Effect of Red Cabbage Sprouts Treating with Organic Acids on the Content of Polyphenols, Antioxidant Properties and Colour Parameters

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Abstract: In recent years, there has been a great deal of consumer interest in consuming vegetables in the form of sprouts, characterized by high nutritional value. The disadvantage of sprouts is the loss of bioactive compounds during storage and the relatively short shelf life, due to the fact that they are a good medium for microorganisms, especially yeasts and molds. The aim of the study was to compare the content of polyphenols, antioxidant properties, color and microbiological quality of red cabbage sprouts preserved by the use of mild organic acids: Citric, ascorbic, lactic, acetic and peracetic. In the study, the content of polyphenols and antioxidant properties of sprouts was examined using the spectrophotometric method, instrumental color measurement was done using an “electronic eye” and the content of mold, yeast and the total number of mesophilic microorganisms was determined using the plate inoculation method. Taking into account the content of polyphenols and the antioxidant potential of sprouts, it was found that the addition of all organic acids contributed to the preservation of the tested compounds during their 14-day storage under refrigerated conditions, depending on the type of organic acid used, from 71 to 86% for polyphenols and from 75 to 96% for antioxidant properties. The best results were obtained by treating the sprouts with peracetic acid and ascorbic acid, respectively, at a concentration of 80 ppm and 1%. The conducted research on the possibility of extending the storage life and preserving the bioactive properties of fresh sprouts showed that the use of peracetic acid in the form of an aqueous solution during pre-treatment allows to reduce the content of microorganisms by one logarithmic order. Ascorbic acid did not reduce the content of microorganisms in the sprout samples tested. Considering the content of bioactive ingredients, as well as the microbiological quality of fresh sprouts, it can be said that there is a great need to use mild organic acids during the pre-treatment of sprouts in order to maintain a high level of health-promoting ingredients during their storage, which may also contribute to their prolongation durability.

Keywords: sprouts; organic acids; bioactive compounds; microbiological quality

1. Introduction

In recent years, there has been a great deal of consumer interest in minimally processed food with preserved natural quality features, high sensory quality and nutritional value, as well as low energy value. Native to the Mediterranean region of Europe, red cabbage (Brassica oleracea var. Capitata f. Rubra) is a widely used component in ready-to-eat products. It is one of the cruciferous vegetables that is distinguished by high levels of anthocyanins, glucosinolates and sulforaphane [1]. There are reports that sulforaphane may be a chemopreventive agent, supporting the removal of toxins and carcinogenic substances from the body [2–5]. Recently, there has been widespread consumer interest in consuming vegetables in the form of sprouts, which have become popular ingredients in fresh vegetable salads [6]. During seed germination, many structural and metabolic
changes take place, contributing to the removal of anti-nutrients such as enzyme inhibitors from seeds, making most types of sprouts safe for human consumption, although some sprouts, e.g., quinoa sprouts, require a prior heat treatment to inactivate the toxic type 1 ribosome-inactivating protein (RIP) [7]. High nutritional and low energy value of various types of sprouts are valued, and numerous literature data confirm their high pro-health properties [8–16]. Sprouts exhibit anti-inflammatory and antioxidant properties, inhibit pathogenic microorganisms, including *Helicobacter pylori*, support fat burning and support the treatment of diabetes [17–25].

The disadvantage of vegetables, including sprouts, produced in a fresh, unprocessed form are the loss of bioactive compounds during their storage and relatively short shelf life, due to the fact that they are a good breeding ground for microorganisms, especially yeasts and molds [26–28]. Sprouts are characterized by a delicate texture and they are easily damaged and deteriorate in color. Hence, difficulties with their disinfection may arise [29,30]. Recently, extensive research has been carried out on the possibility of extending the shelf life of vegetables through the use of organic acids, UV radiation and others; however, these studies are usually carried out on hard-textured vegetables and lettuces [31–41].

The research undertaken in the work is important for scientific and application reasons due to the need to develop a method of producing sprouts from various seeds, which would be characterized by highly preserved health-promoting properties, of high microbiological purity with the use of disinfecting agents approved by sanitary authorities, which at the same time will not have a significant impact on the deterioration of their pro-health and sensory values. The available scientific literature lacks data on the method of sprout production, which allows the preservation of polyphenolic compounds and the antioxidant potential during storage, while affecting the higher microbiological purity of the preserved raw materials.

