IMPACT OF CLARIFYING AGENTS ON THE BIOACTIVE COMPOUNDS, ANTIOXIDANT LEVELS AND COLOR PROPERTIES OF ARONIA JUICE

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Abstract

The demand for fruit juices is increasing, prompting interest in natural clarification methods to enhance clarity and visual appeal. *Aronia melanocarpa* (Michx.) Elliott, rich in anthocyanins, phenolics, and antioxidants, is valuable in juice production. This study evaluated three positively charged clarifying agents: gelatin-based (Erbigel) and plant-based (Litto Fresh, Flora Claire) in combination with two negatively charged agents (Klar Super, Klar Sol30) on Aronia juice. Samples were analysed for total anthocyanin content (TAC), total phenolic content (TPC),2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH), and colour properties. Results showed no significant differences in TAC and TPC across treatments, indicating the stability of anthocyanins and phenolics following clarification. However, DPPH assay revealed that some agents, particularly Klar Sol30 with Litto Fresh and Klar Super with Erbigel, significantly reduced antioxidant activity ($P < 0.05$), whereas others preserved it. Colour measurements demonstrated that all clarifying agents altered juice colour, with plant-based agents causing the most noticeable changes ($\Delta E^* > 6$).

Keywords: Aronia, Clarification, Anthocyanins, Phenolics, Antioxidants.

Introduction

Aronia melanocarpa (Michx.) Elliott, which belongs to the *Rosaceae* family, is a species of North American flora, known also as Black Chokeberry. It is highly resistant to diseases, fungi, pesticides, herbicides, synthetic fertilizers, and environmental pollutants, including those from industry and traffic, as it does not accumulate heavy metals. This resilience is largely attributed to its rich polyphenolic content, particularly the ability of procyanidins to bind with proteins (Oziembłowski et al. 2022). Black chokeberry and its derived products are highly valued for their rich content of bioactive

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compounds, which offer significant biological and nutritional benefits. These include flavonoids (notably anthocyanins, proanthocyanidins, flavanols, and flavonols), phenolic acids (such as chlorogenic, neochlorogenic, and caffeic acids), and tannins. (Oziembłowski et al. 2022).

Black chokeberry fruits contain approximately 0.3% tannins, including catechins and their dimers, quercetin, and other compounds responsible for the slightly bitter taste. As a result, fresh chokeberries are rarely eaten directly and are instead commonly consumed in processed forms like jams or juices (Kobus et al. 2019). Since the fruit can be consumed in various forms, its biochemical composition particularly its antioxidant content can vary. This variation is influenced by factors such as the chokeberry variety, ripeness, cultivation practices, soil and climate conditions, and especially processing methods like heating or drying (Olechno et al. 2023).

The growing global demand for fruit juices has driven a surge in product variety and processing techniques. The market of today offers a wide range of options minimally processed juices, extra pulp variants, concentrates, pasteurized and unpasteurized types, as well as functional juice drinks all tailored to meet diverse consumer preferences (Rai et al. 2022). In industrial juice production, maximizing extraction efficiency is a key goal. However, for consumers, the primary quality indicators are the juice's health benefits and sensory attributes. Thus, production technologies should be optimized to balance both efficiency and quality (Kobus et al. 2019).

Haze and sediment commonly form in juices during storage, diminishing sensory quality and consumer appeal. Removing these haze-forming compounds is essential yet challenging. One effective approach to achieve juice clarification is through the use of clarifying agents (Chen et al. 2019). Clarifying agents are commonly added during or near the end of beverage production such as wine, beer, or non-alcoholic juices to remove organic compounds. Their main functions include improving clarity, adjusting flavour, and enhancing aroma. In fruit juices like apple or grape juice, clarification involves eliminating suspended particles to achieve better clarity and colloidal stability (Ahamad et al. 2023). This process can be achieved through clarifiers, enzymes, natural precipitation, or physical treatments like centrifugation and filtration. Among these methods, clarifying agents stand out for their high efficiency, simplicity, and low cost (Wongmaneepratip et al. 2023). While clarification is widely used in other fruit juices, limited data are available on how different types of clarifying agents affect the retention of bioactive compounds and colour stability in fruit juices and especially Aronia juice.

