0.015 and 0.03% on relative weight as shown in Table 1. 20 mL of samples were placed into plastic bags and vacuum sealed. The foil pouches filled with strawberry juices were treated by RESATO FPU 100-2000 high hydrostatic pressure unit at 400, 500 and 600 MPa pressure values for 5, 12.5 and 20 minutes. The treatments were made at room temperature.

Table 1. The examined factor levels in the experimental design

| Factor | Minimum (-1) | Center (0) | Maximum (+1) |
|--------|--------------|------------|--------------|
| (A) Ascorbic acid addition (%) | 0 | 0.015 | 0.03 |
| (B) ºBrix | 10 | 15 | 20 |
| (C) Pressure (MPa) | 400 | 500 | 600 |
| (D) Treatment time (min) | 5 | 12.5 | 20 |

After treatment strawberry juices were stored in water bath controlled by thermostat (Contraves C40 P) for 21 days at 20 °C. During this period and at this storage temperature, changes were visually perceptible but the aspect was still acceptable from the commercial viability point of view.

2.2. Determination of anthocyanin content

To determine the anthocyanin content of the differently treated, supplemented and stored samples, 1 g of each sample was measured into 50 mL volumetric flask. This flask was filled with hydrochloric-ethanol without loss and well shaken. Thereafter they were kept in a dark place for half an hour then filtered through glass wool, and the absorbance was measured by a spectrophotometer (Hitachi U-2900) at 530 nm wherein hydrochloride alcohol was the blank sample.

Hydrochloride alcohol preparation: 20 mL of conc. HCl was measured into 1000 mL volumetric flask and filled with 96% ethanol to the mark.
2.3. Statistical analyses

The measurements were compiled on the basis of type 2\textsuperscript{n} full factorial experimental design, and the measurements were evaluated by Unscrambler 9.0 software. The implementation of the 2\textsuperscript{n} full factorial experimental design determines a response function which allows interpolating all internal points of the factor space with the expected amount of the preserved anthocyanin content. We gave a central point of the experiment plan for two reasons: to check the stability of the process and measure the inherent variability and besides these to monitor the curvature (different than linear function). The factors have effects independently from each other in the rarest cases. The change in the settings and effects of one factor can entirely change the effects of other factors. It is therefore necessary to change all the factors together in the same time, and not one by one to search optimum.

The response functions of the four factors:

\[ R(A,B,C,D) = M + \beta_A \cdot A + \beta_B \cdot B + \beta_C \cdot C + \beta_D \cdot D + \beta_{AB} \cdot A \cdot B + \beta_{AC} \cdot A \cdot C + \beta_{AD} \cdot A \cdot D + \beta_{BC} \cdot B \cdot C + \beta_{BD} \cdot B \cdot D + \beta_{CD} \cdot C \cdot D \]

where:
- \( M \): average effect (Intercept)
- \( \beta_A, \beta_B, \beta_C, \beta_D \): \( \beta \)-coefficients describing the main effects of the factors
- \( A, B, C, D \): the factors
- \( \beta_{AB}, \beta_{AC}, \beta_{AD}, \beta_{BC}, \beta_{BD}, \beta_{CD} \): \( \beta \)-coefficients of pairwise interactions of factors

3. Results and discussion

As the addition of sugar and ascorbic acid changes the absolute amount of anthocyanin content in the strawberry juice the change of the relative anthocyanin content was monitored in our assessment. On 0 day the post-HHP treatment status was compared to pre-treatment condition (always the cohesive sugar and ascorbic acid combinations), and in the case of the stored samples the 21 day results were compared to the results of the 0 day treated sample.

It could be observed on the residual anthocyanin contents that the direct effect of high hydrostatic pressure treatment was much smaller compared to the changes due to 21 days storage at 20 °C. While on 0 day maximum 20% decrease was observed in the amount of total anthocyanin content, the reduction was approx. 30-55% during storage at room temperature. It draws attention to the high rate of changes that occur during storage and that how important it is to store the products under appropriate conditions after the processing.

If we evaluate the factorial experimental design we can quantify the effect of each factor. We obtained the results shown in Table 2. The table includes the statistical results of both examined periods. The p-values refer to the significance level of the impact of each factor while the \( \beta \)-coefficients indicate the direction and magnitude of the effects. The established model and the factors are significant if the associated p-value is less than 0.05 (i.e., smaller than the type I error). The factors where significant effects were found are highlighted in the table by bold characters.

If we examine the fitting of the post-treatment model (shown in Figure 1 marked with rhombus) we can see that the correlation between the measured and calculated data was only \( r^2 = 0.76 \). Besides, slope (that is ideally one, but here is 0.6), offset (which is 0.39 instead of the ideal zero) and the value of calibration error (which is also non-zero) are also indicated in Figure 1 to show the goodness of fitting. It can be seen that in some cases these values vary widely from the expected ideal values. The fitted model was not significant as it can be seen in Table 2., since the p-value of the post-treatment model was much higher than 0.05 (\( p = 0.4037 \)). When we assess the effect of the factors (Table 2) we can observe that only the ascorbic acid addition had significant effect (\( p = 0.033 \)). In case of a significant factor the \( \beta \)-coefficient value is also important because it suggests what kind of changes (positive or negative) can be expected if we change that given factor. In this case the \( \beta \)-coefficient is positive thus
we can say that ascorbic acid supplementation had a positive impact on the residual anthocyanin content of strawberry juice samples. Consequently increased ascorbic acid content preserved greater amounts of anthocyanins. Changes were not significantly affected by the other factors and their interactions.