The aim of the study was to compare the content of polyphenols, antioxidant properties, color and microbiological quality in red cabbage sprouts preserved by the use of mild disinfectants in the form of citric, ascorbic, lactic, acetic and peracetic acids approved for use in organic products.

2. Materials and Methods
2.1. Materials
The research material consisted of red cabbage and red cabbage sprouts from a horticultural and livestock farm located in the Mazowieckie Voivodeship (Warsaw, Poland), approved for consumption by sanitary authorities, showing no pathogenic microorganisms. After soaking (8 h) and rinsing several times with water, the seeds of red cabbage were thoroughly drained and then sown in a shallow tray filled with a 3 cm thick layer of standard garden soil. After abundant wetting, the substrate with the sown sprouts was covered with a transparent plastic cover, ensuring the maintenance of high relative humidity. After about 3 days of incubation, at the temperature of 22 °C, relative humidity of 80% and moderate sunlight, the first sprouts that appeared were intensively sprinkled (every 8 h) for the rest of their growth. After approximately 8 days of growth and reaching maturity (i.e., the height of the sprouts approx. 6–7 cm), the red cabbage sprouts were cut at a height of approximately 1 cm above the growing medium and intended for laboratory tests. The research was carried out on sprouts stored (7 and 14 days) and not stored. One percent of organic acids (Avantor Performance Materials Poland S.A., Gliwice, Poland), such as citric acid, ascorbic acid, lactic acid, acetic acid and peracetic acid, at a concentration of 80 mg/L were used for the study. The procedure of gentle disinfection of sprouts was applied with the use of various organic acids, which did not affect the perceptibility of sour taste.

The sprouts were assessed for their antioxidant properties, polyphenol content and color immediately after the addition of individual organic acids and after 7 and 14 days of storage. Additionally, in selected samples, the level of mesophilic bacteria, yeasts and molds was determined after 14 days of storage, compared to the non-stored sample.
2.2. Methods

Samples preparation for analytical and microbiological tests: sprout samples were weighed (20.0 ± 0.01 g) into sterile plastic containers with a screw cap, then treated with solutions (10.0 ± 0.01 mL) of individual organic acids by spraying them, and then the excess fluids were drained. The samples were stored in containers in a sterile cooler (temperature 4 °C, relative humidity 85%). After the end of the storage period, analytical and microbiological tests were performed.

Microbiological analysis: The microbiological analysis of sprouts was carried out in accordance with the PN-EN ISO 4833-1:2013-12 standard [42], which was used to determine the total number of microbial colonies at 30 °C, time of analysis: 3 days. The number of yeasts and molds was determined in accordance with the PN-ISO 21527-2: 2009 standard [43] at 25 °C, time of analysis: 5 days.

Extraction of bioactive ingredients: 5.0 g of fresh red cabbage sprouts were scaled (with 0.001g accuracy) (AS 220/X, Radwag, Radom, Poland) into a plastic, falcone tubes with a screw top (50.0 mL capacity) and 30.0 mL of the water-methanol mixture in the volume of 30:70 was added. It was shaken in a vortex shaker (Wizard Advanced IR Vortex Mixer, VELP Scientifica Srl, Usmate, Italy) for 60 s. After that, the samples were incubated in a shaking incubator in 60 °C (IKA KS 4000i Control, IKA Ltd., Warsaw, Poland) for 2 h. Then the samples were shaken anew in a vortex for 60 s to receive a thorough mixture, centrifuged in a refrigerated centrifuge (MPW-380 R, MPW Med. Instruments, Warsaw, Poland) for 15 min (4 °C, 10.000 rpm) and finally the obtained supernatant was collected and used for the determination of the antioxidant capacity and the total content of polyphenols. The same procedure was used to obtain extracts in red cabbage.

Determination of antioxidant properties: Antioxidant activity of red cabbage sprouts extracts were determined by using ABTS•+ (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic) acid) (Sigma-Aldrich, Poznań, Poland) radical cation assay according to modified Re et al. (1999) method [44]. The principle of the method is based on the measurement of the ability to deactivate synthetic ABTS•+ cation species by antioxidant compounds contained in the tested material. A certain quantity of the tested extracts’ solution (determined by a designated dilution scheme) was drawn into 10 mL glass test tubes. After that, 3.0 mL of radical cations ABTS•+ in PBS (Phosphate Buffer Solution, Sigma-Aldrich, Poznań, Poland) solution were added. The absorbance measurements were made after exactly 6 min of incubation the samples in about 20 °C. The absorbance were measured at the wave length of λ = 734 nm, with the use of spectrophotometer (UV/Vis UV-6100A, Metash Instruments Co., Ltd., Shanghai, China). The results were calculated based on the calibration curve (y = −5.6017x + 0.7134, R² = 0.9998) for Trolox (Sigma-Aldrich, Poznań, Poland) and represented as μM TEAC (Trolox Equivalent Antioxidant Capacity) per 1g of fresh weight (f. w.) red cabbage sprouts. The same procedure was used to determine the antioxidant properties in red cabbage.