Therefore, the aim of this study was to evaluate the impact of three different positively charged clarifying agents: gelatin-based (Erbigel) and two plant-based agents (Litto Fresh and Flora Claire) in combination with two negatively charged agents (Klar Super, Klar Sol30) on the quality of Aronia juice. The juice was evaluated in terms of total anthocyanin content (TAC), total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH), and colour properties.

Material and Methods

Plant material

Aronia (variety of Nero) was collected near Lajosmizse (47°02'44.4"N 19°35'14.8"E), Hungary in 2022 in full ripening stage. The sample was stored at -18 °C until further processing and analysis.

Sample preparation

Aronia juice was prepared using a laboratory pressing machine and treated enzymatically with Pectinex Ultra Colour. The juice was then heated in a water bath at 80–85°C and cooled to 45°C. It was divided into seven samples: one control (without clarifying agents) and six, treated with three positively charged agents gelatin-based (Erbigel) and plant-based (Litto Fresh, Flora Claire) in combination with two negatively charged agents silica acid-based (Klar Super, Klar Sol30). Samples were analysed for total anthocyanin content (TAC), total polyphenol content (TPC), 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH), and colour parameters. All treatments and measurements were conducted in triplicate.

Total anthocyanins content (TAC)

Total anthocyanin content was determined using the pH differential method, following the Association of Official Agricultural Chemists (AOAC) Method 2005.07 (AOAC, 2023). Absorbance was measured spectrophotometrically at 520 nm and 700 nm using pH 1.0 and pH 4.5 buffer solutions. Results were expressed as mg cyanidin-3-glucoside equivalents per L (mg CGE/L).

Total phenolic content (TPC)

The amount of total phenolic content was determined by following the colourimetric method, as described by Singleton and Rossi (1965). The absorbance of the samples was measured against the blank solution at 760 nm with a spectrophotometer (Hitachi U-2900, Budapest, Hungary). The results are given in mg gallic acid equivalents per L (mg GAE/L).

2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH)

DPPH was determined according to the binding of the stable DPPH radical method of Blois (1958). In short, samples (40 µl) were mixed with distilled water and the DPPH solution and left to react in the dark for 30 minutes before absorbance measurements. The absorbance of the samples was measured against distilled water as a blank sample at 517 nm. The absorbance was measured at 517 nm. The results are given as mg/L

Colour measurement

Colour coordinates were determined according to C.I.E.LAB system using a digital tristimulus colourimeter (Konica Minolta CR 410, Minolta Canada Inc.). The colour difference (ΔE*) was calculated by following equation:

$$\Delta E_{1;2}^{*} = \sqrt[2]{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2}$$

where L1* and L2 * are lightness of sample and control, respectively; a1* and a2 * are redness of sample and control, respectively; b1* and b2 * are yellowness of sample and control, respectively.

The noticeability in the colour change (ΔE*) was defined as classified by Lukács, 1982 as follows: 0–0.5 = not noticeable, 0.5–1.5 = slightly noticeable, 1.5–3.0 = noticeable, 3.0–6.0 = clearly visible, and 6.0–12.0 = great visibility.

Statistical analysis

The obtained results underwent statistical analysis using IBM SPSS statistics software version 27 (IBM Corp., New York, NY 10022, USA, 2020), and mean differences between factors were assessed using one-way analysis of variances (ANOVA). Significant differences were considered if P-value<0.05.

Results and discussion

Total anthocyanins content (TAC)

The results presented in Figure 1. showed no significant difference (P>0.05) in total anthocyanin content (TAC) between the control (44.88 mg/L) and the treated samples. However, some treatments exhibited slight increases in TAC, with percentage increases ranging from 4.70% to 12.86%. Notably, CA3 (Klar Super + Flora Claire) and CA4 (Klar Sol30 + Erbigel) showed the highest increases of 12.86%, while CA6 (Klar Sol30 + Flora Claire), CA5 (Klar Sol30 + Litto Fresh), CA1 (Klar Super + Erbigel), and CA2 (Klar Super + Litto Fresh) demonstrated increases of 12.46%, 6.69%, and 4.70%, respectively. Despite these variations, the differences were not statistically significant, suggesting that the clarifying agents did not substantially impact the anthocyanin content in Aronia juice.