Table 2. Statistical results of the 2^n full factorial experimental design

| Main effects and interactions | After HHP treatment | After 21 day of storage |
|-------------------------------|---------------------|------------------------|
|                               | p-value             | β -coefficient         | p-value     | β -coefficient |
| Model                         | 0.4037              |                       | 0.009       |               |
| Intercept                     | 0                   | 0                      | 0.553       |               |
| Ascorbic acid addition (A)    | 0.033               | 0.482                  | 0.0087      | -0.303        |
| °Brix (B)                     | 0.7828              | -0.0016                | 0.0035      | 0.0108        |
| Pressure (C)                  | 0.8537              | -0.0000535             | 0.0728      | 0.000272      |
| Treatment time (D)            | 0.6112              | -0.00198               | 0.1844      | 0.00255       |
| AB                            | 0.2703              | -0.0296                | 0.0556      | -0.0262       |
| AC                            | 0.2877              | 0.0284                 | 0.0807      | 0.0224        |
| AD                            | 0.5541              | -0.0154                | 0.0145      | 0.0364        |
| BC                            | 0.9727              | 0.000882               | 0.4703      | 0.00889       |
| BD                            | 0.2035              | -0.0346                | 0.0406      | 0.0286        |
| CD                            | 0.8669              | -0.00432               | 0.8608      | 0.00212       |

Figure 1. Fitting of the calculated residual anthocyanin contents to the original measurement data, assessed by the established models of the 2^n full factorial experimental design

The results after the 21 days of storage show (Figure 1. marked with square) that the fitted model is considered to be significant (p = 0.009). The correlation coefficient calculated between the measured and calculated data highly increased (r^2 = 0.939), and the slope was also closer to the ideal value of one and the offset to zero. The calibration error was also reduced to 0.03%. In view of the main effects, the effect of added ascorbic acid was the most interesting one. The effect was significant (p = 0.0087),
however the β-coefficient was negative (-0.303). So, contrary to the direct effects of the pressure treatments, the storage period had a negative effect on the preservation of the anthocyanin content. This dominates if we examine the combined effect of the sugar content and ascorbic acid (AB) which is very close to be significant (p = 0.06). Cao and his co-workers also determined the same negative effect of added ascorbic acid during storage [21]. They found correlation r = 0.923 and r = 0.954 between the ascorbic acid and anthocyanin content decrease in fibrous strawberry juice, and r = 0.882 and r = 0.967, respectively, for strawberry juice without fiber in case of storage at 4 and 25 °C. Torres and his co-workers found correlation between ascorbic acid and cyanide-3-glucoside in blood orange juice which were r = 0.79 and r = 0.9 at 4 and 20 °C storage temperature indicating a strong interaction during the refrigerated storage [22]. As for the main effects, only the ascorbic acid supplementation and the sugar supplementation had significant effect with positive β-coefficient (p = 0.0035, β-coeff. = 0.0108) in our experiment. However it should be noted that the effect of pressure treatment remained only marginal (p = 0.07) from the statistically proved level. If we evaluate the interaction effects, the interaction of ascorbic acid supplementation and the treatment time and also the interaction of sugar supplementation and treatment time had significant effects. Thus to estimate the anthocyanin content occurring during 21 days we can define the following equation:

$$R(A,B,C,D) = 0.553 + (-0.303*A) + 0.0108*B + 0.0364*A*D + 0.0268*B*D$$

The graphical representation of the model created by the prescribed correlation is shown in Figure 2. The parallel contour lines illustrate that the effect of the pressure values during the treatments are negligible (Figure 2a) and the presence of interactions can be assumed from the twisting of the response surfaces (Figure 2b).

![Figure 2](image.png)

**Figure 2.** The pairwise comparisons of changes in the middle point of the examined factor space, assessed by the prescribed model based on the significant effects of the factorial experimental design (ascorbic acid supplementation: 0.015%, °Brix: 15%, pressure: 500 MPa, treatment time: 12.5 min)
4. Conclusions
We can conclude that after the high pressure treatments the various pressure levels, treatment times and the sugar supplementations did not have significant effects on the decrease of the anthocyanin content in strawberry juices. But the addition of ascorbic acid has influenced the anthocyanin preservation. It was observed that samples with higher ascorbic acid content had also higher anthocyanin content after the treatments. The statistical analysis also showed it was significant. Thus it can be established, that in case of strawberry juices the ascorbic acid has a protective role in the preservation of the initial anthocyanin content during the high pressure treatments.

After 21 days of storage at 20 °C, the anthocyanin content of the samples considerably decreased, often nearly halved compared to the initial anthocyanin content. The different pressure levels and treatment times had no significant effect on these changes based on statistical analysis, in contrast of added ascorbic acid and sugar supplementation. Interestingly, during storage the added ascorbic acid significantly reduced the anthocyanin content that remained after pressure treatment of strawberry juices. Consequently the ascorbic acid content has an antagonist effect on anthocyanin content in strawberry juices, after high pressure treatment helps to preserve the initial anthocyanin content, but during storage reduces that.

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