Determination of the content of polyphenolic compounds: Total polyphenols content in red cabbage sprouts were measured by the reaction between polyphenol compounds with Folin–Ciocalteu and sodium carbonate (Sigma-Aldrich, Poznań, Poland) reagents using a modified method of Singleton and Rossi (1965) [45]. The method is based on the reduction of molybdenum (VI) to molybdenum (V) contained in the Foli–Ciocalteu reagent by polyphenol-like compounds present in the tested samples in an alkaline environment of sodium carbonate. A defined amount of red cabbage sprouts extracts, based on the dilution scheme, were collected into 50 mL graduated flasks, then 2.5 mL of Folin–Ciocalteu reagent and 5.0 mL of 20% sodium carbonate solution were added and made up to the mark with distilled water. The samples were incubated for 60 min at about 20 °C and protected from light. The absorbances were measured at a wavelength of λ = 720 nm using a spectrophotometer (UV/Vis UV-6100A, Metash Instruments Co., Ltd., Shanghai, China). The results were calculated based on the calibration curve (y = 2.1297x + 0.1314, R² = 0.9994) for gallic acid (Sigma-Aldrich, Poznań, Poland) and represented as mg GAE (Gallic Acid Equivalent) per 1g of fresh weight (f. w.) red cabbage sprouts.
Equivalent) per 1g of fresh weight (f. w.) of the tested samples. The same procedure was used to determine the content of polyphenolic compounds in red cabbage.

Colour analysis: Instrumental color measurement was performed by the computer image analysis using the “electronic eye” color analyzer (visual analyzer 400 IRIS, Alpha M.O.S., Toulouse, France) combined with data processing software (Alpha M.O.S., Toulouse, France). Photos of the samples were taken in a closed chamber (420 × 560 mm), which guarantees controlled light conditions, without the influence of external light on the visual analysis. Both top and bottom lighting, preventing the shadow effect (2 × 2 LED assembly lights, 6700 K color temperature and IRC = 98, near D65: daylight during a cloudy day at 12 AM) was applied. The Basler (acA2500-14gc; BaslerAG, Ahrensburg, Germany) camera with a lens of 16 mm diameter was used to take the pictures of the samples. After calibrating the instrument with a certified color scale, the samples were placed in a removable white tray, diffusing uniform light inside the device’s lockable light chamber. Measurements were made in the CIE L* a* b* system (L-brightness, +a-red, -a-green, +b-yellow, -b-blue) and RGB system (R–red, G–green, B–blue). The values for parameters L*, a*, b*, R, G, B were calculated as weighted averages, taking into account the frequency of appearance of individual color code. Measurements were performed in triplicate.

Statistical analysis: The statistical analysis of the obtained results was performed using the Statistica 10.0 program (Statsoft). One-way ANOVA was performed using Duncan’s significance test at a significance level of $p < 0.05$. The results of the statistical analyses are presented in the tables with the appropriate numbers in order to note the homogeneous groups.

3. Results and Discussion

3.1. Effect of Organic Acids Addition on the Content of Polyphenols and Antioxidant Properties in Red Cabbage Sprouts

Figure 1 shows the results of the total polyphenol content (TPC) and antioxidant activity (ABTS) of fresh red cabbage and fresh red cabbage sprouts. The tested fresh sprouts immediately after production were characterized by a higher content of polyphenols (17.43 ± 0.17 mg GAE/g f. w.) compared to the samples of fresh red cabbage (12.83 ± 0.14 mg GAE/g f. w.), while the antioxidant activity of the tested samples was comparable (347.36 ± 2.52 µM TEAC/1 g and 345.67 ± 2.32 µM TEAC/g f.w., respectively).