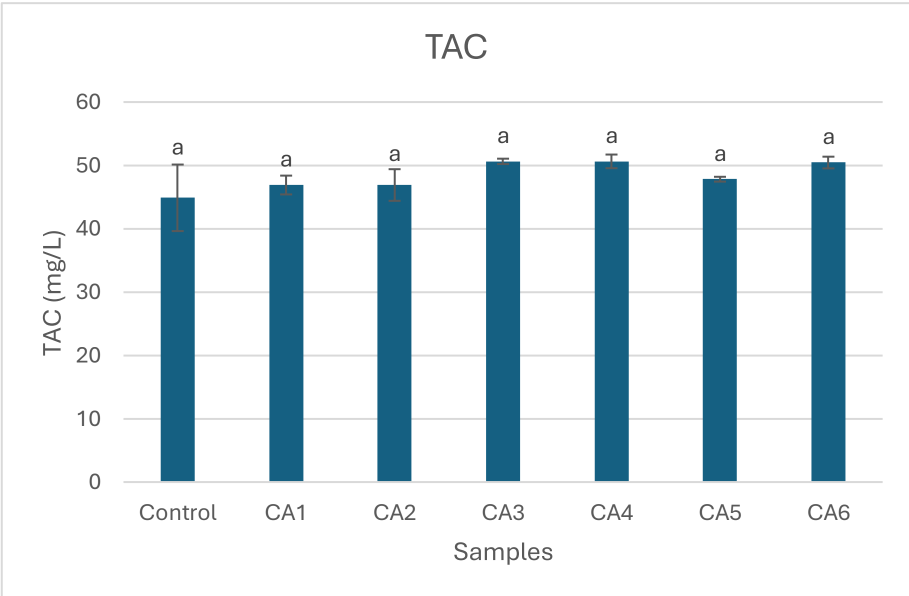

Figure1. Total Anthocyanins Content (TAC), Different letters denote significantly different groups. CA1: Klar Super+ Erbigel, CA2: Klar Super +Litto fresh, CA3: Klar Super +Flora claire. CA4: KLar Sol30 +Erbigel, CA5: Klar Sol30 + Litto fresh, CA6: Klar Sol30 + Flora claire.

Total phenolic content (TPC)

The results shown in Figure 2. indicated no significant difference (P>0.05) in total phenolic content (TPC) between the control (6997.06 mg/L) and the treated samples. Some treatments exhibited slight increases in TPC, with percentage increases ranging from 7.26% to 12.02%. Specifically, CA1 (Klar Super + Erbigel) and CA4 (Klar Sol30 + Erbigel) showed the highest increases of 12.02% and 11.57%, respectively, while CA2 (Klar Super + Litto Fresh), CA3 (Klar Super + Flora Claire), CA5 (Klar Sol30 + Litto Fresh), and CA6 (Klar Sol30 + Flora Claire) demonstrated increases of 11.01%, 9.82%, 9.08%, and 7.26%, respectively. Despite these variations, the differences were not statistically significant, indicating that the phenolic composition of Aronia juice remained relatively stable regardless of the clarifying agents used.