The available literature lacks studies comparing the content of polyphenols and the antioxidant activity of sprouts and vegetables at full maturity, especially in red cabbage. However, there are data on the comparison of the content of nutrients and other compounds showing health-promoting properties. In the studies by Drozdowska et al., 2020 [46], the content of nutrients between young shoots of red-headed cabbage and a fully ripe vegetable was compared. The authors showed that red cabbage was characterized by a significantly higher content of dry matter and total carbohydrates, including digestible carbohydrates, compared to the young shoots. In turn, young shoots in the phase of intensive growth were a better source of protein, minerals and glucosinolates. The higher content of polyphenols in sprouts may be related to the germination process, during which a reactivation of seed metabolism (according to higher activity of enzymes) takes place, promoting the hydrolysis of compounds with health-promoting properties [47].
Figure 1. Total polyphenol content (TPC) and antioxidant activity (ABTS) of fresh red cabbage and fresh red cabbage sprouts. The graph shows the mean values and standard deviation ($n = 3$).

Figures 2 and 3 as well as Tables 1–6 show the results of the total polyphenol content and antioxidant properties (ABTS) of red cabbage sprouts treated with the tested organic acids, not stored and stored for 7 and 14 days at refrigerated temperature, compared to the control sample, without preservation.

Figure 2. Total polyphenol content (TPC) in red cabbage sprouts with the addition of solutions of various organic acids subjected to 7- and 14-day storage. The graph shows the mean values and standard deviation ($n = 3$).
Figure 3. Antioxidant properties (ABTS) of red cabbage sprouts with the addition of solutions of various organic acids subjected to 7- and 14-day storage. The graph shows the mean values and standard deviation ($n = 3$).

Table 1. Group homogeneity test ($\alpha = 0.05$) comparing the effect of applied organic acids on the content of polyphenols (TPC) in red cabbage sprouts not subjected to storage.

| Type of Samples                              | Homogeneous Groups |
|----------------------------------------------|--------------------|
| Red cabbage                                  |                    |
| Sprouts treated with 1% acetic acid          | ****               |
| Sprout treated with 1% citric acid           | ****               |
| Sprouts treated with 1% lactic acid          | ****               |
| Not treated sprouts                          | ****               |
| Sprouts treated with 80 mg/L peracetic acid  | ****               |
| Sprouts treated with 1% ascorbic acid        | ****               |

Table 2. Group homogeneity test ($\alpha = 0.05$) comparing the effect of applied organic acids on the content of polyphenols (TPC) in red cabbage sprouts subjected to 7-day storage.

| Type of Samples                              | Homogeneous Groups |
|----------------------------------------------|--------------------|
| Not treated sprouts                          | ****               |
| Sprouts treated with 1% acetic acid          | ****               |
| Sprout treated with 1% citric acid           | ****               |
| Sprouts treated with 1% lactic acid          | ****               |
| Sprouts treated with 80 mg/L peracetic acid  | ****               |
| Sprouts treated with 1% ascorbic acid        | ****               |
The content of polyphenols during the tested storage period of unfixed sprouts decreased by approximately 32% after 7 days of storage and by approximately 38% after 14 days in relation to the sample not subjected to storage. All the tested organic acids contributed to a much higher conservation of polyphenols during their 14-day storage, compared to the unpreserved sample, depending on the type of organic acid used.
The highest beneficial effect for the stored sprouts was shown by peracetic acid (5 and 14% losses after 7 and 14 days of storage, respectively), and the greatest losses were for the sprouts treated with acetic acid (17 and 29% losses after 7 and 14 days of storage, respectively). The sprouts treated with citric, L-ascorbic and lactic acid showed a similar behavior of the polyphenol content at the level of 93-95% after 7 days of storage and 85–88% after 14 days.

Similar relationships to those obtained in this study were obtained in the study of Zeng et al. (2017) [48], where the content of polyphenols in tea was examined under various pH conditions and different temperatures during storage of aqueous solutions for 24 h. In this work, tea polyphenols were pH-sensitive: The lower the pH, the more stable the tea polyphenols during storage [48]. The stability of polyphenols is dependent on both temperature and pH. It was noted that total polyphenols content is faster degraded with the increase of either pH, oxygen concentration or temperature [49]. Hong et al. (2002) [50] reported that several factors, including pH, concentration of proteins, antioxidant levels and the presence of metal ions, could affect the stability of catechin of which pH is probably the most critical. Polyphenols are unstable in neutral and alkaline solutions and decomposed in a few minutes, whereas they are relatively stable under acidic conditions [51].