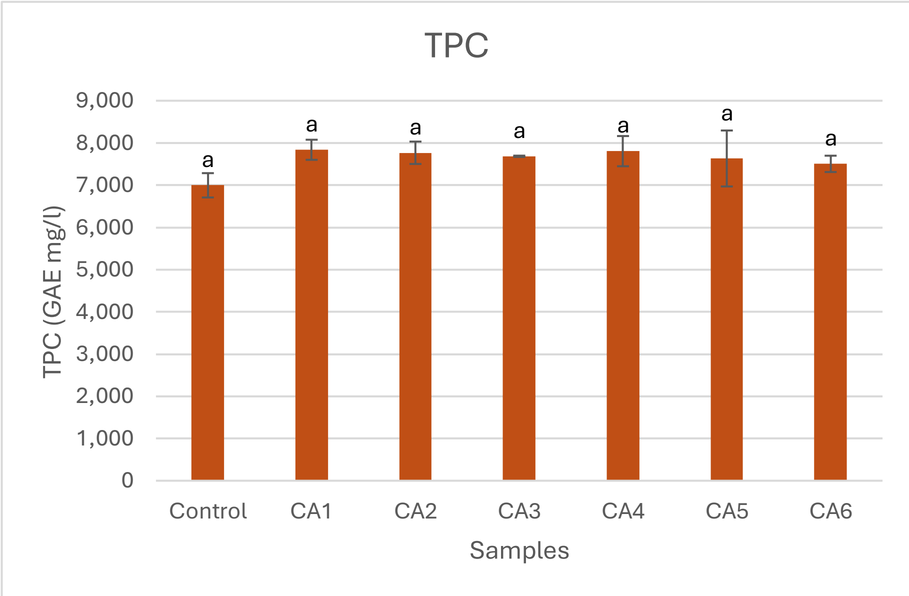

Figure2. Total Phenolic Content (TPC), Different letters denote significantly different groups. CA1: Klar Super+ Erbigel, CA2: Klar Super +Litto fresh, CA3: Klar Super +Flora claire. CA4: KLar Sol30 +Erbigel, CA5: Klar Sol30 + Litto fresh, CA6: Klar Sol30 + Flora claire.

2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH)

The results shown in Figure 3 indicated significant differences ($P<0.05$) in DPPH levels among the various clarifying agents used in Aronia juice. Notably, CA5 (Klar Sol30 + Litto Fresh) and CA1 (Klar Super + Erbigel) exhibited significantly lower antioxidant activity, with decreases of 13.73% and 12.52%, respectively, compared to the control (7305.93 mg/L). This suggests that these clarifying agents may not be as effective in preserving the antioxidant properties of Aronia juice. In contrast, CA3 (Klar Super + Flora Claire) showed a slight increase of 0.42%, while CA2 (Klar Super + Litto Fresh) displayed a marginal decrease of 1.50%, and CA4 (Klar Sol30 + Erbigel) and CA6 (Klar Sol30 + Flora Claire) showed decreases of 3.23% and 5.12%, respectively. Despite these variations, the control, and Klar Super +Litto fresh, Klar Super +Flora claire treatments displayed relatively higher and similar antioxidant activity, indicating that these agents either maintain or slightly improve the juice's antioxidant potential. These findings underscore the importance of selecting appropriate clarifying agents based on their ability to preserve or enhance the bioactive properties of Aronia juice, particularly its antioxidant potential.

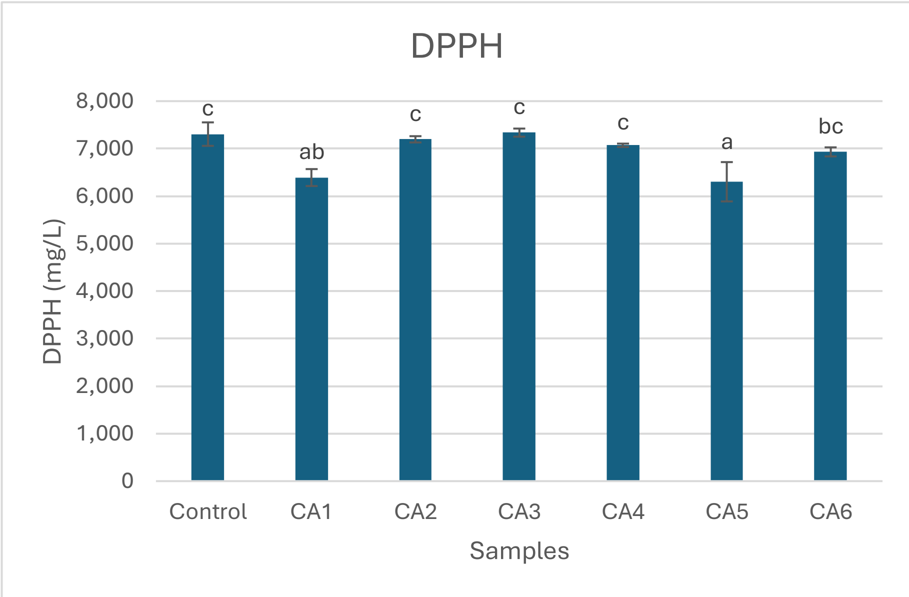

Figure3. 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH), Different letters denote significantly different groups. CA1: Klar Super+ Erbigel, CA2: Klar Super +Litto fresh, CA3: Klar Super +Flora claire. CA4: KLar Sol30 +Erbigel, CA5: Klar Sol30 + Litto fresh, CA6: Klar Sol30 + Flora claire.