The antioxidant activity of sprouts not fixed during storage decreased by approximately 19 and 24% after 7 and 14 days of storage, respectively. All tested sprouts fixed with organic acids were characterized by higher antioxidant activity compared to fresh, non-stored sprouts. The highest preservation of antioxidant properties was noted for sprouts with the addition of peracetic, acetic and lactic acid (losses of about 1–4% after 14 days of storage), and the lowest for citric and ascorbic acids (7–9% after 7 days of storage, 10–25% after 14 days).

A similar tendency in studies on the influence of lactic acid bacteria on the antioxidant properties of fermented red cabbage sprouts was demonstrated by Hunaefi et al. (2013) [52-54]. They found a significant (almost twofold) increase in the antioxidant activity of sprouts fermented with three strains of lactic acid bacteria (Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus rhamnosus) compared to non-fermented sprouts. According to the authors, the applied fermentation processes may be a method for improving the durability and antioxidant properties of red cabbage sprouts.

In many studies, on the basis of the determined dependence of the antioxidant properties of the tested phenolic compounds on the environmental pH, it was shown that the pH of the environment in which the free radical oxidation process takes place is an important factor determining the antioxidant properties of the tested polyphenols [55-60]. The higher antioxidant properties of sprouts treated with organic acids could be due to the increase in antioxidant properties of individual groups of polyphenols present in the tested sprouts. In the case of the discussed values, similar relationships were obtained as those obtained for polyphenols. Additionally, the highest results were obtained in sprouts treated with ascorbic acid, which could be related to the fact that ascorbic acid, in other words vitamin C, has a strong antioxidant effect [61]. Similar relationships to those obtained in this study were also obtained in the work of Walkowiak-Tomczak et al. (2007) [62], where changes in the antioxidant activity of black chokeberry juice concentrate solutions during storage were studied. Changes in antioxidant activity in black chokeberry concentrate solutions were also related to the pH value of the product, especially in samples stored at 30 °C, where the greatest decrease in activity was recorded at pH 5.0. According to Azizah et al. (1999) [63], the pH value of a solution influences the antioxidant activity of products and its changes during storage. In that study, the anthocyanin content decreased with increasing pH value during storage. Anthocyanins showed the highest stability at pH 3.0 or lower when present in the form of a flavilic cation, and an increase in pH above this level resulted in increasing degradation of dyes, especially when heated or stored at temperatures without cooling [64,65].
3.2. Effect of Organic Acids Addition on the Colour of Red Cabbage Sprouts

Table 7 shows the color parameters in the CIE L* a* b* and RGB spaces of red cabbage sprouts stored for 14 days, while Figure 4 shows the values of the $\Delta E^*$ color parameter, which indicates the differences between the color of sprouts during storage. Group homogeneity test comparing the effect of the organic acids used on the $\Delta E^*$ values of red cabbage sprouts subjected to 7-day and 14-day storage are shown in Tables 8 and 9, respectively. The greatest changes in color during the 14-day storage of sprouts occurred for the control sample, not fixed ($\Delta E^*$ approximately 8) and for the sample treated with citric acid ($\Delta E^*$ approximately 7), and the smallest for the sprouts treated with peracetic acid ($\Delta E^*$ approximately 3). Similar values of the $\Delta E^*$ parameter were found for sprouts treated with ascorbic acid and lactic acid ($\Delta E^*$ approximately 6), slightly lower values were obtained for the sample treated with acetic acid ($\Delta E^*$ approximately 5). Taking into account the results given in Table 6, it can be seen that the main changes in the color of the sprouts were associated with the development of a darker color with lower intensity of red, yellow, green and blue. Depending on the individual ability of the human eye to judge the differences in color, three different ranges can be distinguished to distinguish between changes in the color value, i.e., $\Delta E^* < 1$, imperceptible to the human eye; $1.0 < \Delta E^* < 3.3$, only noticeable by a specialist, clinically acceptable; $\Delta E^* > 3.3$, easy to observe, these color changes are not clinically acceptable. In this study, the values from the last range were obtained for all tested samples, except for sprouts treated with acetic acid. This means that the color changes were noticeable, and the samples differed significantly. For acetic acid-treated sprouts, the color change was only noticeable by a skilled person, clinically acceptable ($\Delta E^* < 3.3$). Color changes of sprouts that occurred during storage may result from changes in the content of anthocyanins, the main color-imparting compounds, present in significant amounts in red cabbage and sprouts [66]. In research by Walkowiak-Tomczak et al., (2016) [63] on changes during storage of elderberry juice concentrate solutions, pigment content decreased in each tested sample as the effect of temperature and pH. In that study, the lowest changes in color parameters were noticed at the lowest tested pH of 3, which proved the high stability of anthocyanin dyes in this pH range. At the highest tested pH, 5, the elderberry juice concentrate was darker, which was related to the brownish-red shade of the tested samples. It resulted, inter alia, from the accumulation of anthocyanin degradation products. In this study, the greatest color changes during storage were obtained in samples not treated with individual acids (the highest value of $\Delta E^*$). Taking into account the obtained values of the individual color components, it can be seen that after 14 days of storage, the sprouts not treated with organic acids were significantly darkest in color, compared to all samples treated with solutions of individual acids, which is not noticed immediately after treating the sprouts with acids and after 7 days of storage (in these cases, only some of the sprouts were lighter in color compared to the control sprouts). In this study, as well as in Walkowiak-Tomczak et al., (2016) [62], the values of the color parameters changed during the storage of the tested samples. In the cited work, the brightness of the tested samples increased with the increasing of pH, storage time and temperature, while the values of the a* (red color) and C* (color saturation) color parameters decreased. The b* parameter values (share of yellow color) decreased with increasing pH during storage. Similar relationships for the color parameters a* and b*, regardless of the pH, were also obtained in this study.
Table 7. Color parameters of red cabbage sprouts treated with organic acids subjected to 14-day storage.