Colour measurement

Figure 4. presents the colour difference values (ΔE*), derived from the L*, a*, and b* parameters, comparing the control sample to those treated with various clarifying agents. Colour differences were interpreted based on the visibility thresholds described in the *Materials and Methods* section. The results revealed that treatments with Klar Super+ Litto Fresh, Klar Super +Flora Claire, Klar Sol30+ Litto Fresh, and Klar Sol30 + Flora Claire all plant-based agents resulted in great visibility colour differences compared to the control (ΔE* > 6). Additionally, a clearly visible colour difference (ΔE* ranging from 3 to 6) was observed in samples treated with Klar Super+ Erbigel and Klar Sol30+ Erbigel, the gelatin-based agents. These findings indicate that all tested clarifying agents had a measurable impact on juice colour, with plant-based treatments producing the most pronounced changes.

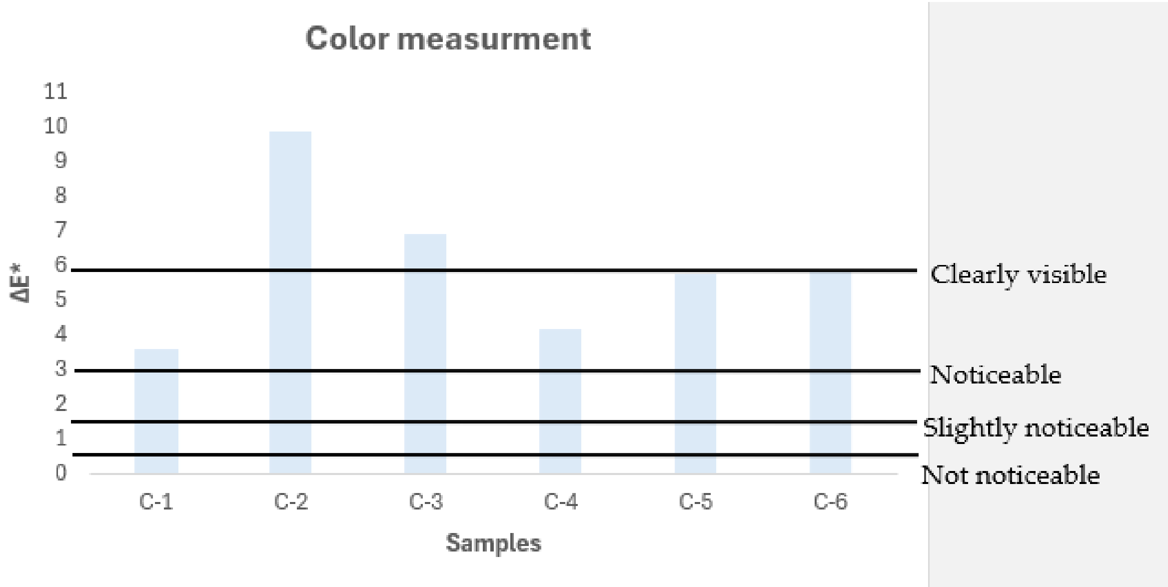

Figure4. Colour differences between the control samples and the samples treated with the claryfing agents. CA1: Klar Super+ Erbigel, CA2: Klar Super +Litto fresh, CA3: Klar Super +Flora claire. CA4: KLar Sol30 +Erbigel, CA5: Klar Sol30 + Litto fresh, CA6: Klar Sol30 + Flora claire.

Conclusion

In conclusion, these findings demonstrate that while the use of clarifying agents effectively preserves total anthocyanins and phenolics in Aronia juice, some agents like Erbigel can reduce antioxidant activity. All treatments led to perceptible colour changes, with plant-based agents causing more noticeable differences. Notably, plant-based agents (Litto Fresh and Flora Claire) proved to be more effective than the gelatin-based Erbigel in maintaining antioxidant activity while also producing more pronounced colour alterations. This suggests that although clarification improves clarity and visual appeal, the choice of agent has a significant impact on the nutritional and sensory qualities of the juice. Careful selection is therefore essential to balance clarity with the preservation of health-promoting properties, especially for premium juice markets.

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