| Sprout Samples                      | Color Parameters | Not Treated Sprouts | Sprout Treated with 1% Citric Acid | Sprouts Treated with 1% Ascorbic Acid | Sprouts Treated with 1% Lactic Acid | Sprouts Treated with 1% Acetic Acid | Sprouts Treated with 80 mg/L Peracetic Acid |
|-------------------------------------|------------------|---------------------|-------------------------------------|---------------------------------------|-------------------------------------|-------------------------------------|---------------------------------------------|
| Sprouts not stored                  | L*               | 44.6 ± 0.8 c        | 43.3 ± 0.9 bc                      | 42.6 ± 0.9 b                         | 40.7 ± 0.8 a                        | 40.2 ± 0.9 a                        | 41.3 ± 1.0 a                               |
|                                     | a*               | 8.1 ± 0.1 b         | 9.2 ± 0.2 e                        | 8.9 ± 0.8 d                         | 8.6 ± 0.1 c                         | 9.5 ± 0.1 f                         | 7.5 ± 0.1 a                                |
|                                     | b*               | 0.9 ± 0.0 b         | 3.4 ± 0.0 e                        | 0.8 ± 0.1 a                         | 1.4 ± 0.0 c                         | 3.3 ± 0.2 e                         | 2.4 ± 0.1 d                                |
|                                     | R                | 120.2 ± 1.0 c       | 119.4 ± 0.6 c                      | 116.6 ± 0.7 b                       | 112.2 ± 1.8 a                       | 111.2 ± 1.1 a                       | 111.3 ± 1.2 a                              |
|                                     | G                | 101.8 ± 0.9 e       | 97.7 ± 0.7 d                       | 96.7 ± 0.8 d                        | 90.1 ± 1.0 a                        | 93.8 ± 1.1 b                        | 85.5 ± 1.1 c                               |
|                                     | B                | 105.3 ± 0.3 e       | 97.9 ± 0.2 c                       | 101.0 ± 1.0 d                       | 90.9 ± 1.2 b                        | 94.6 ± 0.9 a                        | 95.2 ± 1.6 b                               |
| Sprouts stored for 7 days           | L*               | 39.9 ± 2.0 b        | 37.9 ± 1.1 ab                      | 36.7 ± 1.0 a                        | 38.1 ± 1.0 ab                       | 36.4 ± 0.7 a                        | 37.9 ± 1.0 ab                               |
|                                     | a*               | 6.9 ± 0.1 b         | 6.3 ± 0.1 a                        | 6.3 ± 0.2 a                         | 6.8 ± 0.1 b                         | 8.1 ± 0.0 c                         | 6.5 ± 0.13 a                               |
|                                     | b*               | 1.8 ± 0.0 a         | 2.9 ± 0.1 d                        | 2.1 ± 0.1 b                         | 2.9 ± 0.1 d                         | 2.3 ± 0.0 c                         | 2.9 ± 0.0 d                                |
|                                     | R                | 107.1 ± 2.0 d       | 101.7 ± 0.9 ab                     | 98.3 ± 0.7 a                        | 102.8 ± 1.2 c                       | 99.9 ± 1.0 a                        | 102.0 ± 1.5 ab                              |
|                                     | G                | 91.2 ± 1.1 d        | 86.8 ± 1.5 c                       | 83.9 ± 1.0 b                        | 86.8 ± 1.0 c                        | 81.7 ± 1.0 a                        | 86.8 ± 0.9 c                               |
|                                     | B                | 92.5 ± 0.6 c        | 85.9 ± 1.1 ab                      | 84.3 ± 1.5 ab                       | 86.2 ± 0.9 b                        | 83.0 ± 0.1 a                        | 86.1 ± 1.0 ab                               |
| Sprouts stored for 14 days          | L*               | 35.3 ± 0.8 a        | 37.1 ± 1.0 b                       | 37.5 ± 0.6 b                        | 37.7 ± 1.32 b                       | 37.9 ± 1.1 b                        | 38.5 ± 0.6 b                               |
|                                     | a*               | 6.2 ± 0.2 d         | 5.10 ± 0.1 b                       | 5.9 ± 0.1 c                         | 4.0 ± 0.1 a                         | 6.6 ± 0.1 e                         | 6.3 ± 0.1 d                                |
|                                     | b*               | 0.7 ± 0.0 a         | 3.2 ± 0.2 c                        | 1.7±0.0 b                           | 3.5 ± 0.1 b                         | 6.8 ± 0.1 e                         | 1.7 ± 0.0 b                                |
|                                     | R                | 93.9 ± 0.9 a        | 97.9 ± 1.2 b                       | 99.2 ± 0.9 b                        | 97.6 ± 1.5 b                        | 105.5 ± 1.6 C                       | 102.5 ± 0.6 c                              |
|                                     | G                | 80.6 ± 0.9 a        | 85.6 ± 1.4 b                       | 86.0 ± 1.3 bc                       | 87.3 ± 1.6 bc                       | 85.9 ± 1.0 bc                       | 88.3 ± 1.3 c                               |
|                                     | B                | 83.3 ± 0.8 b        | 83.6 ± 0.8 b                       | 86.9 ± 0.9 c                        | 84.2 ± 1.8 b                        | 79.1 ± 2.0 a                        | 89.4 ± 1.4 d                               |

a–f—average values denoted by different letters, significantly different from each other ($\alpha = 0.05$).

Figure 4. Color changes ($\Delta E^*$ values) of red cabbage sprouts with the addition of solutions of various organic acids subjected to 14-day storage. The graph shows the mean values and standard deviation ($n = 3$).
Table 8. Group homogeneity test (α = 0.05) comparing the effect of the organic acids used on the ∆E* values of red cabbage sprouts subjected to 7-day storage.

| Type of Samples | Homogeneous Groups |
|-----------------|--------------------|
| Sprouts treated with 1% ascorbic acid | **** |
| Sprout treated with 1% citric acid | **** | **** |
| Sprouts treated with 80 mg/L peracetic acid | **** | **** |
| Sprouts treated with 1% lactic acid | **** |
| Not treated sprouts | **** |
| Sprouts treated with 1% acetic acid | **** |

Table 9. Group homogeneity test (α = 0.05) comparing the effect of the organic acids used on the ∆E* values of red cabbage sprouts subjected to 14-day storage.

| Type of Samples | Homogeneous Groups |
|-----------------|--------------------|
| Sprouts treated with 80 mg/L peracetic acid | **** |
| Sprouts treated with 1% acetic acid | **** |
| Sprouts treated with 1% lactic acid | **** | **** |
| Sprouts treated with 1% ascorbic acid | **** | **** |
| Sprout treated with 1% citric acid | **** | **** |
| Not treated sprouts | **** |

3.3. Effect of Organic Acids Addition on the Microbiological Quality of Red Cabbage Sprouts

The obtained test results (Table 10) regarding the microbiological quality of the tested types of sprouts immediately after production and after 14 days of refrigerated storage in a package with limited air access indicate that they contain a high content of the microorganisms, i.e., the total content of bacteria, yeast and mold. These studies suggest that it is advisable to carry out disinfecting treatments during their pre-treatment. The use of disinfection may be indicated at various stages, i.e., immediately after the end of the germination process, when rinsing the sprouts with water or removing the seed coat.

Table 10. Microbiological quality of red cabbage sprouts treated with aqueous solutions of ascorbic and peracetic acids after 14-day storage (n = 3).

| Type of Samples | Molds (log CFU/g) | Yeast (log CFU/g) | Total Number of Colonies (log CFU/g) |
|-----------------|------------------|-----------------|----------------------------------|
| Not treated sprouts | 3.53 ± 0.02 c | 4.75 ± 0.01 c | 8.87 ± 0.16 b |
| Sprouts treated with 1% ascorbic acid | 3.25 ± 0.04 b | 4.09 ± 0.07 b | 8.58 ± 0.27 b |
| Sprouts treated with 80 mg/L peracetic acid | 2.18 ± 0.07 a | 3.59 ± 0.05 a | 7.13 ± 0.14 a |

a–c—average values denoted by different letters, significantly different from each other (α = 0.05).

In this study, the microbiological quality was examined on sprouts treated with ascorbic and peracetic acids, which showed the highest polyphenol preservation and antioxidant activity of all the acids tested. The obtained results indicate that the use of a low 1% concentration of ascorbic acid had no significant effect on the level of total number of microorganism’s colonies, but significantly decreased the level of molds (by about almost 8%) and yeast (by about almost 14%). Significant differences for all groups of microorganisms were obtained when the sprouts were treated with peracetic acid used at a very low concentration (80 mg/L) for disinfecting fresh red cabbage sprouts. Peracetic acid does not change the taste of preserved raw materials and can be used for disinfecting vegetables from organic production. The available scientific literature lacks data on the use of the tested organic acids to improve the properties and microbiological quality of sprouts obtained from vegetable seeds. Sprouts are a specific group of products with a delicate character, brittle texture, and they are also a very good breeding ground for
microorganisms due to the high content of valuable nutrients. The extensive literature on the disinfection of fresh vegetables mainly focuses on the effects of various disinfectants, including ozone, peracetic acid or lactic acid, on individual strains of microorganisms, mainly pathogenic [67–69].

4. Conclusions

Taking into account the content of the tested bioactive ingredients and the antioxidant potential of the sprouts, it was found that the addition of all organic acids improved the behavior of the tested compounds during 14-day storage in the refrigerator. The best results were obtained by treating the sprouts with peracetic acid and ascorbic acid at a concentration of 80 ppm and 1%, respectively. Such an addition of acids allowed for the maintenance of the high level of bioactive properties and antioxidant potential of sprouts during the 2-week period of storage in refrigerated conditions. The conducted research on the possibility of extending the shelf life and preserving the bioactive properties of fresh sprouts showed that the use of an aqueous solution of peracetic acid during pre-treatment (washing, spraying) allows to significantly reduce the content of microorganisms. Peracetic acid used in a very low concentration (80 mg/L), permitted by EFSA for disinfecting fresh vegetables, does not change the taste of products, so it can be used to disinfect vegetables, also from organic production. Additionally, peracetic acid caused the smallest color changes of the tested sprout samples. Compared to peracetic acid, ascorbic acid showed lower antimicrobial activity in the examined sprout samples.

Summing up, it can be said that it is possible to obtain fresh sprouts with high-quality features and a longer shelf life compared to those produced so far. In the case of sprouts with a low initial microbial content, the use of mild disinfectants such as 1% aqueous ascorbic acid solutions may be recommended, as it allows for high preservation of their valuable bioactive properties with a moderate reduction of microbes during their 2-week storage. In the case of sprouts with a higher level of microbiological contamination, peracetic acid should be used in a concentration of 80 mg per 1 l of water with simultaneous gentle disinfection of the seeds. These treatments should extend the shelf life of sprouts, including sprouts with a higher content of the microorganisms. These data require confirmation in subsequent studies. This type of research may be an indication for sprout producers to modify the existing production technology or develop a new one.

Author Contributions: Conceptualization, A.S. and K.N.; methodology, A.S. and K.N.; formal analysis, K.N.; investigation, A.S.; resources, A.S. and K.N.; data curation, A.S. and K.N.; writing—original draft preparation, A.S. and K.N.; writing—review and editing, A.S. and K.N.; visualization, A.S. and K.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research was prepared as part of the statutory activity of the Department of Functional and Ecological Food.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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